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NIDA 20528

Food and Drug Administration
Rockville MD 20857

APR 26 1996

NDA 20-528

Knoll Pharmaceutical Company
Attention: Robert W. Ashworth, Ph.D.
3000 Continental Drive - North
Mount Olive, NJ 07828-1234

Dear Dr. Ashworth:

Please refer to your September 28, 1994 new drug application (NDA) submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Mavik (trandolapril) 1, 2, and 4 mg Tablets.

We acknowledge receipt of your correspondence and amendments dated October 23 and December 1, 1995; January 4, February 7, 20, and 23, March 15, 19, and 22, and April 4 (two), 16, 18, 19 (two), and 22, 1996.

The new drug application provides for the use of trandolapril in the treatment of hypertension.

We have completed the review of this application, including the submitted draft labeling, and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the enclosed marked-up draft. Accordingly, the application is approved effective on the date of this letter.

The final printed labeling (FPL) must be identical to the enclosed marked-up draft labeling. Marketing the product with FPL that is not identical to this draft labeling may render the product misbranded and an unapproved new drug.

Please submit sixteen copies of the FPL as soon as it is available, in no case more than 30 days after it is printed. Please individually mount ten of the copies on heavy weight paper or similar material. For administrative purposes this submission should be designated "FINAL PRINTED LABELING" for approved NDA 20-528. Approval of this submission by FDA is not required before the labeling is used.

Should additional information relating to the safety and effectiveness of the drug become available, revision of that labeling may be required.

Validation of the regulatory methods has not been completed. At the present time, it is the policy of the Center not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any problems that may be identified.

We note that you have agreed to an interim dissolution specification of Q= in 45 minutes. Please continue your stability studies with sampling at 30 and 45 minutes.

Please submit one market package of the drug when it is available.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions, please contact:

Ms. Kathleen Bongiovanni
Regulatory Health Project Manager
(301) 594-5334

Sincerely yours,

Robert Temple, M.D.
Director
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Enclosure

at

Food and Drug Administration
Rockville MD 20857

NDA 20-528

SEP 25 1995

Knoll Pharmaceutical Company
Attention: Robert W. Ashworth, Ph.D.
30 North Jefferson Road
Whippany, NJ 07981

Dear Dr. Ashworth:

Please refer to your September 28, 1994 new drug application submitted on September 29, 1994 under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Mavic (trandolapril) 1, 2, and 4 mg Tablets.

We acknowledge receipt of your amendments and correspondence dated September 27, October 11 and 25, November 23 and 28 (two), December 8, 13 (two), 19, 21, and 29, 1994; January 4, 5, 11, 13 (two), 17, 19, 24 and 31, February 6 and 7, March 8, 13, 14, 15, 17, 20, 22 and 23, April 7, 13, 24, 26 and 28, May 2 (two), 18 and 24, June 1 and 30, July 7 and 13, August 16, 28, and 29, 1995.

We have completed the review of this application as submitted with draft labeling and it is approvable. Before the application may be approved, however, it will be necessary for you to submit the following information:

It will also be necessary for you to submit final printed labeling (FPL) for the drug. The labeling should be identical in content to the enclosed draft. If additional information relating to the safety or effectiveness of this drug becomes available, revision of the FPL may be required.

Please note that we have recently reviewed data on adverse experiences involving pemphigus reported with the use of ACE inhibitors and we recommend that the **ADVERSE REACTIONS** section of the package inserts of ACE inhibitor products be revised to include pemphigus; this is included in the enclosed draft.

The review of these cases by the Reports Evaluation Branch of the Office of Epidemiology and Biostatistics is enclosed.

Please submit fifteen copies of the printed labels and other labeling, ten of which are individually mounted on heavy weight paper or similar material.

In addition, please submit three copies of the introductory promotional material that you propose to use for this product. All proposed materials should be submitted in draft or mock-up form, not final print. Please send one copy to the Division of Cardio-Renal Drug Products and two copies of both the promotional material and the package insert directly to:

Food and Drug Administration
Division of Drug Marketing, Advertising and Communications, HFD-240
5600 Fishers Lane
Rockville, Maryland 20857

Within 10 days after the date of this letter, you are required to amend the application, notify us of your intent to file an amendment, or follow one of your other options under 21 CFR 314.110. In the absence of such action FDA may take action to withdraw the application.

The drug may not be legally marketed until you have been notified in writing that the application is approved.

Should you have any questions, please contact:

Ms. Kathleen Bongiovanni
Regulatory Health Project Manager
Telephone: (301) 594-5334

Sincerely yours,

Robert Temple, M.D.
Director
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Enclosures

FINAL PRINTED LABELING HAS NOT BEEN SUBMITTED TO THE FDA.

DRAFT LABELING IS NO LONGER BEING SUPPLIED SO AS TO ENSURE
ONLY CORRECT AND CURRENT INFORMATION IS DISSEMINATED TO THE
PUBLIC.

K. Bongiovanni

NDA 20-528
HFD-110
HFD-110/RWolters/JShorr
HFD 111 / K.Bongiovanni

DEC 29 1994
Consult # 382

REQUEST FOR TRADEMARK REVIEW

TO: Labeling and Nomenclature Committee
Attention: Ms. Yana Mille, Chair, (HFD-500) MPN II

FROM: Division of Cardio-Renal Drug Products HFD- 110
Attention: Robert Walters Phone 4-5376

DATE: ~~December 1, 1994~~

SUBJECT: Request for Assessment of a Trademark for a Proposed Drug Product

Proposed Trademark: Mavik NDA/~~XXXXX~~ 20-528

Company Name: Knoll

Established name, including dosage form: Trandolapril Tablets
1 mg, 2 mg, & 4 mg

Other trademarks by the same firm for companion products:

Indications for Use (may be a summary if proposed statement is lengthy):
Treatment of hypertension ACE inhibitor

Initial comments from the submitter: (concerns, observations, etc.)

NOTE: Meetings of the Committee are scheduled for the 4th Tuesday of the month. Please submit this form at least one week ahead of the meeting. Responses will be as timely as possible.

Consult #382 (HFD-110)

MAVIK

Trandolapril Tablets
1 mg, 2 mg, and 4 mg

A review revealed no names which sound like or look like the proposed name.

Other than the concern described above, the Committee has no reason to find the proposed name unacceptable.

CDER Labeling and Nomenclature Committee

Jana Ruth Miller, Chair 12/20/94

COMPLETED

Trade Name Mavac Tablets Generic Name trandolapril

Applicant Name Knoll Pharmaceuticals HFD # 110

Approval Date If Known _____

PART I IS AN EXCLUSIVITY DETERMINATION NEEDED?

1. An exclusivity determination will be made for all original applications, but only for certain supplements. Complete PARTS II and III of this Exclusivity Summary only if you answer "yes" to one or more of the following question about the submission.

a) Is it an original NDA? YES / / NO / /

b) Is it an effectiveness supplement? YES / / NO / /

If yes, what type? (SE1, SE2, etc.) _____

c) Did it require the review of clinical data other than to support a safety claim or change in labeling related to safety? (If it required review only of bioavailability or bioequivalence data, answer "no.")

YES / / NO / /

* If your answer is "no" because you believe the study is a bioavailability study and, therefore, not eligible for exclusivity, EXPLAIN why it is a bioavailability study, including your reasons for disagreeing with any arguments made by the applicant that the study was not simply a bioavailability study.

If it is a supplement requiring the review of clinical data but it is not an effectiveness supplement, describe the change or claim that is supported by the clinical data:

d) Did the applicant request exclusivity?

YES / / NO / /

If the answer to (d) is "yes," how many years of exclusivity did the applicant request?

IF YOU HAVE ANSWERED "NO" TO ALL OF THE ABOVE QUESTIONS, GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. Has a product with the same active ingredient(s), dosage form, strength, route of administration, and dosing schedule, previously been approved by FDA for the same use?

YES / / NO / /

If yes, NDA # _____ Drug Name _____.

IF THE ANSWER TO QUESTION 2 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

3. Is this drug product or indication a DESI upgrade?

YES / / NO / /

IF THE ANSWER TO QUESTION 3 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8 (even if a study was required for the upgrade).

PART II FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES

(Answer either #1 or #2 as appropriate)

1. Single active ingredient product.

Has FDA previously approved under section 505 of the Act any drug product containing the same active moiety as the drug under consideration? Answer "yes" if the active moiety (including other esterified forms, salts, complexes, chelates or clathrates) has been previously approved, but this particular form of the active moiety, e.g., this particular ester or salt (including salts with hydrogen or coordination bonding) or other non-covalent derivative (such as a complex, chelate, or clathrate) has not been approved. Answer "no" if the compound requires metabolic conversion (other than deesterification of an esterified form of the drug) to produce an already approved active moiety.

YES / / NO / /

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA # (s).

NDA# _____
NDA# _____
NDA# _____

2. Combination product.

If the product contains more than one active moiety (as defined in Part II, #1), has FDA previously approved an application under section 505 containing any one of the active moieties in the drug product? If, for example, the combination contains one never-before-approved active moiety and one previously approved active moiety, answer "yes." (An active moiety that is marketed under an OTC monograph, but that was never approved under an NDA, is considered not previously approved.)

YES / ___ / NO / ___ /

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA # (s).

NDA# _____
NDA# _____
NDA# _____

IF THE ANSWER TO QUESTION 1 OR 2 UNDER PART II IS "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8. IF "YES" GO TO PART III.

PART III THREE-YEAR EXCLUSIVITY FOR NDA'S AND SUPPLEMENTS

To qualify for three years of exclusivity, an application or supplement must contain "reports of new clinical investigations (other than bioavailability studies) essential to the approval of the application and conducted or sponsored by the applicant." This section should be completed only if the answer to PART II, Question 1 or 2 was "yes."

1. Does the application contain reports of clinical investigations? (The Agency interprets "clinical investigations" to mean investigations conducted on humans other than bioavailability studies.) If the application contains clinical investigations only by virtue of a right of reference to clinical investigations in another application, answer "yes," then skip to question 3(a). If the answer to 3(a) is "yes" for any investigation referred to in another application, do not complete remainder of summary for that investigation.

YES /___/ NO /___/

IF "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. A clinical investigation is "essential to the approval" if the Agency could not have approved the application or supplement without relying on that investigation. Thus, the investigation is not essential to the approval if 1) no clinical investigation is necessary to support the supplement or application in light of previously approved applications (i.e., information other than clinical trials, such as bioavailability data, would be sufficient to provide a basis for approval as an ANDA or 505(b)(2) application because of what is already known about a previously approved product), or 2) there are published reports of studies (other than those conducted or sponsored by the applicant) or other publicly available data that independently would have been sufficient to support approval of the application, without reference to the clinical investigation submitted in the application.

(a) In light of previously approved applications, is a clinical investigation (either conducted by the applicant or available from some other source, including the published literature) necessary to support approval of the application or supplement?

YES /___/ NO /___/

If "no," state the basis for your conclusion that a clinical trial is not necessary for approval AND GO DIRECTLY TO SIGNATURE BLOCK ON PAGE 8:

(b) Did the applicant submit a list of published studies relevant to the safety and effectiveness of this drug product and a statement that the publicly available data would not independently support approval of the application?

YES /___/ NO /___/

(1) If the answer to 2(b) is "yes," do you personally know of any reason to disagree with the applicant's conclusion?

YES /___/ NO /___/

If yes, explain: _____

(2) If the answer to 2(b) is "no," are you aware of published studies not conducted or sponsored by the applicant or other publicly available data that could independently demonstrate the safety and effectiveness of this drug product?

YES /___/ NO /___/

If yes, explain: _____

(c) If the answers to (b)(1) and (b)(2) were both "no," identify the clinical investigations submitted in the application that are essential to the approval:

Studies comparing two products with the same ingredient(s) are considered to be bioavailability studies for the purpose of this section.

3. In addition to being essential, investigations must be "new" to support exclusivity. The agency interprets "new clinical investigation" to mean an investigation that 1) has not been relied on by the agency to demonstrate the effectiveness of a previously approved drug for any indication and 2) does not duplicate the results of another investigation that was relied on by the agency to demonstrate the effectiveness of a previously approved drug product, i.e., does not redemonstrate something the agency considers to have been demonstrated in an already approved application.

a) For each investigation identified as "essential to the approval," has the investigation been relied on by the agency to demonstrate the effectiveness of a previously approved drug product? (If the investigation was relied on only to support the safety of a previously approved drug, answer "no.")

Investigation #1 YES /___/ NO /___/

Investigation #2 YES /___/ NO /___/

If you have answered "yes" for one or more investigations, identify each such investigation and the NDA in which each was relied upon:

b) For each investigation identified as "essential to the approval", does the investigation duplicate the results of another investigation that was relied on by the agency to support the effectiveness of a previously approved drug product?

Investigation #1 YES /___/ NO /___/

Investigation #2 YES /___/ NO /___/

If you have answered "yes" for one or more investigation, identify the NDA in which a similar investigation was relied on:

c) If the answers to 3(a) and 3(b) are no, identify each "new" investigation in the application or supplement that is essential to the approval (i.e., the investigations listed in #2(c), less any that are not "new"):

4. To be eligible for exclusivity, a new investigation that is essential to approval must also have been conducted or sponsored by the applicant. An investigation was "conducted or sponsored by" the applicant if, before or during the conduct of the investigation, 1) the applicant was the sponsor of the IND named in the form FDA 1571 filed with the Agency, or 2) the applicant (or its predecessor in interest) provided substantial support for the study. Ordinarily, substantial support will mean providing 50 percent or more of the cost of the study.

a) For each investigation identified in response to question 3(c): if the investigation was carried out under an IND, was the applicant identified on the FDA 1571 as the sponsor?

Investigation #1 !
 IND # _____ YES /___/ ! NO /___/ Explain: _____
 !
 ! _____

Investigation #2 !
 IND # _____ YES /___/ ! NO /___/ Explain: _____
 !
 ! _____

(b) For each investigation not carried out under an IND or for which the applicant was not identified as the sponsor, did the applicant certify that it or the applicant's predecessor in interest provided substantial support for the study?

Investigation #1 !
 YES /___/ Explain _____ ! NO /___/ Explain _____
 !
 ! _____
 ! _____

Investigation #2 !
 YES /___/ Explain _____ ! NO /___/ Explain _____
 !
 ! _____
 ! _____

(c) Notwithstanding an answer of "yes" to (a) or (b), are there other reasons to believe that the applicant should not be credited with having "conducted or sponsored" the study? (Purchased studies may not be used as the basis for exclusivity. However, if all rights to the drug are purchased (not just studies on the drug), the applicant may be considered to have sponsored or conducted the studies sponsored or conducted by its predecessor in interest.)

YES / ___ / NO / ___ /

If yes, explain: _____

Kathleen F. Bruggemann
Signature
Title: Regulatory Health
Project Manager

May 5, 1995
Date

Lepisky
Signature of Office/
Division Director

9/4/95
Date

cc: Original NDA

Division File

HFD-85 Mary Ann Ward

EXCLUSIVITY DETERMINATION CHECKLIST

NDA # _____ SUPPL. # _____ APPLICANT _____ TR. NAME _____

ACTIVE INGRED. _____ POTENCY _____ DOSAGE FORM/ROUTE _____

APPROVAL DATE _____

TYPE OF APPLICATION: FULL NDA _____ 505(b) (2) _____ EFFIC. SUPP. _____ OTHER (SPECIFY) _____

EXCLUSIVITY REQUESTED: 5 YR _____ 3 YR _____ NONE _____

QUALIFICATIONS FOR 5 YR EXCLUSIVITY:

Approved for NCE, no salt or ester of which previously approved

QUALIFICATIONS FOR 3 YR EXCLUSIVITY:

Approval based on clinical study (other than B10)?	Y _____	N _____	
New Studies:			
Previously relied on by Agency for efficacy?		Y _____	N _____
Essential for Approval:			
Approval could have been based on literature?		Y _____	N _____
Previously approved in another application?		Y _____	N _____
Studies conducted by or for applicant:			
Not sponsored by applicant?	Y _____	N _____	
Confirmation of principal sponsor?	Y _____	N _____	

NOTE: If any checks appear in shaded area, it is likely that exclusivity should not be granted. Any exclus. recommendations should be explained below:

EXCLUSIVITY RECOMMENDED: 5 YR _____ 3 YR _____ NONE _____

CONCUR _____
NON CONCUR _____)

SIGNED _____
DIRECTOR, OFFICE OF GENERIC DRUGS

000001

MAVIK® (Trandolapril) NDA 20-528

Patent Information

For the purpose of assisting FDA in the publication of patent information relative to trandolapril, Knoll Pharmaceutical Company certifies that the primary U.S. patent covering this new chemical entity was issued on June 12, 1990 and was assigned Patent Number 4,933,361. A copy of the patent is provided herein.

[54] DERIVATIVES OF BICYCLIC AMINOACIDS AGENTS CONTAINING THESE COMPOUNDS AND THEIR USE

[75] Inventors: Hansjörg Urbach, Kronberg/Tannus; Rainer Henning, Frankfurt am Main; Volker Teetz, Hofheim am Taunus; Rolf Geiger, Frankfurt am Main; Reinhard Becker, Wiesbaden; Holger Gaul, Niederneisen, all of Fed. Rep. of Germany

[73] Assignee: Hoechst Aktiengesellschaft, Frankfurt am Main, Fed. Rep. of Germany

[21] Appl. No.: 346,339

[22] Filed: Apr. 28, 1989

Related U.S. Application Data

[63] Continuation of Ser. No. 453,092, Dec. 27, 1982, abandoned.

[30] Foreign Application Priority Data

Dec. 29, 1981 [DE] Fed. Rep. of Germany 3151690
Mar. 24, 1982 [DE] Fed. Rep. of Germany 3210701

[51] Int. Cl.³ A61K 31/405; C07D 209/12

[52] U.S. Cl. 514/419; 548/486; 548/492; 546/158; 546/164

[58] Field of Search 548/492; 514/419

[56] References Cited

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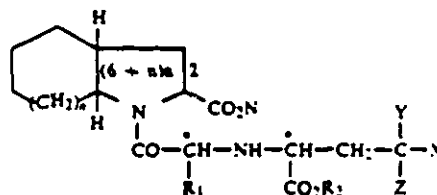
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(List continued on next page.)

Primary Examiner—Glennon H. Hollrah
Assistant Examiner—James H. Turnipseed
Attorney, Agent, or Firm—Curtis, Morris & Safford

[57] ABSTRACT

Disclosed are cis, exo- and trans-compounds of the formula I



in which n denotes 0, 1 or 2. R₁ denotes hydrogen, (C₁-C₆)-alkyl which can optionally be substituted by amino, (C₁-C₄)-acyl- or bezoylamino, (C₂-C₆)-alkenyl, (C₃-C₆)-cycloalkyl, (C₃-C₆)-cycloalkenyl, (C₃-C₇)-cycloalkyl-(C₁-C₄)-alkyl, aryl or partially hydrogenated aryl, which can, in each case, be substituted by (C₁-C₂)-alkyl, (C₁-C₂)-alkoxy or halogen, aryl-(C₁-C₄)-alkyl, /which can be substituted as defined previously in the aryl radical/, a monocyclic or bicyclic sulfur or nitrogen and/or nitrogen heterocyclic radical, or a side chain of naturally occurring aminoacid. R₂ denotes hydrogen, (C₁-C₆)-alkyl, (C₂-C₆)-alkenyl or aryl-(C₁-C₄)-alkyl, Y denotes hydrogen or hydroxyl. Z denotes hydrogen or Y and Z together denote oxygen. X denotes (C₁-C₆)-alkyl, (C₂-C₆)-alkenyl, (C₂-C₆)-cycloalkyl or aryl which can be mono-, di- or tri- substituted by (C₁-C₄)-alkyl, (C₁-C₄)-alkoxy, hydroxyl, halogen, nitro, amino, (C₁-C₄)-alkylamino, di-(C₁-C₄)-alkylamino or methylenedioxy, or indol-3-yl, or physiologically acceptable salts thereof, a process of the preparation thereof, agents containing them, their use as a medicine and intermediates for the preparation thereof.

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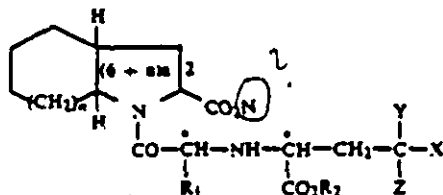
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DERIVATIVES OF BICYCLIC AMINOACIDS
AGENTS CONTAINING THESE COMPOUNDS
AND THEIR USE

This application is a continuation of application Ser. No. 453,092, filed Dec. 27, 1982, now abandoned.

The present invention relates to new derivatives of the bicyclic aminoacids of the formula I



in which the hydrogen atoms on the bridgehead C atoms 3a and (6+n)a have the cis or trans configuration relative to one another, wherein, in the case of the cis configuration, the carboxyl group on C atom 2 is oriented exo to the bicyclic ring system and wherein n denotes 0, 1 or 2, R₁ denotes hydrogen, (C₁-C₆)-alkyl which can optionally be substituted by amino, (C₁-C₄)-acylamino or benzoylamino, (C₂-C₆)-alkenyl, (C₅-C₉)-cycloalkyl, (C₅-C₉)-cycloalkenyl, (C₅-C₇)-cycloalkyl-, (C₁-C₄)-alkyl, aryl or partially hydrogenated aryl, which can, in each case, be substituted by (C₁-C₂)-alkyl, (C₁-C₂)-alkoxy or halogen, aryl-(C₁-C₄)-alkyl, [the aryl radical of which can be substituted as defined previously], a monocyclic or bicyclic heterocyclic radical having 5 to 7 or 8 to 10 ring atoms respectively, of which 1 to 2 ring atoms are sulfur or oxygen atoms and/or of which 1 to 4 ring atoms are nitrogen atoms, or a side chain of a naturally occurring amino acid. R₂ denotes hydrogen, (C₁-C₆)-alkyl, (C₂-C₆)-alkenyl or aryl-(C₁-C₄)-alkyl. Y denotes hydrogen or hydroxyl. Z denotes hydrogen or Y and Z together denote oxygen. X denotes (C₁-C₆)-alkyl, (C₂-C₆)-alkenyl, (C₅-C₉)-cycloalkyl or aryl which can be mono-, di- or tri-substituted by (C₁-C₄)-alkyl, (C₁-C₄)-alkoxy, hydroxyl, halogen, nitro, amino, (C₁-C₄)-alkylamino, di-(C₁-C₄)-alkylamino or methylenedioxy, or indol-3-yl, and their physiologically acceptable salts.

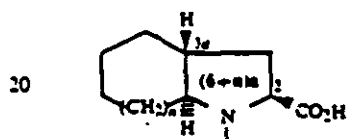
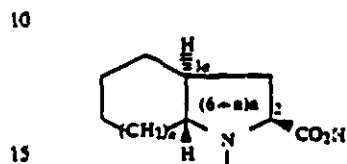
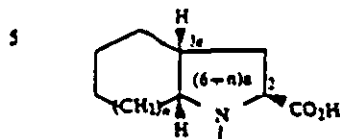
Particularly suitable salts are alkali metal and alkaline earth metal salts, salts with physiologically tolerated amines and salts with inorganic or organic acids such as, for example, HCl, HBr, H₂SO₄, maleic acid and fumaric acid.

In this context and in the following text, aryl is to be understood as meaning phenyl or naphthyl. Alkyl can be straight-chain or branched.

In the case of the trans configuration of the H atoms on C-3a and C-(6+n)a, there are two possible configurations of the bicycle, and these are the 2β, 3α, (6+n)αβ configuration (part-formula Ib) and the 3β, 3aβ, (6+n)αα configuration (part-formula Ic), whilst in the case of the cis configuration of the H atoms, the carboxyl group must be oriented in the exo-position (=β-position).

The exo-position (=β-position) of the carboxyl group on C-2 is defined so that the carboxyl group is oriented in the direction of the relevant H atoms, i.e. faces away from the concave side of the bicycle. For example corresponding to part-formula Ia. (For the

definition of α and β, cf. Fieser and Fieser, Steroids, page 2, 1961).



Compounds of the formula I have chiral C atoms in positions C-2, C-3a, C-(6+n)a, and in the C atoms labeled with an asterisk in the side chain. The invention relates to both the R and also the S configurations at all centers. The compounds of the formula I can thus be in the form of optical isomers, as diastereomers, as racemates or as mixtures thereof. Compounds of the formula I are preferred in which the C atom 2 in the bicyclic ring system and the C atoms labeled with an asterisk in the side chain have the S configuration.

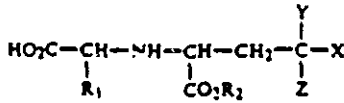
Preferred compounds of the formula I are those in which R₁ denotes hydrogen, (C₁-C₃)-alkyl, (C₂-C₃)-alkenyl, benzyl or 4-aminobutyl, R₂ denotes hydrogen, (C₁-C₄)-alkyl or benzyl and X denotes phenyl which can be mono- or disubstituted or, in the case of methoxy, tri-substituted by (C₁-C₂)-alkyl, (C₁-C₂)-alkoxy, hydroxyl, fluorine, chlorine, bromine, amino, (C₁-C₄)-alkylamino, di-(C₁-C₄)-alkylamino, nitro or methylenedioxy.

Compounds of the formula I are particularly preferred in which R₁ denotes methyl and X denotes phenyl and in which R₂ denotes hydrogen or ethyl.

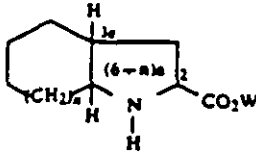
Compounds of the formula I to which special attention is drawn are N-(1S-carboethoxy-3-phenyl-propyl)-S-alanyl-2S,3aR,6aR-octahydrocyclopenta[b]pyrrole-2-carboxylic acid, N-(1S-carboxy-3-phenyl-propyl)-S-alanyl-2S,3aR,7aR-octahydroindole-2-carboxylic acid, N-(1S-carboxy-3-phenyl-propyl)-S-alanyl-2S,3aR,6aR-octahydrocyclopenta[b]pyrrole-2-carboxylic acid, N-(1S-carboxy-3-phenyl-propyl)-S-alanyl-2S,3aR,7aS-octahydroindole-2-carboxylic acid and N-(1S-carboxy-3-phenyl-propyl)-S-alanyl-2S,3aS,7aR-octahydroindole-2-carboxylic acid, but especially N-(1S-carboethoxy-3-phenyl-propyl)-S-alanyl-2S,3aR,7aR-octahydroindole-2-carboxylic acid, N-(1S-carboethoxy-3-phenyl-propyl)-S-alanyl-2S,3aR,7aS-octahydroindole-2-carboxylic acid and N-(1S-carboethoxy-3-phenyl-propyl)-S-alanyl-2S,3aS,7aR-octahydroindole-2-carboxylic acid.

The invention further relates to processes for the preparation of the compounds of the formula I. One variant of the process comprises reacting, by methods for amide formation known in peptide chemistry, a compound of the formula II

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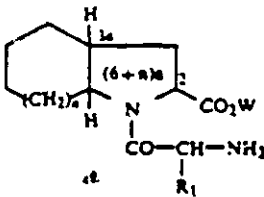


wherein R₁, R₂, X, Y and Z have the meanings as in formula I, with a compound of the formula III



in which the H atoms on the C atoms 3a and (6+n)a have the cis or trans configuration relative to one another, wherein, in the case of the cis configuration, the group —CO₂W is oriented exo to the bicyclic ring system and wherein n denotes 0, 1 or 2 and W denotes a radical which can be split off by hydrogenolysis or by acid, in particular a benzyl or a tert.-butyl radical, and then splitting off the radical W by catalytic hydrogenation or acid treatment and, where appropriate, also splitting off the radical R₂ by additional acid or base treatment, the free carboxylic acids being obtained in each case.

Further synthetic processes for the preparation of the compounds of the formula I, in which Y and Z together denote oxygen comprise reacting in a known manner in a Michael reaction (Organikum, 6th edition, page 492, 1967) a compound of the formula IV



wherein the H atoms on the C atoms 3a and (6+n)a have the cis or trans configuration relative to one another, wherein in the case of the cis configuration, the group —CO₂W is oriented exo to the bicyclic ring system, and wherein n and R₁ have the meanings as in formula I and W has the meaning as in formula III, with a compound of the formula V,



wherein R₂ and X have the meanings as in formula I, and splitting off the radical W and, if appropriate, the radical R₂ as described above, or comprise reacting in a known manner in a Mannich reaction (Bull. Soc. Chim. France 1973, page 625) a compound of the abovementioned formula IV with a compound of the general formula VI, wherein R₂ has the meaning as in formula I, and with a compound of the general formula VII

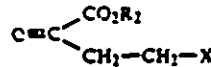


wherein X has the meaning as in formula I, and subsequently splitting off the radical W and, if appropriate, the radical R₂ as described above to form the free carboxyl groups.

II

Furthermore, compounds of the formula I with Y and Z being hydrogen can also be prepared in such a manner that a compound of the abovementioned formula IV is reacted in accordance with the procedure described in J. Amer. Chem. Soc. 93 2897 (1971) with a compound of the formula VIII

III



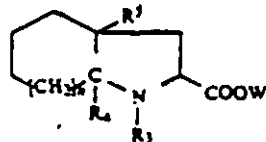
wherein R₂ and X have the meanings as in formula I, and the Schiff's bases obtained are reduced and subsequently the radical W and, if appropriate, the radical R₂ are split off as described above to form the free carboxyl groups, or that a compound of the formula I, in which Y and Z together denote oxygen, obtained according to the above procedures is reduced catalytically with hydrogen. The reduction of the Schiff's bases can be carried out catalytically, electrolytically or with reducing agents, such as, for example, sodium borohydride or sodium cyanoborohydride.

Compounds of the formula I with Y being hydroxyl and Z being hydrogen can also be obtained for example, by reduction of a compound I with Y and Z together being oxygen obtained according to the above procedure. This reduction can be carried out catalytically with hydrogen or with another reducing agent, such as, for example, sodium borohydride.

The invention further relates to compounds of the formula III', in which n represents a whole number from 0 to 10, W' represents hydrogen, alkyl having 1 to 18 C atoms or aralkyl having 7 to 10 C atoms and R₃, R₄ and/or R₅

IV

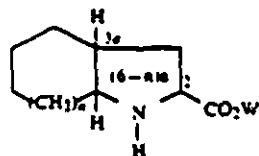
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represent hydrogen, or R₃ and R₄ or R₄ and R₅ together each denote a chemical bond.

Preferred compounds of the formula III' are compounds of the formula III''

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wherein C-2, C-3a and C-(6+n)a have the same configuration as in formula III, n denotes 0, 1 or 2 and W' has the meaning of W in formula III and also denotes hydrogen. According to the invention, these compounds serve as starting materials for the synthesis of compounds of the formula I and can be prepared according to the invention by the following procedures.

One synthetic variant starts with a compound of the formula IX

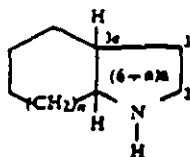
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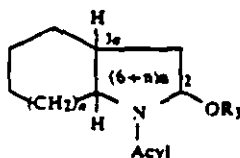
wherein i have the other, wh group — and when purificatu of recryst: drolized manner to W' = hyd: In particu HCl or H



wherein the H atoms on the C atoms 3a and (6+n)a have the cis or trans configuration relative to one another and wherein n denotes the number 0, 1 or 2.

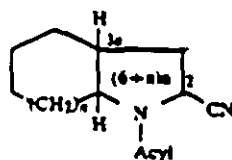
Compounds of the formula IX with n being 0 are known from Booth et al., *J. Chem. Soc.* 1959, page 1050, those with n being 1 are known from King et al., *J. Chem. Soc.* 1953, pages 250 and 253 and those with n being 2 are known from Ayerst et al., *J. Chem. Soc.* 1960, page 3445.

These compounds of the formula IX are acylated in a known manner, an aliphatic or aromatic acyl radical, preferably an acetyl or benzoyl radical, being bonded to the nitrogen atom, and the N-acylated compounds obtained are anodically oxidized (in analogy with Liebigs *Ann. Chem.* 1978 page 1719) in an aliphatic alcohol, preferably an alkanol having 1 to 4 C atoms, in particular methanol, in the presence of a conducting salt, preferably at temperatures in the range from 0° to -40° C. with formation of a compound of the formula X, wherein n denotes 0, 1 or 2 and



R₃ denotes (C₁-C₄)-alkyl.

The compound of the general formula X obtained is reacted with trimethylsilyl cyanide in accordance with Tetrahedron Letters 1981 page 141 in an aprotic organic solvent, such as, for example, in a hydrocarbon, halogenated hydrocarbon, in ether or in THF at temperatures in the range from -60° C. to +20° C., preferably -40° C. to ±0° C. in the presence of a Lewis acid, such as, for example, ZnCl₂, SnCl₂, SnCl₄, TiCl₄ or BF₃ etherate, preferably BF₃ etherate, and the compound of the formula XI obtained

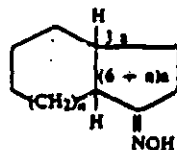


wherein the H atoms on the C atoms 3a and (6+n)a have the cis or trans configuration relative to one another, wherein in the case of the cis configuration, the group -CN is oriented exo to the bicyclic ring system and wherein n has the abovementioned meaning, after purification and separation from by-products by means of recrystallization or column chromatography, is hydrolyzed by the action of acid or bases in a known manner to give a compound of the formula III having W' = hydrogen, and the latter is esterified if appropriate. In particular, in the acid hydrolysis of the nitrile group, HCl or HBr is used as the acid. In this instance as in the

IX

following, the esterification is carried out by the procedures usual in amino-acid chemistry.

Compounds of the general formula III' can also be prepared by converting, in a Beckmann rearrangement analogous to *Helv. Chim. Acta* 46, 1190 (1963), a compound of the formula XII

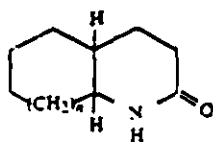


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wherein the H atoms on the C atoms 3a and (6+n)a have the cis or trans configuration and n has the abovementioned meaning, into a compound of the formula XIII, wherein n has the abovementioned meaning.

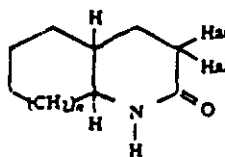
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and the latter is halogenated to give a compound of the formula XIV

X



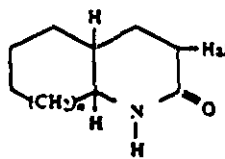
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wherein n has the abovementioned meaning and Hal denotes a halogen atom, preferably chlorine or bromine. Examples of suitable halogenating agents are halides of inorganic acid, such as PCl₅, SO₂Cl₂, POCl₃, SOCl₂, PBr₃ or halogens, such as bromine. It is advantageous to use PCl₅ or POCl₃ combined with SO₂Cl₂. The intermediate initially formed is an imide halide, which, with the halogenating agent mentioned and subsequent hydrolysis under basic conditions, preferably with aqueous alkali metal carbonate, reacts further to give a compound of the formula XIV.

XI

The compounds of the formula XIV are subsequently reduced catalytically in a protic solvent, such as, for example, an alcohol, preferably ethanol, or a carboxylic acid, such as, for example, acetic acid, with the addition of an acid acceptor, such as, for example, sodium acetate or triethylamine, to give a compound of the formula XV

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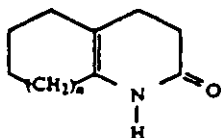


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wherein n and Hal have the abovementioned meanings. Examples of suitable catalysts are Raney nickel or platinum on animal charcoal. Compounds of the formula XV can also be prepared directly by halogenation of the compounds of the formula XIII by using smaller amounts of the abovementioned halogenating agent.

Compounds of the formula XV are converted, in accordance with the known Favorskii reaction in the presence of a base, into a compound of the formula III' with W' being hydrogen, and the latter is esterified if appropriate. The abovementioned Favorskii reaction is carried out in an alcoholic solvent, such as methanol, ethanol or tert.-butanol, or in water or in mixtures thereof at temperatures in the range from 20° to 140° C., preferably between 60° and 100° C. Bases which are advantageously employed are alkali metal or alkaline earth metal hydroxides, such as sodium, potassium or barium hydroxide or alkali metal alcoholates, such as, for example, sodium methylate or potassium tert.-butanolate.

Furthermore, the compounds of the formula III', wherein the H atoms on the C atoms 3a and (6-n)a have the cis configuration, can be prepared from the compound of the formula XVI, wherein a denotes 0, 1 or 2.

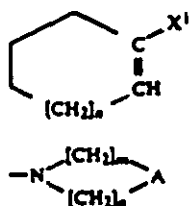


XVI

by reducing the latter by means of platinum oxide/acetic acid in accordance with Ann. Chim. 62, 200 (1972) to give a compound of the abovementioned formula XIII and reacting the latter in accordance with the procedures described above. Compounds of the formula XVI are known from J. Org. Chem. 29, 2780 (1964).

Compounds of the formula III', in which the H atoms on the C atoms 3a and (6+n)a have the trans configuration, can also be prepared from a compound of the abovementioned formula XVI by reducing the latter with sodium formate/formic acid in accordance with Bull. Soc. Chim. Belg. 85 11 (1976) to give a compound of the formula XIII and reacting the latter further in accordance with the procedures described above.

Trans-configured 2-azabicycloalkane-3-carboxylic acids of the formula III', in which a denotes a whole number from 0 to 10 and W' represents hydrogen, and their alkyl and aralkyl esters can be prepared from enamines of the formula XVII in which a has the previous meaning and X' represents dialkylamino having 2 to 10 C atoms or a radical of the formula XVIIa, wherein m and o denote a whole number from 1 to 3, (m+o) ≥ 3 and A denotes CH₂, NH, O or S (Organikum, 6th edition, page 370, 1967),

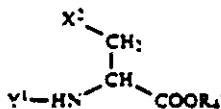


XVII

XVIIa

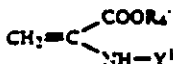
together with N-acylated β-halogeno-α-aminopropionates of the formula XVIII (for the free amino compound, cf. Helv. Chim. Acta 40, 1541 (1957)), in which X² represents halogen, preferably chlorine or bromine, Y¹ represents alkanoyl having 1 to 5 C atoms, aroyl having 7 to 9 C atoms or other protective groups which are customary in peptide chemistry and which can be

split off by acid, and R₁' represents alkyl having 1 to 5 C atoms or aralkyl having 7 to 9 C atoms.



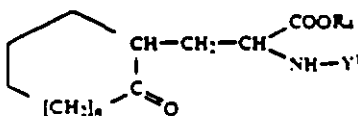
XVIII

or together with acrylates of the formula XIX (Chem. Ber. 91 2427 (1958)), in which Y¹ and R₁' have the previous meaning



XIX

to give compounds of the formula XX, in which R₁' and Y¹ have the previous meaning.

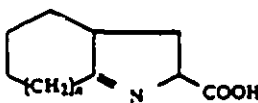


XX

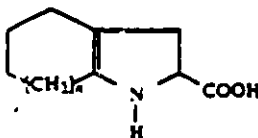
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the latter are cyclized, with acylamide and ester cleavage, by means of strong acids to give compounds of the formula XXIIa, which can also be in the tautomeric form of the formula XXIIb, and in which n has the previous meaning



XXIIa



XXIIb

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and the compounds of the formula XXIIa or b, if appropriate after conversion into their C₁-C₁₈-alkyl or C₇-C₁₀-aralkyl esters, are converted by catalytic hydrogenation in the presence of transition metal catalysts or by reduction with borane-amine complexes or complex borohydrides, into trans-configured compounds of the formula III', in which a has the previous meaning and W' represents hydrogen, alkyl having 1 to 18 C atoms or aralkyl having 7 to 10 C atoms, and esters of the formula III' are saponified if appropriate, and the latter, if W' is hydrogen, esterified, if appropriate, to give compounds of the formula III' in which a has the previous meaning and W' represents alkyl having 1 to 18 C atoms or aralkyl having 7 to 10 C atoms.

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The reaction of 3-bromopropylamine with an enamine of cyclohexanone is described in Am. Soc. 81 (1956) 2596. It is also stated that a free NH₂ group is necessary for successful alkylation. Thus it is extremely surprising that even halogenopropionic acid derivatives having an acylated amino group, as do the compounds of the formula XVII described previously, can be employed for the alkylation of enamines. Enamines of cyclohexanone or cycloheptanone are preferably employed for this purpose. Examples of suitable amine

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components are diethylamine, pyrrolidine, piperidine or morpholine. However, other secondary amines are also suitable. Pyrrolidinocycloalkylenes are preferred.

Particularly suitable groups Y¹ in the β-bromo- or chloro-α-aminocarboxylates of the formula XVIII are formyl, acetyl, propionyl or benzoyl, or other protective groups which can be split off with acid, such as, for example, tert-butyloxycarbonyl. The C₁-C₃-alkyl or the benzyl esters are preferably employed.

The acrylic acid derivatives of the formula XIX, which are produced as intermediates from the β-halogeno-α-aminopropionates under the basic conditions of the experiment, are also suitable as the starting compounds. They are prepared, for example, by treatment of the halogenoaminopropionic acid derivatives or the analogous o-tosylserine derivatives with bases. Tertiary organic bases, such as, for example, triethylamin are preferably used. It is advantageous to carry out the reaction with the addition of small amounts of polymerization inhibitors, such as, for example, hydroquinone in organic solvents. The acrylic acid derivatives of the formula XIX can be employed instead of the halogenopropionic acid derivatives under identical reactions conditions.

Suitable solvents for the enamine synthesis are organic solvents which cannot be alkylated, such as, for example, dimethylacetamide, DMSO, THF or toluene. Dimethylformamide is particularly suitable.

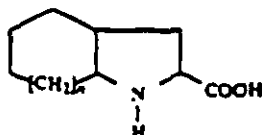
It is advisable to employ the enamines of the formula XVII in excess in order to avoid impurities of N-acylacrylates in the final product.

The hydrolysis of the N-acyl group necessary for cyclization is generally brought about at the same time as cleavage of the ester function by strong aqueous mineral acids, such as sulfuric acid or, preferably, hydrochloric acid. In the case of the N-tert-butyloxycarbonyl derivatives of the formula XVII, it is possible, for example, on using dioxane/HCl or anhydrous trifluoroacetic acid, to retain the ester functionality and to isolate the esters of the dehydrocarboxylic acids XXIa or XXIb. The latter can be converted by hydrogenation in the presence of metal catalysts or reaction with borane-amine complexes or complex borohydrides into the trans-configured 2-azabicycloxylenes of the formula III'.

Noble metal or nickel catalysts are suitable for catalytic hydrogenation. The isomeric ratio occurring on catalytic hydrogenation depends on the reaction conditions and the type of catalyst used.

It is possible to shorten the reaction time by increasing the pressure of hydrogen, but the temperature should be kept low. Examples of suitable solvents for the catalytic hydrogenation are ethanol, methanol, ethyl acetate, dioxane, glacial acetic acid or mixtures of these solvents.

Compounds of the formula III'b, in which n represents a whole number from 0 to 10 and W' represents hydrogen,



can also be obtained by reduction of compounds of the formulae XXIa or b, in which n has the previous mean-

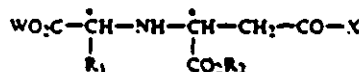
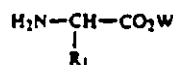
ing, with borane-amine complexes or complex borohydrides in lower alcohols. The preferred reducing agent is sodium borohydride in alcohols, in particular in methanol, ethanol or isopropanol. Amine-borane complexes in glacial acetic acid can equally be used.

Isolation of the pure trans compounds of the formula III'b can, for example, be carried out by chromatographic processes or crystallization processes.

The pure trans compound of the formula III'b is advantageously separated out of the mixture of diastereomers from the amine-borane-complex or boronate reduction by fractional crystallization.

The compounds of the formula III' can, if appropriate, be converted into the C₁-C₁₈-alkyl or C₇-C₁₀-aryl-alkyl esters by methods which are described, for example, in Houben-Weyl, volume VIII (1952).

The compounds of the formula II with Y and Z being hydrogen, R₁ being methyl and R₂ being methyl or ethyl and X being phenyl which are used as starting materials for the preparation of the compounds of the formula I are known (European Patent Application No 37,231). The compounds of the formula II can be prepared by various procedures. One synthetic variant starts from a ketone of the abovementioned formula VII, which is reacted by known procedures in a Mannich reaction with a compound of the abovementioned formula VI together with aminoacid esters of the formula XXII



wherein R₁ and W have the abovementioned meanings, to give a compound of the formula XXIII, wherein R₂, X and W have the abovementioned meanings, with the proviso that in the case where W denotes a radical which can be split off by hydrogenolysis, in particular benzyl, R₂ may not have the meaning of W. If the radical W is split off by hydrogenolysis using, for example, palladium, compounds of the formula II with Y and Z being hydrogen are obtained. If the radical W is split off with acids, such as, for example, trifluoroacetic acid or hydrochloric acid in an inert organic solvent, such as, for example, dioxane, compounds of the formula II with Y and Z together being oxygen are obtained.

Compounds of the formula XXIII can also be obtained by Michael addition of a compound of the abovementioned formula V with a compound of the abovementioned formula XXII by known procedures. Preferentially, this process is suitable for the preparation of those compounds of the formula XXIII in which R denotes methyl, R₂ denotes ethyl and X denotes aryl.

The compounds of the formula XXIII are obtained as mixtures of diastereomers. Preferred diastereomers of the formula XXIII are those in which the chiral C atoms labeled with an asterisk are each in the S configuration. These can be separated out by recrystallization or by chromatography, for example on silica gel. The configurations of the chiral C atoms are maintained during the subsequent splitting off of the radical W.

The compounds of the abovementioned formula IV used as starting materials for the preparation of the compounds of the formula I are obtained by known

procedures from compounds of the abovementioned formula III by reaction with an N-protected 2-aminocarboxylic acid of the formula XXIV



wherein V is a protective group and R₁ has the abovementioned meaning. Examples of suitable protective groups V, which are split off again after completion of the reaction, are the groups benzyloxycarbonyl or tert-butoxycarbonyl.

The reaction of a compound of the formula II with a compound of the formula III to prepare a compound of the formula I is carried out by a condensation reaction known in peptide chemistry, the condensing agent added being, for example, dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. In the subsequent removal of the radical W by hydrogenolysis, the catalyst used is preferably palladium, while the acids employed for the acid removal of the radical W are preferably trifluoroacetic acid or hydrogen chloride.

In the reactions described above for the preparation of the compounds of the formulae III', IV and I, the configurations at the bridgehead C atoms 3a and (6+n)a in the immediate products are retained in each case. In the case of the trans configuration of the H atoms on the C atoms 3a and (6+n)a, the relevant compounds are obtained, in some cases, as pure diastereomers, and in other cases, as mixtures of diastereomers, which have the 2β, 3αα, (6+n)αβ or the 2β, 3αβ, (6+n)αα configuration as indicated above. These isomers can easily be separated by recrystallization or chromatography. If, for the preparation of the compounds of the formulae III', IV or I, corresponding starting materials having the cis configuration of the H atoms on C-3a and C-(6+n)a are employed, the exo (or β) isomers are obtained almost exclusively, and small proportions of the other isomers can be removed by recrystallization or by chromatography.

The compounds of the formula III' obtained according to the procedures described above are produced as racemic mixtures and can be employed as such in the further syntheses described above. However, they can also be employed as the pure enantiomers after separation of the racemates into the optical antipodes by customary methods, for example, via salt formation with optically active bases or acids.

If the compounds of the formula I are produced as racemates, these can also be resolved into their enantiomers by customary methods, such as, for example, via salt formation with optically active bases or acids.

The compounds of the formula I according to the invention are in the form of internal salts. Since they are amphoteric compounds, they can form salts with acids or bases. These salts are prepared in a customary manner by reaction with one equivalent of acid or base.

The compounds of the formula I and their salts have long-lasting and powerful hypotensive activities. They are strong inhibitors of the angiotensin converting enzyme (ACE inhibitors). They can be employed to control high blood pressure of various etiologies. It is also possible to combine them with other compounds having hypotensive, vasodilator or diuretic activity. Typical representatives of these classes of active compounds are described, for example, in Erhardt-Ruschig, *Arzneimittel*

(Drugs), 2nd edition, Weinheim, 1972. They can be used intravenously, subcutaneously or perorally.

The dosage on oral administration is 1-100 mg, preferably 1-40 mg, for a single dose for an adult patient of normal weight. This can also be increased in severe cases, since no toxic properties have been observed hitherto. A decrease in the dose is also possible and is particularly appropriate when diuretics are administered concurrently.

The compounds according to the invention can be administered orally or parenterally in an appropriate pharmaceutical formulation. For a form for oral use, the active compounds are mixed with the additives customary for this purpose, such as vehicles, stabilizers or inert diluents and converted by customary methods into suitable forms for administration, such as tablets, coated tablets, hard capsules, aqueous, alcoholic or oily suspensions or aqueous, alcoholic or oily solutions. Inert vehicles which can be used are, for example, gum arabic, magnesium carbonate, potassium phosphate, lactose, glucose or starch, particularly corn starch. In this context, the formulation can either be as dry or as moist granules. Examples of suitable oily vehicles or solvents are plant and animal oils, such as sunflower oil or cod-liver oil.

For subcutaneous or intravenous administration, the active compounds or their physiologically tolerated salts are converted into solutions, suspensions or emulsions, if desired together with the substances customary for this purpose such as solubilizers, emulsifiers or other auxiliaries. Examples of suitable solvents for the new active compounds and the corresponding physiologically tolerated salts are: water, physiological saline or alcohols, for example ethanol, propanediol or glycerol, additionally also sugar solutions, such as glucose or mannitol solutions, or also a mixture of the various solvents mentioned.

The extremely high activity of the compounds according to formula I is demonstrated by the pharmacological data in the following tables: Intraduodenal administration to the anesthetized rat, 50% inhibition of the pressor reaction induced by 310 ng of angiotensin I 30 min. After administration in the dose . . . = ED₅₀:

TABLE I

(H atoms on C-3a and C-6 = all in formula I have the cis configuration)						
n	X	Y	Z	R ₂	R ₁	ED ₅₀ (μg/kg)
0		H	H	C ₂ H ₅	CH ₃	40
0		H	H	H	CH ₃	100
1		H	H	C ₂ H ₅	CH ₃	50
1		H	H	H	CH ₃	100

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TABLE I-continued

(H atoms on C-3a and C-(6-n) in formula I have the cis configuration)						
n	X	Y	Z	R ₁	ED ₅₀ (μg/kg)	
1		H	H	C ₂ H ₅	CH ₃	130
2		H	H	H	CH ₃	840
1		-O-		C ₂ H ₅	CH ₃	390

The symbols n, X, Y, Z, R₁ and R₂ relate to the compound of the formula I.

TABLE II

(H atoms on C-3a and C-(6-n) have the trans configuration: X = phenyl, R ₁ = methyl)					
n	Y	Z	R ₂	Configuration of the bicycle	ED ₅₀ (μg/kg)
0	H	H	H	2S, 3aR, 6aS	700
0	H	H	C ₂ H ₅	2S, 3aR, 6aS	40
1	H	H	H	2S, 3aR, 7aS	800
1	H	H	C ₂ H ₅	2S, 3aR, 7aS	55
1	H	H	H	2S, 3aS, 7aR	850
1	H	H	C ₂ H ₅	2S, 3aS, 7aR	65
2	H	H	H	2S, 3aR, 8aS	1080
2	H	H	C ₂ H ₅	2S, 3aR, 8aS	110
1	O		C ₂ H ₅	2S, 3aR, 7aS	180

The symbols m, X, Y, Z, R₁ and R₂ relate to the compounds of the formula I.

The following examples serve to illustrate the invention but do not restrict it to the compounds mentioned as representatives:

EXAMPLE 1

2β,3aβ,7aβ-Octahydroindole-2-carboxylic acid

(a) N-Acetyl-3aβ,7aβ-octahydroindole

3.5 g of platinum oxide were added to a solution of 77 g of indole in 700 ml of glacial acetic acid. The compound was initially hydrogenated under 100 atmospheres at 20° to 25° C. for 16 hours and then at 20° to 25° C. under normal pressure until the uptake of hydrogen was complete. The catalyst was filtered off with suction and the solvent was distilled off in vacuo. The residue was taken up in water and made alkaline with saturated potassium carbonate solution. After saturation with sodium chloride, the aqueous phase was extracted four times with methylene chloride and the organic phase was dried and evaporated.

The residue was taken up in 250 ml of pyridine. 93 ml of acetic anhydride were added and the mixture was allowed to react at 20° to 25° C. for 12 hours. After distilling off the pyridine, water was added to the residue and the mixture was made alkaline with concentrated aqueous sodium hydroxide. The aqueous phase was extracted with methylene chloride, the organic phase obtained was washed with 2N hydrochloric acid and with water. After drying and concentrating the solution, the residue was distilled.

Yield: 85 g; b.p.: 91° to 95° C./0.2 mm Hg

(b) N-Acetyl-2-methoxy-3aβ,7aβ-octahydroindole

54 g of N-acetyl-3aβ,7aβ-octahydroindole were anodically oxidized in methanol, with the addition to tetramethylammonium tetrafluoroborate, according to the details in Liebigs Ann. Chem. 1978, page 1790. The solvent was distilled off and the residue was filtered through 500 g of silica gel by means of ethyl acetate. 53.6 g of the abovementioned product were obtained from the ethyl acetate solution after evaporation. R_f value (thin-layer chromatogram): 0.33 (silica gel, ethyl acetate).

(c) N-Acetyl-2-(3aβ,7aβ-octahydroindole-2-carbonitrile

25 g of trimethylsilyl cyanide in 50 ml of methylene chloride were added dropwise to a solution of 49.3 g of N-acetyl-2-methoxy-3aβ,7aβ-octahydroindole in 250 ml of methylene chloride at -40° C. Subsequently, 35.9 g of boron trifluoride etherate were added dropwise so that the temperature of the reaction mixture did not exceed -20° C. After 2 hours of reaction at -20° C., the temperature was slowly raised to 0° C., the mixture was stirred overnight at 0° C. and then for one hour at 20° to 25° C. Water was added to the mixture and this was stirred 10 min. The aqueous phase was extracted three times with methylene chloride. The combined organic extracts were dried, concentrated and the residue was triturated with diisopropyl ether.

Yield: 47 g; m.p. 128° to 130° C.

(d) 2β,3aβ,7aβ-Octahydroindole-2-carboxylic acid

10 g of N-acetyl-2β,3aβ,7aβ-octahydroindole-2-carbonitrile in 30 ml of concentrated hydrogen bromide were heated to boiling for 2 hours. After distilling off the hydrogen bromide, the residue was stirred with a little acetone and filtered off with suction.

An aqueous solution of the product was adjusted to a pH of 6.0 with a weakly basic ion exchanger. After filtration, the solution was evaporated and the residue was filtered through silica gel with a mixture of methylene chloride, methanol, glacial acetic acid and water in the ratio 20:10:0.5:0.5. The eluate was concentrated and the residue was triturated with diisopropyl ether.

Yield: 7.6 g

¹H NMR spectrum*: 1.0-2.5 (m, 11H); 3.4-3.9 (m, 1H); 4.0-4.5 (m, 1H); 7.5-8.3 (broad s, exchangeable with D₂O).

*Here and in the following text, the ¹H NMR data were obtained in CDCl₃ and are reported in ppm.

When the process was carried out in analogy to the procedures described under Example 1, the compounds mentioned under Example 2 and Example 3 in the following text were obtained:

EXAMPLE 2

2β,3aβ,6aβ-Octahydrocyclopenta[b]pyrrole-2-carboxylic acid

(a) N-Acetyl-cis-octahydrocyclopenta[b]pyrrole

¹H NMR data: 1.0 to 2.3 (m, 9H); 2.0 (d, 3H); 3.3 to 4.2 (m, 3H).

(b) Acetyl-2-methoxy-cis-octahydrocyclopenta[b]pyrrole

¹H NMR data: 0.9 to 2.6 (m, 9H); 2.1 (s, 3H); 3.3 (s, 3H); 3.8-4.3 (m, 1H); 4.7-5.3 (m, 1H).

(c) N-Acetyl-2-cyano-cis-octahydrocyclopenta[b]pyrrole

¹H-NMR data: 1.0-3.0 (m, 9H); 2.1 (d, 3H); 3.5-4.1 (m, 1H); 4.4-4.7 (m, 1H).

(d) 2 β ,3 $\alpha\beta$, 6 $\alpha\beta$ -Octahydrocyclopenta[b]pyrrole-2-carboxylic acid
¹H NMR data: 1.0-2.3 (m, 9H); 3.5-3.9 (m, 1H); 4.0-4.6 (m, 1H); 7.7-8.4 (broad s, exchangeable with D₂O).

EXAMPLE 3

2 β ,3 $\alpha\beta$,

8 $\alpha\beta$ -Decahydrocyclohepta[b]pyrrole-2-carboxylic acid

(a) N-Acetyl-cis-decahydrocyclohepta[b]pyrrole
¹H NMR data: 0.9-2.5 (m, 13H); 2.1 (s, 3H); 3.1-4.1 (m, 3H).

(b) N-Acetyl-2-methoxy-cis-decahydrocyclohepta[b]pyrrole
¹H NMR data: 0.9-2.7 (m, 13H); 2.1 (s, 3H); 3.2 (s, 3H); 3.7-4.2 (m, 1H); 4.7-5.3 (m, 1H).

(c) N-Acetyl-2-cyano-cis-decahydrocyclohepta[b]pyrrole
¹H NMR data: 0.9-3.1 (m, 13H); 2.1 (s, 3H); 3.5-4.1 (m, 1H); 4.3-4.7 (m, 1H).

(d) 2 β ,3 $\alpha\beta$,8 $\alpha\beta$ -Decahydrocyclohepta[b]pyrrole-2-carboxylic acid

¹H NMR data: 0.8-2.4 (m, 13H); 3.5-3.9 (m, 1H); 4.1-4.6 (m, 1H); 7.6-8.3 (broad s, exchangeable with D₂O).

EXAMPLE 4

2 β ,3 $\alpha\beta$,7 $\alpha\beta$ -Octahydroindole-2-carboxylic acid

(a) 3,4,5,6,7,8-Hexahydro-1H-quinolin-2-one

392 g of cyclohexanone and 212 g of acrylonitrile together with 20 g of cyclohexylamine, 4 g of glacial acetic acid and 0.4 g of hydroquinone were heated under reflux for 4 hours up to a final temperature of 200° C. After distillation at 100° to 150° C./0.5 mm Hg, the residue remaining, which contained the desired product, was recrystallized from n-hexane.

The distillate was heated with 10 ml of 10% strength acetic acid at 200° C. for 2 days. After cooling down, further product was obtained which was crystallized from methanol/water.

A total of 460 g of title compound was obtained, m.p.: 143°-144° C.

(b) Cis-octahydro-1H-quinolin-2-one

One gram of platinum(IV) oxide was added to a solution of 80 g of 3,4,5,6,7,8-hexahydro-1H-quinolin-2-one and this was hydrogenated at 20° to 25° C. under normal pressure. After filtration of the reaction solution, it was evaporated and the residue was fractionally crystallized from n-hexane. 35 g of cis-octahydro-1H-quinolin-2-one having a melting point of 123° to 126° C. were obtained.

(c) 3,3-Dichloro-cis-octahydro-1H-quinolin-2-one

28.8 g of phosphorus pentachloride were added to a solution of 23 g of cis-octahydro-1H-quinolin-2-one in 350 ml of anhydrous chloroform. To this were added dropwise 43.1 g of sulfuryl chloride in 45 ml of anhydrous chloroform at 20° to 30° C. within 30 min and the reaction mixture was stirred at the boiling point for 5 hours. After allowing to stand overnight, the mixture was neutralized with aqueous potassium carbonate cooled to 0° C. The aqueous phase was extracted twice with methylene chloride. The combined organic phases were dried over sodium sulfate and concentrated. The residue was recrystallized from ethanol with the addition of active charcoal. 32 g of pale yellow crystals, having a melting point of 176° to 177° C., were obtained.

(d) 3-Chloro-cis-octahydro-1H-quinolin-2-one

15.9 g of 3,3-dichloro-cis-octahydro-1H-quinolin-2-one in one liter of ethanol, with the addition of 10 ml of triethylamine and Raney nickel, were hydrogenated at 20° to 25° C. under normal pressure until one mole-equivalent had been taken up. After filtration, the solution was evaporated, the residue was taken up in ethyl acetate, the solution was extracted twice with water and dried over sodium sulfate. After removal of the solvent, the product was triturated with diisopropyl ether and filtered off with suction. Colorless crystals, having a melting point of 185° C., were obtained.

(e) 2 β ,3 $\alpha\beta$,7 $\alpha\beta$ -Octahydroindole-2-carboxylic acid

3.75 g of 3-chloro-cis-octahydro-1H-quinolin-2-one were added to a boiling solution of 6.63 g of barium hydroxide octahydrate in 120 ml of water. After heating under reflux for 3.5 hours, 0.9 ml of concentrated sulfuric acid was added to the reaction mixture and this was heated to boiling for a further hour and then allowed to stand overnight.

The precipitate was filtered off with suction and the filtrate was adjusted to a pH of 6.5 with 1N sodium hydroxide and evaporated to dryness. The residue was extracted with boiling ethanol, concentrated and induced to crystallize.

Yield: 3.1 g

The compounds 2 β -cis-octahydrocyclopenta[b]pyrrole-2-carboxylic acid (corresponds to the compound from Example 2 d) and 2 β ,3 $\alpha\beta$,8 $\alpha\beta$ -decahydrocyclohepta[b]pyrrole-2-carboxylic acid (corresponds to the compound in Example 3 d) may be prepared in a manner analogous to that described in Example 4.

EXAMPLE 5

Benzyl 2 β ,3 $\alpha\beta$,7 $\alpha\beta$ -octahydroindole-2-carboxylate hydrochloride

3 ml of thionyl chloride were added dropwise to 25 ml of benzyl alcohol. 3 g of 2 β ,3 $\alpha\beta$,7 $\alpha\beta$ -octahydroindole-2-carboxylic acid hydrochloride were added to this mixture. The reaction mixture was allowed to stand at 5° C. for 2 days, after which a clear solution had formed. After evaporation, diisopropyl ether was added to the residue obtained and this was filtered off with suction. 3.8 g of the title compound, having a melting point of 150° C. (with decomposition), were obtained.

The following ester compounds in Examples 6 and 7 may be prepared in a manner analogous to that described in Example 5:

EXAMPLE 6

Benzyl

2 β ,3 $\alpha\beta$,6 $\alpha\beta$ -octahydrocyclopenta[b]pyrrole-2-carboxylate hydrochloride

¹H NMR data: 1.0-2.3 (m, 9H); 3.4-3.9 (m, 1H); 4.1-4.6 (m, 1H); 5.1 (s, 2H); 7.2 (s, 5H).

EXAMPLE 7

Benzyl

2 β ,3 $\alpha\beta$,8 $\alpha\beta$ -decahydrocyclohepta[b]pyrrole-2-carboxylate hydrochloride

¹H NMR data: 0.9-2.3 (m, 13H); 3.5-3.9 (m, 1H); 4.2-4.7 (m, 1H); 5.2 (s, 2H); 7.2 (s, 5H).

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EXAMPLE 8

Tert.-butyl 2 β ,3 $\alpha\beta$,7 $\alpha\beta$ -octahydroindole-2-carboxylate hydrochloride

10 ml of concentrated sulfuric acid and 50 g of isobutylene were added to a solution of 10 g of 2 β ,3 $\alpha\beta$,7 $\alpha\beta$ -octahydroindole-2-carboxylic acid in 100 ml of dioxane cooled down to -10° C. The reaction mixture was slowly warmed to 20° to 25° C. in an autoclave and stirred at this temperature for 20 hours.

The mixture was added to ice-cold 50% strength aqueous sodium hydroxide and extracted with methylene chloride. The combined organic phases were washed with water, dried with sodium sulfate and concentrated. The residue was taken up in ether and the pH was adjusted to 2.0 to 3.0 by means of ethereal hydrogen chloride. The mixture was evaporated to dryness and the product was triturated with diisopropyl ether. 7.3 g of the title compound were obtained after filtering off with suction.

¹H NMR data: 1.0-2.5 (m, 11H); 1.3 (s, 9H); 3.4-3.9 (m, 1H); 4.0-4.5 (m, 1H).

The following ester compounds of Examples 9 and 10 can be prepared in analogy to the procedure described in Example 8:

EXAMPLE 9

Tert.-butyl 2 β ,3 $\alpha\beta$,6 $\alpha\beta$ -octahydrocyclopenta[b]pyrrole-2-carboxylate hydrochloride

¹H NMR data: 1.0-2.7 (m, 9H); 1.3 (s, 9H); 3.4-3.9 (m, 1H); 4.0-4.5 (m, 1H).

EXAMPLE 10

Tert.-butyl 2 β ,3 $\alpha\beta$,8 $\alpha\beta$ -decahydrocyclohepta[b]pyrrole-3-carboxylate hydrochloride

¹H NMR data: 0.8-2.9 (m, 13H); 1.3 (s, 9H); 3.4-3.9 (m, 1H); 4.0-4.5 (m, 1H).

EXAMPLE 11

Benzyl N-(1S-carboethoxy-3-phenylpropyl)-S-alanyl-2S,3 α R,7 α R-octahydroindole-2-carboxylate (=diastereomer A 11) and benzyl

N-(1S-carboethoxy-3-phenylpropyl)-S-alanyl-2R,3 α S,7 α S-octahydroindole-2-carboxylate (=diastereomer B 11)

2.5 g of N-(1S-carboethoxy-3-phenylpropyl)-S-alanine, 1.22 g of 1-hydroxybenzotriazole, 2.5 g of benzyl (d,l)-2 β ,3 $\alpha\beta$,7 $\alpha\beta$ -octahydroindole-2-carboxylate hydrochloride, 1.25 ml of N-ethylmorpholine and 2 g of dicyclohexylcarbodiimide were added to 20 ml of dimethylformamide at 0° C.

The mixture was stirred at 0° C. for 1 hour, then slowly warmed to room temperature and stirred at 20° to 25° C. overnight.

25 ml of ethyl acetate were added to the reaction mixture and precipitated urea was filtered off with suction. After evaporation of the solution, the residue obtained was taken up in 50 ml of ether; the ethereal solution was washed with saturated aqueous sodium bicarbonate and water, dried and concentrated. A mixture of the abovementioned diastereomers A 11 and B 11 was obtained which was separated over silica gel using a mixture of cyclohexane and ethyl acetate (4:1).

R_v value for diastereomer A 11: 0.36

R_v value for diastereomer B 11: 0.34

The following compounds in Examples 12 to 16 were obtained in a procedure analogous to that described in Example 11.

EXAMPLE 12

Tert.-butyl N-(1S-carboethoxy-3-phenylpropyl)-S-alanyl-2S,3 α R,7 α R-octahydroindole-2-carboxylate (=diastereomer A 12).

¹H NMR data: 1.25 (d+t, 6H); 1.35 (s, 9H); 1.3-3.6 (m, 18H); 4.2 (q, 2H); 4.4 (m, 1H); 7.3 (s, 5H).

EXAMPLE 13

Benzyl N-(1S-carboethoxy-3-phenylpropyl)-S-alanyl-2S,3 α R,6 α R-octahydrocyclopenta[b]pyrrole-2-carboxylate (=diastereomer A 13)

¹H NMR data: 1.1 (d, 3H); 1.3 (t, 3H); 1.3-2.4 (m, 10H); 2.3-3.4 (m, 6H); 4.1 (q, 2H); 4.55 (d, 1H); 5.2 (s, 2H); 7.2 (s, 5H); 7.4 (s, 5H).

EXAMPLE 14

Benzyl N-(1S-carboethoxy-3-phenylpropyl)-S-alanyl-2S,3 α R,8 α R-decahydrocyclohepta[b]pyrrole-2-carboxylate (=diastereomer A 14)

¹H NMR data: 1.2 (d+t, 6H); 1.3-2.4 (m, 14H); 2.3-3.4 (m, 6H); 4.2 (q, 2H); 4.6 (m, 1H); 5.2 (s, 2H); 7.2 (s, 5H); 7.4 (s, 5H).

EXAMPLE 15

Tert.-butyl N-(1S-carboethoxy-3-phenylpropyl)-S-alanyl-2S,3 α R,6 α R-octahydrocyclopenta[b]pyrrole-2-carboxylate (=diastereomer A 15)

¹H NMR data: 1.1 (d, 3H); 1.3 (t, 3H); 1.3-2.4 (m, 10H); 1.4 (s, 9H); 2.3-3.4 (m, 6H); 4.2 (q, 2H); 4.6 (m, 1H); 7.2 (s, 5H).

EXAMPLE 16

Tert.-butyl N-(1S-carboethoxy-3-phenylpropyl)-S-alanyl-2S,3 α R,8 α R-decahydrocyclohepta[b]pyrrole-2-carboxylate (=diastereomer A 16)

¹H NMR data: 1.1 (d, 3H); 1.3 (t, 3H); 1.4 (s, 9H); 1.4-2.5 (m, 14H); 2.3-3.4 (m, 6H); 4.1 (q, 2H); 4.7 (m, 1H); 7.3 (s, 5H).

EXAMPLE 17

N-(1S-Carboethoxy-3-phenylpropyl)-S-alanyl-2S,3 α R,7 α R-octahydroindole-2-carboxylic acid hydrochloride
Method A:

0.7 g of diastereomer A 11 from Example 11 were hydrogenated in 25 ml of ethanol with 100 ml of palladium-animal charcoal (10%) at 20° to 25° C. under normal pressure. After removal of the catalyst, the solution was treated with 0.5N ethanolic hydrogen chloride until the reaction was acid. The solution was concentrated in vacuo and the residue was triturated with diisopropyl ether. 400 mg of the title compound, having a melting point of 198° to 200° C., were obtained.

¹H NMR data of the free base: 1.0-3.0 (m, 19H); 3.0-3.3 (t, 1H); 3.3-3.9 (m, 3H); 3.9-4.4 (q, 2H); 4.4-4.7 (broad s, 4H); 7.2 (s, 5H).

Method B:

A solution of 0.8 g of diastereomer A 12 from Example 12 in 5 ml of methylene chloride was saturated with dry hydrogen chloride gas and allowed to stand at 20° to 25° C. for 16 hours. The solution was concentrated in vacuo, the residue was triturated with diisopropyl ether and filtered off with suction.

Yield: 550 mg.

EXAMPLE 18

N-(1S-Carboethoxy-3-phenylpropyl)-S-alanyl-2S,3aR,6aR-octahydrocyclopenta[b]pyrrole-2-carboxylic acid hydrochloride

This compound was obtained from the diastereomer A 13 in Example 13 in a procedure analogous to method A in Example 17.

¹H NMR data: 1.2 (d, 3H); 1.3 (t, 3H); 1.2-3.8 (m, 16H); 4.15 (q, 2H); 4.2-4.6 (m, 4H); 7.2 (s, 5H).

EXAMPLE 19

N-(1S-Carboethoxy-3-phenylpropyl)-S-alanyl-2S,3aR,8aR-decahydrocyclohepta[b]pyrrole-2-carboxylic acid hydrochloride

This compound was prepared from diastereomer A 14 in Example 14 in analogy to the method A described in Example 17.

¹H NMR data: 1.2 (d, 3H); 1.3 (t, 3H); 1.2-3.8 (m, 20H); 4.2 (q, 2H); 4.3-4.7 (m, 4H); 7.2 (s, 5H).

The compounds of Examples 18 and 19 can also be prepared from the diastereomers A 15 and A 16 respectively by the method B described in Example 17.

EXAMPLE 20

N-(1S-Carboxy-3-phenylpropyl)-S-alanyl-2S,3aR,7aR-octahydroindole-2-carboxylic acid

Two equivalents of potassium hydroxide and a 10% excess of 4N potassium hydroxide were added to a solution of 1 g of N-(1S-carboethoxy-3-phenylpropyl)-S-alanyl-2S,3aR,7aR-octahydroindole-2-carboxylic acid hydrochloride in 10 ml of water. After stirring for 8 hours at 20° to 25° C., the reaction solution was adjusted to a pH of 4.0 with 2N hydrochloric acid and concentrated in vacuo. The residue was taken up in ethyl acetate and precipitated salt was filtered off. The ethyl acetate solution was concentrated and the residue was triturated with diisopropyl ether and filtered off with suction.

Yield: 0.6 g

¹H NMR data: 1.2 (d, 3H); 1.2-3.8 (m, 18H); 4.0-4.6 (m, 4H); 7.2 (s, 5H).

EXAMPLE 21

N-(1S-Carboxy-3-phenylpropyl)-S-alanyl-2S,3aR,6aR-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

This compound was prepared from the compound in Example 18 in analogy to the process described in Example 20.

¹H NMR data: 1.2 (d, 3H); 1.2-3.8 (m, 16H); 4.05-4.7 (m, 4H); 7.2 (s, 5H).

EXAMPLE 22

N-(1S-Carboxy-3-phenylpropyl)-S-alanyl-2S,3aR,8aR-decahydrocyclohepta[b]pyrrole-2-carboxylic acid

This compound was prepared from the compound in Example 19 in analogy to the process described in Example 20.

¹H NMR data: 1.2 (d, 3H); 1.3-3.9 (m, 20H); 4.0-4.7 (m, 4H); 7.2 (s, 5H).

EXAMPLES 23

Tert-butyl

N-(1S-carboethoxy-3-phenyl-3-oxopropyl)-S-alanyl-2β,3aβ,7aβ-octahydroindole-2-carboxylate

2.5 g of N-(1S-carboethoxy-3-phenyl-3-oxopropyl)-S-alanine, together with 1.2 g of 1-hydroxybenzotriazole, 2.5 g of tert-butyl (d,l)2β,3aβ,7aβ-octahydroindole-2-carboxylate hydrochloride, 1.25 ml of N-ethylmorpholine and 2 g of dicyclohexylcarbodiimide, were added to 20 ml of dimethylformamide. The mixture was stirred at 0° C. for 1 hour and then at 20° to 25° C. for 12 hours.

The reaction solution was diluted with 25 ml of ethyl acetate and precipitated urea was filtered off with suction. After concentration in vacuo, the residue obtained was taken up in ether, the ethereal solution was washed with saturated aqueous sodium bicarbonate and with water, dried and evaporated. 3 g of the title compound were obtained as a mixture of diastereomers.

¹H NMR data: 1.2 (s, 9H); 0.9-2.6 (m, 18H); 3.5-5.1 (m, 6H); 7.2-8.2 (m, 5H).

The mixture of diastereomers can be separated over silica gel into the optically pure compounds using cyclohexane/ethyl acetate as the eluant.

EXAMPLES 24 TO 28

The following compounds of Examples 24 to 28 may be prepared in a procedure analogous to Example 23 using the appropriate bicyclic carboxylic acid ester compounds.

EXAMPLE 24

Tert-butyl

N-(1S-carboethoxy-3-phenyl-3-oxopropyl)-S-alanyl-2β,3aβ,6aβ-octahydrocyclopenta[b]pyrrole-2-carboxylate

¹H NMR data: 1.2 (s, 9H); 0.9-2.5 (m, 16H); 3.5-5.1 (m, 6H); 7.2-8.2 (m, 5H).

EXAMPLE 25

Tert-butyl

N-(1S-carboethoxy-3-phenyl-3-oxopropyl)-S-alanyl-2S,3aR,8aR-decahydrocyclohepta[b]pyrrole-2-carboxylate

¹H NMR data: 1.4 (s, 9H); 1.0-2.8 (m, 20H); 3.4-5.1 (m, 6H); 7.2-8.2 (m, 5H).

EXAMPLE 26

Benzyl

N-(1S-carboethoxy-3-phenyl-3-oxopropyl)-S-alanyl-2β,3aβ,7aβ-octahydroindole-2-carboxylate

¹H NMR data: 1.2 (d, 3H); 1.3 (t, 3H); 1.4-2.4 (m, 10H); 2.4-3.9 (m, 6H); 4.2 (g, 2H); 4.3-4.8 (m, 1H); 5.2 (s, 2H); 7.2 (s, 5H); 7.4-8.0 (m, 5H).

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EXAMPLE 27

Benzyl

N-(1S-carboethoxy-3-phenyl-3-oxopropyl)-S-alanyl-2 β ,3 $\alpha\beta$,6 $\alpha\beta$ -octahydrocyclopenta[b]pyrrole-2-carboxylate

¹H NMR data: 1.25 (d+t, 6H); 1.4-2.4 (m, 8H); 2.4-3.8 (m, 6H); 4.2 (q, 2H); 4.3-4.8 (m, 1H); 5.2 (s, 2H); 7.2 (s, 5H); 7.4-8.0 (m, 5H).

EXAMPLE 28

Benzyl

N-(1S-carboethoxy-3-phenyl-3-oxopropyl)-S-alanyl-2 β ,3 $\alpha\beta$,8 $\alpha\beta$ -decahydrocyclohepta[b]pyrrole-2-carboxylate

¹H NMR data: 1.2 (d, 3H); 1.3 (t, 3H); 1.4-2.5 (m, 12H); 2.4-3.8 (m, 6H); 4.2 (q, 2H); 4.3-4.8 (m, 1H); 5.2 (s, 2H); 7.2 (s, 5H); 7.4-8.0 (m, 5H).

EXAMPLE 29

N-(1S-Carboethoxy-3-phenyl-3-oxopropyl)-S-alanyl-2 β ,3 $\alpha\beta$,7 $\alpha\beta$ -octahydroindole-2-carboxylic acid trifluoroacetate

2.6 g of the mixture of diastereomers from Example 23 were stirred in 15 ml of trifluoroacetic acid at 20° to 25° C. for 2 hours. The solution was concentrated in vacuo, the residue was triturated with diisopropyl ether and filtered off with suction.

Yield: 0.8 g

¹H NMR data: 1.2 (d+t, 6H); 1.3-3.6 (m, 16H); 4.2 (q, 2H); 4.1-4.6 (m, 4H); 7.3-8.1 (m, 5H).

EXAMPLE 30

N-(1S-Carboethoxy-3-phenyl-3-oxopropyl)-S-alanyl-2 β ,3 $\alpha\beta$,6 $\alpha\beta$ -octahydrocyclopenta[b]pyrrole-2-carboxylic acid trifluoroacetate

This compound was prepared in analogy to the procedure described in Example 29.

¹H NMR data: 1.2 (d+t, 6H); 1.3-3.6 (m, 14H); 4.15 (q, 2H); 4.0-4.6 (m, 4H); 7.3-8.0 (m, 5H).

EXAMPLE 31

N-(1S-Carboethoxy-3-phenyl-3-oxopropyl)-S-alanyl-2 β ,3 $\alpha\beta$,8 $\alpha\beta$ -decahydrocyclohepta[b]pyrrole-2-carboxylic acid

1 g of tert-butyl N-(1S-carboethoxy-3-phenyl-3-oxopropyl)-S-alanyl-2 β ,3 $\alpha\beta$,8 $\alpha\beta$ -decahydrocyclohepta[b]pyrrole-2-carboxylate was dissolved in 10 ml of methylene chloride, the solution was saturated with hydrogen chloride gas and allowed to stand at 20° to 25° C. for 16 hours. The solution was concentrated in vacuo and the residue was triturated with diisopropyl ether and filtered off with suction.

Yield: 0.4 g

¹H NMR data: 1.3 (d+t, 6H); 1.3-3.8 (m, 18H); 4.2 (q, 2H); 4.0-4.7 (m, 4H); 7.2-8.1 (m, 5H).

The carboxylic acids described in the foregoing Examples 29 to 31 can also be prepared from the corresponding benzyl esters by catalytic hydrogenolysis (10% palladium on charcoal, ethanol, 20° to 25° C.).

EXAMPLE 32

N-(1S-Carboxy-3-phenyl-3-oxopropyl)-S-alanyl-2 β ,3 $\alpha\beta$,7 $\alpha\beta$ -octahydroindole-2-carboxylic acid

For the preparation of this compound, 1 g of N-(1S-carboethoxy-3-phenyl-3-oxopropyl)-S-alanyl-2 β ,3 $\alpha\beta$,

7 $\alpha\beta$ -octahydroindole-2-carboxylic acid was reacted with potassium hydroxide in analogy to the procedure described in Example 19.

¹H NMR data: 1.2 (d, 3H); 1.3-3.6 (m, 16H); 4.1-4.7 (m, 4H); 7.2-8.1 (m, 5H).

EXAMPLE 33

N-(1S-Carboethoxy-3-phenyl-3-hydroxypropyl)-S-alanyl-2 β ,3 $\alpha\beta$,7 $\alpha\beta$ -octahydroindole-2-carboxylic acid

1 g of N-(1S-carboethoxy-3-phenyl-3-oxopropyl)-S-alanyl-2 β ,3 $\alpha\beta$,7 $\alpha\beta$ -octahydroindole-2-carboxylic acid was dissolved in 50 ml of anhydrous ethanol and hydrogenated with 1 mole equivalent of hydrogen and 50 mg of palladium/charcoal at 20° to 25° C. under normal pressure. After removal of the catalyst by filtration, the solution was evaporated and the residue was triturated with diisopropyl ether and filtered off with suction.

Yield: 0.7 g

¹H NMR data: 1.2 (d, 3H); 1.3 (t, 3H); 1.3-3.3 (m, 16H); 4.2 (q, 2H); 4.1-4.6 (m, 4H); 4.7 (d, 1H); 7.1-7.4 (m, 5H).

EXAMPLE 34

N-(1S-Carboethoxy-3-phenyl-3-hydroxypropyl)-S-alanyl-2 β ,3 $\alpha\beta$,6 $\alpha\beta$ -octahydrocyclopenta[b]pyrrole-2-carboxylic acid

This compound was prepared according to the procedure described in Example 33 from the compound described in Example 29.

¹H NMR data: 1.2 (d, 3H); 1.3 (t, 3H); 1.4-3.9 (m, 14H); 4.2 (q, 2H); 4.1-4.7 (m, 4H); 4.7 (d, 1H); 7.0-7.4 (m, 5H).

EXAMPLE 35

N-(1S-Carboethoxy-3-phenyl-3-hydroxypropyl)-S-alanyl-2 β ,3 $\alpha\beta$,8 $\alpha\beta$ -decahydrocyclohepta[b]pyrrole-2-carboxylic acid

This compound was prepared according to the procedure described in Example 33 from the compound described in Example 31.

¹H NMR data: 1.25 (d+t, 6H); 1.3-4.0 (m, 13H); 4.2 (q, 2H); 4.1-4.6 (m, 4H); 4.8 (d, 1H); 7.0-7.5 (m, 5H).

EXAMPLE 36

Benzyl

S-alanyl-2S,3 α R,7 α R-octahydroindole-2-carboxylate

(a) Benzyl N-tert-butoxycarbonyl-S-alanyl-2S,3 α R,7 α R-octahydroindole-2-carboxylate

13 ml of N-ethylmorpholine, 13.5 g of 1-hydroxybenzotriazole and 29.6 g of benzyl 2 β ,3 $\alpha\beta$,7 $\alpha\beta$ -octahydroindole-2-carboxylate hydrochloride were added to a solution of 19 g of Boc-Ala-OH in 100 ml of DMF. The mixture was cooled in an ice bath and 31 g of dicyclohexylcarbodiimide were added. The mixture was stirred at 20° to 25° C. for 15 hours. The precipitated urea was filtered off with suction, the filtrate was evaporated in vacuo and taken up in ethyl acetate. The organic phase was extracted 3 times in each case with aqueous potassium bisulfate, potassium bicarbonate and sodium chloride, dried and concentrated. The residue was chromatographed on silica gel with ethyl acetate/cyclohexane (1:3). The first fraction contained the desired product.

Yield: 21 g

¹H NMR data: 1.3 (d, 3H); 1.45 (s, 9H); 1.1-2.4 (m, 12H); 3.2-3.9 (m, 2H); 5.3 (s, 2H); 7.4 (s, 5H).

(b) Benzyl S-alanyl-2S,3aR,7aR-octahydroindole-2-carboxylate

20.5 g of benzyl N-tert-butoxycarbonyl-S-alanyl-2S,3aR,7aR-octahydroindole-2-carboxylate were dissolved in 50 ml of trifluoroacetic acid. After a reaction time of 10 min., the solution was concentrated in vacuo, and the residue was triturated several times with diisopropyl ether and then dried in vacuo.

Yield: 14 g.

EXAMPLES 37 AND 38:

These compounds were prepared in analogy to the procedures described in Example 36 under (a) and (b).

EXAMPLE 37

Benzyl

S-alanyl-2S,3aR,6aR-octahydrocyclopenta[b]pyrrole-2-carboxylate

¹H NMR data: 1.3 (d, 3H); 1.1-2.4 (m, 10H); 3.2-3.9 (m, 2H); 5.2 (s, 2H); 7.4 (s, 5H).

EXAMPLE 38

Benzyl

S-alanyl-2S,3aR,8aR-decahydrocyclopenta[b]pyrrole-2-carboxylate

¹H NMR data: 1.3 (d, 3H); 1.1-2.4 (m, 14H); 3.2-3.9 (m, 2H); 5.2 (m, 2H); 7.4 (s, 5H).

EXAMPLE 39

N-(1S-Carboethoxy-3-phenylpropyl)-S-alanyl-2S,3aR,7aR-octahydroindole-2-carboxylic acid

(a) Benzyl N-(1S-carboethoxy-3-phenylpropyl)-S-alanyl-2S,3aR,7aR-octahydroindole-2-carboxylate

10 mmoles of benzyl S-alanyl-2S-3aR,7aR-octahydroindole-2-carboxylate were dissolved in 30 ml of anhydrous ethanol. The solution was adjusted to a pH of 7.0 by means of ethanolic potassium hydroxide and 1 g of powdered molecular sieve (4 Å) and then 10 mmoles of ethyl 2-keto-4-phenylbutyrate were added. A solution of 1 g of sodium cyanoborohydride in 10 ml of anhydrous ethanol was slowly added dropwise. After a reaction time of 20 hours at 20° to 25° C., the reaction solution was filtered and the solvent was distilled off. The residue was taken up in ethyl acetate/water. After concentration of the ethyl acetate phase, the residue was chromatographed on silica gel with ethyl acetate/cyclohexane (1:4). The ¹H NMR data agree with the data of the compound from Example 11.

(b) The compound obtained above was reacted further as described in Example 17, method A to give the desired compound.

EXAMPLE 40

Benzyl

N-(1S-carboethoxy-3-oxo-3-phenylpropyl)-S-alanyl-2β,3aβ,6aβ-octahydrocyclopenta[b]pyrrole-2-carboxylate

10 mmoles of benzyl S-alanyl-2S,3aR,6aR-octahydrocyclopenta[b]pyrrole-2-carboxylate were dissolved in 100 ml of anhydrous ethanol together with 10 mmoles of ethyl 3-benzoylacrylate and 10 mmoles of triethylamine and the mixture was stirred at 20° to 25° C. for 24 hours. It was then neutralized with 1N hydrochloric acid, evaporated to dryness and the residue was taken up with ethyl acetate/water. The ethyl acetate

phase was dried, evaporated and chromatographed on silica gel.

EXAMPLE 41

Benzyl

N-(1S-carboethoxy-3-oxo-3-phenylpropyl)-S-alanyl-2β,3aβ,8aβ-decahydrocyclopenta[b]pyrrole-2-carboxylate

10 mmoles of acetophenone, 10 mmoles of ethyl glyoxylate and 10 mmoles of benzyl S-alanyl-2S,3aR,8aR-decahydrocyclohepta[b]pyrrole-2-carboxylate were heated at 45° C. in 30 ml of glacial acid for 36 hours. After concentration in vacuo, the mixture was made alkaline with aqueous sodium bicarbonate and extracted with ethyl acetate. The ethyl acetate phase was concentrated and chromatographed on silica gel.

EXAMPLE 42

N-(1S-Carboethoxy-3-phenylpropyl)-S-alanyl-2S,3aR,7aS-octahydroindole-2-carboxylic acid

(a) Benzyl DL-2β,3aβ,7aα-octahydroindole-2-carboxylate hydrochloride

1.4 g of DL-2β,3aβ,7aα-octahydroindole-2-carboxylic acid were added, at -10° to 0° C., to a solution of 1.4 ml of thionyl chloride in 14 ml of benzyl alcohol prepared at -5° to 0° C. The mixture was stirred at 0° C. for 1 hour and was then allowed to stand overnight at 20° to 25° C. The benzyl alcohol was distilled off at 50° C. under high vacuum and the residue was triturated with diisopropyl ether. 2.5 g of colorless crystals, m.p. 154° C., were obtained.

(b) Benzyl N-(1S-carboethoxy-3-phenylpropyl)-S-alanyl-2β,3aβ,7aα-octahydroindole-2-carboxylate

1.06 g of 1-hydroxybenzotriazole, 2.2 g of benzyl DL-2β,3aβ,7aα-octahydroindole-2-carboxylate hydrochloride and 1.08 ml of N-ethylmorpholine were added to a suspension of 2.16 g of N-(1S-carboethoxy-3-phenylpropyl)-S-alanine in 8.6 ml of anhydrous dimethylformamide and then 1.7 g of dicyclohexylcarbodiimide were added at 0° C. After stirring at 20° to 25° C. for 3.5 hours, the reaction mixture was diluted with 20 ml of ethyl acetate and the precipitated dicyclohexylurea was filtered off. After concentration in vacuo, the residue was taken up in ether, washed twice with saturated aqueous sodium bicarbonate, dried over sodium sulfate and evaporated. After chromatography on silica gel using ethyl acetate-cyclohexane as the eluant, 2 pale yellow oils were obtained in the ratio 1:1, and these each contain one isomer of the desired compound.

¹H NMR data of the isomer having the 2R,3aR,7aS configuration: 7.35 (s, 5H); 7.2 (s, 5H); 5.18 (s, 2H); 4.55 (d, 1H); 4.1 (q, 2H); 3.4-2.3 (m, 6H); 2.4-1.3 (m, 12H); 1.3 (t, 3H); 1.1 (d, 3H).

¹H NMR data of the isomer having the 2S,2aS,7aR configuration: 7.35 (s, 5H); 7.2 (s, 5H); 5.16 (s, 2H); 4.9-4.2 (m, 1H); 4.2 (q, 2H); 3.9-2.4 (m, 6H); 2.4-1.4 (m, 12H); 1.25 (d+l, 6H).

(c) N-(1S-Carboethoxy-3-phenylpropyl)-S-alanyl-2S,3aR,7aS-octahydroindole-2-carboxylic acid

1.7 g of the isomer having the 2S,3aS,7aR configuration obtained under (b) were hydrogenated under normal pressure in 60 ml of anhydrous ethanol with the addition of 200 mg of palladium-charcoal (10%) at 20° to 25° C. for 2 hours. The catalyst was filtered off and the filtrate was evaporated. 1.2 g of the title compound were obtained as a colorless foam.

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¹H NMR data: 7.2 (s, 5H); 4.4 (m, 4H); 4.2 (q, 2H); 3.6-1.3 (m, 18H); 1.28 (d+t, 6H).

The hydrochloride of the abovementioned compound was obtained as a colorless amorphous powder.

EXAMPLES 43 TO 47

The compounds mentioned in these examples were prepared in analogy to the procedures described in Example 42.

EXAMPLE 43

N-(1*S*-Carboethoxy-3-phenylpropyl)-*S*-alanyl-2*S*,3*aS*-7*aR*-octahydroindole-2-carboxylic acid

This compound was obtained from the mixture of products from Example 51, which were further reacted according to the procedure of Example 42. The isomers were separated by column chromatography on silica gel before hydrogenation in accordance with Example 42c.

¹H NMR data: 7.2 (s, 5H); 4.0-4.6 (m, 4H); 4.15 (q, 2H); 3.8-1.2 (m, 18H); 1.3 (t, 3H); 1.2 (d, 3H).

The hydrochloride was obtained as a colorless amorphous powder.

EXAMPLE 44

N-(1*S*-Carboethoxy-3-phenylpropyl)-*S*-alanyl-2*S*,3*aR*-6*aS*-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

¹H NMR data: 7.25 (s, 5H); 4.35 (m, 4H); 4.2 (q, 2H); 3.9-1.4 (m, 16H); 1.35 (t, 3H); 1.15 (d, 3H).

The hydrochloride was obtained as a colorless amorphous powder.

EXAMPLE 45

N-(1*S*-Carboethoxy-3-phenylpropyl)-*S*-alanyl-2*S*,3*aS*-6*aR*-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

¹H NMR data: 7.3 (s, 5H); 4.3 (m, 4H); 4.2 (q, 2H); 3.8-1.3 (m, 16H); 1.3 (t+d, 6H).

The hydrochloride was obtained as a colorless amorphous powder.

EXAMPLE 46

N-(1*S*-Carboethoxy-3-phenylpropyl)-*S*-alanyl-2*S*,3*aR*-8*aS*-decahydrocyclohepta[b]pyrrole-2-carboxylic acid

¹H NMR data: 7.15 (s, 5H); 4.4 (m, 4H); 4.2 (q, 2H); 3.8-1.3 (m, 20H); 1.3 (t+d, 6H).

EXAMPLE 47

N-(1*S*-Carboethoxy-3-phenylpropyl)-*S*-alanyl-2*S*,3*aS*-8*aR*-decahydrocyclohepta[b]pyrrole-2-carboxylic acid

¹H NMR data: 7.2 (s, 5H); 4.4 (m, 4H); 4.25 (q, 2H); 3.8-1.2 (m, 20H); 1.3 (t, 3H); 1.15 (d, 3H).

EXAMPLE 48

2*β*,3*aβ*,7*aα*-Octahydroindolecarboxylic acid

(a) Methyl 3-chloro-*N*-acetylaniline

181 g of methyl 3-chloroaniline hydrochloride were heated under reflux with 163.9 g of acetyl chloride in 1.5 l of anhydrous toluene for about 5 hours until a clear solution was obtained. This was evaporated to dryness and the residue was crystallized from ethyl acetate/petroleum ether. 170 g of product of m.p. 104° C. were obtained.

(b) Methyl 3-(2-oxocyclohexyl)-*N*-acetylaniline

160 g of methyl 3-chloro-*N*-acetylaniline and 171.9 g of 1-pyrrolidinocyclohexene were dissolved in 1.2 l of

absolute DMF and the mixture was allowed to stand at 20° to 25° C. for 3 days.

The solution was concentrated under high vacuum, the residue obtained was taken up in 600 ml of water and the pH was adjusted to 2.0 with concentrated hydrochloric acid. The aqueous solution was extracted with ethyl acetate and the organic phase was dried over sodium sulfate and evaporated. The product was obtained as a yellow oil.

(c) 3,3*a*,4,5,6,7-2*H*-Hexahydroindole-2-carboxylic acid hydrochloride

220 g of the compound obtained under (b) were heated to boiling under reflux with 1 l of 2*N* of hydrochloric acid for 2 hours. The reaction mixture was extracted with ethyl acetate and the aqueous phase was concentrated. Residual amounts of water were removed by evaporation in vacuo with the addition of toluene three times. 210 g of the product were obtained as a yellow oil which crystallized on being left to stand.

(d) 2*β*,3*aβ*,7*aα*-Octahydroindole-2-carboxylic acid

128 g of 3,3*a*,4,5,6,7-2*H*-hexahydroindole-2-carboxylic acid hydrochloride were hydrogenated under normal pressure in 700 ml of glacial acetic acid with the addition of 4 g of platinum/charcoal (10%) at room temperature. The catalyst was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in 500 ml of hot ethanol and cooled down to -20° C. This caused the 2*β*,3*aβ*,7*aβ*-isomer of the title compound to precipitate out. The product was obtained from the solution by concentrating and adding isopropanol, in the form of colorless crystals of m.p. 280° C.

EXAMPLE 49

2*β*,3*aβ*,8*aα*-Decahydrocyclohepta[b]pyrrole-2-carboxylic acid

This compound was prepared in analogy to the procedures (b) to (d) of Example 48 starting from pyrrolidinocycloheptene.

EXAMPLE 50

2*β*,3*aβ*,6*aα*-Octahydrocyclopenta[b]pyrrole-2-carboxylic acid

This compound was prepared in analogy to the procedures (b) to (d) of Example 48, starting from pyrrolidinocyclopentene.

EXAMPLE 51

2*β*,3*aα*,7*aβ*-Octahydroindole-2-carboxylic acid

(a) 3,4,5,6,7,8-Octahydro-1*H*-quinolin-2-one

392 g of cyclohexanone and 212 g of acrylonitrile, together with 20 g of cyclohexylamine, 4 g of glacial acetic acid and 0.4 g of hydroquinone, were heated under reflux for 4 hours up to a final temperature of 200° C. The distillate obtained after distillation at 100° to 150° C./0.5 mm Hg was heated with 10 ml of 50% strength acetic acid at 200° C. for 2 days. After cooling down, the reaction mixture was recrystallized from methanol/water. Combined with the residue from distillation, obtained previously, which was recrystallized from n-hexane, 460 g of the product of m.p. 143° to 144° C. were obtained.

(b) Trans-octahydro-1*H*-quinolin-2-one

A mixture of 25 g of the product obtained under (a) and 70 g of sodium formate in 120 ml of formic acid were heated to boiling under reflux for 18 hours. The reaction solution was made alkaline with 20% strength

aqueous sodium hydroxide and extracted with ethyl acetate. The organic phase was dried over sodium sulfate and concentrated. The residue was recrystallized from cyclohexane and the product was obtained having an m.p. of 152° C.

(c) 3,3-Dichloro-trans-octahydro-1H-quinolin-2-one

A solution of 36.4 g of sulfuryl chloride in 40 ml of chloroform was added dropwise at 20° to 30° C. over 30 minutes to a solution of 19.4 g of the compound obtained under (b) and 24.3 g of phosphorus pentachloride in 300 ml of anhydrous chloroform. The mixture was heated to boiling for 6 hours and allowed to stand overnight at 20° to 25° C.

The mixture was neutralized with ice-cold saturated aqueous potassium carbonate, extracted with methylene chloride, and the organic phase was dried over sodium sulfate and then concentrated. After recrystallization of the product from ethanol, with the addition of active charcoal, 25 g of the product, of m.p. 195° to 198° C., were obtained.

(d) 3-Chloro-trans-octahydro-1H-quinolin-2-one

15.6 g of the compound obtained under (c) were hydrogenated under normal pressure in 1 l of ethanol and 9.7 ml of triethylamine, with the addition of Raney nickel, at 20° to 25° C. until 1 mole-equivalent of hydrogen had been taken up.

After the catalyst had been filtered off, the filtrate was evaporated to dryness and the residue was taken up in ethyl acetate. The organic phase was washed twice with water, dried over sodium sulfate and evaporated. The residue was triturated with diisopropyl ether, filtered off with suction and dried. The product was obtained in the form of pale yellow crystals of m.p. 115°-120° C.

(e) 2β,3α,7αβ-Octahydroindole-2-carboxylic acid

3.75 g of the compound obtained under (d) were added to a boiling solution of 6.63 g of barium hydroxide octahydrate in 120 ml of water. After heating under reflux for 4 hours, 0.9 ml of concentrated sulfuric acid were added, heating under reflux was continued a further hour, the reaction solution was filtered and the filtrate was adjusted to a pH of 6.5 with 1N sodium hydroxide. After evaporation of the solution, the residue was heated in ethanol, again filtered and the filtrate was evaporated to a small volume. On cooling down, a crystalline 1:1 mixture of the title compound and the compound of Example 48, of m.p. 275°-276° C. was obtained.

EXAMPLE 52

2β,3αβ,6αα-Octahydrocyclopenta[b]pyrrole-2-carboxylic acid

(a) 1,2,3,4,6,7-Hexahydro-5H-1-pyridin-2-one

A mixture of 1 mole of cyclopentanone, 1 mole of acrylonitrile, 0.05 mole of ammonium acetate and 3 ml of 30% strength ammonia were heated in a pressure vessel for 3 hours at 220° C. The mixture was filtered through silica gel with ethyl acetate/cyclohexane (1:1) and the residue obtained after evaporation of the filtrate was recrystallized from cyclohexane. The product melted at 118° to 120° C.

¹H NMR data: 9.4 (broad s, 1H); 3.2-2.0 (m, 12H).

(b) Octahydro-trans-5H-1-pyridin-2-one

This compound was prepared in analogy to the procedure described in Example 51 under (b).

¹H NMR data: 7.8 (broad s, 1H); 2.9 (broad s, 1H); 2.6-2.2 (m, 2H); 2.1-1.0 (m, 8H).

(c) 3,3-Dichlorooctahydro-trans-5H-1-pyridin-2-one

This compound was prepared in analogy to the procedure described in Example 51 under (c).

¹H NMR data: 7.9 (broad s, 1H); 3.8 (broad s, 1H); 3.2-2.0 (m, 2H); 2.1-1.0 (m, 6H).

(d) 3-Chlorooctahydro-trans-5H-1-pyridin-2-one

This compound was prepared in analogy to the procedure described in Example 51 under (d).

¹H NMR data: 7.8 (broad s, 1H); 4.6-4.3 (m, 1H); 3.3-3.0 (m, 1H); 2.1 (d, J=6 Hz, 2H); 1.8-1.1 (m, 6H).

(e) 2β,3αβ,6αα-Octahydrocyclopenta[b]pyrrole-2-carboxylic acid

This compound was prepared in analogy to the procedure described in Example 51 under (e).

¹H NMR data: 4.7-4.4 (m, 1H); 3.0-0.9 (m, 10H).

EXAMPLE 53

2β,3αβ,8αα-Decahydrocyclohepta[b]pyrrole-2-carboxylic acid

(a) 1,2,3,4,5,6,7,8-Octahydrocyclohepta[b]pyridin-2-one

This compound was prepared in analogy to the procedure described in Example 51 under (a), starting from cycloheptanone.

¹H NMR data: 9.4 (broad s, 1H); 3.2-2.0 (m, 14H).

(b) Trans-Decahydrocyclohepta[b]pyridin-2-one

This compound was prepared in analogy to the procedure described in Example 51 under (b), starting from the compound previously mentioned under (a).

¹H NMR data: 7.9 (broad s, 1H); 2.9 (broad s, 1H); 2.6-2.2 (m, 2H); 2.1-1.0 (m, 12H).

(c) 3,3-Dichloro-trans-decahydrocyclohepta[b]pyridin-2-one

This compound was prepared in analogy to the procedure described in Example 51 under (c).

¹H NMR data: 7.9 (broad s, 1H); 3.8 (broad s, 1H); 3.2-2.0 (m, 2H); 2.1-1.0 (m, 10H).

(d) 3-Chloro-trans-decahydrocyclohepta[b]pyridin-2-one

This compound was prepared in analogy to the procedure described in Example 51 under (d).

¹H NMR data: 7.8 (broad s, 1H); 4.6-4.3 (m, 1H); 3.3-3.0 (m, 1H); 2.1 (d, J=6 Hz, 2H); 1.8-1.1 (m, 10H).

(e) 2β,3αβ,8αα-Decahydrocyclohepta[b]pyrrole-2-carboxylic acid

This compound was prepared in analogy to the procedure described in Example 51 under (e).

¹H NMR data: 4.7-4.4 (m, 1H); 3.2-1.1 (m, 1H).

EXAMPLE 54

Tert-butyl 2β,3αα,7αβ-octahydroindole-2-carboxylate hydrochloride

This compound was prepared from 2β,3αα,7αβ-octahydroindole-2-carboxylic acid in analogy to the procedure described in Example 8.

¹H NMR data: 4.7-4.4 (m, 1H); 3.2-1.1 (m, 12H); 1.2 (s, 9H).

The compounds in the following Examples 55 to 59 were prepared in analogy to the procedure described in Example 8.

EXAMPLE 55

Tert-butyl 2β,3αβ,7αα-octahydroindole-2-carboxylate hydrochloride

¹H NMR data: 4.7-4.3 (m, 1H); 3.4-1.1 (m, 12H); 1.2 (s, 9H).

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EXAMPLE 56

Tert-butyl
2 β ,3 α ,6 $\alpha\beta$ -octahydrocyclopenta[b]pyrrole-2-carboxylate hydrochloride

¹H NMR data: 4.8-4.4 (m, 1H); 3.4-1.2 (m, 10H); 1.2 (s, 9H).

EXAMPLE 57

Tert-butyl
2 β ,3 $\alpha\beta$,6 $\alpha\alpha$ -octahydrocyclopenta[b]pyrrole-2-carboxylate hydrochloride

¹H NMR data: 4.8-4.4 (m, 1H); 3.5-1.2 (m, 10H); 1.2 (s, 9H).

EXAMPLE 58

Tert-butyl
2 β ,3 $\alpha\alpha$,8 $\alpha\beta$ -decahydrocyclohepta[b]pyrrole-2-carboxylate hydrochloride

¹H NMR data: 4.7-4.4 (m, 1H); 3.3-1.0 (m, 14H); 1.2 (s, 9H).

EXAMPLE 59

Tert-butyl
2 β ,3 $\alpha\beta$,8 $\alpha\alpha$ -decahydrocyclohepta[b]pyrrole-2-carboxylate hydrochloride

¹H NMR data: 4.7-4.3 (m, 1H); 3.2-0.8 (m, 14H); 1.2 (s, 9H).

EXAMPLE 60

Tert-butyl
N-(S-alanyl)-2S,3 α R,7 α S-octahydroindole-2-carboxylate hydrochloride

(a) Tert-butyl N-(N'-benzyloxycarbonyl-S-alanyl)-2S,3 α R,7 α S-octahydroindole-2-carboxylate
This compound was prepared from Z-alanine in analogy to the procedure described in Example 36 under (a), and separated from its isomer as described there.

¹H NMR data: 7.4 (s, 5H); 5.3-4.8 (m, 1H); 4.2-3.7 (m, 2H); 3.1-1.4 (m, 11H); 1.3 (d, J=7 Hz, 3H); 1.2 (s, 9H).

(b) The compound mentioned above under (a) was hydrogenated with palladium/barium sulfate in 1N ethanolic hydrogen chloride, the desired title compound being obtained.

¹H NMR data: 5.2-4.8 (m, 1H); 4.2-3.7 (m, 2H); 3.1-1.4 (m, 11H); 1.3 (d, J=7 Hz, 3H); 1.2 (s, 9H).

The compounds in the following Examples 61 to 65 were prepared in analogy to the procedures described in Example 60 under (a) and (b).

EXAMPLE 61

Tert-butyl
N-(S-alanyl)-2S,3 α S,7 α R-octahydroindole-2-carboxylate hydrochloride

¹H NMR data: 5.2-4.7 (m, 1H); 4.4-3.8 (m, 2H); 3.1-1.4 (m, 11H); 1.25 (d, J=7 Hz, 3H); 1.2 (s, 9H).

EXAMPLE 62

Tert-butyl
N-(S-alanyl)-2S,3 α R,6 α S-octahydrocyclopenta[b]pyrrole-2-carboxylate hydrochloride

¹H NMR data: 5.3-4.7 (m, 1H); 4.4-3.8 (m, 2H); 3.1-1.4 (m, 9H); 1.3 (d, J=7 Hz, 3H); 1.2 (s, 9H).

EXAMPLE 63

Tert-butyl
N-(S-alanyl)-2S,3 α S,6 α R-octahydrocyclopenta[b]pyrrole-2-carboxylate hydrochloride

¹H NMR data: 5.3-4.7 (m, 1H); 4.4-3.8 (m, 2H); 3.1-1.4 (m, 9H); 1.3 (d, J=7 Hz, 3H); 1.2 (s, 9H).

EXAMPLE 64

Tert-butyl
N-(S-alanyl)-2S,3 α R,8 α S-decahydrocyclopenta[b]pyrrole-2-carboxylate hydrochloride

¹H NMR spectrum: 5.1-4.6 (m, 1H); 4.5-3.7 (m, 2H); 3.1-1.4 (m, 13H); 1.3 (d, J=7 Hz, 3H); 1.2 (s, 9H).

EXAMPLE 65

Tert-butyl
N-(S-alanyl)-2S,3 α S,8 α R-decahydrocyclohepta[b]pyrrole-2-carboxylate hydrochloride

¹H NMR data: 5.1-4.6 (m, 1H); 4.5-3.7 (m, 2H); 3.1-1.4 (m, 13H); 1.3 (d, J=7 Hz, 3H); 1.2 (s, 9H).

EXAMPLE 66

N-(1S-Carboethoxy-3-phenylpropyl)-S-alanyl-2S,3 α R,7 α S-octahydroindole-2-carboxylic acid

This compound was prepared in analogy to the procedure described in Example 39 under (a), starting from tert-butyl N-(S-alanyl)-2S,3 α R,7 α S-octahydroindole-2-carboxylate. The tert-butyl group was split off with trifluoroacetic acid.

EXAMPLE 67

N-(1S-Carboethoxy-3-keto-3-phenylpropyl)-S-alanyl-2S,3 α R,7 α S-octahydroindole-2-carboxylic acid

This compound was prepared in analogy to the procedure described in Example 40, starting from tert-butyl N-(S-alanyl)-2S,3 α R,7 α S-octahydroindole-2-carboxylate. The tert-butyl group was split off with trifluoroacetic acid.

¹H NMR data: 1.2 (d+t, 6H); 1.3-3.6 (m, 16H); 4.2 (q, 2H); 4.1-4.6 (m, 4H); 7.3-8.1 (m, 5H).

EXAMPLE 68

N-(1S-Carboethoxy-3-keto-3-phenylpropyl)-S-alanyl-2S,3 α S,7 α R-octahydroindole-2-carboxylic acid

This compound was prepared in analogy to the procedure described in Example 40, starting from tert-butyl N-(S-alanyl)-2S,3 α S,7 α R-octahydroindole-2-carboxylate. The tert-butyl group was split off with trifluoroacetic acid.

¹H NMR data: 1.2 (d+t, 6H); 1.3-3.6 (m, 16H); 4.2 (q, 2H); 4.1-4.6 (m, 4H); 7.3-8.1 (m, 5H).

EXAMPLE 69

N-(1S-Carboethoxy-3-keto-3-phenylpropyl)-S-alanyl-2S,3 α R,6 α S-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

This compound was prepared in analogy to the procedure described in Example 40, starting from tert-butyl N-(S-alanyl)-2S,3 α R,6 α S-octahydrocyclopenta[b]pyrrole-2-carboxylate. The tert-butyl group was split off with trifluoroacetic acid.

¹H NMR data: 1.1 (d, 3H); 1.35 (t, 3H); 1.0-1.7 (m, 14H); 4.2 (q, 2H); 4.0-4.7 (m, 4H); 7.3-8.0 (m, 5H).

EXAMPLE 70

N-(1S-Carboethoxy-3-keto-3-phenylpropyl)-S-alanyl-2S,3aS,6aR-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

This compound was prepared in analogy to the procedure described in Example 41, followed by splitting off the tert.-butyl group with trifluoroacetic acid.

¹H NMR data: 1.1 (d, 3H); 1.35 (t, 3H); 1.0-3.8 (m, 14H); 4.2 (q, 2H); 4.0-4.7 (m, 4H); 7.2-8.1 (m, 5H).

EXAMPLE 71

N-(1S-Carboethoxy-3-keto-3-phenylpropyl)-S-alanyl-2S,3aR,8aS-decahydrocyclohepta[b]pyrrole-2-carboxylic acid

This compound was prepared in analogy to the reaction procedure described in Example 41, following by splitting off the tertiary butyl group with trifluoroacetic acid.

¹H NMR data: 1.2 (d, 3H); 1.4 (t, 3H); 1.2-3.8 (m, 18H); 4.1 (q, 2H); 4.0-4.6 (m, 4H); 7.2-8.2 (m, 5H).

EXAMPLE 72

N-(1S-Carboethoxy-3-keto-3-phenylpropyl)-S-alanyl-2S,3aS,8aR-decahydrocyclohepta[b]pyrrole-2-carboxylic acid

This compound was prepared in analogy to the procedure described in Example 41, followed by cleaving off the tert.-butyl group with trifluoroacetic acid.

¹H NMR data: 1.2 (d+t, 6H); 1.2-3.8 (m, 18H); 4.2 (q, 2H); 4.0-4.6 (m, 4H); 7.1-8.1 (m, 5H).

EXAMPLE 73

N-(1S-Carboethoxy-3-hydroxy-3-phenylpropyl)-S-alanyl-2S,3aR,7aS-octahydroindole-2-carboxylic acid

This compound was prepared in analogy to the procedure described in Example 33.

¹H NMR data: 1.2 (d, 3H); 1.3 (t, 3H); 1.3-3.8 (m, 16H); 4.2 (q, 2H); 4.0-4.6 (m, 4H); 4.7 (d, 1H); 7.1-7.4 (m, 5H).

EXAMPLE 74

N-(1S-Carboethoxy-3-hydroxy-3-phenylpropyl)-S-alanyl-2S,3aS,6aR-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

This compound was prepared in analogy to the procedure described in Example 33.

¹H NMR data: 1.2 (d+t, 3H); 1.1-3.9 (m, 14H); 4.2 (q, 2H); 4.0-4.7 (m, 4H); 4.8 (d, 1H); 7.1-7.4 (m, 5H).

EXAMPLES 75 TO 80

The compounds mentioned in these examples were prepared in analogy to the procedure described in Example 20.

EXAMPLE 75

N-(1S-Carboxy-3-phenylpropyl)-S-alanyl-2S,3aR,7aS-octahydroindole-2-carboxylic acid

¹H NMR data: 1.2 (d, 3H); 1.2-3.8 (m, 18H); 4.0-4.6 (m, 4H); 7.2 (s, 5H).

EXAMPLE 76

N-(1S-Carboxy-3-phenylpropyl)-S-alanyl-2S,3aS,7aR-octahydroindole-2-carboxylic acid

¹H NMR spectrum: 1.2 (d, 3H); 1.2-3.8 (m, 18H); 4.0-4.6 (m, 4H); 7.2 (s, 5H).

EXAMPLE 77

N-(1S-Carboxy-3-phenylpropyl)-S-alanyl-2S,3aR,6aS-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

¹H NMR data: 1.2 (d, 3H); 1.1-3.7 (m, 16H); 4.0-4.6 (m, 4H); 7.2 (s, 5H).

EXAMPLE 78

N-(1S-Carboxy-3-phenylpropyl)-S-alanyl-2S,3aS,6aR-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

¹H NMR spectrum: 1.2 (d, 3H); 1.1-3.8 (m, 16H); 4.0-4.6 (m, 4H); 7.2 (s, 5H).

EXAMPLE 79

N-(1S-Carboxy-3-phenylpropyl)-S-alanyl-2S,3aR,6aS-decahydrocyclohepta[b]pyrrole-2-carboxylic acid

¹H NMR data: 1.2 (d, 3H); 0.9-3.6 (m, 20H); 4.0-4.6 (m, 4H); 7.2 (s, 5H).

EXAMPLE 80

N-(1S-Carboxy-3-phenylpropyl)-S-alanyl-2S,3aS,8aR-decahydrocyclohepta[b]pyrrole-2-carboxylic acid

¹H NMR data: 1.2 (d, 3H); 0.8-3.6 (m, 20H); 4.0-4.6 (m, 4H); 7.2 (s, 5H).

EXAMPLE 81

N-(1S-Carboxy-3-keto-3-phenylpropyl)-S-alanyl-2S,3aR,7aS-octahydroindole-2-carboxylic acid

¹H NMR data: 1.2 (d, 3H); 0.9-3.6 (m, 16H); 3.9-4.7 (m, 4H); 7.1-8.2 (m, 5H).

EXAMPLE 82

N-(1S-Carboxy-3-keto-3-phenylpropyl)-S-alanyl-2S,3aS,7aR-octahydroindole-2-carboxylic acid

¹H NMR data: 1.2 (d, 3H); 0.9-3.6 (m, 16H); 3.9-4.7 (m, 4H); 7.2-8.1 (m, 5H).

EXAMPLE 83

N-(1S-Carboxy-3-keto-3-phenylpropyl)-S-alanyl-2S,3aR,6aS-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

¹H NMR data: 1.2 (d, 3H); 1.1-3.7 (m, 14H); 3.9-4.6 (m, 4H); 7.2-8.1 (m, 5H).

EXAMPLE 84

N-(1S-Carboxy-3-keto-3-phenylpropyl)-S-alanyl-2S,3aS,6aR-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

¹H NMR data: 1.2 (d, 3H); 1.1-3.7 (m, 14H); 3.9-4.6 (m, 4H); 7.2-8.1 (m, 5H).

EXAMPLE 85

N-(1S-Carboxy-3-keto-3-phenylpropyl)-S-alanyl-2S,3aR,8aS-decahydrocyclohepta[b]pyrrole-2-carboxylic acid

¹H NMR data: 1.2 (d, 3H); 1.1-3.7 (m, 18H); 3.9-4.6 (m, 4H); 7.3-8.2 (m, 5H).

N-(1S-Carboxy-3-phenylpropyl)-S-alanyl-2S,3aR,6aS-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

N-(1S-Carboxy-3-phenylpropyl)-S-alanyl-2S,3aR,8aS-decahydrocyclohepta[b]pyrrole-2-carboxylic acid

N-(1S-Carboxy-3-phenylpropyl)-S-alanyl-2S,3aR,6aS-decahydrocyclohepta[b]pyrrole-2-carboxylic acid

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EXAMPLE 86

N-(1S-Carboxy-3-keto-3-phenyl)-alanyl-2S,3aS,8aR-decahydrocyclohepta[b]pyrrole-2-carboxylic acid

¹H NMR data: 1.2 (d, 3H); 1.1-3.7 (m, 18H); 3.9-4.6 (m, 4H); 7.3-8.2 (m, 5H).

EXAMPLE 87

N-(1S-Carboxy-3-hydroxy-3-phenylpropyl)-S-alanyl-2S,3aR,7aS-octahydroindole-2-carboxylic acid

¹H NMR spectrum: 1.2 (d, 3H); 1.1-3.7 (m, 16H); 3.8-4.6 (m, 4H); 4.7 (m, 1H); 7.1-7.4 (m, 5H).

EXAMPLE 88

N-(1S-Carboxy-3-hydroxy-3-phenylpropyl)-S-alanyl-2S,3aS,6aR-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

¹H NMR data: 1.2 (d, 3H); 1.1-3.7 (m, 14H); 3.9-4.5 (m, 4H); 4.7 (d, 1H); 7.1-7.4 (m, 5H).

EXAMPLE 89

2β, 3aβ, 7αα-octahydroindole-2-carboxylic acid

290 g of the acetylamino derivative obtained in accordance with Example 1 were heated with 2N hydrochloric acid as indicated in Example 2. The mixture was evaporated in vacuo, the residue was taken up in 1 l of isopropanol and reduced with about 35 g of NaBH₄ which was added in portions in the course of 30 minutes. The reaction temperature was kept at 40°-50° C. The reaction was allowed to continue for about 4 hours, then the mixture was evaporated in vacuo, the pH was adjusted to 6.5 with dilute hydrochloric acid, solid sodium chloride was used to saturate, and the aminoacids were extracted several times with n-butanol. The residue remaining after concentration of the organic phase was fractionally crystallized from chloroform/diisopropyl ether as described in Example 2.

Yields: 40-60 g of 2β, 3a, 7αα-product, 20 g of a mixed fraction, 100-130 g 2β, 3aβ, 7αα-product.

In the same manner, 2β, 3aβ, 7αα-2-azabicyclo[5.3.0]-decane-3-carboxylic acid and 2β, 3aβ, 7αα-2-azabicyclo[6.3.0]-undecane-3-carboxylic acid can be prepared.

EXAMPLE 90

N-(1S-Carbethoxy-3-phenyl-propyl)-O-ethyl-S-tyrosyl-2S,3aR,7aS-octahydroindole-2-carboxylic acid

(a) N-(1-R,S-Carbethoxy-3-phenyl-propyl)-O-ethyl-S-tyrosylbenzylester

24 g of benzoylacrylic acid ethyl ester are reacted in 100 ml of ethanol with 30 g of O-ethyl-S-tyrosinebenzylester in the presence of 0.5 ml of triethylamine, and after concentration of the solution and digestion of the residue with diethyl ether/petroleum ether (1:1) as well as drying in vacuo, 42 g of the compound indicated in the heading.

(b) N-(1-R,S-Carbethoxy-3-phenyl-propyl)-O-ethyl-S-tyrosine

40 g of the compound obtained according to Example 90a are hydrogenated in 800 ml of glacial acetic acid and 4 g of Pd/C (10%) at 100 bar and room temperature. After chromatography on silica gel with ethyl acetate/cyclohexane (1:3) as eluent and drying of the evaporation residue, 25 g of compound of the heading, m.p. 205°-213° C., nearly completely homogeneous according to thin-layer chromatography.

C₂₃H₂₉NO₃(399.5) calc. C 69.15, H 7.31, N 3.50. found C 69.5, H 7.4, N 3.3.

(c) N-(1-S-Carbethoxy-3-phenyl-propyl)-O-ethyl-S-tyrosyl-2S,3aR,7aS-octahydroindole-2-carboxylic acid

According to the operation mode described in Example 42b, 1.5 g of the benzyl ester described in Example 42a are reacted with 2.5 g of N-(1-R,S-carbethoxy-3-phenyl-propyl)-O-ethyl-S-tyrosine, 1.3 g of dicyclohexylcarbodiimide and 0.8 g of 1-hydroxybenzotriazole. Chromatography of the crude product on silica gel with cyclohexane/ethyl acetate (1:1) as eluent gives 1 g of the compound of the heading in the form of a colorless oil. The ¹H-NMR data and the mass spectrum correspond to the indicated structure.

The benzyl ester is hydrogenated according to the operation mode described in Example 42a. 0.6 g of the compound of the heading is obtained as colorless, amorphous powder.

¹H-NMR data: 7.3 (s, 5H); 7.1-6.5 (2d, 4H); 4.4-4.0 (m, 4H); 3.9-3.0 (m, 4H); 2.9-1.2 (m, 17H); 1.4 (t, 3H); 1.25 (t, 3H).

EXAMPLE 91

N-(1-S-carbethoxy-3-phenyl-propyl)-O-methyl-S-tyrosyl-2S,3aR,7aS-octahydroindole-2-carboxylic acid

This compound is obtained according to the operation mode described in Example 90 with the use of O-methyl-tyrosine-benzyl ester instead of O-ethyl-tyrosine benzyl ester in the step analogous to 90a).

¹H-NMR data: 7.2 (s, 5H); 7.1-6.5 (2d, 4H); 4.4-4.0 (m, 3H); 3.9-3.0 (m, 3H); 3.5 (s, 3H); 2.9-1.2 (m, 17H); 1.3 (t, 3H).

EXAMPLE 92

N-(1-S-carbethoxy-3-phenyl-propyl)-O-ethyl-S-tyrosyl-2S,3aS,7aR-octahydroindole-2-carboxylic acid

Prepared according to the operation mode of Example 90 from the amino acid described in Example 51. The ¹H-NMR data correspond to the indicated structure.

EXAMPLE 93

N-(1-S-carbethoxy-3-phenyl-propyl)-O-methyl-S-tyrosyl-2S,3aS,7aR-octahydroindole-2-carboxylic acid

Prepared according to the operation mode described in Example 91 with the use of the amino acid described in Example 51. The ¹H-NMR data correspond to the indicated structure.

EXAMPLE 94

N-(1-S-carbethoxy-3-phenyl-propyl)-O-ethyl-S-tyrosyl-2S,3aR,6aS-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

Prepared according to the operation mode of Example 90 with the use of the amino acid described in Example 52.

¹H-NMR data: 7.3 (s, 5H); 7.2-6.6 (2d, 4H); 4.4-3.9 (m, 4H); 3.9-3.0 (m, 4H); 2.9-1.2 (m, 15H); 1.35 (t, 3H); 1.25 (t, 3H).

EXAMPLE 95

N-(1-S-carbethoxy-3-phenyl-propyl)-O-methyl-S-tyrosyl-2S,3aR,6aS-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

Prepared according to the operation mode of Example 91 with the use of the amino acid described in Example 52. The ¹H-NMR-data correspond to the indicated structure.

EXAMPLE 96

N-(1-S-carbethoxy-3-phenyl-propyl)-O-ethyl-S-tyrosyl-2S,3aS,6aR-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

Prepared according to the operation mode described in Example 90. The analysis data correspond to the indicated structure.

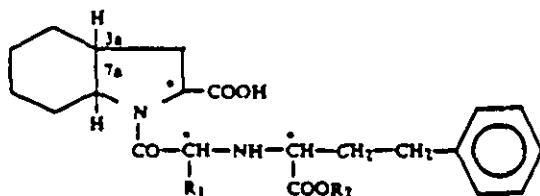
EXAMPLE 97

N-(1-S-carbethoxy-3-phenyl-propyl)-O-methyl-S-tyrosyl-2S,3aS,6aR-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

Prepared according to the operation mode described in Example 91. The analysis data correspond to the indicated structure.

We claim:

1. A compound of the formula



or a physiologically acceptable salt thereof, wherein the hydrogen atoms on the bridgehead C atoms in the 3a-position and 7a-position have the trans-configuration

relative to one another and those carbon atoms labelled with an asterisk all have the (S) configuration;

R₁ is (C₁-C₃)-alkyl; and

R₂ is hydrogen or (C₁-C₄)-alkyl.

2. A compound or salt as in claim 1 wherein the C atom in the 2-position of the bicyclic ring system and those C atoms in the side chain labeled with an asterisk all have the S configuration.

3. A compound or salt as in claim 1 wherein the hydrogen atoms on the bridgehead C atom in the 3a-position and on the C atom in the 2-position of the bicyclic ring system are on the same side of the reference plane of the ring atoms.

4. A compound or salt as in claim 1 wherein the hydrogen atoms on the bridgehead C atom in the 3a-position and on the C atom in the 2-position of the bicyclic ring system are on opposite sides of the reference plane of the ring atoms.

5. A compound or salt as in claim 1 wherein R₁ is methyl and X is phenyl.

6. A compound or salt as in claim 1 wherein R₂ is hydrogen or ethyl.

7. A compound as in claim 1 which is N-(1S-carbethoxy-3-phenylpropyl)-S-alanyl-2S,3aS,7aR-octahydroindole-2-carboxylic acid or a physiologically acceptable salt thereof.

8. A compound as in claim 1 which is N-(1S-carbethoxy-3-phenylpropyl)-S-alanyl-2S,3aR,7aS-octahydroindole-2-carboxylic acid or a physiologically acceptable salt thereof.

9. A method for treating high blood pressure in a patient suffering therefrom which comprises administering to said patient a hypotensively effective amount of a compound or salt as in claim 1.

10. A composition for the treatment of high blood pressure comprising a hypotensively effective amount of a compound or salt as in claim 1 and a pharmaceutically acceptable excipient therefor.

11. A method as in claim 9 wherein said compound or salt is administered together with a diuretic.

12. A composition as in claim 10 which additionally comprises a diuretic.

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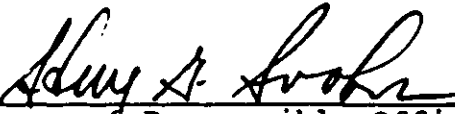
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CERTIFICATION IMPOSED BY
GENERIC DRUG ENFORCEMENT ACT

Knoll Pharmaceutical Company hereby certifies that we did not and will not use in any capacity the services of any person or firm convicted or debarred under Section 306 (a) or (b) in connection with this application.

No affiliated persons responsible for the development or submission of this application have been convicted as described in Section 306 (a) or (b), within the last five years.


Signature of Responsible Official

Vice President
Regulatory and Technical Affairs
Title

November 14, 1994
Date

DRUG STUDIES IN PEDIATRIC PATIENTS
(To be completed for all NME's recommended for approval)

SEP 28 1995

NDA # 26 528 Trade (generic) names Mobic (triamolone) Tablets

Check any of the following that apply and explain, as necessary, on the next page:

1. A proposed claim in the draft labeling is directed toward a specific pediatric illness. The application contains adequate and well-controlled studies in pediatric patients to support that claim.
2. The draft labeling includes pediatric dosing information that is not based on adequate and well-controlled studies in children. The application contains a request under 21 CFR 210.58 or 314.126(c) for waiver of the requirement at 21 CFR 201.57(f) for A&W studies in children.
- a. The application contains data showing that the course of the disease and the effects of the drug are sufficiently similar in adults and children to permit extrapolation of the data from adults to children. The waiver request should be granted and a statement to that effect is included in the action letter.
- b. The information included in the application does not adequately support the waiver request. The request should not be granted and a statement to that effect is included in the action letter. (Complete #3 or #4 below as appropriate.)
3. Pediatric studies (e.g., dose-ranging, pharmacokinetic, adverse reaction, adequate and well-controlled for safety and efficacy) should be done after approval. The drug product has some potential for use in children, but there is no reason to expect early widespread pediatric use (because, for example, alternative drugs are available or the condition is uncommon in children).
- a. The applicant has committed to going such studies as will be required.
- (1) Studies are ongoing.
- (2) Protocols have been submitted and approved.
- (3) Protocols have been submitted and are under review.
- (4) If no protocol has been submitted, on the next page explain the status of discussions.
- b. If the sponsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request.
4. Pediatric studies do not need to be encouraged because the drug product has little potential for use in children.

___ 5. If none of the above apply, explain.

Explain, as necessary, the foregoing items: _____

Multiple horizontal lines for handwritten explanation.

[Handwritten Signature]
Signature of Preparer:

9/5/95
Date

cc: Orig NDA
HFD-___/Div File
NDA Action Package

101
APR 24 1996

BIOPHARMACEUTICS / PHARMACOKINETICS REVIEW

NDA: 20-528 (Amendment)

SUBMISSION DATES: April 19, 1996

Priority: 1 S

MAVIK® (Trandolapril)

Tablets (1, 2 and 4 mg)

KNOLL PHARMACEUTICALS.

REVIEWER: Emmanuel O. Fadiran, Ph.D.

TYPE OF SUBMISSION: Revised Dissolution Specification - Sponsor's Response

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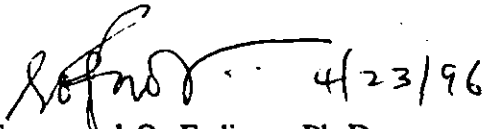
BACKGROUND:

The joint Biopharmaceutics-Statistics-Medical review of NDA 20-528 was completed on July 7, 1996 and an approvable letter was issued on September 25, 1996. Based on the dissolution data submitted to the NDA it was recommended that the dissolution specification should be changed from Q= in 30 minutes to Q= in 30 minutes. The sponsor has now submitted stability data at 6 and 12 months (attached) that shows that future batches of the product might not meet the proposed specification during their expiry period.

RECOMMENDATION:

The Division of Pharmaceutical Evaluation I has reviewed the the dissolution stability data submitted by the sponsor and after consultation with Dr. Bob Waters (Chemist Team Leader) has made the following conclusions:

1. An interim dissolution specification of Q= in 45 minutes is recommended.
2. The sponsor should continue the stability study and sampling at 30 minutes and 45 minutes.
3. The dissolution specification will be reviewed with the annual report.


Emmanuel O. Fadiran, Ph.D.
Pharmacokinetics Evaluation Branch I

FT Initialed by A. Parekh, Ph.D.


Ameeta Parekh
4/24/96

cc: NDA 20-528, HFD-110, HFD-860 (Fadiran, Mehta, Malinowski), HFD-880 (Fleischer),
HFD-870 (M. Chen), HFD-870: Chron, Drug, Review Files (Clarence Bott, PKLN Rm
13B-31), FOI (HFD-19), HFD-340 (Vish).

MAR 12 1996

DIVISION OF CARDIO-RENAL DRUG PRODUCTS MEDICAL OFFICER'S REVIEW

NDA: 20-528 (Mavik®, trandolapril)
Sponsor: Knoll Pharmaceutical Company
Submission: Response to reviewers' requests
Submission date: 7 February 1996
Receipt date: 8 February 1996
Review date: 11 March 1996
Reviewers: N. Stockbridge, M.D., Ph.D.

1. Resume

During the course of the review of this NDA, it became clear that no study reports were provided for an number of trandolapril studies conducted by the sponsor in Europe. The sponsor provided adequate descriptions of pivotal U.S. trials. The adequacy of European safety data could not be ascertained. The sponsor provided a list of previously undescribed studies, and, as a condition for approval, has now provided updated safety information pertaining to deaths and withdrawals.

2. Extent of coverage

The sponsor provided a list of studies covered by this submission. Included in this list were studies for which there are no reported withdrawals or deaths. This list was compared with the submission of 30 June 1995, which was the list of unreported studies. The following studies were listed in the earlier submission, but have no mention in the present submission:

2.1. Studies said to have been included in the original NDA safety summary

2.1.1. Short-term studies

<i>F/86/570/10</i>	<i>n=18</i>	<i>8, 16 mg</i>	<i>R, DB, PC</i>	<i>7 days</i>	<i>tolerance</i>
<i>FF/87/570/02</i>	<i>n=169</i>	<i>4</i>	<i>R, DB, AC</i>	<i>?</i>	<i>comparison with enalapril</i>
<i>FF/87/570/03</i>	<i>n=180</i>	<i>4</i>	<i>R, DB, AC</i>	<i>?</i>	<i>comparison with captopril</i>
<i>FF/87/570/05</i>	<i>n=295</i>	<i>2, 4</i>	<i>R, DB, AC</i>	<i>?</i>	<i>comparison with atenolol</i>
<i>FF/87/570/06</i>	<i>n=158</i>	<i>2</i>	<i>R, DB, AC</i>	<i>?</i>	<i>factorial with nifedipine</i>
<i>UK/89/570/01</i>	<i>n=236</i>	<i>1, 2</i>	<i>R, DB, AC</i>	<i>?</i>	<i>comparison with lisinopril</i>
<i>J/90/570/110</i>	<i>efficacy study; "[f]ull report not available."</i>				

2.1.2. Long-term studies

FFR/92/570/164 *Follow-on to FF/89/570/49 and FF/90/570/98; "[f]ull report not available."*

2.1.3. Studies in special populations

FF/89/570/44 *Elderly, long term; "[f]ull report not available."*
FF/89/570/51 *LVH, comparison with enalapril; "[f]ull report not available."*
FFR/90/570/107 *Chronic renal failure; "[f]ull report not available."*
F/91/570/149 *O.d. dosing, comparison with nitrendipine; "[f]ull report not available."*
J/90/570/09 *Elderly, severe hypertension; "[f]ull report not available."*

2.1.4. Other hypertension studies

UK/89/570/05 *n=64* *1, 2* *R, DB, UC* *? days* *ABPM*
FFR/92/570/177 *Compliance; "[f]ull report not available."*

J/90/570/11	Lipid profiles; "[f]ull report not available."
7-22	No information provided; "[f]ull report not available."
61-1	No information provided; "[f]ull report not available."
42-1	No information provided; "[f]ull report not available."
58-1	No information provided; "[f]ull report not available."
56-1	No information provided; "[f]ull report not available."

2.2. Studies excluded from the sponsor's integrated summary of safety

2.2.1. Clinical pharmacology

F/90/570/112	n=459 0.125-32 PC variable summary of >1 studies?
F/85/570/02	Pharmacokinetics, pharmacodynamics; "[f]ull report not available."
F/86/570/12	Absolute bioavailability; "[f]ull report not available."
F/87/570/12	Transcutaneous dosing; "[f]ull report not available."
UK/88/570/03	Response to bradykinin; "[f]ull report not available."
F/88/570/36	Transcutaneous dosing; "[f]ull report not available."
F/88/570/37	Cirrhosis; "[f]ull report not available."
F/89/570/56	n=? 4 DB, PC, XO 1 day renal function; "[f]ull report not available."
GB/89/570/71	Hemodynamics with a diuretic; "[f]ull report not available."
J/90/570/101	Acute hemodynamics and endocrine effects; "[f]ull report not available."
J/90/570/104	Bioequivalence; "[f]ull report not available."
J/90/570/105	Bioequivalence; "[f]ull report not available."
J/91/570/114	Renal function; "[f]ull report not available."
J/91/570/115	Renal impairment, pharmacokinetics; "[f]ull report not available."
J/91/570/125	Bioequivalence; "[f]ull report not available."
J/91/570/126	Renal impairment; "[f]ull report not available."
J/91/570/131	Renal impairment; "[f]ull report not available."
UK/92/570/171	CHF, pharmacokinetics; "[f]ull report not available."
ZA/93/570/184	Bioequivalence; "[f]ull report not available."
D/89/570/85	LVH; "[f]ull report not available."
CDN/90/570/97	Autonomic effects; "[f]ull report not available."
I/91/570/127	Hemodynamics, comparison with nifedipine; "[f]ull report not available."
B/91/570/141	LVH, comparison with atenolol; "[f]ull report not available."

2.2.2. Hypertension studies

F/86/570/13	Once-a-week for 8 weeks; "[f]ull report not available."
J/88/570/04	Initial dose; "[f]ull report not available."
J/88/570/05	Diurnal effects; "[f]ull report not available."
J/88/570/08	Long-term; "[f]ull report not available."
J/89/570/66	Open dose-ranging; "[f]ull report not available."
J/89/570/67	Open dose-ranging; "[f]ull report not available."
I/89/570/87	Coronary blood flow; "[f]ull report not available."
J/90/570/10	Renal disorders; "[f]ull report not available."
CDN/90/570/97	Effect on autonomic function; "[f]ull report not available."
I/91/570/127	Hemodynamics, comparison with nifedipine; "[f]ull report not available."
B/91/570/141	LVH, comparison with atenolol; "[f]ull report not available."
GR/92/570/173	Follow-on to FFR/89/570/55; "[f]ull report not available."

D/92/570/175 LVH; "[f]ull report not available."
 CR/92/570/176 Efficacy; "[f]ull report not available."
 FFR/92/570/178 Self-measurement, comparison with perindopril; "[f]ull report not available."

2.2.3. Miscellaneous studies

2.3. Newly reported studies

The following studies were not part of the original NDA submission and were not mentioned in the amendment filed 30 June 1995, but appear in the list of deaths and withdrawals in the present submission:

F/88/570/32
 GB/89/570/72

3. Deaths

Table 1 below is a list of deaths by study protocol. Deaths previously described in the NDA review are not included in this listing. In addition to those shown, there was one subject in Study FF/88/570/29 who died 3 months after the last dose of study drug and for whom the CRFs are not "available". CRFs were missing for two deaths in Study FFR/92/570/166. Thirteen events from this study have been narratively summarized. All of these studies were undertaken by Knoll.

Table 1. Previously unreported deaths.

Study	n	Study	n
	2	UK/89/570/70	1
FF/89/570/44	7	FFR/92/570/166	13/15
FF/89/570/49	3	Total	27/29
FFR/89/570/59	1		

Study FF/89/570/44 was a long-term study in the elderly hypertensive population.

Study FF/89/570/49 was a long-term study in subjects on combined treatment with diuretics and/or calcium channel blocker.

FFR/89/570/59 was a study in subjects with non-insulin-dependent diabetes mellitus.

Study FFR/92/570/166 was a study in the elderly hypertensive population.

Brief descriptions of deaths are shown in Table 2 below.

Table 2. Descriptions of previously undescribed deaths.

	Subject	Event
	0164/0004	75 year old Caucasian female with 10 year history of heart failure. Death from heart failure on study day 12. Investigator indicated improbable relationship to study drug.
	0164/0011	65 year old Caucasian male with 2 month history of heart failure. Subject discontinued because of skin rash. Also had episode of ventricular tachycardia. Death 10 days post-treatment was from terminal heart failure. Investigator did not attribute events to study drug.
FF/89/570/44	0089/0004	73 year old Caucasian male with 2 year history of hypertension. Subject on treatment for 376 days prior to unexpected death from pulmonary infection and sudden septic shock. Investigator considered events unrelated to study drug.
FF/89/570/44	0089/0009	76 year old Caucasian female with 15 year history of hypertension. Subject withdrawn from study for acute lower-limb arterial obstruction, diabetes, hyponatremia, and pulmonary infection. Death 78 days after withdrawal was from septicemia. Investigator considered events unrelated to study drug.
FF/89/570/44	0089/0014	83 year old Caucasian female. Death from cerebrovascular accident after 372 days on study drug. Investigator considered events unrelated to study drug.
FF/89/570/44	0089/0023	Subject died from "pneumonia with septic shock and hydroelectric disorder" after 290 days on study drug. Investigator considered events unrelated to study drug.
FF/89/570/44	0089/0035	82 year old caucasian female. Subject died from intestinal obstruction and gastric aspiration after 220 days on study drug (14 days after discontinuation). Investigator considered events unrelated to study drug.
FF/89/570/44	2518/0362	80 year old Caucasian female with 3 year history of hypertension. Subject died from myocarditis after 420 days on study drug. Investigator considered events unrelated to study drug.
FF/89/570/44	2629/0434	70 year old Caucasian female with 12 year history of hypertension. Subject discontinued for abdominal pain and died 14 days later from pancreatic carcinoma. Investigator considered events unrelated to study drug.
FF/89/570/49	0000/0139	71 year old Caucasian male with 1 year history of hypertension. Subject with hip fracture and surgery died from pulmonary embolism 14 days post-surgery and 6 days after withdrawal from study drug. Investigator considered event improbably related to study drug.
FF/89/570/49	1825/0484	51 year old 'other' female with 5 year history of hypertension. Subject died from cardiac arrest while hospitalized for acute hemorrhagic pancreatitis after 546 days on study drug. Investigator considered events improbably related to study drug.
FF/89/570/49	0072/0096	66 year old Caucasian female with 7 year history of hypertension. Subject with "asthenia, headache, fall, sudden death" after 843 days on study drug. Investigator did not comment on relationship to study drug.
FFR/89/570/59	0068/0098	77 year old Caucasian female with history of myocardial infarction. Subject with exertional dyspnea, epigastric pain, collapse and tachycardia discontinued and died from pulmonary embolism 5 days later. Investigator considered events improbably related to study drug.
UK/89/570/70	0000/0048	63 year old Caucasian male with 5 year history of coronary artery disease and prior myocardial infarction. Subject experienced sudden death after 4 days on study drug. Investigator considered events improbably related to study drug.
FFR/91/570/166	1702	81 year old Caucasian female with 3 year history of hypertension. Subject died after myocardial infarction, CABG, and mitral valve repair after 62 days on study drug. Investigator considered events improbably related to study drug.
FFR/91/570/166	2361	73 year old Caucasian female with newly diagnosed hypertension. Subject died from lung carcinoma after 2 months on study drug. Investigator considered events unrelated to study drug.
FFR/91/570/166	4937	63 year old Caucasian male with 10 year history of hypertension. Subject died from cerebrovascular accident after 48 days on study drug. Investigator considered events improbably related to study drug.
FFR/91/570/166	7409	72 year old Caucasian male with newly diagnosed hypertension. Subject died from cerebral embolus following cerebral hemorrhage after 35 days on study drug. Investigator considered events unrelated to study drug.
FFR/91/570/166	10398	80 year old Caucasian female with diabetes mellitus and 3 year history of hypertension. Subject died from sudden death and cerebral embolus after 49 days on study drug. Investigator considered event unrelated to study drug.
FFR/91/570/166	11272	84 year old Caucasian male with 12 year history of hypertension. Subject discontinued because of femoral neck fracture and died 1 month later from suppurative infection and bronchitis. Investigator considered events unrelated to study drug.
FFR/91/570/166	11296	78 year old Caucasian male with inadequately documented history. Death from suspected cerebrovascular accident was subsequent to inguinal hernia repair. Investigator did not comment on relationship between events and study drug.
FFR/91/570/166	15080	72 year old Caucasian male with diabetes mellitus and 9 year history of hypertension. Subject died from carcinoma of the lung after 27 days on study drug. Investigator considered events unrelated to study drug.
FFR/91/570/166	15531	68 year old Caucasian male with newly diagnosed hypertension. Subject committed suicide after 44 days on study drug. Investigator considered events improbably related to study drug.
FFR/91/570/166	15898	74 year old Caucasian male with 12 year history of hypertension. Subject died with carcinoma of the larynx and sepsis after 63 days on study drug. Investigator considered events unrelated to study drug.
FFR/91/570/166	16020	62 year old Caucasian male with 4 year history of hypertension. Subject died from myocardial infarction after 13 days on study drug. Investigator considered events unrelated to study drug.
FFR/91/570/166	17913	74 year old Caucasian male with 12 year history of hypertension. Subject died from hypokalemia, attributed to laxative abuse, after 28 days on study drug. Investigator considered events unrelated to study drug.
FFR/91/570/166	18152	82 year old Caucasian female with diabetes mellitus and 22 year history of hypertension. Subject died a sudden death after 8 days on study drug. Investigator considered events possibly related to study drug. Blood pressure was poorly controlled.

No mortal event was suspected as being more than possibly related to study drug. All events were of a nature (and perhaps frequency) not unexpected in a large hypertensive and heart failure population.

4. Adverse events leading to withdrawal

The sponsor has counted 501 subjects newly reported as withdrawals for adverse events. Case report forms for these subjects have been provided but were not reviewed. The sponsor's brief narrative summaries for causes for withdrawal were reviewed. Events were not tabulated by type, but the common events were familiar: cough, headache, non-specific gastrointestinal symptoms.

5. Summary

- 5.1. The present submission covers only a portion of the information requested concerning studies not reported with the original NDA submission. Since the numbers of subjects in each trial has not been part of the material submitted with either of the pertinent submissions, it is unclear what fraction of the estimated 19,000 subjects in foreign non-IND studies has been included in the summaries provided.
- 5.2. The sponsor has provided some information concerning the existence of two previously undisclosed studies conducted in 1988 and 1989. The issue of how many more such studies there may be needs to be revisited. Perhaps the Division should consider involving DSI.
- 5.3. Of the data submitted thus far, there are no indications of novel safety concerns with trandolapril.


N. Stockbridge, M.D., Ph.D.

cc: NDA 20-528
HFD-110
HFD-110/CSO
HFD-110/Stockbridge

et

**DIVISION OF CARDIO-RENAL DRUG PRODUCTS
MEDICAL OFFICER'S REVIEW**

JUL 18 1995

NDA: 20-528 (Mavik®, trandolapril)

Sponsor: Knoll Pharmaceutical Company

Submission: Response to reviewers' requests

Submission date: 30 June 1995

Receipt date: 5 July 1995

Review date: 14 July 1995

Reviewers: N. Stockbridge, M.D., Ph.D.

1. Resume: During the course of the review of this NDA, it became clear that no study reports were provided for an unknown number of trandolapril studies conducted by the sponsor in Europe. The sponsor provided adequate descriptions of pivotal U.S. trials. The adequacy of European safety data could not be ascertained. In a letter dated 6 April 1995, the sponsor was asked to provide a list of unreported studies. The present submission lists preclinical and clinical studies; only the clinical study information is summarized in this review.

2. Studies said to have been included in the sponsor's NDA summary of safety, but with no study report submitted

2.1. Short-term studies

<i>F/87/403/01</i>	<i>n=18</i>	<i>4, 8 mg</i>	<i>R, DB, PC</i>	<i>1 day</i>	<i>tolerance</i>
<i>F/86/570/10</i>	<i>n=18</i>	<i>8, 16</i>	<i>R, DB, PC</i>	<i>7 days</i>	<i>tolerance</i>
<i>F/86/570/11</i>	<i>n=27</i>	<i>1, 2, 4</i>	<i>R, DB, PC</i>	<i>1 day</i>	<i>i.v. tolerance</i>
<i>ZA/89/570/16</i>	<i>n=10</i>	<i>4</i>	<i>open</i>	<i>10 days</i>	<i>pharmacokinetics</i>
<i>ZA/89/570/52</i>	<i>n=18</i>	<i>2</i>	<i>R, SB, XO</i>	<i>1 day</i>	<i>bioequivalence</i>
<i>ZA/89/570/58</i>	<i>n=10</i>	<i>1</i>	<i>R, OL, XO</i>	<i>10 days</i>	<i>pharmacokinetics</i>
<i>F/89/570/61</i>	<i>n=12</i>	<i>2</i>	<i>R, OL, XO</i>	<i>1 day</i>	<i>absolute bioavailability</i>
<i>F/86/570/Q3</i>	<i>n=12</i>	<i>8</i>	<i>R, OL, XO</i>	<i>1 day</i>	<i>food effect</i>
<i>FF/88/570/31</i>	<i>use with nifedipine, "[f]ull report not available."</i>				
<i>ZA/87/570/15</i>	<i>n=12</i>	<i>4</i>	<i>OL</i>	<i>1 day</i>	<i>pharmacokinetics in elderly</i>
<i>I/91/570/138</i>	<i>use in idiopathic dilated cardiomyopathy, "[f]ull report not available."</i>				
<i>FF/87/570/02</i>	<i>n=169</i>	<i>4</i>	<i>R, DB, AC</i>	<i>?</i>	<i>comparison with enalapril</i>
<i>FF/87/570/03</i>	<i>n=180</i>	<i>4</i>	<i>R, DB, AC</i>	<i>?</i>	<i>comparison with captopril</i>
<i>FF/87/570/05</i>	<i>n=295</i>	<i>2, 4</i>	<i>R, DB, AC</i>	<i>?</i>	<i>comparison with atenolol</i>
<i>FF/87/570/06</i>	<i>n=158</i>	<i>2</i>	<i>R, DB, AC</i>	<i>?</i>	<i>factorial with nifedipine</i>
<i>UK/89/570/01</i>	<i>n=236</i>	<i>1, 2</i>	<i>R, DB, AC</i>	<i>?</i>	<i>comparison with lisinopril</i>
<i>FFR/92/570/167</i>	<i>comparison with peridopril; ABPM; "[f]ull report not available."</i>				
<i>CDN/91/570/128</i>	<i>R, DB, PC, use with HCTZ; "[f]ull report not available."</i>				
<i>J/90/570/110</i>	<i>efficacy study; "[f]ull report not available."</i>				

2.2. Long-term studies

<i>FF/87/570/07</i>	<i>Pooled study with n=1042, including n=169 on diuretic, n=52 on calcium antagonist, and n=40 on diuretic and calcium antagonist. "Pooled report not available."</i>
<i>FF/87/570/08</i>	
<i>FF/87/570/22</i>	
<i>FF/87/570/29</i>	<i>Follow-on to FF/87/570/07, -08, -22 and FF/88/570/29-33. "[f]ull report not available."</i>
<i>FF/87/570/33</i>	
<i>FF/89/570/49</i>	
<i>FF/90/570/98</i>	<i>Follow-on to FF/89/570/49; "[f]ull report not available."</i>

FFR/92/570/164 Follow-on to FF/89/570/49 and FF/90/570/98: "[f]ull report not available."
 FF/89/570/55 "Full report not available."

2.3. Studies in special populations

FFR/89/570/59 Non-insulin-dependent diabetes: "[f]ull report not available."
 FF/89/570/44 Elderly, long term; "[f]ull report not available."
 FF/89/570/51 LVH, comparison with enalapril: "[f]ull report not available."
 I/89/570/65 LVH, use with enalapril: "[f]ull report not available."
 FFR/90/570/107 Chronic renal failure; "[f]ull report not available."
 FFR/90/570/108 Elderly, comparison with nitredipine: "[f]ull report not available."
 D/90/570/93 Non-insulin-dependent diabetes; "[f]ull report not available."
 F/91/570/149 O.d. dosing, comparison with nitrendipine; "[f]ull report not available."
 FFR/92/570/166 Elderly; "[f]ull report not available."
 J/90/570/09 Elderly, severe hypertension: "[f]ull report not available."

2.4. Other hypertension studies

I/89/570/62 n=27 1, 2 mg R, DB, UC ? days ABPM
 UK/89/570/05 n=64 1, 2 R, DB, UC ? days ABPM
 UK/88/570/01 With indomethacin: "[f]ull report not available."
 FFR/92/570/165 Blood pressure self-measurement; "[f]ull report not available."
 FFR/92/570/177 Compliance: "[f]ull report not available."
 J/90/570/11 Lipid profiles; "[f]ull report not available."
 7-22 No information provided; "[f]ull report not available."
 61-1 No information provided; "[f]ull report not available."
 42-1 No information provided; "[f]ull report not available."
 58-1 No information provided; "[f]ull report not available."
 56-1 No information provided; "[f]ull report not available."

3. Studies not included in the sponsor's efficacy summary and with no study report submitted

3.1. Clinical pharmacology

CDN/90/570/95 n=34 1 mg DB, XO 4 weeks b.i.d. vs. q.d., ABPM
 ZA/91/570/117 n=18 2 R, DB, XO 7 days interaction with glibenclamide
 F/90/570/112 n=459 0.125-32 PC variable summary of >1 studies?
 F/85/570/02 Pharmacokinetics, pharmacodynamics; "[f]ull report not available."
 F/86/570/12 Absolute bioavailability; "[f]ull report not available."
 F/87/570/12 Transcutaneous dosing; "[f]ull report not available."
 UK/88/570/03 Response to bradykinin; "[f]ull report not available."
 F/88/570/36 Transcutaneous dosing; "[f]ull report not available."
 F/88/570/37 Cirrhosis; "[f]ull report not available."
 F/89/570/56 n=? 4 DB, PC, XO 1 day renal function; "[f]ull report not available."
 GB/89/570/71 Hemodynamics with a diuretic; "[f]ull report not available."
 CDN/89/570/83 Appearance in breast milk; "[f]ull report not available."

J/90/570/101	Acute hemodynamics and endocrine effects; "[f]ull report not available."
J/90/570/104	Bioequivalence; "[f]ull report not available."
J/90/570/105	Bioequivalence; "[f]ull report not available."
GB/90/570/113	Effect on cognitive deficit; "[f]ull report not available."
J/91/570/114	Renal function; "[f]ull report not available."
J/91/570/115	Renal impairment, pharmacokinetics; "[f]ull report not available."
ZA/91/570/118	Interaction with atenolol; "[f]ull report not available."
J/91/570/125	Bioequivalence; "[f]ull report not available."
J/91/570/126	Renal impairment; "[f]ull report not available."
J/91/570/131	Renal impairment; "[f]ull report not available."
FFR/91/570/147	ACE inhibition, comparison with enalapril; "[f]ull report not available."
ZA/91/570/154	CHF, pharmacokinetics; "[f]ull report not available."
UK/91/570/171	CHF, pharmacokinetics; "[f]ull report not available."
GB/92/570/179	Urinary metabolites; "[f]ull report not available."
ZA/93/570/184	Bioequivalence; "[f]ull report not available."
F/87/570/10	Arterial and venous compliance; "[f]ull report not available."
F/89/570/50	Arterial compliance; "[f]ull report not available."
I/89/570/63	Hemodynamics; "[f]ull report not available."
D/89/570/85	LVH; "[f]ull report not available."
I/89/570/87	LVH, coronary hemodynamics; "[f]ull report not available."
CDN/90/570/97	Autonomic effects; "[f]ull report not available."
I/91/570/127	Hemodynamics, comparison with nifedipine; "[f]ull report not available."
B/91/570/141	LVH, comparison with atenolol; "[f]ull report not available."

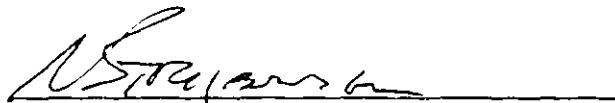
3.2. Hypertension studies

UK/88/570/02	n=17	1, 2 mg	R, DB	? days	ABPM
FFR/89/570/60	n=57	0.5, 1	R, DB, PC	1 day	sodium-depleted subjects
I/89/570/64	n=15	2	OL	? days	renal hemodynamics
I/89/570/76	n=15	2	OL	? days	effect on LVH
I/89/570/77	n=60	2	R, DB, PC	? days	ABPM
CDN/90/570/100	n=65	0.5-4	R, DB, AC	? days	ABPM, comparison with enalapril
FFR/90/570/103	n=23	2	OL	? days	ABPM
F/86/570/13	Once-a-week for 8 weeks; "[f]ull report not available."				
J/88/570/04	Initial dose; "[f]ull report not available."				
J/88/570/05	Diurnal effects; "[f]ull report not available."				
J/88/570/08	Long-term; "[f]ull report not available."				
FF/88/570/32	Lipid profile, comparison with atenolol; "[f]ull report not available."				
J/89/570/66	Open dose-ranging; "[f]ull report not available."				
J/89/570/67	Open dose-ranging; "[f]ull report not available."				
I/89/570/68	Diabetes mellitus; "[f]ull report not available."				
D/89/570/85	LVH; "[f]ull report not available."				
I/89/570/87	Coronary blood flow; "[f]ull report not available."				
J/90/570/10	Renal disorders; "[f]ull report not available."				
F/90/570/94	Effect on "blood pressure variation"; "[f]ull report not available."				
CDN/90/570/97	Effect on autonomic function; "[f]ull report not available."				
FFR/90/570/107	Chronic renal failure; "[f]ull report not available."				
I/91/570/127	Hemodynamics, comparison with nifedipine; "[f]ull report not available."				
B/91/570/141	LVH, comparison with atenolol; "[f]ull report not available."				
F/91/570/145	ABPM, comparison with enalapril; "[f]ull report not available."				
I/91/570/152	ACE inhibition, comparison with enalapril; "[f]ull report not available."				
D/91/570/158	Organic brain syndrome; "[f]ull report not available."				
GR/92/570/173	Follow-on to FFR/89/570/55; "[f]ull report not available."				
D/92/570/175	LVH; "[f]ull report not available."				
CR/92/570/176	Efficacy; "[f]ull report not available."				
FFR/92/570/178	Self-measurement, comparison with perindopril; "[f]ull report not available."				

3.3. Miscellaneous studies

5. Summary

- 5.1. There are no fewer than 157 studies with trandolapril, mostly non-IND, for which study reports were not submitted with NDA 20-528. Of these, 97 were excluded from the sponsor's summary of safety.
- 5.1.1. The sponsor indicates that 87 foreign studies contributed to the NDA safety summary, but no study summary was provided for 60 of these studies. Of these 60, the sponsor denies a final study report is available for 43. It is unclear whether reports are available for the other 17 studies.
- 5.1.2. The sponsor indicates that an additional 80 foreign studies were not included in the NDA safety summary. The sponsor claims no study report is available for 70 of these studies. It is unclear whether reports are available for the other 10 studies.
- 5.2. The U.S. monotherapy trials were adequate to conclude that trandolapril is antihypertensive and to establish a reasonable set of instructions for use. The safety data for the U.S. trials is also probably adequate to describe the risks of treatment. Nothing among the *reported* foreign trials raised novel safety concerns. The reviewed studies, although only 20% of those conducted by the sponsor, in the context of a well-studied class of drugs, can form a reasonable basis for regulatory action.



N. Stockbridge, M.D., Ph.D.

cc: NDA 20-528

HFD-110

HFD-110/CSO

HFD-110/Stockbridge



Food and Drug Administration

Steven M. Rodin, M.D.

Medical Officer
Division of Cardio-Renal Drugs

Medical Review of Protocol

GENERAL INFORMATION

NDA #: 20-528
Drug: trandolapril (Mavac®)
sponsor: Knoll Pharmaceutical Co.

Protocol #: FF 89-570-45
Protocol objective: pharmacokinetics in the elderly

Pharmacologic type: angiotensin converting enzyme inhibitor
Proposed use: antihypertensive

Date of NDA submission: 30 September 1994

Reviewer: Steven M. Rodin, M.D.
Review last revised: 22 December 1994

Review objective:

As per Dr. Stockbridge's request of 12/21/94 this medical review of a pharmacokinetic (PK) study will not include PK results, but will describe the study design and evaluate safety outcomes.

A.1. Study FF-895-570-45: "Pharmacokinetics and pharmacodynamics of trandolapril after repeated doses of 2 mg in elderly patients with mild to moderate arterial hypertension" (study report vols 1.102 and 1.103; case report forms were not submitted).

A1.1. Investigators:

A. Wade; Glasgow, U.K.
P. Amer; Huddinge, Sweden

A1.2. Study dates:

Nov. '89- April '90

A1.3. Drug formulation:

trandolapril 2 mg capsule (size 4): batch MB 22327-163

Trandolapril (ND20-528, Knoll)

Study FF 89-570-45

A1.4. Study Design:

This single-blind, nonrandomized, baseline placebo-controlled study administered trandolapril 2 mg once-daily for 10 days to 14 fasting subjects (elderly patients (mean age 69 years) with mild-to-moderate arterial hypertension), subsequent to a 14 day placebo run-in. On days 1 and 10 the timing of post-drug meals was standardized. After discontinuation of drug therapy patients underwent placebo washout (one capsule once-daily) for two ~~days~~ /s.

Enrolled subjects were adults of both sexes ~~with~~ with age ≥ 65 years and uncomplicated mild- to-moderate hypertension (diastolic blood pressure (BP) ≥ 95 mm Hg and ≤ 115 mm Hg). The exclusion criteria were conventional for a pharmacokinetic study of a cardiovascular agent (see appendix 1 if details are of interest).

A1.4.1. Endpoints:

The study objectives were to characterize pharmacokinetics, pharmacodynamics, and safety after single and multiple trandolapril doses in elderly patients. BP determinations were made 15 minutes before dosing, and 1, 2, 3, 4, 6, 8, 12, and 48 hours post-dosing on days 1 and 10 as well as immediately before dosing on days 2, 3, 5, 7, and 9.

Blood sampling was undertaken up to 16 hours post-dose on days 1 and 10. Trough plasma drug concentration was defined as the observed C_{min} . Drug accumulation ratios (R) were calculated as the ratio of AUC on day 10 to AUC on day 1 [unless otherwise noted the AUCs were calculated up to the last time point where the plasma concentration was detectable].

Laboratory hematology, chemistry and urinalysis endpoints ^{were observed} pre-treatment and 24 hours after the last drug dose. Adverse events (AE) were to have been recorded after spontaneous reporting by patients or in answer to non-leading questions. Clinical followup was undertaken one and two weeks after the final drug doses.

A1.4.2. Statistical methods:

Statistical significance was defined at an alpha level of 0.05. A 2-way analysis of variance (ANOVA) was used to test for differences in PK parameters between treatment days (except for T_{max} , which was tested using the rank sign test). Changes from pre-treatment BP were assessed using the t test. Drug concentrations below the limit of quantification (0.1 ng/ml) were imputed to be zero.

Trandolapril (ND20-528, Knoll)

Study FF 89-570-45

A1.5 Results:

See the table below for a description of demographic and pre-treatment characteristics of subjects.

Table: 1

**Demographic and Pre-treatment characteristics of subjects
(study FF-895-570-45)**

male	5
female	9
mean age (yr) range	69 65-76
caucasian other	14 0
mean height (cm)	162
mean weight (kg) range	70 48-90
mean pre-treatment systolic BP (mm Hg)	167.6
mean pre-treatment diastolic BP (mm Hg)	100.6

Two patients were excluded from the analyses because of noncompliance. Two patients (202 and 207) violated the protocol by receiving concomitant beta-blockers, but they were included in the analysis.

Trandolapril (ND20-528, Knoll)

Study FF 89-570-45

The mean hemodynamic changes from pre-treatment are shown in the table below.

Table: 2

Mean systolic BP, diastolic BP and heartrate (HR) changes from pre-treatment, observed in supine posture (study FF-895-570-45)

Visit	Time / dosing (h:m)	SYST BP Δ		DIAST. BP Δ		HR Δ	
		N	MEAN	MEAN	MEAN	MEAN	
Day 1	Before dosing	12	0.0	0.0	0.0		
	1:00	12	-1.4	-3.3	-8.3		
	2:00	12	-6.6	-7.3	-6.6		
	3:00	12	-20.3	-15.8	-1.3		
	4:00	12	-23.9	-17.5	-3.7		
	6:00	12	-24.6	-18.3	-1.1		
	8:00	12	-17.7	-12.8	-5.1		
	12:00	12	-20.1	-15.0	-0.5		
Day 2	Before dosing	12	-15.7	-8.0	-2.8		
Day 3	Before dosing	12	-14.0	-12.3	-4.3		
Day 5	Before dosing	12	-15.4	-8.3	-4.3		
Day 7	Before dosing	12	-9.8	-7.8	-1.9		
Day 9	Before dosing	12	-11.3	-9.8	-4.8		
Day 10	1:00	12	-19.3	-11.3	-9.6		
	2:00	12	-20.1	-10.3	-8.3		
	3:00	12	-30.3	-20.9	-2.1		
	4:00	12	-28.6	-19.8	-4.8		
	6:00	12	-25.3	-18.8	-2.2		
	8:00	12	-22.0	-15.8	-5.2		
Day 10	12:00	12	-15.0	-11.9	-0.3		
Day 11	no dose given	12	-16.5	-10.8	-2.5		
Day 12	48 hr post-dose	12	-8.5	-9.5	-4.1		
Day 17		10	-8.7	-6.8	-0.8		

(source: modified photocopies of tables on pgs 330-332, appendix X1, vol 103)

The pretreatment observations were at the end of a 2 week placebo run-in. There were no concurrent placebo observations with which to correct the above data. The day 11 and 12 observations were during a placebo washout period.

Trandolapril (ND20-528, Knoll)

Study FF 89-570-45

A1.6 Safety outcomes:

Case report forms were not submitted for evaluation.

There were no deaths or dropouts described. One patient (210) reportedly had several episodes of asymptomatic orthostatic hypotension (systolic BP difference of >25 mm Hg between supine and standing positions) which resolved within 2 minutes of assuming the standing position. One patient (202) reportedly had mild palpitations 8 days after discontinuing trandolapril. A beta-blocker was administered and the symptom did not recur.

There were no clinically significant changes from pre-treatment hematology and laboratory chemistry data [as reported in appendix XI-2, pg 333]. One patient had a small (2 µmol/L) reduction in serum total bilirubin.

A1.7 Summary:

Following 2 mg p.o. trandolapril administration for 10 days to 14 elderly hypertensive patients there was reportedly one case of asymptomatic orthostatic hypotension without other clinically significant adverse events.

A1.8 Comment

This study provides only a nonconcurrent placebo-controlled, small sample from which one could plot the relationship of diastolic BP to plasma trandolapril or trandolaprilat level. This relationship, and the duration of antihypertensive effects would be appropriate subjects of further scrutiny if it becomes evident that there aren't more adequate data upon which to base such analyses.


Steven M. Rodin, MD _____ 12/22/94
Medical Officer Date

cc: HFD-110 division file, CSO (HFD-110), NStockbridge (HFD-110); * no copy to S.Rodin

1 appendix attached

Trandolapril (ND20-528, Knoll)

Study FF 89-570-45

Appendix 1: Exclusion criteria

- Related to hypertension :

- . Cerebral haemorrhage or ischaemia.
- . Retinal haemorrhage or thrombosis (grade III or IV retinopathy).
- . Ischaemic heart disease, e.g. angina or myocardial infarction.
- . Coronary insufficiency.
- . Active arteriopathy.
- . Mesenteric arteriopathy.
- . Renal insufficiency (serum creatinine greater than 150 $\mu\text{mol/l}$).
- . Heart failure and cardiomegaly (cardiothoracic ratio greater than 0.8).

Other diseases,

- . Any severe chronic disease, particularly cardiopulmonary, hepatic, metabolic or neurological and psychiatric disease; and alcoholism.
- . Insulin dependent diabetes.
- . History of hypersensitivity to drugs in particular to angiotensin converting enzyme inhibitors.
- . Collagen diseases.
- . Neutrocytopenia ($\leq 2500/\text{mm}^3$).
- . Proteinuria.
- . Blood haemoglobin less than 80 g/l.

- Secondary hypertension with a well defined cause :

- . Uni or bilateral stenosis of the renal artery.
- . Primary hyperaldosteronism.
- . Pheochromocytoma.
- . Acromegaly.
- . Cushing's syndrome.
- . Coarctation of the aorta.

- Major rhythm disorders.
- Hyperkalaemia (serum potassium $> 5.5 \text{ mmol/l}$, whatever the cause).
- Overweight ($> 130 \%$ over normal weight, see table 1 appendix 2 of protocol).

- Administration of one of the following long term treatments :

In the month preceding the study and during the study itself :

- . enzyme inducing drugs (see appendix 4 of protocol, drug with at least 3 $\leftarrow$),
- . calcium blockers,
- . vasodilators,
- . cimetidine, ranitidine,
- . chronic administration of non steroidal anti-inflammatory drugs,
- . glucocorticoids,
- . digitalis glycosides,
- . oestrogen, progestarone compounds,
- . angiotensin converting enzyme inhibitors.

In the two weeks preceding the study and during the study itself :

- . β blockers,
- . diuretics.

- Other exclusion criteria :

- . Subjects unable to follow the therapeutic or dietary recommendations.
- . Patients who have participated in another clinical trial within the last 8 weeks before the preselection.

[source: modified photocopy of pages 16-17, vol 102]

HFD-110/C 50

JUL - 7 1995



DIVISION OF CARDIO-RENAL DRUG PRODUCTS
BIOPHARMACEUTICAL—STATISTICAL—MEDICAL REVIEW

NDA: 20-528 (Mavik®, trandolapril; IND

Sponsor: Knoll Pharmaceutical Company.

Submission	Volumes	Submission date	Receipt date
Original NDA	1.1 to 1.418	28 September 1994	29 September 1994
Clinical Audit Package	2.1	27 September 1994	28 September 1994
Response to Reviewer	3.1	28 November 1994	1 December 1994
New biopharm study	4.1	19 December 1994	20 December 1994
Response to reviewer	5.1	29 December 1994	3 January 1995
Response to reviewer	5.1	11 January 1995	12 January 1995
Response to reviewer	5.1	13 January 1995	17 January 1995
Response to reviewer	5.1	17 January 1995	18 January 1995
Response to reviewer	5.1	19 January 1995	20 January 1995
Response to reviewer	5.1	24 January 1995	25 January 1995
Response to reviewer	—	31 January 1995	2 February 1995
Response to reviewer	5.1	6 February 1995	7 February 1995
PK data on diskette	6.1	14 March 1995	15 March 1995
Response to reviewer	6.1	15 March 1995	16 March 1995
120-day safety update, report for study TR-22-HTN	6.1 to 6.27	13 March 1995	14 March 1995
Response to reviewer	8.1	22 March 1995	23 March 1995
Response to reviewer	8.1	23 March 1995	24 March 1995
Response to reviewer	8.1	7 April 1995	10 April 1995
Response to reviewer	8.1	13 April 1995	14 April 1995
Response to reviewer	9.1	28 April 1995	2 May 1995
Response to reviewer	9.1	2 May 1995	3 May 1995
Response to reviewer	9.1	24 May 1995	25 May 1995
Response to reviewer	9.1	1 June 1995	2 June 1995

Review date: 6 July 1995.

- Reviewers:
- I. Cerny, Pharm.D., HFD-110
 - L. Cui, Ph.D., HFD-713
 - E. Fadiran, Ph.D., HFD-426
 - S. Rodin, M.D., HFD-110
 - N. Stockbridge, M.D., Ph.D., HFD-110

John C. Cerny, Pharm.D. 7/6/95
Ling Cui, Ph.D. 7/7/95
E. Fadiran 7/6/95
S. Rodin, MD
N. Stockbridge, MD, PhD

Concurrences:

G. Chi, Ph.D., HFD-713

S. Dubey, Ph.D., HFD-713

A. Parekh, Ph.D., HFD-426

[Handwritten signatures and dates]
7/7/95
7-7-95
7/6/95

Distribution:

NDA 20-528

HFD-110

HFD-110/Cerny

HFD-713/Cui

HFD-426/Fadiran

HFD-110/Rodin

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HFD-110/CSO

HFD-426/Fleischer

HFD-427/Chen

HFD-713/Chi

HFD-713/Dubey

HFD-19 (FOI)

HFD-340/Vish

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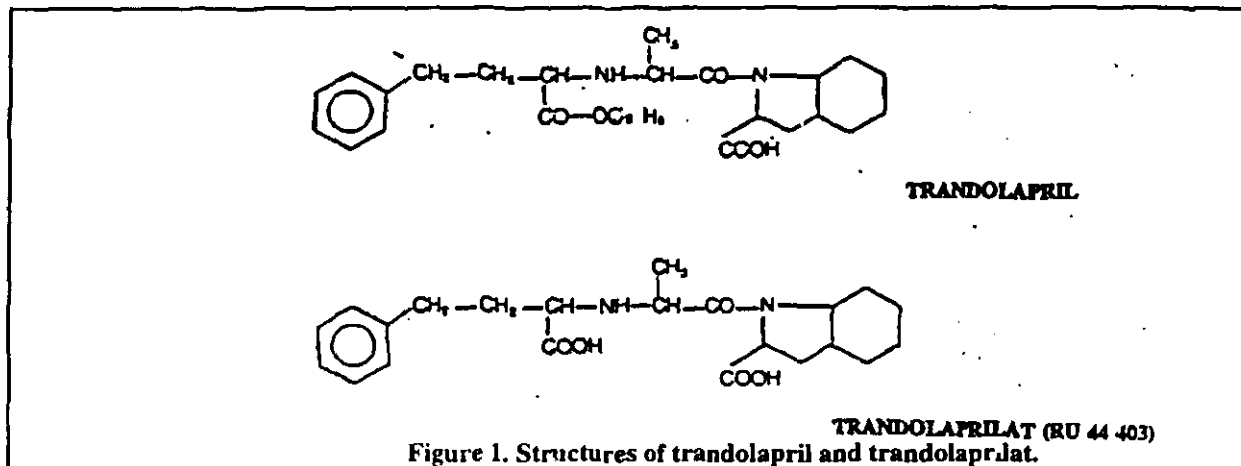
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1. General information

- 1.1. **Name of drug** The structure of trandolapril¹ and its active metabolite, trandolaprilat are shown in Figure 1 below. The trade name, Mavik®, has been found acceptable by the Labeling and Nomenclature Committee.



- 1.2. **Pharmacological category** Angiotensin Converting Enzyme (ACE) inhibitor. ACE catalyzes the conversion of angiotensin I to angiotensin II. Angiotensin II causes vasoconstriction and increased aldosterone secretion, the latter leading to increased renal sodium and water reabsorption.
- 1.3. **Proposed indication** The proposed indication is essential hypertension.
- 1.4. **Dosage and administration** The formulation proposed for marketing is a tablet, with 1, 2, or 4 mg trandolapril. The sponsor recommends a starting dose of 2 mg once daily in patients not receiving a diuretic, and titration to 4 mg, if necessary, after at least 1 week. When blood pressure is not adequately controlled on trandolapril alone, the sponsor recommends addition of verapamil or a diuretic. The recommended starting dose for patients on a diuretic and those with hepatic cirrhosis or creatinine clearance <30 ml/min is 1 mg daily.

¹ (2S-(1(R*(R*)),2a,3aα,7aβ))-1-(2-((1-(ethoxycarbonyl)-2-phenylpropyl)amino)-1-oxypropyl)octahydro-1H-indole-2-carboxylic acid.

2. Manufacturing controls²

The following issues remain unresolved at the present time:

- (1) The final drug product is a
- (2) Other final testing needs to be specified for determination of heavy metals and the diacid degradation product of trandolapril.
- (3) Two stress-test degradation products remain to be characterized.
- (4) The method of selecting an expiration date was not acceptable.
- (5) There were inadequate descriptions of the toxicity of potential synthesis impurities.
- (6) EER has not been returned.
- (7) Environmental Assessment was found unacceptable and additional information was requested.
- (8) No methods validation has been requested.

The unresolved manufacturing issues are considered minor deficiencies.

4.

² Information here is based upon Chemistry Reviews dated 16 December 1994 and 14 April 1995.

3. Pharmacology³

3.1. ACE inhibition

3.1.1. Binding studies

Binding affinities of ACE inhibitors for human renal ACE were compared in competition with ³H-quinaprilat. The order of increasing IC₅₀ was trandolaprilat < lisinopril < trandolapril < captopril < enalaprilat << enalapril. Similar binding studies were performed on various isolated rat tissues with ordering as shown below⁴

Lung	perindoprilat	enalaprilat	ramiprilat	trandolaprilat	captopril	perindopril	trandolapril	ramipril
Heart	trandolaprilat	perindoprilat	enalaprilat	ramiprilat	captopril	perindopril	trandolapril	enalapril
Kidney	trandolaprilat	ramiprilat	enalaprilat	perindoprilat	captopril	trandolapril	ramipril	enalapril
Brain	enalaprilat	trandolaprilat	ramiprilat	perindoprilat	captopril	perindopril	ramipril	trandolapril
Adrenal	perindoprilat	ramiprilat	trandolaprilat	enalaprilat	captopril	perindopril	ramipril	trandolapril

Human plasma ACE inhibition was studied *in vitro*. Trandolaprilat produced approximately 90% ACE inhibition at 10⁻⁸ M; near-complete inhibition required concentrations some 30-fold higher. This is consistent with the clinical data demonstrating less than complete plasma ACE inhibitions at doses which are clearly above those at which antihypertensive effects are manifest.

ACE activity was assessed in various tissues of rats administered oral trandolapril. Inhibition, lasting more than 24 hours, was seen in heart, kidney, adrenal glands, lung, abdominal aorta, and serum, but not basal ganglia.

3.1.2. Physiological effects

In vivo studies demonstrated the ability of intravenous trandolapril and trandolaprilat and oral trandolapril to block the pressor response to exogenous angiotensin I, but not to exogenous angiotensin II, in ganglion-blocked Sprague-Dawley rats and in dogs. The dose required to block the effect of angiotensin I was 10-fold higher than that necessary to block plasma ACE activity. There was no comparable study reported in man.

Dose-related antihypertensive effects were found with genetically hypertensive rats.

Dose-dependent reductions in blood pressure were also obtained in dogs pre-treated over 3 days with HCTZ. Other hemodynamic findings in these animals included reduction in total peripheral resistance, left ventricular work, and stroke work.

Spontaneously hypertensive rats treated with oral trandolapril or enalapril for 2 or 4 weeks demonstrated less cardiac hypertrophy and less thickening of major artery media than did control animals.

3.1.3. Specificity

Inhibition of prostaglandins by indomethacin had no effect on antihypertensive effects of oral trandolapril in spontaneously hypertensive rats. Antihypertensive effects of oral trandolapril were blocked by nephrectomy, presumably because of the lack of renin-dependence of blood pressure in animals so treated.

Carageenan and dextran produce subplantar edema when injected into foot pads of rats. The carageenan response is thought to be bradykinin-mediated. Animals pretreated with trandolapril or enalapril had much greater swelling in response to carageenan, but not to dextran. Trandolapril and trandolaprilat potentiated the effect of bradykinin on guinea-pig ileum *in vitro*.

³ Information here is based upon the draft Pharmacology Review, and has been edited by the 3 involved Pharmacology Reviewers.

⁴ The affinities are ordered for the higher affinity site. Diacid inhibitors (perindoprilat, trandolaprilat, ramiprilat, and enalaprilat) bind to a second site on ACE with IC₅₀ about two orders of magnitude higher (but also about two orders of magnitude lower than the IC₅₀ for the corresponding ester).

Prostaglandin $F_{2\alpha}$ -contracted canine femoral arteries in vitro were relaxed by trandolapril only in the presence of bradykinin, adenosine, or angiotensin I.

- 3.2. General pharmacology** Single oral doses of trandolapril to 30 mg/kg demonstrated no CNS symptomatology in mice. Intravenous trandolapril to 10 mg/kg demonstrated no antagonism of acetylcholine- or histamine-induced bronchospasm in guinea pigs. Oral trandolapril ≥ 1 mg/kg delayed gastric emptying in rats. Oral trandolapril produced a diuresis in rats at 30 mg/kg. Oral doses of trandolapril to 30 mg/kg did not affect blood glucose levels in fasting mice. Oral doses of trandolapril to 30 mg/kg did not produce analgesia in mice. A trandolapril solution had no local anesthetic activity on the guinea pig cornea. Trandolapril had no effect on platelet aggregation in vitro (rabbit) or prothrombin time (rats).
- 3.2.1. Congestive heart failure** Rats surviving myocardial infarction from coronary artery occlusion develop congestive heart failure. Animals given trandolapril 0.1 to 1.0 mg/kg/day had increased survival, at least for the first 6 months. Aged spontaneously hypertensive rats also develop congestive heart failure. These animals had better survival on trandolapril.
- 3.2.2. Cardiac hypertrophy** Angiotensin I and angiotensin II induce expression of growth-stimulating proto-oncogenes in rabbit heart and aorta. Trandolapril inhibited the expression of these genes in rabbits and reduced cardiac hypertrophy in spontaneously hypertensive rats.
- 3.2.3. Renal function** Intravenous trandolapril 1 and 10 mg/kg produced diuresis and increased excretion of electrolytes in anesthetized dogs. There were no observed changes in creatinine or PAH clearance.
- 3.3. Toxicology**
- 3.3.1. Acute toxicology** The acute LD_{50} in dog, mouse, and rat was >1 g/kg for both oral and intraperitoneal exposure. Associated signs in dogs were marked weight loss, hypotonicity, hypomotility, weak pulse, and respiratory difficulties.
- 3.3.2. Subacute toxicology** A 15-day study in Sprague-Dawley rats with oral trandolapril doses to 500 mg/kg/day showed no mortality, but decreased weight and functional and anatomic renal disease. Dose-dependent renal tubule lesions were also seen in a 30-day study in rats given trandolapril 4 to 100 mg/kg/day.
- A 15-day study in dogs given oral trandolapril to 250 mg/kg/day demonstrated no effects on mortality or histopathology. However, increased alkaline phosphatase, urea, and cholesterol were seen at 250 mg/kg/day. The no-effect dose was 10 mg/kg/day. A similar 30-day study demonstrated a few minor renal tubular lesions in all dose groups (10 to 250 mg/kg/day).
- 3.3.3. Chronic toxicology** Sprague-Dawley rats received oral trandolapril 0.25 to 25 mg/kg/day for 26 weeks followed by a 6-week recovery period. About 5% of animals in the 2.5 and 25 mg dose groups died from what were thought to be drug-related reasons. Relative heart weights were less in the higher dose animals. Increased renal weight was associated with glomerulonephritis (2.5 and 25 mg/kg/day).
- Sprague-Dawley rats were administered oral trandolapril 0.25 to 9 mg/kg/day for 78 weeks, with no dose-related mortality or histopathological effects observed.
- Dogs received oral trandolapril 2.5 to 125 mg/kg/day for 26 weeks, followed by a 6-week recovery period. A 250 mg/kg dose group was sacrificed early because of marked toxicity. The 125 and 250 mg/kg doses were associated with hypotonia, vomiting, diarrhea, and dehydration. Small changes in enzyme levels and urinary electrolytes normalized by the end of the recovery period. Renal tubular effects were seen in few animals after the recovery period.

A 52-week study in dogs with oral trandolapril 0.25 to 25 mg/kg/day demonstrated no systemic toxicity.

3.3.4. Carcinogenicity

Rats administered oral trandolapril 0.25 to 8 mg/kg/day for 104 weeks had no apparent dose-related mortality or palpable tumors. There was a positive linear trend in the incidence of pituitary chromophobe adenomas in female rats ($p=0.0043$), but a pair-wise comparison between control and high-dose missed conventional levels of statistical significance ($p=0.0816$). This finding warrants some kind of follow-up, perhaps blinded re-evaluation of female rat histopathology slides.

Oral trandolapril 1 to 25 mg/kg/day was given to mice for 78 weeks. There was no apparent dose-related mortality. Palpable tumors were found in 2 of 60 male controls and 1 of 60 low-dose females. This study was criticized by the statistician for failure to use a maximum tolerated dose. This issue will need to be addressed by the CAC.

3.3.5. Reproductive toxicology

Fertility and reproduction were unaffected in rats by oral trandolapril to 100 mg/kg/day. Offspring in the 100 mg/kg dose group showed renal structural abnormalities which resolved with growth. Minor visceral defects were found in offspring of rats given 1000 mg/kg/day. No treatment-related malformations were found in rabbits at doses of trandolapril producing maternal toxicity.

Cynomolgus monkeys showed dose-related increased abortions at 10 to 250 mg/kg/day. High mortality and fetal loss prevented adequate description of teratogenic potential.

A segment III peri- and post-natal period study in rats showed no effects only at the lowest studied dose (1 mg/kg/day). This dose is about 12 times (on a weight-adjusted basis) or 0.4 times (on a surface area-adjusted basis) the maximum recommended human dose.

3.3.6. Mutagenicity and clastogenicity

Trandolapril was subjected to Ames testing in 5 histidine-requiring strains of *Salmonella typhimurium* in the presence and absence of S-9 metabolic activation. There was no mutagenic activity (spontaneous reversion) at 5 mg/dish in any strain.

Gene conversion was tested in *Saccharomyces cerevisiae* D4 cells with and without metabolic activation with S-9. There was no increase in gene conversion with trandolapril to 2 mg/ml compared with negative controls.

Trandolapril to 0.5 mg/ml was not mutagenic in an HPRT gene mutation assay with V79 Chinese hamster cells.

Clastogenic activity was assessed in cultured human lymphocytes. There was no increase in chromosomal aberrations with trandolapril up to 1 mg/ml. Chromosomal aberrations were sought with V79 Chinese hamster lung fibroblasts in culture. No dose-dependent effects were seen with trandolapril to 4 mg/ml. Chromosomal aberrations were not found with Chinese hamster ovary cells in culture with trandolapril to 0.5 mg/ml. Micronuclei were sought in mouse bone marrow cells following a single oral dose of trandolapril 1 or 2 g/kg. There was no increase compared with control animals.

3.4. Summary of pharmacological and toxicological findings

Trandolapril and trandolaprilat were shown to be inhibitors of angiotensin converting enzyme, with in vitro and in vivo activities demonstrably consistent with other members of the same class. No reported series of experiments excluded other possible receptor-mediated activities. Toxicity was also similar to other members of the class. No mutagenic or clastogenic potential was demonstrated.

4. Clinical background

Papers mentioning trandolapril are reviewed in Appendix C, on page 348. Literature references to studies reported in the NDA were generally consistent with the sponsor's reports. Several studies are described for which no corresponding NDA report was identified; these are briefly outlined below.

4.1. Hypertension

Backhouse et al. (1994; reviewed on page 348) report interim results of an uncontrolled foreign trial (may be one of the full study reports that was not submitted to FDA) in 960 subjects. Diastolic blood pressure results and adverse events appear similar to NDA data.

Cesarone et al. (1994; reviewed on page 348) report 24-hour ambulatory blood pressure results in 25 subjects on either trandolapril 1 mg or 2 mg. The 2 mg dose trended toward producing a greater effect. There is hardly any information regarding adverse events.

Mancia et al. (1992; reviewed on page 348) reported another 24-hour ambulatory blood pressure placebo-controlled study in 62 subjects (42 on trandolapril). There is little discussion of adverse events.

Pauly and Safar (1994; reviewed on page 349) reported a fixed-dose study in 180 subjects comparing trandolapril 2 mg daily to captopril 50 mg twice daily. Trandolapril had a greater effect in lowering diastolic pressure. Adverse events are not discussed in much detail, but were reportedly similar in the two groups.

Poirier et al. (1993b; reviewed on page 350) reported a 24-hour ambulatory blood pressure study in 31 subjects comparing trandolapril 1 mg once daily to 0.5 mg twice daily. The blood pressure effects of the two regimens were similar. Again, adverse events are not thoroughly discussed but appeared similar in the two groups.

Poirier et al. (1993a; reviewed on page 350) reported a 24-hour ambulatory blood pressure study in 65 subjects using titrated doses of trandolapril (up to 4 mg/day) and enalapril (up to 20 mg/day). The efficacy was similar. There is no discussion of adverse events.

Ravogli et al. (1994; reviewed on page 350) reported a placebo-controlled 24-hour ambulatory blood pressure study in 62 subjects. There is no discussion of adverse events.

Steiner et al. (1994; reviewed on page 350) compared trandolapril 2 mg/day to a sustained release nifedipine product and to the combination. Although the combination produced a greater antihypertensive effect than did trandolapril, adverse events of the combination were reported to be significantly higher. However, these events are hardly discussed.

4.2. Microalbuminuria

Banduceau et al. (1994; reviewed on page 351) evaluated the effects of trandolapril in 67 non-insulin-dependent diabetic subjects. In a subgroup of 13 subjects with significant microalbuminuria, 11 normalized. Adverse events were similar to events seen in the NDA with exception of one subject's with plantar mal perforans. One patient died from pulmonary edema.

4.3. Post-myocardial infarction

The TRACE study (discussed on page 351) is on-going randomized double-blind placebo-controlled trial in Denmark and plans to study the effects of trandolapril administered to post-myocardial infarction subjects with reduced left ventricular function as shown by echo wall motion index <1.2.

5. Human pharmacokinetics

A separate appendix with individual subject data and statistical analyses is being retained in the Division of Biopharmaceutics, and can be obtained upon request.

- 5.1. Synopsis** The sponsor has adequately studied the pharmacokinetics (single and multiple dose), metabolism and excretion of trandolapril, a new angiotensin converting enzyme (ACE) inhibitor to include the dosing range covered in the proposed package insert. Acceptable studies have also been performed to study renal and liver impairment as well as age and gender effects. Drug interaction between the drug and cimetidine, digoxin and furosemide were studied. Food effect was studied. An acceptable in vitro dissolution method has been provided.
- 5.2. Recommendation** The Division of Biopharmaceutics recommends that the sponsor's NDA 20-528 be accepted for meeting the bioavailability requirements of CFR 320, provided the comments below (*Comments to be sent to the firm on page 11*) are satisfactorily addressed by the sponsor.
- 5.3. Background** Trandolapril is a synthetic non-sulphydryl ACE inhibitor with structure shown in Figure 1 on page 1. It is a prodrug which is hydrolyzed in the liver to its active diacid, trandolaprilat. It is one of the most lipophilic among the presently available ACE inhibitors. It is proposed to be used for the treatment of hypertension. Headache, cough, dizziness and diarrhea are the major side effects. The sponsor has submitted 18 pharmacokinetics studies in support of the NDA and 16 studies were reviewed. The recommended dosing is 2 to 4 mg once a day.
- Trandolapril is an acidic highly lipophilic compound with a solubility of >100 mg/ml in chloroform, dichloromethane and methanol. The molecular weight of trandolapril is 430.54. The lipophilic index determined by reversed phase HPLC at pH 7.4 was 1.487 ± 0.113 for trandolapril (compared to ramipril, perindopril, and captopril, ramipril is the most lipophilic molecule followed by trandolapril) and 1.461 ± 0.117 for trandolaprilat (compared to ramiprilat, perindoprilat, and enalaprilat, trandolaprilat is the most lipophilic).
- 5.4. Summary of bioavailability, pharmacokinetics, and pharmacodynamics**
- 5.4.1. Bioavailability and bioequivalence**
- 5.4.1.1. Absolute bioavailability** With reference to a 2 mg i.v. bolus dose, the absolute bioavailability of trandolapril given as 2 mg oral solution averaged 10.7% (CV 22.7%) while the absolute bioavailability as trandolaprilat¹ averaged 71.3% (CV 24.8%)².
- 5.4.1.2. Bioequivalence** Bioequivalence was evaluated on log-transformed parameters and 90% confidence intervals were reported. Study TR-4-CP³ compared the 1 mg and 4 mg capsule formulations used in Phase I, II and III studies to the 1 mg and 4 mg future-market tablet formulations. The two one-sided test procedure on the AUC (0.90 to 1.24 for 1 mg, 0.79 to 1.04 for 4 mg), C_{max} (0.87 to 1.29 for 1 mg, 0.76 to 1.08 for 4 mg) for trandolapril, and AUC (0.80 to 1.13 for 1 mg, 0.87 to 1.04 for 4 mg), C_{max} (0.86 to 1.04 for 1 mg, 0.87 to 0.97 for 4 mg) for trandolaprilat show that the clinically tested capsules and to-be-marketed tablet formulations are bioequivalent with respect to trandolapril but bioequivalent with respect trandolaprilat.
- 5.4.1.3. Food effect** The effect of food on the absorption of trandolapril administered in the morning as single 1 mg capsule was evaluated⁴. After co-administration of a standard meal (caloric content = 820 Kcal), the T_{max} increased from 0.6 to 1.8 hours and AUC

¹ Calculated from the ratio of AUC's for trandolaprilat after oral and intravenous administration.

² Study GB/89/570/48, reviewed on page 186.

³ Study TR-4-CP, reviewed on page 214.

⁴ Study F/89/570/47, reviewed on page 109.

increased by 17% for trandolapril; T_{max} increased from 5.4 to 8.2 hours for trandolaprilat, but there was no effect on C_{max} for trandolapril, AUC or C_{max} for trandolaprilat, or plasma ACE inhibition.

5.4.2. Pharmacokinetics

Single⁵- and multiple⁶-dose (4 mg) pharmacokinetic parameters for trandolapril and trandolaprilat are summarized in Table 1 below. The accumulation factor was 1.02 for

Table 1. Single- and multiple-dose pharmacokinetics after 4 mg dosing.

	Trandolapril		Trandolaprilat	
	Single	Multiple	Single	Multiple
C_{max} (ng/ml)	6.1 (51 ^a)	6.2 (70)	6.0 (40)	9.0 (33)
AUC (ng·h/ml)	9.8 (34)	8.2 (73)	192 (29)	101 (33) ^b
T_{max} (h)	1.1 (57)	0.7 (37)	4.4 (12)	2.4 (22)
$T_{1/2}$ (h)	6.6 (99)	8.4 (71)	47 (51)	115 (101)
CL/F (L/h)	546 (92)	751 (70)	23 (35)	47 (59)

a. %C.V.

b. $AUC_{0-\tau}$

trandolapril and 1.50 for trandolaprilat⁷. On multiple dose administration of 4 mg capsules q.d. for 8 days, the oral clearance for trandolapril increased from 546 (C.V. 92%) to 751 l/h (C.V. 70%); mean formation clearance for trandolaprilat increased from 23 (C.V. 35%) to 47 l/h (C.V. 59%)⁸. On multiple dose administration of 4 mg capsules q.d. for 8 days, the mean terminal half-life for trandolapril increased from 6.6 (C.V. 99%) to 8.4 hours (C.V. 71%); mean terminal half-life for trandolaprilat increased from 47.4 (C.V. 51%) to 115.0 hours (C.V. 101%)⁹. Greater than 59% of the total radioactivity was recovered from urine (33.3%) and feces (60.9%) over 168 hours after administration of an 8-mg trandolapril solution; of the urine recovery, 2.6% was trandolapril and 16.0% was trandolaprilat¹⁰. The volume of distribution was about 18 liters and the systemic clearance was 52 l/h for trandolapril; the systemic clearance was 7 l/h and the renal clearance was 4.3 l/h for trandolaprilat¹¹. Following i.v. administration of 2 mg trandolapril, 23.6% of the dose was recovered in urine as trandolaprilat; following i.v. administration of 1.87 mg trandolaprilat, 69.8% of the dose was recovered in urine as trandolaprilat over 56 hours¹².

5.4.3. Metabolism

Metabolic profiling trandolapril confirms the presence of at least seven metabolites in urine and feces, the principle metabolic transformations were either by conjugation with glucuronic acid or de-esterification. Trandolaprilat is the major active metabolite in urine and feces. Other metabolites that have been identified include the glucuronides of trandolapril and trandolaprilat, diketopiperazine of trandolapril and trandolaprilat¹³. The percentages of the i.v. and p.o. trandolapril dose converted to the active metabolite were 36% and 39%, respectively¹⁴.

⁵ Study TR-2-CP, reviewed on page 209.

⁶ Study TR-2-CP, reviewed on page 209.

⁷ Study TR-2-CP, reviewed on page 209.

⁸ Study TR-2-CP, reviewed on page 209.

⁹ Study TR-2-CP, reviewed on page 209.

¹⁰ Study V1GB-101, reviewed on page 193.

¹¹ Study GB/89/570/48, reviewed on page 186.

¹² Study GB/89/570/48, reviewed on page 186.

¹³ Study V1GB-101, reviewed on page 193.

¹⁴ Study GB/89/570/48, reviewed on page 186.

5.4.4. Dose and dosage form proportionality The C_{max} and AUC of trandolapril were dose proportional for 1 to 4 mg single oral doses, but the C_{max} and AUC of trandolaprilat were not dose proportional for 1 to 4 mg single oral doses as determined by linear regression¹⁵. The pharmacokinetics of trandolapril and trandolaprilat are non-linear following single or multiple doses of trandolapril between 1 and 16 mg¹⁶.

5.4.5. Special populations

5.4.5.1. Renal impairment When renally insufficient subjects with creatinine clearances ranging from 10 to ≥ 80 ml/min were administered a single 4 mg oral dose of trandolapril in the morning, the pharmacokinetic parameters shown in Table 2 below were obtained. When renally

Table 2. Pharmacokinetic parameters in renally impaired subjects.

	Hemodialysis ^a	CL_{CR} (ml/min)				
		<10	10 to 30	31 to 50	51 to 80	Normal ^b
Trandolapril						
C_{max} (ng/ml)	12.1±2.3	18.6±7.4	10.5±1.3	22.7±11.3	9.6±2.1	6.6±1.6
AUC (ng·h/ml)	17.1±0.2	29.8±2.3	14.0±1.8	38.9±19.4	15.5±2.4	11.1±5.2
Trandolaprilat						
C_{max} (ng/ml)	19.8±3.6	24.4±8.0	16.6±1.3	10.0±2.02	8.7±0.9	9.6±2.3
AUC (ng·h/ml)	550±105	570±198	327±17	29±35	196±14	178±15
CL_R (L/h)	0.03±0.01	0.12±0.12	0.73±0.11	2.44±0.35	3.68±0.14	5.26±0.71

a. Study F/87/570/26, reviewed on page 161.

b. Study FF/87/570/26, reviewed on page 154.

insufficient subjects with creatine clearance ranging from 7 to ≥ 80 ml/min were administered multiple 2 mg oral doses of trandolapril in the morning for 10 days, C_{max} increased (1.6 to 2.7 ng/ml for trandolapril and 4.7 to 9.9 ng/ml for trandolaprilat); AUC increased (2.0 to 3.8 ng·h/ml for trandolapril and 108.4 to 184.3 ng·h/ml for trandolaprilat), and the renal clearance for trandolaprilat decreased from 4.2 to 0.7 l/h in subjects with creatine clearance between 7 and 30 ml/min compared with subjects with normal renal function¹⁷. The maximum inhibition (I_{max}) of ACE activity by trandolapril was not modified by renal failure. Trandolaprilat is dialyzable. Adjustment to lower doses of trandolapril to patients with creatine clearance <30 ml/min should be considered.

5.4.5.2. Hepatic impairment Subjects with hepatic insufficiency were administered a single oral dose of 2 mg trandolapril capsule¹⁸. Compared to normal subjects¹⁹, the C_{max} and AUC increased (about 9-fold for trandolapril and about 2-fold for trandolaprilat), but inhibition of ACE activity by trandolapril was not affected by hepatic impairment. Adjustment to lower doses of trandolapril to patients with hepatic insufficiency should be considered.

5.4.6. Elderly and by gender Hypertensive subjects (5 males and 9 females) aged >65 years were administered single morning doses of 2 mg trandolapril capsule for 10 days²⁰. Compared to young subjects²¹, the C_{max} and the AUC of trandolapril following oral administration in the elderly increased by 43% and 65%, respectively, while the C_{max} and the AUC of trandolaprilat increased between 11% and 18%, respectively, but inhibition of ACE activity by trandolapril was not affected by age.

¹⁵. Study F/89/570/54, reviewed on page 113.

¹⁶. Study TR-2-CP, reviewed on page 209.

¹⁷. Study FF/88/570/39, reviewed on page 173.

¹⁸. Study FF/88/570/38, reviewed on page 167.

¹⁹. Study F/89/570/54, reviewed on page 113.

²⁰. Study F/89/570/45, reviewed on page 180.

²¹. Study S/87/570/23, reviewed on page 201.

Analysis of the data for the elderly subjects by gender shows that the pharmacokinetics of trandolapril and trandolaprilat and the inhibition of ACE activity by trandolapril were similar in elderly male and female subjects.

5.4.7. Drug interactions

5.4.7.1. Digoxin

Study F/87/570/24²² had a study design in which subjects received 0.50 mg digoxin tablet on day 1 (loading dose) then 0.25 mg digoxin tablet on days 2 to 15 and 4 mg trandolapril capsule on days 8 to 15. The mean C_{min} and C_{2h} for digoxin were similar on days 7 to 8 and days 8 to 15, showing no effect of trandolapril on the pharmacokinetics of digoxin.

5.4.7.2. Cimetidine

The results of Study F/91/570/132²³, in which volunteers received a single dose of trandolapril (2 mg) with and without prior and concurrent administration of cimetidine (400 mg), indicated that with co-administration of cimetidine there is an increase of about 44% in C_{max} of trandolapril but no difference in the pharmacokinetics of trandolaprilat or in ACE inhibition.

5.4.7.3. Furosemide

Co-administration of furosemide tablet (40 mg) with trandolapril capsule (2 mg) did not affect the pharmacokinetics of furosemide or trandolapril, but the renal clearance of trandolaprilat increased by about 25%²⁴. Furosemide did not affect the inhibition of ACE activity by trandolapril.

5.4.8. Pharmacokinetic/pharmacodynamic relationship

Inhibition of ACE has been used as a pharmacodynamic measurement of the activity of trandolapril. A plot of the concentration of trandolaprilat against inhibition of ACE activity (% basal value) shows that maximum inhibition of ACE activity is achieved with a concentration of 2 ng/ml of trandolaprilat²⁵.

5.4.9. Formulation

The three tablet formulations to be marketed (1, 2 and 4 mg) are not compositionally proportional. Their compositions are shown in Table 3 below.

Table 3. Proposed composition of to-be-marketed formulations.

	1 mg	2 mg	4 mg
Trandolapril	1.0	2.0	4.0
Iron oxide, yellow			
Iron oxide, red			
Corn starch			
Lactose, hydrous			
Hydroxypropyl methylcellulose			
Croscarmellose sodium			
Povidone			
Sodium stearyl fumarate			
Total			

5.4.10. Dissolution

The proposed dissolution method²⁶ of USP Apparatus II at 50 rpm in water is acceptable, but the specification should be changed from Q= at 45 minutes to Q= at 30 minutes.

5.4.11. Assay

The sponsor used validated methods to measure trandolapril and trandolaprilat in all the studies. Overall, the analytical validation was satisfactory.

²² Study F/87/570/24, reviewed on page 105.

²³ Study F/91/570/132, reviewed on page 118.

²⁴ Study ZA/89/570/43, reviewed on page 298.

²⁵ Study ZA/89/570/43, reviewed on page 298, and Study FF/89/570/45, reviewed on page 180.

²⁶ Appendix D. Dissolution on page 362.

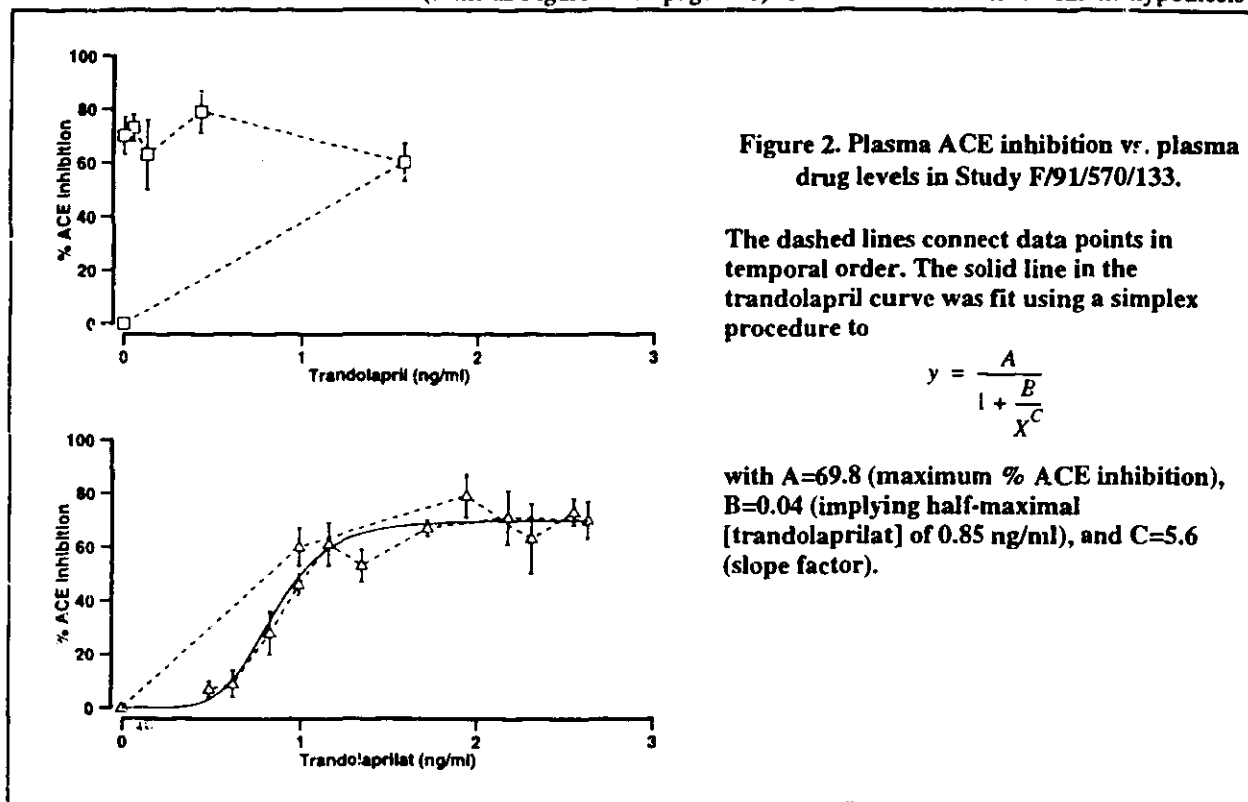
- 5.4.12. Plasma protein binding** The serum protein binding of trandolapril is >80% and is independent of concentration. Plasma protein binding of trandolaprilat varies from 94% at 0.1 ng/ml to 65% at 1000 ng/ml, indicating saturation of binding at high concentration.²⁷
- 5.4.13. Labeling** The clinical pharmacology section of the labeling is deficient and the firm has been advised to modify it accordingly.
- 5.4.14. Influence of race** The pharmacokinetics of trandolapril and trandolaprilat have not been described in various race groups.
- 5.5. Comments to be sent to the firm**
1. Follow up to the reviewer's telephone conversations with the firm, the sponsor is requested to send the following information/data to the Agency: Assay validation for plasma ACE activity for Study GB/89/570/48.
 2. Dissolution: Based on the dissolution data submitted by the sponsor, the dissolution specification should be changed from Q= in 45 minutes to Q= in 30 minutes.

²⁷ Study HAG 006472, reviewed on page 192.

6. Human pharmacodynamics

6.1. Mechanism of action

6.1.1. ACE inhibition Trandolaprilat is known, from animal and in vitro work, to be an angiotensin converting enzyme inhibitor. Plasma ACE inhibition was measured in numerous clinical trials. The time course following dosing was studied in many of these cases¹. Some of these studies had measurements of plasma trandolapril and trandolaprilat levels, permitting the characterization of hysteresis curves for ACE inhibition, an example of which (following a single dose in normal volunteers) is shown in Figure 2 below (same as Figure 67 on page 128). The data are consistent with the hypothesis



that ACE inhibition is a function of trandolaprilat, but not trandolapril. The time course for ACE activity thus reflects the long terminal half-life of trandolaprilat, as shown in Figure 3 below (same as Figure 66 on page 127; from the same single-dose study) after a single dose and after repeated dosing².

Plasma ACE inhibition was not 100%, even at high doses. Most studies showed a maximal effect of about 85 to 90% inhibition. In vitro plasma ACE inhibition was complete, but required trandolaprilat levels approximately 100-fold greater than that required to produce 90% inhibition³.

The relationship between plasma ACE inhibition and diastolic pressure reduction is shown (as a hysteresis curve) in Figure 4 below (left, same as Figure 83 on page 171;

¹ Study CH/86/570/01, reviewed on page 90, Study F/89/570/47, reviewed on page 109, Study F/91/570/132, reviewed on page 118, Study F/91/570/133, reviewed on page 122, Study FF/87/570/25, reviewed on page 154, Study FF/87/570/26, reviewed on page 161, Study FF/88/570/38, reviewed on page 167, Study FF/88/570/39, reviewed on page 173, Study FF/89/570/45, reviewed on page 180, Study GB/89/570/48, reviewed on page 186, Study S/87/570/23, reviewed on page 201, and Study ZA/89/570/43, reviewed on page 298.

² Study FF/89/570/45, reviewed on page 180.

³ See Figure 12 on page 21 of the Pharmacology Review.

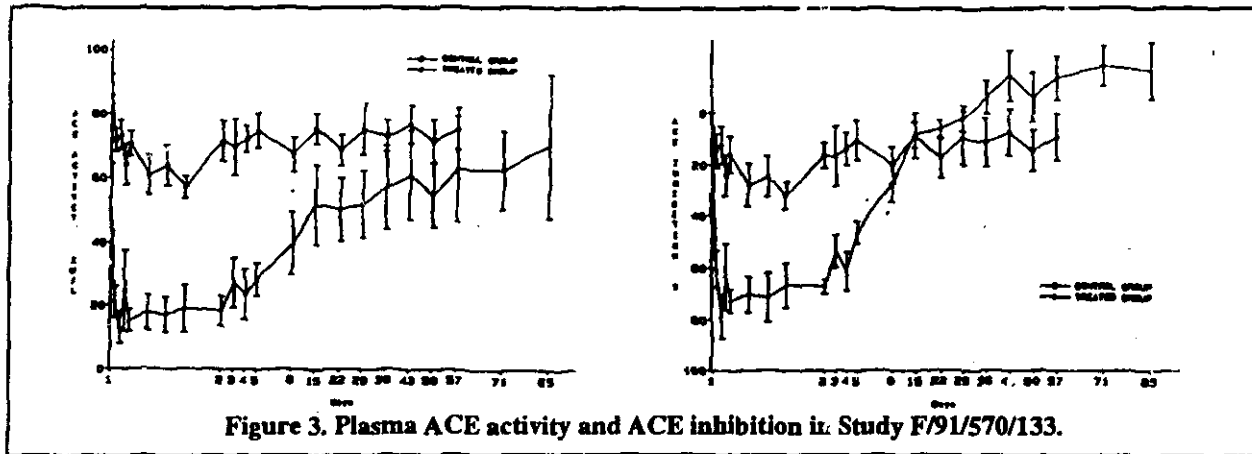


Figure 3. Plasma ACE activity and ACE inhibition in Study F/91/570/133.

right, same as Figure 76 on page 159). A blood pressure effect was seen with >80% plasma ACE inhibition. Some hysteresis is evident, suggesting that the active sites for tissue ACE are less accessible than sites on plasma ACE.

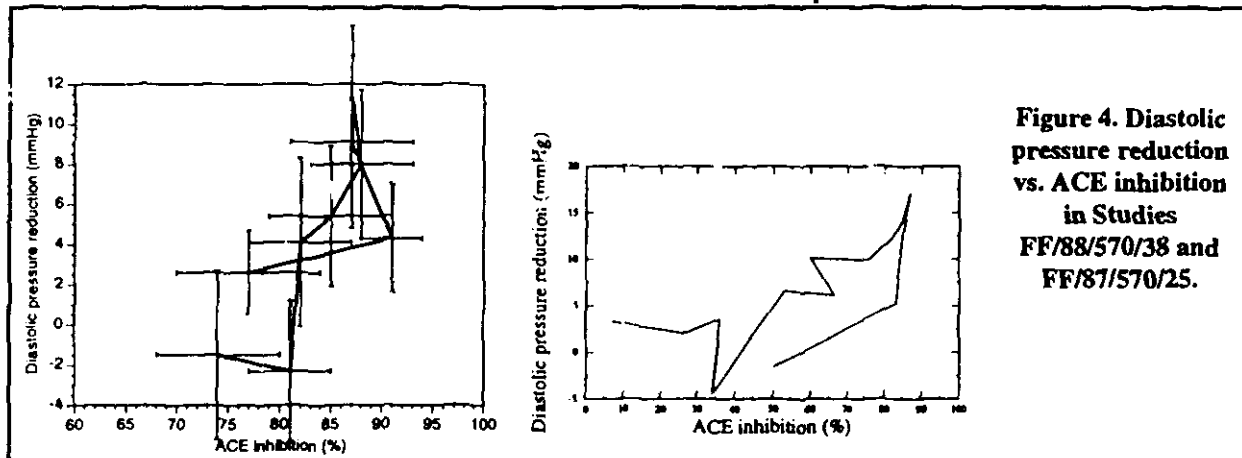


Figure 4. Diastolic pressure reduction vs. ACE inhibition in Studies FF/88/570/38 and FF/87/570/25.

6.1.2. Concomitant hormonal changes

Some of the studies in which ACE inhibition was monitored also collected renin activity and aldosterone levels. One study⁴ collected data at multiple time points after a single dose, but this was in a hepatic impairment population, so the results cannot be extrapolated to the general hypertensive population. One study⁵ in normal volunteers had measurements of renin activity and aldosterone at the times 0, as well as angiotensin I and angiotensin II measurements. This suggests a dose-dependent increase (doses of 0.5, 2, and 3 mg) in renin activity at 4 hours after dosing, but little effect on trough renin levels or aldosterone levels at either time. The ratio of angiotensin II to angiotensin I fell during dosing in a dose-dependent manner, and was only about 50% back to baseline 3 days after the last dose. Other studies⁶ showed similar effects with respect to increasing renin activity and no consistent effect on aldosterone levels.

The observed rise in renin activity is a predicted consequence of ACE inhibition, as is the fall in angiotensin II levels. Angiotensin II stimulates adrenocortical synthesis of aldosterone, so a fall in aldosterone levels would be expected, but was generally not observed in these trials and is, with other ACE inhibitors a small effect, possibly because of compensatory stimuli of steroidogenesis⁷.

⁴ Study FF/88/570/38, reviewed on page 167.

⁵ Study CH/86/570/01, reviewed on page 90.

⁶ Study FF/87/570/21, reviewed on page 149, Study FF/88/570/38, reviewed on page 167, Study TR-20-HTN, reviewed on page 218, Study TR-20A-HTN, reviewed on page 229, Study TR-21-HTN, reviewed on page 240, and Study TR-21A-HTN, reviewed on page 250.

6.1.3. Response to exogenous angiotensin I and II

There were no clinical studies reported by the sponsor examining the effects of exogenous angiotensin I or angiotensin II⁸.

6.2. Antihypertensive effect

6.2.1. Well-controlled, short-term US trials

Analysis focussed on US studies⁹, all of which shared a common design. These studies had double-blind, randomized, placebo-controlled, multiple-dose, parallel-group designs, with a 4-week single-blind placebo lead-in phase, followed by a 6-week double-blind treatment phase, and finally a 3-week, single-blind, placebo-washout phase. Subjects qualified with mild to moderate hypertension (supine diastolic pressure 95 to 114 mmHg) at the end of the run-in phase. The primary analysis was intent-to-treat change in supine diastolic pressure at the final double-blind visit. Two of these trials enrolled only Black subjects and two enrolled essentially only Caucasians, with a small number of other ethnic groups represented. Two trials covered doses 0.25 to 4 mg and two trials covered 4 to 16 mg.

6.2.1.1. Dose-response

Placebo-subtracted changes in supine diastolic pressure at trough are shown in Figure 5 below for these trials separately, and in Figure 6 below for all trials combined. The error bars in these figures represent 95% confidence limits. These analyses were performed as part of the statistical review. The 0.5 mg dose produces an effect on supine diastolic pressure which is statistically significantly different from that of placebo. Doses from 2 to 16 mg produce effects which are not distinguishable in magnitude.

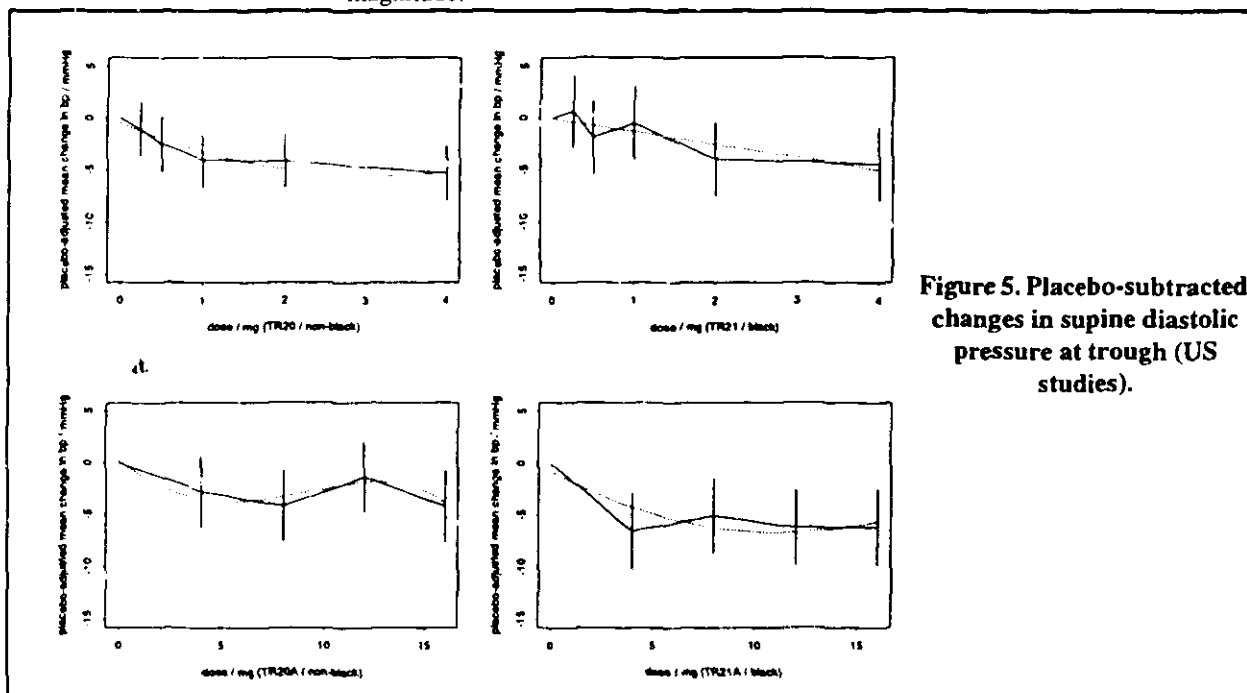


Figure 5. Placebo-subtracted changes in supine diastolic pressure at trough (US studies).

No similar cross-study analysis was done for other efficacy parameters, but the data from the pivotal US studies are summarized in Table 4 below. The dose-response relationship is less clear from any of these parameters, but is generally consistent with the relationship seen for supine diastolic pressure. There does not appear to be an increase in responder rates with doses above 4 mg.

⁷ Goodman & Gillman, 8th edition, 1990, page 758.

⁸ Such studies were done in animals; see Pharmacology Review.

⁹ Study TR-20-HTN, reviewed on page 218, Study TR-20A-HTN, reviewed on page 229, Study TR-21-HTN, reviewed on page 240, and Study TR-21A-HTN, reviewed on page 250.

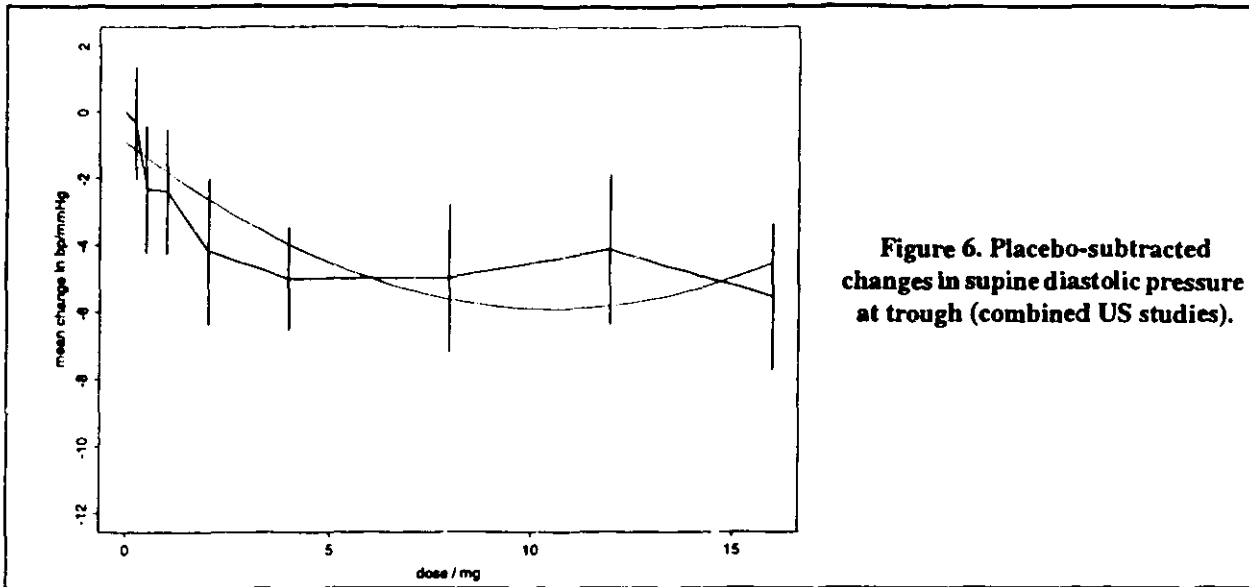


Figure 6. Placebo-subtracted changes in supine diastolic pressure at trough (combined US studies).

Table 4. Secondary efficacy data from pivotal US studies.

	Plcbo	Trandolapril (mg/day)					Plcbo	Trandolapril (mg/day)			
		0.25	0.5	1	2	4		4	8	12	16
Caucasians		Study TR-20-HTN					Study TR-20A-HTN				
n	49	53	53	51	53	53	36	38	40	39	36
Peak/trough	—	0.79	0.61	0.93	0.87	0.65	—	0.68	0.56	0.28	0.50
Δsitting diastolic	—	-1.1	0.7	-3.0	-5.0	-5.8	—	-3.8	-5.2	-0.8	-3.3
Δstanding diastolic	—	-2.4	-1.5	-3.0	-4.7	-4.5	—	-0.9	-2.2	-1.4	-4.1
Δsupine systolic	—	-3.5	-4.7	-9.1	-6.7	-9.1	—	-3.2	-9.6	-4.2	-6.2
Δsitting systolic	—	-3.4	-4.0	-10.0	-8.4	-8.7	—	-1.9	-11.5	-4.3	-5.4
Δstanding systolic	—	-5.0	-4.8	-9.6	-8.8	-10.3	—	-4.1	-8.7	-5.4	-10.2
Responder rate (%)	35	—	—	—	—	62	39	53	58	46	55
Blacks		Study TR-21-HTN					TR-21A-HTN				
n	22	23	22	23	22	23	37	37	38	37	36
Peak/trough	—	-0.14	0.54	0.13	0.48	0.57	—	0.93	0.90	1.10	1.31
Δsitting diastolic	—	2.4	0.1	1.4	-0.7	-3.3	—	-4.3	-3.3	-5.0	-4.5
Δstanding diastolic	—	3.4	0.1	-1.7	-1.9	-3.4	—	-4.1	-3.5	-6.7	-5.8
Δsupine systolic	—	—	—	—	—	—	—	-6.3	-4.4	-5.7	-7.1
Δsitting systolic	—	—	—	—	—	—	—	-3.5	-2.9	-3.5	-4.4
Δstanding systolic	—	—	—	—	—	—	—	-5.9	-5.7	-5.4	-8.0
Responder rate (%)	14	9	18	13	41	43	5	38	24	38	33

6.2.1.2. Time course of effect following a dose

In Study FF/86/570/05¹⁰, hypertensive subjects underwent repeated cuff and 48-hour ambulatory blood pressure monitoring beginning at the time of administration of a single dose of placebo or 1, 4, or 16 mg trandolapril. The cuff data (baseline- and placebo-subtracted) are reproduced in Figure 7 below (same as Figure 68 on page 132). The 4 and 16 mg doses produce comparable effects, peaking at 4 hours post-dosing. The effect is near baseline by 33 hours, but there is a second dip at 36 hours. This

¹⁰ Study FF/86/570/05, reviewed on page 130.

secondary dip is visible in all 3 dose groups, and looks similar to that following multiple dosing¹¹.

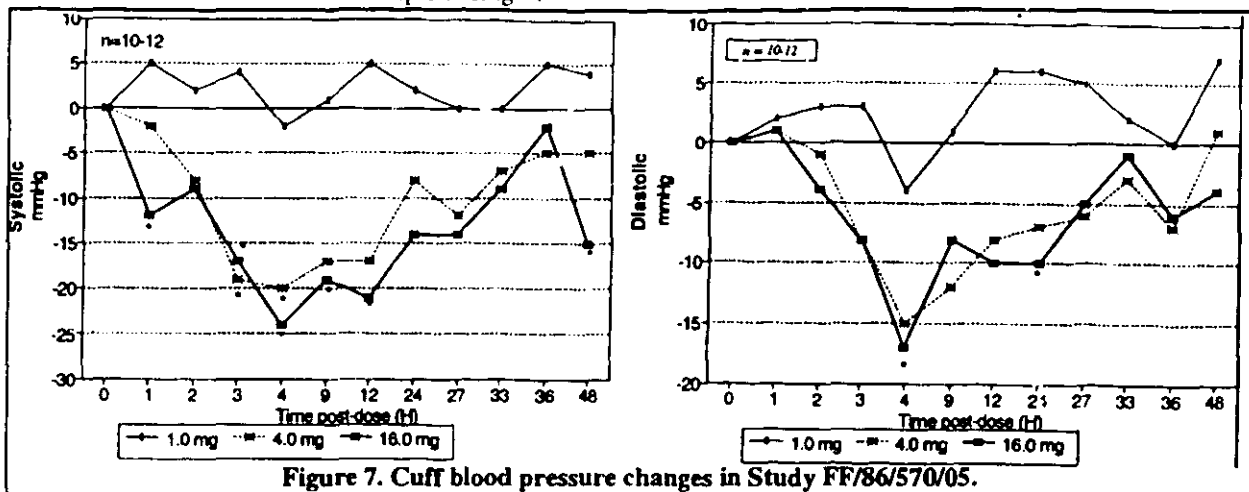


Figure 7. Cuff blood pressure changes in Study FF/86/570/05.

6.2.1.3. Time course of effect with repetitive dosing

The time course for development of an effect on supine diastolic pressure with repetitive dosing can be determined from the major US efficacy trials¹², which had assessments at 1, 2, 4, and 6 weeks. The data from the two high-dose ranging trials are shown in Figure 8 below (same as Figure 114 on page 232) and Figure 9 below (same

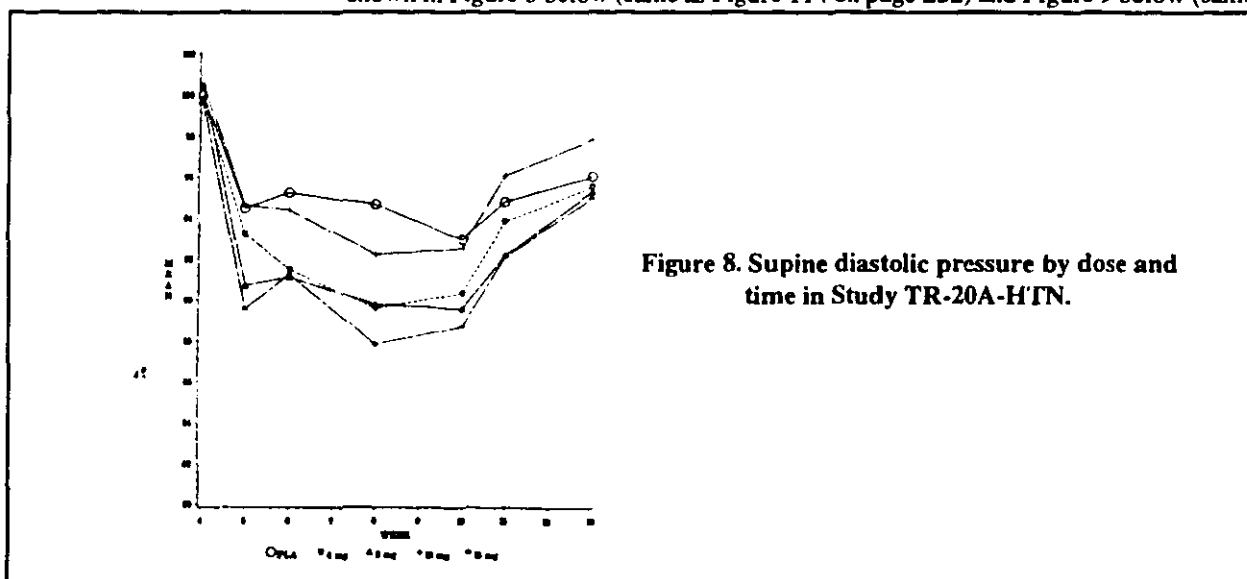


Figure 8. Supine diastolic pressure by dose and time in Study TR-20A-HTN.

as Figure 124 on page 253). The effect appears to be at least nearly fully manifest by the end of the first week of dosing. Similar results were obtained in a small Swedish study¹³ and the US ABPM study¹⁴. In contrast, a European study comparing trandolapril monotherapy, HCTZ monotherapy, and the combination in a 16-week double-blind design¹⁵ shows the effect on blood pressure to progress over 8 weeks (Figure 10 below, same as Figure 70 on page 147), but the lack of a placebo group makes this result less interpretable.

11. See section 6.2.1.4 on page 17.
 12. Study TR-20-HTN, reviewed on page 218, Study TR-20A-HTN, reviewed on page 229, Study TR-21-HTN, reviewed on page 240, and Study TR-21A-HTN, reviewed on page 250.
 13. Study S/87/570/23, reviewed on page 201.
 14. Study TR-42-CP, reviewed on page 282.
 15. Study FF/87/570/04, reviewed on page 145.

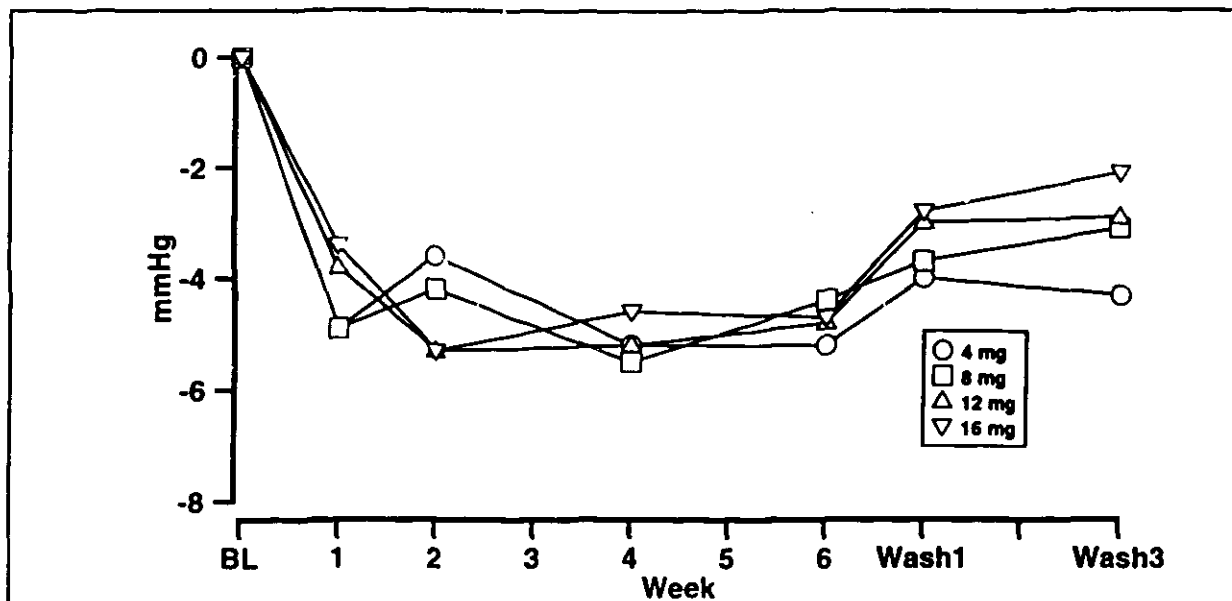


Figure 9. Change in supine diastolic pressure at trough by time in Study TR-21A-HTN.

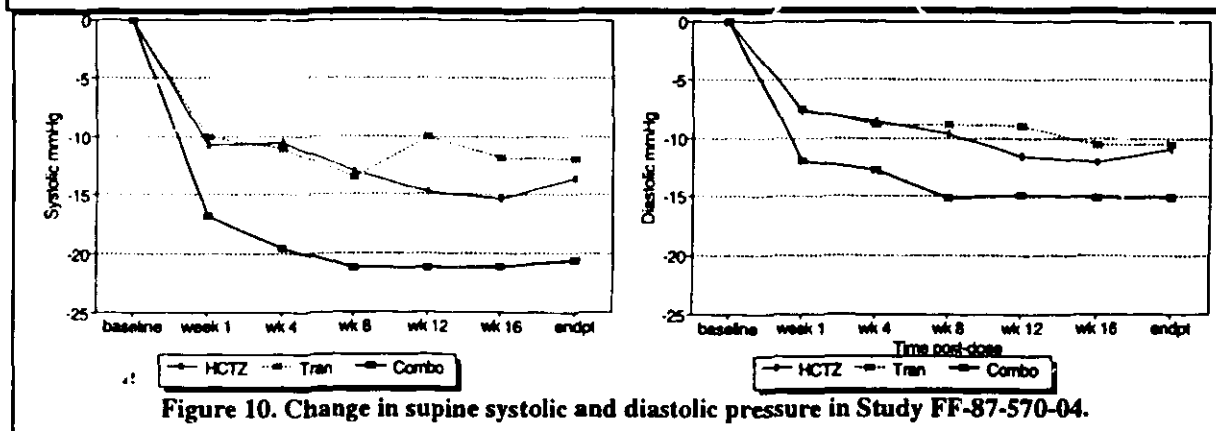


Figure 10. Change in supine systolic and diastolic pressure in Study FF-87-570-04.

6.2.1.4. Time course of effects following multiple dosing

In Study TR-42-CP¹⁶, subjects underwent 48-hour ambulatory blood pressure recordings, ending prior to the first dose and then beginning with the last dose in an 8-week double-blind treatment period. Figure 11 below (same as Figure 136 on page 285) shows baseline- and placebo-subtracted ambulatory blood pressure data following the last dose, as analyzed by the statistical reviewer. The effect of treatment appears to dissipate over about 36 hours, but then reappears, with a larger magnitude in the latter half of the second day. The magnitude aspect of the result is unlikely to be reproducible, but the general result is simply not attributable to a single outlying observation, and strongly suggests a duration of significant effect on blood pressure of at least 48 hours.

Figure 8 (page 16) and Figure 9 (page 17) appear to show incomplete resolution of antihypertensive effects 1 and 3 weeks after a 6-week dosing period, but there is no dose-response to the time course for recovery.

6.2.1.5. Relationship between ACE inhibition and antihypertensive effect

The relationship between ACE inhibition and antihypertensive effect was explored with the data collected in a hepatic impairment population¹⁷, because that was the only study with repeated measures of ACE activity following a dose. The results are discussed in section 6.1.1 on page 12.

¹⁶ Study TR-42-CP, reviewed on page 282.

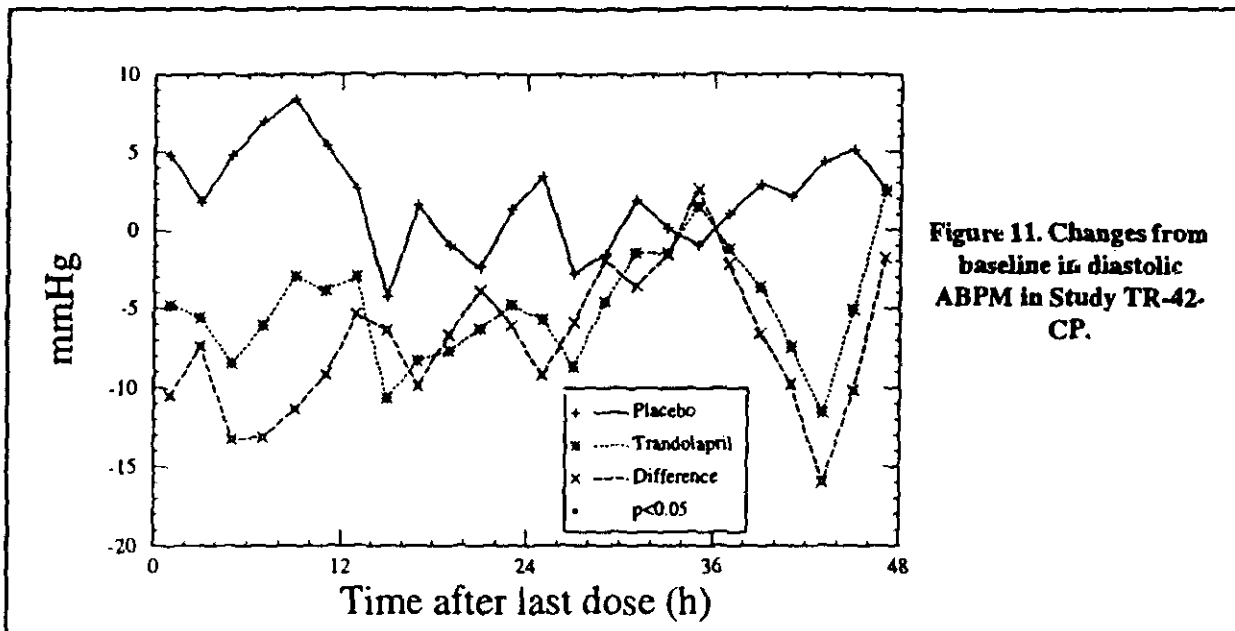


Figure 11. Changes from baseline in diastolic ABPM in Study TR-42-CP.

6.2.2. Subgroup analyses

As part of the statistical review, an ANOVA model was used to assess the effects of gender and race on treatment outcome, change from baseline to end point in trough supine diastolic pressure. In this analysis, the four US studies were combined and the model included treatment, sex, race (Black or Caucasian), and center nested in race as factors. Without placebo adjustment, significant differences in change in blood pressure were found for treatment, race, and center ($p < 0.003$ for each of them), but no treatment-by-sex or treatment-by-race interaction was found. Paired comparisons using two-sample t-tests showed that reductions in supine diastolic pressure at trough at end point for Blacks were significantly smaller than those for Caucasians at placebo, 0.25 mg, 1 mg, 4 mg, 8 mg, and 16 mg.

6.2.2.1. Analysis by race

Table 5 below shows changes in supine diastolic pressure at trough, analyzed by race.

Table 5. Mean changes in supine diastolic pressure ($\pm$ SD) at trough by dose and race (US trials).

	Plcbo	0.25	0.5	1	2	4	8	12	16
Caucasian n=	85	53	53	51	53	91	40	39	38
	-5.1 $\pm$ 6.5	-5.0 $\pm$ 6.1	-6.4 $\pm$ 7.7	-7.9 $\pm$ 6.3	-8.1 $\pm$ 6.6	-9.3 $\pm$ 6.7	-10.8 $\pm$ 7.9	-8.0 $\pm$ 7.3	-10.9 $\pm$ 9.6
Black n=	59	23	22	23	22	60	38	37	36
	-0.7 $\pm$ 7.0	-1.5 $\pm$ 4.7	-4.0 $\pm$ 5.5	-2.7 $\pm$ 6.1	-6.1 $\pm$ 7.9	-6.5 $\pm$ 6.2	-5.0 $\pm$ 9.1	-6.1 $\pm$ 9.8	-6.1 $\pm$ 6.6

These data are shown graphically in Figure 12 below.

Regression method was used to obtain dose-response curves for Caucasian and Black populations. In each case, starting with a model including baseline and a polynomial of dose with order six, a candidate dose-response model was selected by running the SAS step-wise regression procedure. Then the final model was obtained by removing higher-order terms if the resulting model was not rejected by a lack of fit test. The obtained dose-response curves for Caucasians and Blacks were, respectively,

$$\Delta = -5.795 - 0.923 \times Dose + 0.043 \times Dose^2$$

and

¹⁷ Study FF/88/570/38, reviewed on page 167.

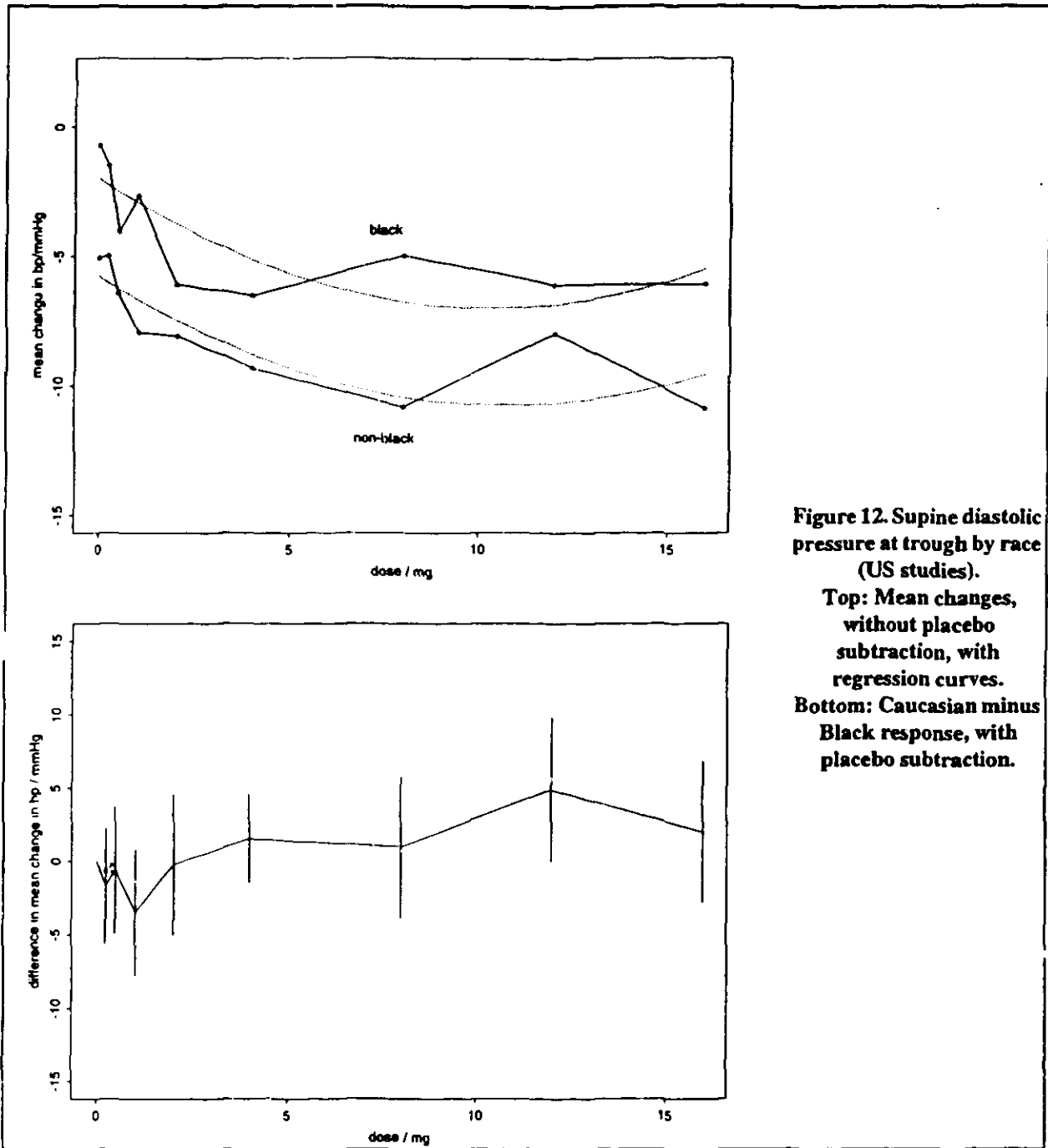


**Figure 12. Supine diastolic pressure at trough by race (US studies).
 Top: Mean changes, without placebo subtraction, with regression curves.
 Bottom: Caucasian minus Black response, with placebo subtraction.**

$$\Delta = -2.023 - 0.969 \times Dose + 0.047 \times Dose^2$$

where Δ denotes change in supine diastolic pressure at trough at end point. The fitted dose-response curves are shown as dotted lines in Figure 12.

To see the net difference in mean change from baseline in supine diastolic pressure at trough at end point, placebo-adjusted means were compared for each dose level. The difference was marginally significant only for the 12 mg group ($p=0.05$). Study-wise, placebo-adjusted dose-response curves (raw and fitted) are given in Figure 5 (page 14), where the vertical bars are 95% confidence intervals of mean change in supine diastolic pressure for each dose group. The difference in placebo-adjusted mean

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change between Blacks and Caucasians is graphically presented in the lower panel of Figure 12.

6.2.2.2. Analysis by sex Table 6 below shows changes in supine diastolic pressure at trough, analyzed by sex.

Table 6. Mean changes in supine diastolic pressure at trough by dose and sex (US trials).

	0.05	0.25	0.5	1	2	4	8	12	16
Male n=	78	47	45	40	46	74	44	46	43
	-2.9±7.2	-3.4±5.9	-5.8±7.6	-6.6±6.0	-8.1±6.9	-8.0±7.0	-7.9±8.3	-7.4±9.0	-8.0±8.8
Female n=	66	29	30	34	29	77	34	30	31
	-3.8±6.9	-4.8±5.8	-5.6±6.7	-5.9±7.5	-6.6±7.1	-8.4±6.3	-8.0±9.8	-6.6±8.1	-9.3±8.3

These data are shown graphically in Figure 13 below.

Further analyses were done by sex, similar to those done by race. The fitted dose-response curves for male and female subjects were, respectively,

$$\Delta = -3.572 - 2.296 \times Dose + 0.299 \times Dose^2 - 0.011 \times Dose^3$$

and

$$\Delta = -4.822 - 0.749 \times Dose + 0.0342 \times Dose^2$$

Point-wise comparison of placebo-adjusted mean changes from baseline in supine diastolic pressure showed no statistically significant differences between males and females. The difference (males minus females) is shown in the lower panel of Figure 13.

6.2.2.3. Analysis by age

Possible effects of age on treatment outcome were examined in several ways as part of this review. (1) Subjects in the 4 pivotal U.S. trials¹⁸ were classified as young or old, using age 60 years as a cutoff. Means and standard deviations were computed for supine diastolic pressure for each combination of race, dose, and age group, as shown in Table 7 below. Using analysis of variance, placebo-adjusted mean changes in supine

Table 7. Mean change in supine diastolic pressure by dose, race, and age (US studies).

	Trandolapril dose (mg/day)								
	Plcbo	0.25	0.5	1	2	4	8	12	16
Caucasian									
Age≤60									
n=	49	37	40	27	30	61	27	22	23
Δ±S.D.	-3.6±6.2	-5.2±6.6	-6.9±7.3	-8.5±6.8	-8.6±6.3	-9.7±6.7	-12.7±7.9	-10.1±6.7	-11.3±7.1
Age>60									
n=	36	16	13	24	23	30	13	17	15
Δ±S.D.	-7.1±6.6	-4.6±5.0	-4.9±9.1	-7.3±5.9	-7.4±7.0	-8.5±6.7	-6.8±6.4	-5.3±7.5	-10.2±12.8
Black									
Age≤60									
n=	44	18	19	16	18	45	24	25	20
Δ±S.D.	-0.0±7.2	-1.6±4.2	-3.5±5.4	-1.8±4.4	-5.9±7.4	-6.5±5.8	-4.5±8.0	-7.3±9.3	-4.9±6.6
Age>60									
n=	15	5	3	7	4	15	14	12	16
Δ±S.D.	-2.8±6.2	-1.1±6.7	-7.2±5.2	-4.7±8.9	-7.2±11.1	-6.5±7.4	-5.8±11.0	-3.6±10.8	-7.5±6.5

¹⁸ Studies TR-20-HTN, TR-20A-HTN, TR-21-HTN, and TR-21A-HTN.

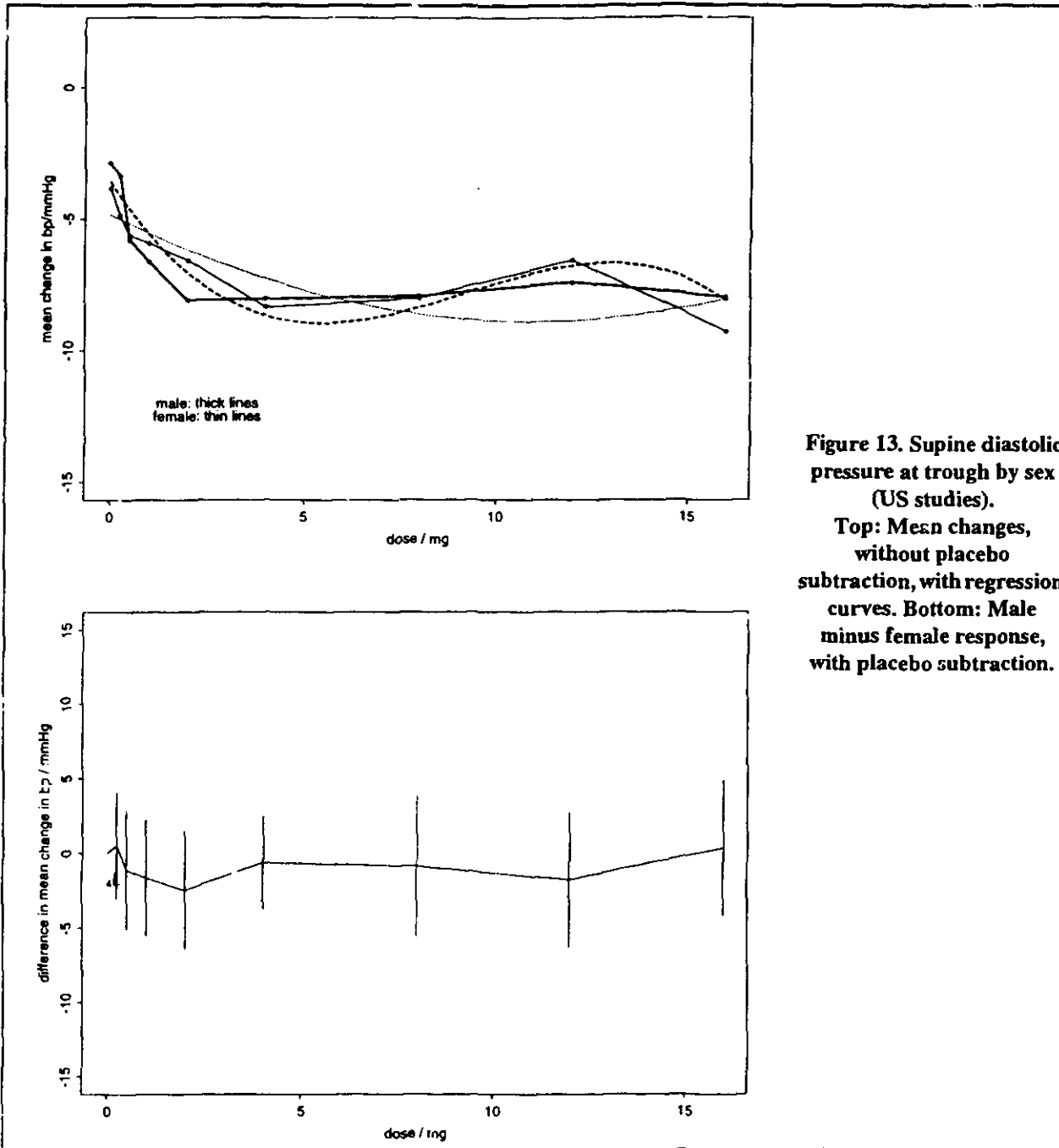


**Figure 13. Supine diastolic pressure at trough by sex (US studies).
Top: Mean changes, without placebo subtraction, with regression curves. Bottom: Male minus female response, with placebo subtraction.**

diastolic pressure at trough and end point were compared between age groups but within the same racial group. Significant treatment differences were found, by testing contrasts¹⁹ within the ANOVA model, for Caucasians at all dose levels except 16 mg. No such differences were observed for Blacks at any dose level. The change from baseline in supine diastolic pressure is shown for each age and racial group, along with the young-minus-elderly difference, in Figure 14 below. There was a larger reduction in placebo-adjusted mean change in supine diastolic pressure in young Caucasians compared with elderly Caucasians. No attempt was made to analyze age effects for a Caucasian plus Black group, because of the inhomogeneity in treatment effect between racial and age groups at several dose levels. (2) Treating age as a continuous variable,

¹⁹ Comparison between placebo-subtracted means.

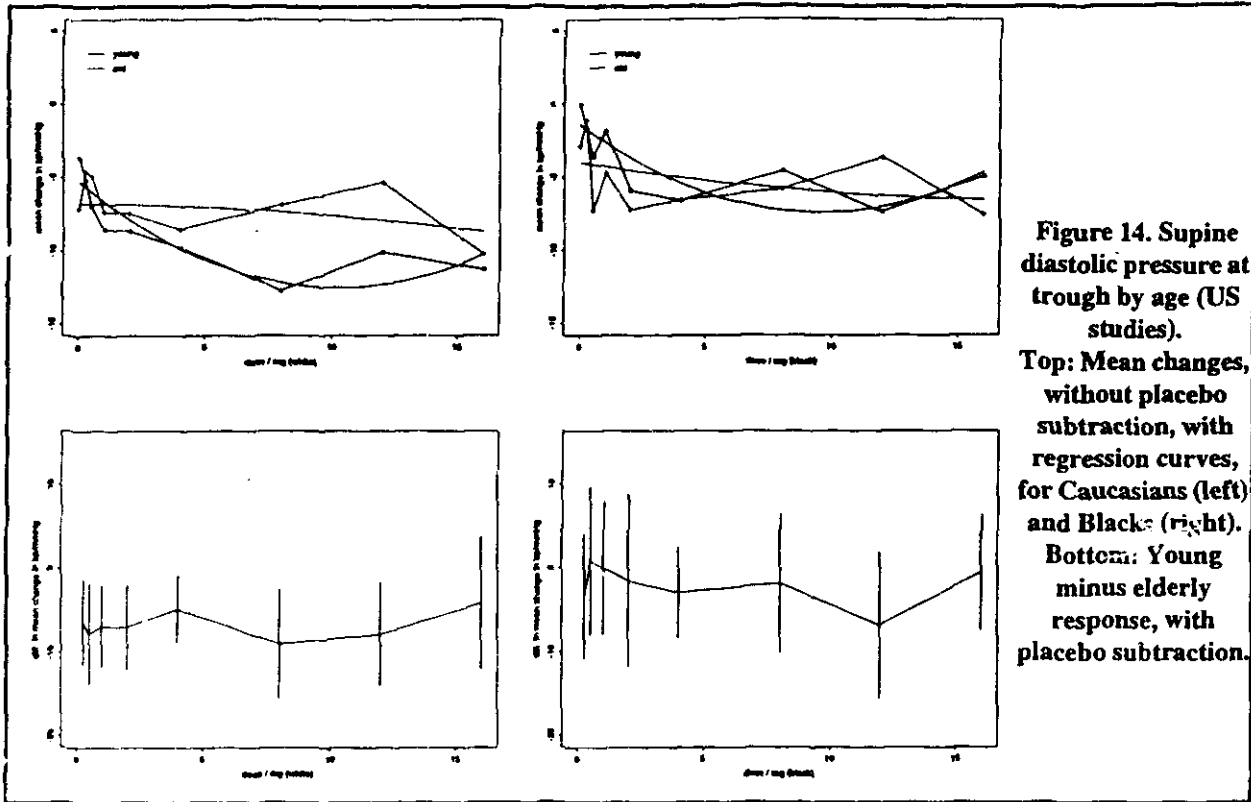


Figure 14. Supine diastolic pressure at trough by age (US studies).
 Top: Mean changes, without placebo subtraction, with regression curves, for Caucasians (left) and Blacks (right).
 Bottom: Young minus elderly response, with placebo subtraction.

regressions were done on change from baseline in supine diastolic pressure against age. The estimated slopes and p-values for testing unequal slopes between active treatment groups and placebo are shown in Table 8 below and Figure 15 below. The

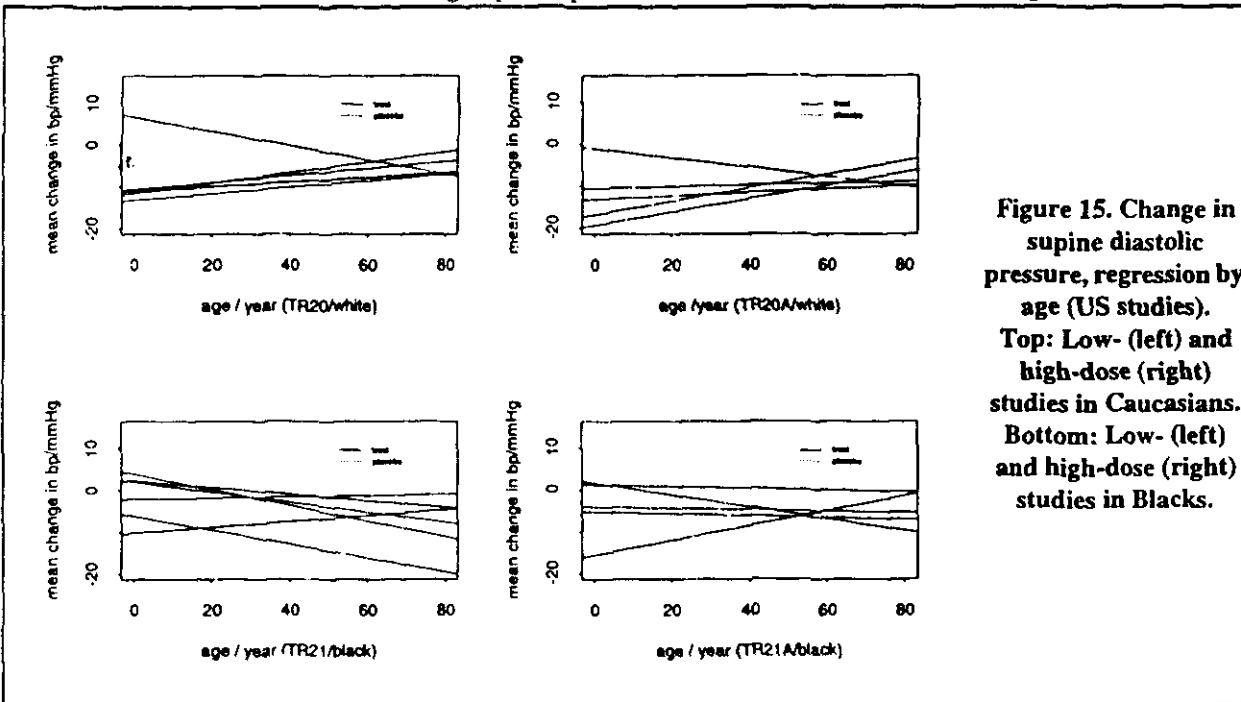


Figure 15. Change in supine diastolic pressure, regression by age (US studies).
 Top: Low- (left) and high-dose (right) studies in Caucasians.
 Bottom: Low- (left) and high-dose (right) studies in Blacks.

estimated slopes indicate a larger placebo effect (negative slope, $p=0.1$) and a smaller treatment effect (positive slopes) for elderly Caucasians. No effect of age was found for Blacks. (3) The overall effect of age was assessed using an analysis of covariance model with treatment, age, and age-by-treatment interaction as terms. For each of the four pivotal studies, the analysis was done by pooling the data from all the active

Table 8. Regression analysis of supine diastolic pressure by dose, race, and age (US studies).

	Trandolapril dose (mg/day)											
	Placebo	0.75	0.5	2.5	4	16	Placebo	4	12	16	12	16
Caucasian												
Intercept	6.46	-11.67	-10.79	-11.13	-11.20	-13.29	-1.15	-10.57	-19.35	-16.88	-13.23	
Slope	-0.17	0.12	0.08	0.06	0.05	0.08	-0.11	0.02	0.16	0.16	0.04	
p-value	—	0.018	0.064	0.059	0.062	0.070	—	0.500	0.053	0.019	0.733	
Black												
Intercept	2.17	-2.19	1.89	6.20	3.88	-10.29	1.17	-5.28	-4.08	-15.65	1.61	
Slope	-0.08	0.01	-0.12	-0.17	-0.19	0.07	-0.02	-0.02	-0.02	0.18	-0.14	
p-value	—	0.566	0.368	0.543	0.435	0.432	—	1.000	1.000	0.238	0.818	

treatment arms. The p-values for the interaction in the 4 studies were 0.029 (low-dose range, Caucasians), 0.065 (high-dose range, Caucasians), 0.906 (low-dose range, Blacks), and 0.801 (high-dose range, Blacks). The small p-values for the studies in Caucasians indicate a significant age-by-treatment interaction, which is consistent with the previously described findings based on grouping and regression methods.

6.2.2.4. Once-daily versus twice-daily dosing

One study²⁰ randomized subjects with mild to moderate hypertension to 6 weeks of double-blind treatment with placebo, 4 mg q.d., or 2 mg b.i.d. Somewhat larger reductions in supine diastolic pressure were seen with b.i.d. dosing, as shown in Figure 16 below (same as Figure 129 on page 263).

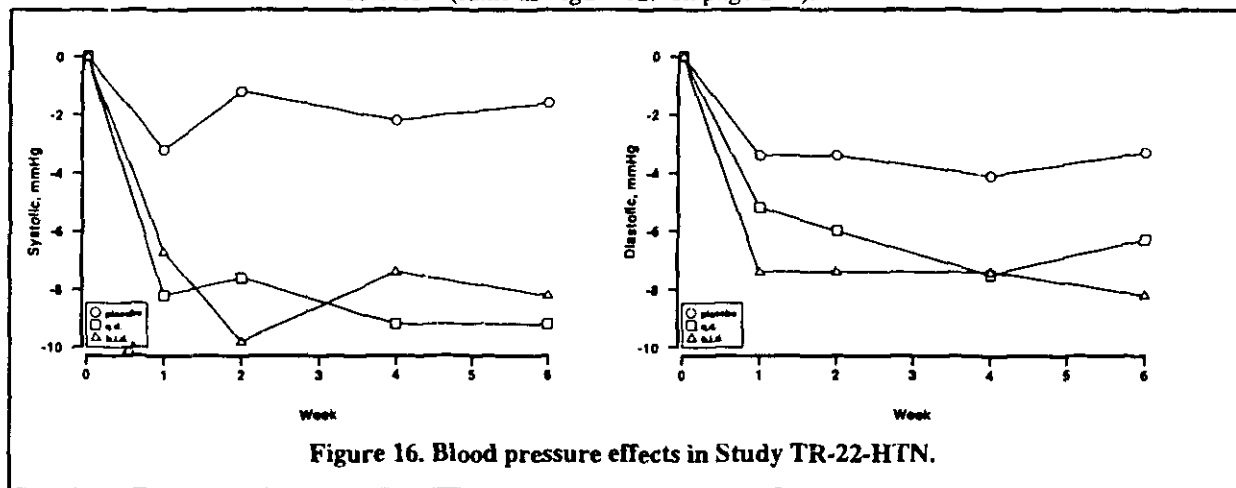


Figure 16. Blood pressure effects in Study TR-22-HTN.

6.2.2.5. Factorial trial with HCTZ

There was one single-dose factorial trial with hydrochlorothiazide²¹. Subjects with mild to moderate hypertension were randomized to 16 weeks on trandolapril 2 mg q.d., HCTZ 25 mg q.d., or the combination in a double-blind manner. The results are shown in Figure 17 below (same as Figure 69 on page 147). The combination had antihypertensive effects greater than that of either agent alone, but less than the sum of the effects of the individual agents. The response-rate data were consistent with this result.

6.2.2.6. Use with verapamil and HCTZ

There was a multi-center, open-label study²² with a 19-week treatment period, during which subjects with a baseline supine diastolic pressure 115 to 135 mmHg were titrated as needed from 1 to 2 to 4 mg trandolapril, 4 mg trandolapril plus 180 mg Isoptin SR (sustained release verapamil), and finally trandolapril, verapamil, and 12.5 to 25 mg HCTZ. Of 90 subjects enrolled, 31 withdrew, 22 for lack of efficacy. End point evaluation of response rate was made for 87 subjects, 70 of whom were receiving all 3 agents at end point (with a response rate of 79%).

²⁰ Study TR-22-HTN, reviewed on page 260.

²¹ Study FF/87/570/04, reviewed on page 145.

²² Study TV-40-HTN, reviewed on page 288.

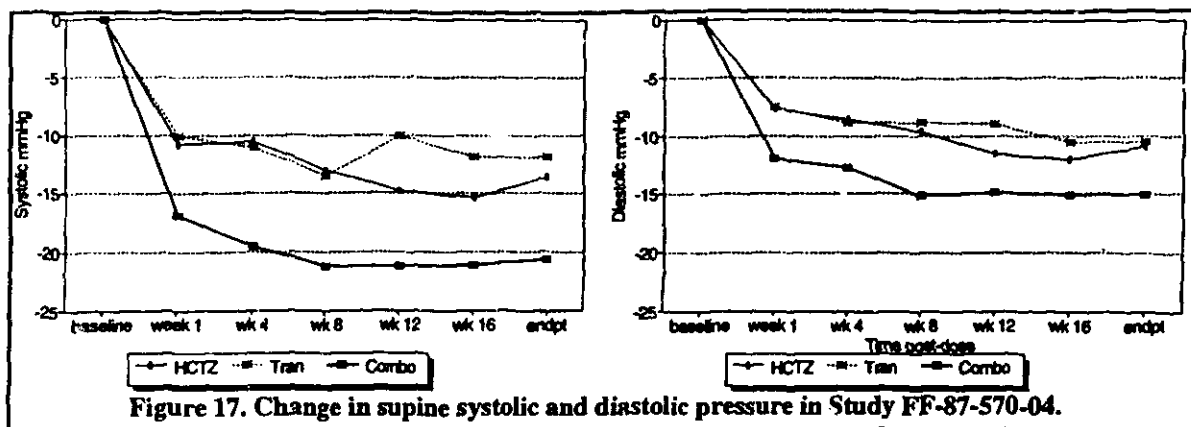


Figure 17. Change in supine systolic and diastolic pressure in Study FF-87-570-04.

Over the same period, there was a single-center, open-label study²³ of subjects with mild to moderate hypertension. There was a 10-week titration period and then an optional 12-month open-label follow-on. The titration sequence was trandolapril 2 to 4 mg, then add Isoptin SR 120 to 180 mg. Ten of 11 subjects completed study, 6 on trandolapril monotherapy, and 4 on trandolapril plus the lower dose of verapamil. Subjects who required both drugs had a higher baseline supine diastolic pressure than the subjects who completed on trandolapril alone. Nine subjects began the long-term study and completed at most 9 months; at the time of withdrawal most subjects were on the highest allowed doses of both agents.

Subjects in US long-term studies (see section 6.2.4.1 on page 24) were also allowed treatment with verapamil.

6.2.2.7. Active control

Factorial trials with verapamil and HCTZ have been described above. No other trials were reported with active controls.

6.2.3. Controlled, short-term foreign trials

Table 9 below provides a brief description of foreign short-term, double-blind, placebo-controlled, parallel-group studies included in the NDA. These studies contribute little; they were technically flawed, too small, or of too short a duration. In addition, these studies represent a sponsor-selected sample of the studies conducted outside the U.S.

6.2.4. Long-term open-label use

6.2.4.1. US long-term trials

Four-hundred forty-four subjects participated in a 12-month, open-label study²⁴; for 280 subjects, this was an extension to short-term US trials. The allowed doses of trandolapril were 1, 2, and 4 mg. Subjects whose blood pressure was not adequately controlled on trandolapril alone could have sustained-release verapamil added. The drop-out rate peaked at 3 to 6 months, with 32% of the withdrawals being for lack of efficacy. About 44% of subjects completed 12 months. At the last visit, 70% of subjects were on trandolapril only.

Subjects on trandolapril only with supine diastolic pressure <90 mmHg at 12 months were eligible for a double-blind, placebo-withdrawal study²⁵. Eighty-five subjects participated in the withdrawal study, in which subjects were randomized to either their normal dose of trandolapril alone or to placebo. Subjects were followed at 2 and 4 weeks after randomization with responder rate being the primary end point. Analyses performed by the sponsor and as part of the NDA review demonstrated no statistically significant difference in response rate between treatment groups at 4 weeks. Analysis

²³ Study TV-41-CP, reviewed on page 292.

²⁴ Study TR-30-HTN, reviewed on page 268.

²⁵ Study TR-30-HTN-Amendment III, reviewed on page 277.

Table 9. Foreign short-term, double-blind, placebo-controlled, parallel-group studies.

Study	Review	n	Doses (mg/day)	Duration	Summary
Study FF/86/570/05 (Study report: vols 1.128, case report tabulations: vols 1.128-1.131; case report forms for deaths and discontinuations for adverse events: none) "Randomized double-blind placebo controlled study of the effect of three single oral doses (1, 4, or 16 mg) oftrandolapril on blood pressure in patients with mild to moderate essential hypertension".	page 130	44	1, 4, 16	1 day	A single oral dose oftrandolapril (1 to 16 mg) was administered to 44 hypertensive subjects in this placebo-controlled study. The mean inhibition of ACE was approximately 25-80% at trough in the actively treated groups. During active treatment, renin levels were increased, aldosterone levels were decreased, and ANP levels were not consistently changed. The sponsor's statistical analysis of cuff data found a significant reduction in mean trough diastolic pressure in thetrandolapril 16 mg group (with a similar trend for systolic pressure). Peak mean effects were seen at 4 hours, and the antihypertensive effect generally disappeared by 27 hours after dosing (with the exception of an apparent delayed systolic pressure lowering at 48 post-dose). Neither 1 mg or 4 mgtrandolapril had statistically distinguishable antihypertensive effect 24 hours after a single administration. ABPM data for 16 mgtrandolapril appeared to show a greater than placebo change from baseline during 36 hours post-dose. A number of laboratory abnormalities were noted, but the sponsor's presentation does not allow differentiation of treatment-emergent from pre-existing abnormalities.
Study FF/86/570/06 (Study report: vols 1.212-1.220; case report tabulations: vols 1.213-1.216; case report forms for deaths and discontinuations for adverse events: vols 1.383) "Evaluation of the effect of repeated doses oftrandolapril (8 days) on blood pressure in patients with mild to moderate essential hypertension: double-blind, placebo-controlled, multicentre study evaluating doses of 2 mg, 4 mg, and 8 mg".	page 136	216	2, 4, 8	8 days	This was the first placebo-controlled, multiple-dose study in man. Hypertensive subjects underwent an 8-day double-blind phase, with fixed doses, and a 7-day washout. Efficacy data are difficult to interpret because of defects in the conduct of the protocol affecting one-third of the subjects. Doses of 2 to 8 mg q.d. appeared to have antihypertensive effects distinguishable from placebo but not from one another. Safety data revealed no events with an apparent relationship to treatment.
Study FF/86/570/14 (Study report: vols 1.221-1.230; case report tabulations: vols 1.223-1.227; case report forms for deaths and discontinuations for adverse events: vols 1.384) "Evaluation of the effect oftrandolapril () in repeated doses (28 days) on blood pressure in patients with mild to moderate essential hypertension: double-blind, placebo-controlled, multicentre study to evaluate 2 mg, 4 mg, and 8 mg doses".	page 140	188	2, 4, 8	28 days	Technical problems marred the conduct of this 4-week double-blind, placebo-controlled trial. For neither the primary end point of responder rate nor for supine diastolic pressure was there a significant difference between treated and placebo groups. This may have been a result of a large placebo effect. No truly serious adverse events were reported. There was no apparent relationship between adverse events or abnormalities in laboratory safety data and dosing group.
Study FF/87/570/21 (Study report: vols 1.231-1.240; case report tabulations: vols 1.233-1.237; case report forms for deaths and discontinuations for adverse events: vols 1.385) "Evaluation of the effect oftrandolapril () in repeated doses (28 days) on blood pressure in patients with mild to moderate essential hypertension: double-blind, placebo-controlled, multicentre study to evaluate 0.5 mg, 1 mg, and 2 mg doses".	page 149	170	0.5, 1, 2	28 days	Technical problems marred the conduct of this 4-week double-blind, placebo-controlled trial. For the primary end point of responder rate and for supine diastolic pressure there was a significant treatment effect which was not accompanied by differences among the actively treated groups. No truly serious adverse events were reported. There was no apparent relationship between adverse events or abnormalities in laboratory safety data and dosing group.

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performed as part of the review did demonstrate statistically significant treatment effects on change from baseline in supine diastolic pressure, for the pooled analysis and for the 1 mg dose.

6.2.4.2. Foreign long-term trials

No foreign long-term open-label studies were submitted for review.

6.3. Summary

6.3.1. Mechanism of action

The clinical data are consistent with the principal mechanism of antihypertensive action being inhibition of angiotensin converting enzyme. There was large hysteresis in the relationship between plasma ACE inhibition and plasmatrandolapril levels, but little hysteresis in the relationship between ACE inhibition andtrandolaprilat levels. Complete plasma ACE inhibition was never obtained in clinical trials, consistent with results expected on the basis of binding studies performed in vitro. No clinical study demonstrated the expected lack of pressor effects of angiotensin I or expected pressor effects of angiotensin II, but such studies were performed in animals. Endogenous angiotensin I, angiotensin II, and renin levels changed in a predictable fashion. Neither trials in man nor in other animals ruled out other possible contributing mechanisms of action, but none is suggested by the clinical data. The long time course of effect oftrandolapril on ACE inhibition and the natural short-term variability in blood pressure make it difficult to interpret the relationship between these two parameters, but it appears that antihypertensive effects are manifest with ACE inhibition exceeding 70%, which is achieved by all studied doses (≥ 0.25 mg) oftrandolapril.

- 6.3.2. Magnitude of antihypertensive effect** Treatment-related changes in supine diastolic pressure at the proposed dosing interval (24 hours) at steady state averaged approximately 5 mmHg for daily doses of 4 to 16 mg. Peak effects on supine diastolic pressure were inadequately ascertained in many trials. Consequently, trough-peak ratios had wide confidence limits (with point estimates often >1) and were not particularly useful. More useful is the observation of clinically significant blood pressure reductions at trough and a low incidence of orthostatic hypotension. Mean peak effects in some special populations (renal and hepatic impairment) were 10 to 15 mmHg.
- 6.3.3. Time course of onset** At least under fasting conditions, doses of 4 mg or greater have demonstrable effects on blood pressure within 1 to 2 hours and have a peak effect at about 4 hours, coincident with the peak plasma levels of trandolaprilat.
- 6.3.4. Duration of effect after a single dose** Cuff and ambulatory blood pressure monitoring demonstrate a gradual loss of effect over 36 to 48 hours after dosing.
- 6.3.5. Once-daily vs. twice-daily dosing** Given the time to peak and the time course of the decline from peak after a dose, a modest effect of twice daily dosing was predictable. Modest effects at trough were seen in the one comparison study.
- Had such a study examined area under the curve for reduction in diastolic pressure (or, equivalently, effect on the daily mean diastolic pressure), more substantial differences would likely have emerged. If blood pressure reduction throughout the day is important, rather than just at trough, then twice-daily dosing would seem to be a reasonable option for administration of trandolapril.
- 6.3.6. Development of effects during multiple dosing** From the clinical experience with other ACE inhibitors, a gradual improvement in efficacy would be expected over a period of weeks with continued dosing. In fact, antihypertensive effects of trandolapril reach a plateau within a week of continued once-daily dosing.
- 6.3.7. Duration of effect during and following multiple dosing** In the pivotal U.S. trials, subjects were dosed once daily for 6 weeks and then underwent single-blind placebo-withdrawal with assessments of blood pressure at 1 and 3 weeks. These studies showed loss of about half the effect at 1 week and incomplete recovery at 3 weeks, with nothing suggesting dose-dependence to the time course for recovery. There was one double-blind placebo-withdrawal study with blood pressure assessments at 2 and 4 weeks for some of the subjects completing 1 year of open-label use. Although the statistical analyses demonstrated little evidence of a treatment effect, there being a small number of subjects per group, the point estimates are consistent with a recovery by about 2 weeks.
- These best estimates of the duration of effect following multiple dosing and of the sustained efficacy with long-term exposure were not considered sufficiently robust to merit inclusion in labeling.
- 6.3.8. Dose-response** The pivotal U.S. trials were all of similar design and showed consistent effects. Together, doses of 0.25 to 16 mg were explored. Doses of 4 to 16 mg had maximal effects on supine diastolic pressure at trough. The lowest dose which is statistically distinguishable from placebo is 0.5 mg, although a smooth curve through these data would suggest that the estimated effect at 0.5 mg was probably less than the observed mean.
- 6.3.9. Efficacy by subgroups** This development program was unusual in having separate pivotal efficacy trials for Blacks and Caucasians. While it has been consistently observed that Blacks respond less well to monotherapy with other ACE inhibitors, no such effect was discernible in the trandolapril data. While it would be of interest to have a similar trial with the positive control of another ACE inhibitor, there would appear to be little incentive (at least for the sponsor of trandolapril).
- Pooled analyses of the pivotal U.S. studies showed no significant differences in supine diastolic pressure at trough between males and females or between subjects less than

and greater than age 60. There was a tendency for a greater effect on blood pressure in young Caucasians than in other groups, but there was no dose-dependence to this observation.

6.3.10. Use with other antihypertensive agents

The sponsor submitted the results of a trial comparing antihypertensive effects of single dosage levels of trandolapril, HCTZ, and the combination. The effect of the combination was greater than that of the components. This study supported the use of the drugs in combination.

There were open-label studies of subjects with mild-to-moderate and severe hypertension, in which sustained-release verapamil was added if there was inadequate efficacy on trandolapril alone. By design and results, these studies did not provide compelling support for the use of trandolapril with verapamil.

7. Safety

7.1. Exposure

7.1.1. US studies

The sponsor has provided summary exposure data only for hypertensive subjects. No normal volunteer or special subject (e.g., renal or hepatic impairment) summary exposure data was provided by the sponsor. Table 10 below features the demographics

Table 10. Number of subjects by extent of exposure in U.S. studies.

	Exposure (weeks)							Overall
	<2	>2 to 5	>5 to 12	>12 to 24	>25 to 40	>40 to 52	>52	
All subjects	35	354	139	121	42	108	204	1003
With verapamil ...and HCTZ	50 10	56 12	19 6	44 45	10 0	18 0	0 0	227 73
Age <65	25	274	110	102	37	89	164	801
Age ≥65	10	80	29	19	5	19	40	202
Males	23	200	84	69	26	72	129	603
Females	12	154	55	52	16	36	75	400
Caucasians	18	193	87	77	32	94	169	670
Blacks	17	161	52	44	10	14	35	333

and numbers of subjects exposed to trandolapril sorted by weeks of exposure. Table 11 below presents the mean number of days of exposure to a dose of trandolapril for the various demographic groups. The N for all subjects in the two tables differs because the sponsor did not provide complete summary exposure data in the 120 day safety update for study TR-22-HTN.

Table 11. Number of subjects and mean exposure time in U.S. monotherapy studies.

	Trandolapril dose (mg)									
	Placebo	0.25	0.5	1	2	4	8	12	16	Any
All subjects										
N	237	76	75	471	494	640	111	77	74	1134
Days	40.1	41.0	39.7	77.0	81.2	77.2	31.7	39.3	41.1	135.9
Age <65										
N	133	62	62	383	405	420	91	56	59	801
Days	40.8	40.9	39.1	70.1	80.7	90.4	31.2	38.9	41.0	137.2
Age ≥65										
N	36	14	13	88	88	88	20	21	15	202
Days	39.9	41.8	42.5	107.4	84.6	71.2	33.8	40.3	41.7	130.9
Males										
N	99	47	45	291	313	312	65	47	43	603
Days	40.7	40.4	39.2	81.5	79.6	83.4	30.6	37.9	40.7	139.0
Females										
N	70	29	30	180	180	196	46	30	31	400
Days	40.6	42.1	40.5	69.8	84.6	92.8	33.2	41.5	41.8	131.2
Caucasians										
N	106	53	53	377	398	354	44	39	38	670
Days	41.2	41.0	40.4	84.1	85.7	95.3	37.9	38.4	41.6	162.1
Blacks										
N	63	23	22	94	95	154	67	38	36	333
Days	39.7	41.0	38.0	48.8	63.6	68.0	27.6	40.3	40.7	83.2

A slightly higher proportion of males versus females was exposed to trandolapril for >39 weeks. Also, numerically, if not statistically, a much higher proportion of Caucasians was exposed to trandolapril >39 weeks as opposed to Blacks. In the sponsor's preferred dosage range of 1 to 4 mg, a greater proportion of Caucasian versus Black subjects was exposed to trandolapril for a greater mean number of days.

7.1.2. European studies

Although there were at least 19,610 subjects enrolled in European hypertension trials for trandolapril, the sponsor compiled exposure data for only for subjects enrolled prior to 31 October 1989 (N=1959). Table 12 below features the numbers of subjects

Table 12. Number of subjects by duration of exposure in selected European studies.

	Exposure (months)						Overall
	≤1	>1 to ≤3	>3 to ≤6	>6 to ≤9	>9 to ≤12	>12	
All subjects	460	270	443	135	193	458	1959

exposed to trandolapril sorted by weeks of exposure whereas Table 13 below presents the mean number of days of exposure to a dose of trandolapril.

Table 13. Number of subjects and mean exposure time in selected European studies.

	Trandolapril dose (mg)									
	Placebo	0.25	0.5	1	2	4	8	12	16	Any
All subjects	—	0	41	209	1490	766	100	0	0	1959
N	—	0	41	209	1490	766	100	0	0	1959
Days	—	—	27.6	47.7	160.4	171.8	16.3	—	—	195.7

Although 1/3 of these subjects were exposed to trandolapril for at least 9 months, very little of this experience is with doses above 4 mg/day.

7.2. Deaths

From the U.S. database, 2 subjects on placebo and 3 subjects on trandolapril died during short- and long-term trials with trandolapril. All these cases are summarized below:

Subject 2013-8 in Study TR-20-HTN, a 59 year old Caucasian female, complained of neck, arm, and chest pain at day 17 of the double-blind phase while receiving trandolapril 4 mg/day. Her symptoms were thought to be related to a known back and neck problem; ECG was normal and unchanged from baseline and CPK enzymes were normal. Subject was found dead at home 2 days later. The subject's private physician stated that the cause of death was cardiorespiratory failure probably the result of myocardial infarction.

Subject 2126-6 in Study TR-21-HTN, a 66 year old Black male had been randomized to 12 mg/day of trandolapril. Twenty-nine days after completion of the study, and 49 days after the last dose of trandolapril, the subject presented to the emergency room with acute shortness of breath. An echocardiogram showed a large clot in the right ventricle. The subject died the next day from a pulmonary embolus. In the investigator's opinion, the pulmonary embolus and death were not related to the HCTZ. The investigator did not comment regarding the relationship of these events to trandolapril.

Subject 3007-2013 in Study TR-30-HTN, a 52 year old Caucasian male, experienced jaw pain, chest pain and diaphoresis after receiving trandolapril 1 mg/day for 26 days, 2 mg/day for 30 days

and then 4 mg/day for 206 days, during the long-term open-label phase. He was admitted to an emergency room, where an EKG revealed an acute anterolateral myocardial infarction. He could not be resuscitated. An autopsy revealed occlusion of the left main coronary artery. This adverse reaction was considered by the investigator to be "definitely not" study drug related.

Subject 2204-19 in Study TR-22-HTN, a 68 year old Caucasian male, experienced an acute cerebral vascular accident with right hemiparesis during the double-blind phase after receiving placebo for 20 days. The subject was hospitalized and discontinued from the study because of the cerebrovascular accident. This adverse reaction was considered by the investigator to be "severe" and "definitely not" related to study drug. The subject died from gastrointestinal bleeding 6 weeks later. The investigator considered the cause of death to be related to the anti-coagulant therapy which was being administered to the subject.

Subject 2101- a 64 year old Black female, died during the placebo run-in phase of study TR-21-HTN. A few days into the run-in period, the subject experienced persistent chest discomfort, and was admitted with a diagnosis of acute myocardial infarction. The cause of death was listed as cerebral hypoperfusion and ventricular pump failure resulting from myocardial infarction. Although the study screening ECG suggested a possible old anteroseptal scar, there were no symptoms of ischemic heart disease.

The 45 deaths in the European database are summarized in Appendix B2.7. Deaths in European hypertension studies on page 337.

7.3. Withdrawals

7.3.1. U.S. hypertension studies

Table 14 below gives the primary reason for discontinuation in U.S. hypertension trials.

Table 14. Reasons for discontinuation from U.S. hypertension studies.

	Placebo n=236	Trandolapril only ^a n=831	Verap/HCTZ Combin ^b n=228	All ^c n=1134
Unsatisfactory response	16	24	65	125
Adverse effects	10	14	14	56
Failure to follow appointment schedule	8	20	8	41
Refusal of treatment	1	3	0	13
Intercurrent medical problems	0	3	1	12
Unacceptable concomitant medications	0	1	0	5
Failure to meet entry criteria	1	2	0	2
Administrative problems	0	1	0	2
Death	2	2	0	3
Other	3	5	11	24
Total	41	75	99	283

a. Randomized monotherapy trials.

b. Trials in combination with verapamil or HCTZ.

c. Includes randomized or open-label studies, alone or in combination.

The placebo and the randomized monotherapy data originate from studies TR-20-HTN¹ (n=263), TR-20A-HTN² (n=155), TR-21-HTN³ (n=113), TR-21A-HTN⁴ (n=149), TR-22-HTN⁵ (n=131), and TR-42-CP⁶ (n=20, for a total n=831). Combination data represent those subjects in studies TR-30-HTN⁷ (n=139, 4 of whom began combination but completed on monotherapy), TV-40-HTN⁸ (n=79), and TV-41-CP⁹ (n=10, for a total n=228) who had verapamil and/or HCTZ in combination with trandolapril. The 'All' data are for subjects in either set of studies (n=831+228=1059), plus those on monotherapy in the combination trials (n=131), minus those subjects who were recruited into open-label trial TR-30-HTN from short-term controlled trials of trandolapril (n=56; so the total is 1059+131-56=1134).

Overall, withdrawals were 17% of placebo subjects (5% for death or adverse events), 9% of trandolapril monotherapy subjects (2% for death or adverse events), 43% of trandolapril combination subjects (6% for death or adverse events), and 25% of all trandolapril subjects (5% for death or adverse events). However, several caveats should be mentioned. First of all, it is not clear how the investigators were to distinguish between an "adverse effect" versus an "intercurrent medical problem" versus an "unsatisfactory response." Secondly, given that all the controlled trials were designed as fixed-dose trials, the true incidence of "unsatisfactory response" may be overestimated. Lastly, the bulk of withdrawals in the "other" category could easily have been classified as "administrative" withdrawals, since most of the "other" withdrawals were the result of subjects moving away, on travel, or withdrawing consent. Of the remaining "other" withdrawals, only two that occurred during the controlled trials are noteworthy: one subject, who had 3x ULN SGPT readings during pre-treatment and baseline, was discontinued from trandolapril when another SGPT reading came back 3x ULN (investigator did not believe the first two readings), and one subject was terminated by Knoll for an unspecified reason.

Appendix B1. *Withdrawals from U.S. hypertension trials* on page 305 contains narrative summaries of subjects who withdrew from U.S. hypertension trials, separated by treatment group. Table 15 below summarizes the adverse events that contributed to withdrawals for these subjects. Some subjects had multiple reasons for withdrawal. Frequent or potentially serious adverse events are discussed below.

Table 15. Adverse events contributing to withdrawal from US hypertension studies.

	Placebo	Monotherapy		Combination	
		Rand	Open-label	+Verap	+Verap +HCTZ
Abdominal swelling	0	0	1	0	0
Anxiety	0	0	1	0	0
Arrhythmia (atrial)	0	1	0	0	0
Angioneurotic edema	0	2	1	0	0
Assault	0	0	1	0	0
AV block (first degree)	0	0	0	1	0

¹ Study TR-20-HTN, reviewed on page 218.

² Study TR-20A-HTN, reviewed on page 229.

³ Study TR-21-HTN, reviewed on page 240.

⁴ Study TR-21A-HTN, reviewed on page 250.

⁵ Study TR-22-HTN, reviewed on page 260.

⁶ Study TR-42-CP, reviewed on page 282.

⁷ Study TR-30-HTN, reviewed on page 268. Four subjects in this study started on combination but ended on monotherapy.

⁸ Study TV-40-HTN, reviewed on page 288.

⁹ Study TV-41-CP, reviewed on page 292.

Table 15. Adverse events contributing to withdrawal from US hypertension studies. (Continued)

	Placebo	Monotherapy		Combination	
		Random	Open label	Verapamil	Verapamil/PGI2
Blurred vision	0	1	0	0	0
Broken heel	1	0	0	0	0
Bundle branch block	0	0	1	0	0
Cancer	0	0	1	0	0
Cerebral vascular accident	1	0	2	0	0
Cholelithiasis	0	0	2	1	0
Constipation	0	0	0	1	0
Cough	1	1	11	3	1
Death	2	2	1	0	0
Diplopia	0	0	1	0	0
Dizziness/lightheadedness	1	3	1	1	0
Dyspnea	0	0	1	0	0
Edema, pedal	1	1	1	0	0
Epigastric pain	0	1	0	0	0
Epistaxis	1	0	0	1	0
Gastrointestinal bleeding	1	0	0	0	0
Gallop (S ₃)	0	1	0	0	0
Headache	4	2	3	1	0
Heartburn	0	0	1	0	0
Hypotension	0	1	0	0	0
Impotence	0	0	1	0	1
Insomnia	0	0	0	1	0
Ischemic Heart disease	1	1	3	1	1
Itching	0	1	0	0	0
Lethargy/weakness	1	0	2	1	0
Liver enzyme elevation	1	1	1	1	0
Lumbar degenerative disease	0	0	1	0	0
Lymphocytosis	0	1	0	0	0
Myocardial infarction	2	1	2	0	0
Mood swings	0	0	0	1	0
Nausea	0	0	2	3	0
Neutropenia	0	0	0	1	0
Palpitations	1	0	0	0	0
Paresthesia	1	0	0	1	0
Periorbital edema	1	0	0	0	0
Pre-syncope	0	0	0	1	0
Perspiration, excessive	0	0	1	0	0
Pulmonary embolus	0	1	0	0	0
Retinopathy, grade 1	0	1	0	0	0
Sex drive, loss of	0	1	0	0	0
Skin rash	0	0	1	0	0

Table 15. Adverse events contributing to withdrawal from US hypertension studies. (Continued)

	Placebo	Monotherapy		Combination	
		Rand	Open	Verin	Verin + (CIZ)
Tachycardia	0	0	1	0	0
Thrombocytopenia	0	1	0	0	0
Transient ischemic attack	0	1	0	0	0
Vertigo	0	0	0	1	0
Vomiting	0	0	2	0	0

7.3.2. European withdrawals and deaths

Analysis of these data is hampered by numerous subject accounting and other problems. First, the sponsor has divided the presentation of the data into three distinct windows: subjects in studies dated before 31 October 1989 (n=2003), subjects in studies between 31 October 1989 and 31 December 1993 (n=17,607), and subjects in studies (where the denominator not stated) conducted between 1 January 1994 and 30 June 1994, with some studies overlapping two of the three time windows. Second, the sponsor has not submitted detailed study reports for the majority of studies referenced in the safety data. Third, there are a number of subject accounting questions in the databases. For example, in a summary of the pre-31 October 1989 data (n=1959 in this case), the sponsor states there were 272 withdrawals: 3 for "abnormal lab value", 65 for "intolerance", 46 for "unsatisfactory response", 61 for "intercurrent medical problems", and 102 for "other" reasons. However, in the sponsor's table of withdrawals for adverse events, 123 subjects are listed. Thus it is not clear what individual components of the 277 withdrawals went into the 123 withdrawals figure. Fourth, the sponsor often has tables of subjects who died and narrative summaries of adverse events where it is unclear what studies were included or what the denominator might be. Fifth, in the two later databases, withdrawals are not addressed: the sponsor presents only "subjects with serious or potentially serious adverse events". This review has counted all of these subjects as withdrawals, realizing that this is probably a slight overestimation.

The pre-31 October 1989 and 31 October 1989 to 31 December 1993 databases for deaths and withdrawals are combined (n=2003+17,607=19,610) in Table 16 below. The sponsor counted each event only once per data time window. Subjects may have had more than one adverse event affecting the same or different body systems. Narrative summaries of withdrawals are presented in Appendix B2. *Withdrawals from foreign hypertension trials* on page 318 and summaries of deaths are provided in Appendix B2.7. *Deaths in European hypertension studies* on page 337.

Table 16. Adverse events contributing to withdrawal in European hypertension studies.

	n		n		n
Cardiovascular	80	Resp system (cont'd)		Hepatic function	6
Aneurysm	2	Pulmonary embolus	4	Hepatitis	1
Aortic stenosis	2	Pneumopathy	2	Hepatic steatosis	1
Atrial fibrillation	5	Sinusitis	1	Liver function test abn	4
AV Block-2nd degree	1	Bronchitis	4	Hematological	5
Cardiomyopathy	1	Laryngeal carcinoma	1	Neutropenia	1
ECG abnormality	1	Lung/bronchial carcinoma	6	Leukemia	1
Edema	2	Nasal polyp removal	1	Leukopenia	1
Heart failure	6	Skin/appendages	21	Decreased hemoglobin	1
Hypertensive crisis	2	Angioedema	2	WBC abnormal	1

Table 16. Adverse events contributing to withdrawal in European hypertension studies. (Continued)

	n		n		n
Hypertension	3	Eczema	1	Metabolic/nutritional	19
Hypotension	5	Facial edema	1	Diabetes (new)	2
Lower limb ischemia	3	Herpes zoster	1	Diabetes (worsened)	5
Myocardial infarct	15	Rash	7	Dehydration	1
Angina pectoris	9	Dry skin	1	Enzymes abnormal	1
Myocarditis	1	Breast carcinoma	4	Hypochloremia	1
Chest pain	5	Skin carcinoma	2	Hypokalemia	1
Palpitations	3	Skin ulceration	2	Hyponatremia	4
Pulmonary hypertension	1	Special senses	10	Hypothyroidism	1
Syncope	5	Cataract	2	Hyperglycemia	2
Supravent Extrasystole	1	Conjunctival edema	1	LDH increased	1
Tachycardia	4	Deafness	1	Renal	15
Arteritis	3	Glaucoma	1	Polycystic renal disease	1
Carcinoma	20	Otitis media	1	Kidney failure	3
Death	45	Retinal thrombosis	2	Nephrotic syndrome	2
Nervous system	71	Tinnitus	1	Pyelonephritis	1
Abnormal feeling	1	Abnormal vision	1	Creatinine increased	6
Asthenia	6	Urogenital system	22	Renal insufficiency	1
Cereb vasc accident	12	Impotence	1	Nephrolithiasis	1
Cerebral abscess	1	Hypermenorrhea	4	Musculoskeletal	21
Dizziness	6	Hysterectomy	4	Arthralgia	3
Depression	2	Ovarian cyst	1	Arthrosis	4
Headache	13	Prostatectomy	1	Disk disorders	2
Hemiplegia	1	Urinary tract infection	1	Bone fracture	10
Hallucinations	1	Uterine fibroids increased	1	Joint edema/swelling	2
Hot flushes	2	Ureterolithiasis	1	Miscellaneous	30
Libido decreased	1	Ureteronephrectomy	1	Unknown surgery	2
Malaise	4	Utero/pelvic prolapse	2	Accident	5
Neuralgia	1	Urogenital neoplasm	3	Viral infection	1
Neuropathy	1	Vasectomy	2	Infection/sepsis	9
Paresthesia	2	Digestive system	33	Unkn lab test abnormality	1
Psychosis	1	Cholecystectomy	5	Thorax pain	1
Sleep disorder	1	Diarrhea	1	Inguinal swelling	1
Suicide/attempt	2	Dry mouth	1	Hernia	2
Sweating increase	1	Dyspepsia	1	Phlebitis	1
Transient ischemic attack	2	Esophagitis	1	Purpura	1
Tremor	1	Intestinal obstruction	3	Thrombophlebitis	3
Vertigo	9	Nausea	7	Peripheral vascular dis	2
Respiratory system	47	Gastric carcinoma	1	Hyperdigoxinemia	1

Table 16. Adverse events contributing to withdrawal in European hypertension studies. (Continued)

	n		n
Cough	17	Gastrointestinal pain	3
Dyspnea	4	Gastrointestinal bleeding	3
Epistaxis	1	Pancreatitis	2
Upper resp infection	1	Rectal disease/bleeding	2
Pneumonia	4	Peptic ulcer	1
Pulmonary Edema	1	Vomiting	2

The incidence of serious events leading to withdrawal appears similar between the US and usable European data and are compared in Table 17 below.

Table 17. Incidence of selected adverse events contributing to withdrawals in US and European studies.

	US studies		Europe n=19,610
	Placebo n=236	trandolapril n=1134	
Myocardial infarction	0.8%	0.3%	0.1%
Cerebrovascular accident	0.4%	0.2%	0.1%
Death	0.8%	0.3%	0.2%
Carcinoma	0.0%	0.1%	0.1%
Cholelithiasis	0.0%	0.3%	0.0%
Hepatic dysfunction	0.4%	0.3%	0.0%
Neutropenia	0.0%	0.1%	0.0%
Angioedema	0.0%	0.3%	0.0%
Cough	0.4%	1.4%	0.1%

Since the withdrawal data in the European four-month update contain no denominator¹⁰, the withdrawal data¹¹ are presented separately in Table 18 below.

Table 18. Adverse events contributing to withdrawal in European hypertension studies (from Safety Update)^a.

	n		n		n
Cardiovascular	3	Hematological	1	Urogenital	3
Cardiomyopathy, dilated	1	Increased CD4:CD8 ratio	1	Prostatic adenoma	1
Coronary angiography	1	Musculoskeletal	1	Renal adenoma	1
Hypertensive crisis	1	Shoulder/hip arthralgia	1	Nephrotic colic	1
Central nervous system	1	Respiratory	2	Miscellaneous	2
Cerebrovascular accident	1	Cough	1	Hernia repair	1
Carcinoma	2	Pharyngitis	1	Hospitalization for check-up	1
Digestive system	1				
Intestinal obstruction	1				

a. Total n is unknown.

¹⁰ The sponsor states only that "the estimated number of subjects enrolled in these studies is similar to that reported in the Mavik NDA with only a small increase in the number of subjects in these studies".

¹¹ Actually the data on "subjects with serious or potentially serious adverse events".

7.3.3. Deaths and withdrawals in European studies other than hypertension

These studies include 7 studies in congestive heart failure subjects¹², one study of coronary hemodynamics¹³, and single-dose studies in subjects with cirrhosis¹⁴ and renal impairment¹⁵. Table 19 below lists the deaths/withdrawals from these studies. Again, that these were all withdrawals is probably an overestimation since the sponsor's data represents "subjects with serious or potentially serious adverse events." And again, no denominator is available.

Table 19. Adverse events contributing to withdrawal in European non-hypertension studies.

	n		n
Cardiovascular	1	Respiratory (continued)	
Adams-Stokes syndrome	2	Cough	1
Angina pectoris	10	Dyspnea	4
Coronary angioplasty	1	Epistaxis	2
Aorto-femoral atherosclerosis	1	Hemoptysis	1
Bradycardia	1	Laryngeal tumor	1
Chest pain	12	Pulmonary edema	10
Atrial fibrillation	8	Pneumonia	4
Atrial flutter	2	Pneumothorax	1
Elective coronary bypass planned	2	Respiratory insufficiency	2
Leg edema	5	Musculoskeletal	3
Heart failure (new/worsening)	37	Bone fracture	3
Hypertension	1	Leg cramps	1
Hypotension	11	Rheumatoid arthritis	1
Myocardial infarction	14	Skin	1
Syncope	2	Rash/pruritus	1
Ventricular tachycardia	8	Special senses	2
Ventricular fibrillation	2	Cataract surgery	2
Ventricular septal rupture	1	Urogenital	3
Central nervous system	2	Kidney failure	5
Cerebrovascular accident	7	Abnormal renal function	1
Depression	2	Creatinine increased	1
Dizziness	2	Dysmenorrhea	1
Transient ischemic attack	4	Renal tumor	1
Epileptic seizure	2	Uterine prolapse	1
Insomnia	1	Prostatic resection	1
Malaise	1	Prostatitis	1
Mood disturbance (aggravation of)	1	Miscellaneous	7
Paresthesia	2	Accident	3
Asthenia	4	Elective cholecystectomy	1
Neurinoma	1	Physical therapy hospitalization	1

¹² Studies UK/89/570/070,

¹³ No reports were provided for these studies.

¹⁴ Study NZ/89/570/84. No report was provided.

¹⁵ Study FF/88/570/38, reviewed on page 167.

¹⁶ Study FF/88/570/39, reviewed on page 173.

Table 19. Adverse events contributing to withdrawal in European non-hypertension studies. (Continued)

	n		n
Carcinoma	3	Planned cardioversion for atrial fib	1
Death	36	Physiotherapy for rheumatoid arthritis	1
Digestive system	1	Cellulitis	1
Abdominal pain	1	Chest infection	3
Cholecystitis	1	Shock	2
Gastroenteritis	1	Vasculitis	2
Pancreatic carcinoma	1	Femoral-popliteal graft occlusion	1
Metabolic/endocrine	1	Anemia	3
Bilirubin increased	1	Leukopenia	1
Diabetes mellitus (worsening)	2	Toe Infection	1
Hyperglycemia	3	Hyperdigoxinemia	1
Hyperkalemia	1	Sepsis	2
Hypokalemia	1	Hospitalization for insulin adjustment	1
Hyponatremia	1	Raynaud's syndrome	1
Dehydration	3	Weight gain	1
Transaminase increase	1	Prothrombin ratio decreased	1
Respiratory	3	Coagulation disorder	1
Asthma	3	Fever	1
Bronchitis	3		

7.3.4. Withdrawals by cause

7.3.4.1. Myocardial ischemia

From the U.S Hypertension database, 2 subjects on placebo (n=236) and 3 subjects on "Any" trandolapril (n=1134) suffered confirmed myocardial infarctions, of which one in each group lead to death. One subject on placebo, and 6 subjects on "Any" trandolapril were diagnosed with ischemic heart disease during the course of their studies. These were generally associated with anginal pain or pressure, but, in one case, a routine ECG noted ischemic changes.

There appears to be little difference between the placebo and trandolapril groups with respect to the development of ischemic heart disease or myocardial infarct. The development of these conditions is more than likely a result of pre-existing coronary artery disease induced by hypertension.

From the European data (n=19,610), 15 subjects had myocardial infarctions, of which 4 were fatal.

7.3.4.2. Angioneurotic edema

Cases of angioneurotic edema were uncommon. A synopsis of each case is presented below:

Subject 2101-22 in Study TR-21-HTN, a 54 year old Black female with a 5-year history of hypertension, experienced angioneurotic edema, urticaria, and pruritus after receiving 0.5 mg/day trandolapril during the double-blind phase for 21 days. This subject was discontinued by the investigator 3 days after onset and the event resolved in 3 days.

Subject 2010-11 in Study TR-20-HTN was a 55 year old Caucasian male with a 15-year history of hypertension who experienced hives and facial and lip edema after receiving 0.25 mg/day trandolapril for 38 days. This subject was discontinued from the study and was placed on diphenhydramine, prednisone and enalapril. At a 2-week follow-up, the subject stated that "he has no further problems".

Subject 3004-2004 in Study TR-30-HTN, a 68 year old Caucasian female with a 5-year history of hypertension, developed angioedema after taking trandolapril 1 mg/day for 5 days during the long-term open-label phase. This subject had swelling and drooping of the right lower face and lips. Upon examination, no breathing or neurological problems were noted and CAT scan and ECG were normal. The subject was terminated from the study by the investigator on the day of onset of the adverse reaction. The event completely resolved within 2 days.

Given that there were only three cases leading to withdrawal, one can merely speculate regarding the relationships of angioedema in these subjects to trandolapril dose, race¹⁶, or sex¹⁷.

There were only two reported cases of angioedema from the European (N=19,610) database, both in males:

Subject 278/109 in Study FF/87/570/07 was a 64 year old male smoker reported to be suffering from asthma before entry into the trial and taking salbutamol (inhaler) and beclomethasone (inhaler). After 5 weeks of trandolapril 2 mg/day, the subject developed an angioneurotic reaction, with swollen lips, tongue and face (intensity: severe). He was examined by his physician who prescribed chlorpheniramine maleate. Treatment with trandolapril was stopped as soon as the symptoms appeared and the symptoms resolved within 2 days.

Subject 99 in Study FF/87/570/21, a 34 year old Caucasian male with a 1-year history of hypertension, discontinued on day 7 of treatment with trandolapril 2 mg/day with facial edema of several days' duration.

7.3.4.3. Cancer

From the U.S. hypertension database, only one subject was diagnosed with cancer during long-term treatment with trandolapril.

Subject 3011-3005 in Study TR-30-HTN was a 66 year old Caucasian male with a history of diabetes mellitus and a 12-year history of hypertension. He experienced a decrease in appetite and unilateral leg pain after receiving trandolapril 1 mg/day for 25 days and then 2 mg/day for 7 days during the long-term open-label phase. Two weeks after withdrawal, following subacute mental status changes, the subject underwent subtotal resection of a glioblastoma multiforme and had a less than 1-year expected life span.

Although these data cannot rule out a relationship to trandolapril, such a relationship appears remote.

From the European database, (n=19,610) 20 cases of carcinoma were reported, descriptions of eight were available.

Subject 72/416 in study FF/87/570/07 was a 56 year old male subject with angina pectoris, osteoarthritis, a lipid disorder and a history of heavy smoking. His concomitant treatment was: pentaerythryl tetranitrate, fenofibrate, paracetamol and vitamin C. One month before inclusion, the subject presented with persistent cough and hemoptysis; X-ray revealed an opacity of the upper left lobe. As a result of this radiological finding, bronchoscopy was

¹⁶ There are 2.2 Caucasian subjects per Black subject in the database.

¹⁷ There are 1.5 males per female in the database.

undertaken 2 months later (the subject had been on trandolapril 2 mg/day for about 3 weeks) and showed a tumor with bronchial obstruction. Spinocellular carcinoma was confirmed by biopsy. The subject died unexpectedly at home a few days later.

Subject 269/469 in study FF/87/570/05 was a 54 year old male who smoked. A chest X-ray performed at inclusion was normal, but his WBC count was high (13.8). One week before starting active treatment, while still taking placebo, the subject had a cold with occasional headaches (intensity: mild), for which paracetamol was prescribed. Recovery occurred within 10 days. At the end of the first month of active treatment, an increased leucocytosis was reported (WBC: 28.3). One week later, the subject experienced nausea and anorexia (intensity: moderate), and a weight loss (-6.5 kg) was noted. He was admitted to hospital for investigation and carcinoma of the bronchus was diagnosed. The investigator decided to discontinue the test drug. He died within 5 months and there was no postmortem examination.

Subject 15080 in Study FFR/91/570/166 was a 72 year old man with a history of hypertension (unspecified beginning date), and non-insulin-dependent diabetes mellitus treated with gliclazide. After one month on trandolapril 2 mg/day, he had to be hospitalized because of aggravation of his diabetes, bronchial infection, and mental status alteration. Bronchial carcinoma was diagnosed and led to death within 3 weeks. In the investigator's opinion, these events were unrelated to study drug.

Subject 2361 in Study FFR/91/570/166 was a 73 year old non-smoking female with no previous relevant history. The subject, experiencing asthenia, was started on trandolapril 2 mg/day. Fifteen days later, blood pressure was 160/80 mmHg, but asthenia persisted and was associated with anorexia, weight loss (5 to 6 kg within 15 days) and cough. The subject was admitted to hospital and carcinoma of lung was diagnosed. One month after beginning treatment, the subject died from her carcinoma. In the investigator's opinion, the carcinoma is unrelated to study drug.

Subject 434 in Study UK/90/570/01 (Local) and Study FF/89/570/44 (International) was an 80 year old female with a medical history of hypertension. The subject started at 1 mg/day. One month later, the dose was increased to 2 mg. After 5 months of treatment, the subject experienced moderate vomiting, which resolved spontaneously over 4 days. Three weeks later, the subject began to complain of severe abdominal pain and trandolapril was withdrawn, after approximately 6 months of treatment. The subject was admitted to hospital and had a laparotomy at which time the subject was diagnosed as having inoperable carcinoma of the pancreas. The subject was discharged from hospital with dexamethasone 1 mg, cyclizine 50 mg and morphine sulphate 10 mg. The subject died from carcinoma of the pancreas, 7 months after starting trandolapril. In the investigator's opinion pancreatic carcinoma is unrelated to trandolapril.

Subject 105/357 in Study FF/87/570/22 was a 70 year old female who after one month of trandolapril 2 mg/day, adenocarcinoma of the caecum was diagnosed. Trandolapril was discontinued, and the subject was admitted for surgery and chemotherapy.

Subject 280/651 in Study FF/87/570/05 was a 55 year old female with no history of smoking. At the beginning of the placebo period, a chest X-ray was taken and showed a lung opacity of the left lower

zone. Tomography confirmed the presence of a tumor, and biopsy performed two-and-a-half months after initiating trandolapril revealed a squamous cells carcinoma in the lower left lobe of the lung. At the subsequent visit scheduled in the protocol, the investigator decided to withdraw trandolapril.

Subject 271/607 in Study FF/87/570/08 was a 65 year old male with no special medical history. One-and-one-half months after taking trandolapril 2 mg/day and bendroflumethiazide 2.5 mg/day in the long-term study, the subject complained of nausea and tiredness with poor concentration (intensity: severe for all these symptoms). Serum calcium was 2.89 mM. One month later, still symptomatic, he stopped trandolapril. He was referred to a hospital where his serum calcium was 5.38 mM. He was treated with prednisolone, suffered cardiac arrest but was successfully resuscitated. Three days later, he underwent surgery where a parathyroid adenoma was found and removed. Following the operation however, he developed renal failure and required dialysis. He died 2 days after surgery. Post mortem examination revealed a severe underlying coronary artery disease, without evidence of acute thrombosis.

7.3.4.4. Cough

Cough was the most frequent cause of adverse event related withdrawal from U.S. long-term studies with trandolapril. However, only one subject from the trandolapril arm of the short-term controlled trials withdrew as a result of cough. To date, of the 16 withdrawals for cough, only 2 reports include descriptions of the nature of the cough (in both cases, the description is the typical ACE-inhibitor cough: dry, non-productive), and only 3 descriptions include information regarding the time course of resolution of the cough. The sponsor has been asked to provide information regarding the time course of resolution of cough in these other 13 subjects. Seventeen subjects withdrew because of cough from the European database (n=19, 610).

7.3.4.5. Headache

From the U.S hypertension database, 4 subjects on placebo, 2 subjects on short-term trandolapril, and 4 subjects on long-term trandolapril discontinued as a result of headache. Thirteen subjects from the European database (n=19, 610) withdrew for headache.

7.3.4.6. Cholelithiasis

Three subjects in the long-term U.S. studies, all Caucasian females, experienced either a worsening of pre-existing cholelithiasis or the onset of new symptoms. Two of the three subjects were to undergo cholecystectomy and the third was to have a gall bladder "surgery". A synopsis of each case is presented below:

Subject 3016-2015 in Study TR-30-HTN, a 69 year old Caucasian female, experienced atypical chest pain and symptoms of reflux esophagitis diagnosed as cholelithiasis, after receiving trandolapril 1 mg/day for 55 days and 2 mg/day for 207 days. The subject continued on 2 mg/day of trandolapril and was then discontinued from the study 26 days after the onset of the adverse event. The adverse event resolved on the same day as the onset. The subject was hospitalized for laparoscopic cholecystectomy and ovarian cystectomy.

Subject 3016-2020 in Study TR-30-HTN, a 43 year old Caucasian female, experienced intermittent epigastric pain after receiving trandolapril 1 mg/day for 27 days and 2 mg/day for 16 days. The subject continued on 2 mg/day of trandolapril and was discontinued from the study 219 days after the onset of the adverse drug reaction. The subject was treated with the following drugs that were ongoing at termination: Alka-Seltzer, Bufferin, Motrin, Regular and Extra Strength Tylenol, Tempo, Tums, and Zantac. This adverse event was ongoing at the time of termination and was considered by the investigator to be 'mild' in severity and 'definitely not' related to study drug. Follow-up notes that the subject is to undergo gall bladder surgery. Subject also complained of back pain secondary to

snow shoveling.

Subject 3016-2013 in Study TR-30-HTN, a 61 year old Caucasian female, experienced worsening cholelithiasis (pre-dated entry into trial) after receiving trandolapril 1 mg/day for 31 days, 2 mg/day for 55 days, 4 mg/day for 27 days, and then 4 mg/day of trandolapril with 180 mg/day of verapamil for 130 days. The subject was then discontinued from the study (17 days after the onset of the adverse event) and hospitalized for a laparoscopic cholecystectomy. This adverse event was considered by the investigator to be "moderate" in severity and "probably not" related to study drug. Subject had multiple other complaints such as cough, back pain, hiatal hernia, vomiting, flu symptoms, right upper quadrant pain which were not considered contributory to withdrawal.

Five cases of cholecystectomy are reported in the European database (n=19,610). No descriptions of these cases are available.

A Medline Express search linking trandolapril with cholelithiasis yielded no articles. Similarly, an article by Wiseman and McTavish¹⁸ did not discuss cholelithiasis. The sponsor's introductory sections of many of the protocols in the NDA note that trandolapril's diacid metabolite (trandolaprilat) is "eliminated partly in the urine and partly in the bile." These cases are not adequate to either confirm or deny an association of trandolapril with cholelithiasis.

7.3.4.7. Hepatic abnormalities

From the U.S. database, one subject on placebo and 3 subjects on any dose of trandolapril experienced liver function test elevations leading to withdrawal. The three trandolapril cases are summarized below:

Subject 2123-27 in Study TR-21A-HTN, a 41 year old Black male with a previous history of hepatic dysfunction (enzymes at baseline: SGOT 98 [normal 0-55] and SGPT 99 [normal 0-55]), experienced increased SGOT and SGPT (3 times upper normal limit) and mild lymphocytosis after receiving 16 mg/day trandolapril during the double-blind phase for 7 days. The subject also admitted to drinking heavily. Enzymes were back to near baseline at 10-day follow-up.

Subject 3019-3001 in Study TR-30-HTN, a 35 year old Caucasian male experienced headaches after receiving trandolapril 1 mg/day for 36 days, 2 mg/day for 35 days, and 4 mg/day for 2 days during the long-term open-label phase. The subject was discontinued from the study by the investigator 12 days after the onset of the adverse reaction and was also dropped because of mildly elevated SGPT and alkaline phosphatase (although both of these elevation were present at baseline and were considered to be related to alcohol abuse).

Subject 3011-3011 in Study TR-30-HTN, a 48 year old Caucasian female, developed elevated SGOT 102 U/L, (baseline was 25 U/L; normal range is 7 to 39 U/L), SGPT of 299 U/L, (baseline 31 U/L; normal range 2 to 54 U/L) and LDH 275 U/L, (baseline 180 U/L; normal range 88 to 230 U/L) after receiving trandolapril 1 mg/day for 26 days, 2 mg/day for 31 days, 4 mg/day for 107 days and then 2 mg/day trandolapril plus 180 mg/day verapamil for 28 days during the long-term open label phase. The subject was discontinued from the study by the investigator 7 days after the onset of the adverse reactions. The SGOT, SGPT and LDH elevations returned to normal ranges at 2-week posttreatment follow-up.

¹⁸ Trandolapril: A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in essential hypertension. *Drugs* 48(1): 71-90, 1994.

The European database (n=19,610) contained a report of one discontinuation because of hepatitis (description below), one for hepatic steatosis (no description available), and 4 discontinuations for liver enzyme elevation:

Subject 42/286 in Study FF/87/570/22 was a 51 year old male. After 3 months of treatment with trandolapril 2 mg/day, persistent high diastolic blood pressure (94 mmHg supine) led to the addition of nifedipine to the subject's treatment. Two weeks earlier, he had begun to experience a daytime cough (intensity: severe). Symptomatic treatment with triprolidine, pseudoephedrine, and paracetamol was ineffective, and the subject was withdrawn after one month. He recovered within 2 weeks. Laboratory tests on withdrawal showed raised LDH (643 IU/L; normal is <480) and GGT (280 IU/L; normal is <43); these values normalized one month after he stopped treatment.

Subject 27 in Study F/86/570/10, a 25-year-old healthy male volunteer, was enrolled to in the 16 mg trandolapril group. Eight days before trandolapril was started, laboratory data were normal. Serologic tests for hepatitis B were negative. The volunteer complained of abdominal pain on day 2 and loose stools on day 3 of trandolapril and reported muscular pain on days 1, 4, and 5. On day 6, mild isolated increase of SGPT (10 2x normal) was noted and the subject was withdrawn from the study. Three days later, SGPT reverted to normal.

Subject 58 in Study FF/86/570/06, a 56 year old male not previously treated for hypertension, experienced elevation of liver enzymes. Enzymes were normal at baseline, but at the end of run-in, SGOT was 73, SGPT was 80, and AlkPhos was 108. At the end of double-blind treatment (day 8), SGOT was 479, SGPT was 828, and AlkPhos was 140. Enzymes climbed further during washout, then declined, but were still not normal range at 3 weeks. Subject tested positive for HBS antibody at 3 weeks.

Subject 267/719 in Study FF/87/570/05 was a 61 year old male who complained of tiredness and lethargy (intensity: moderate) after one day of trandolapril. Laboratory results were within normal ranges. Tiredness persisted to a lesser extent throughout the study. After 12 weeks of active treatment, liver enzymes were raised ASAT 230 (normal is <41), ALAT 152 (normal is <41) and AlkPhos 254 (normal is <115). Despite the absence of symptoms of liver disease, the investigator decided to stop trandolapril and one week after drug withdrawal, enzymes were within the normal range.

7.3.4.8. Neutropenia

Only one case of neutropenia led to withdrawal in the U.S. database:

Subject 3005-3010 in Study TR-30-HTN, a 60 year old Black male, had a severely low neutrophil count of 22% with a WBC count of 4.4 K/ml. At baseline, the subject's neutrophil count was 37% with a WBC count of 4.3 K/ml. The subject had received trandolapril 1 mg/day for 35 days, 2 mg/day for 35 days, and 4 mg/day for 35 days. At the time of the decreased neutrophil count, the subject's trandolapril dose was changed to 2 mg/day with 180 mg/day of verapamil. After 40 days of combination trandolapril/verapamil treatment, the neutrophil count was 27% with a WBC count was 4.1 K/ml. The subject was discontinued from the study at that time. Three days after the last dose of study medication, the neutrophil count was 53% with a WBC count of 4.7 K/ml, and the absolute neutrophil count was 2491.

One subject in each the European database taking trandolapril (n=19,610) and the European placebo database (denominator unknown) reported neutropenia. Additionally, one subject taking trandolapril withdrew because of leukopenia:

Subject 280/456 in Study FF/87/570/05 was 45 year old male who, while taking placebo, presented with influenza from day -15 to day 7, for which Bactrim was prescribed for one week. Since his neutrophil count (1.7 K/ml) on entry was lower than permitted in the protocol, the subject was withdrawn at the sponsor's request, after one week of active treatment. One week later, his neutrophil count was 2.5 K/ml.

Subject 144 in Study UK/89/570/01 was a 63 year old female smoker who had a history of hypertension for an unspecified period. One month prior to starting the study drug, laboratory data showed a slight increase in WBC count (11.3 K/ml) with 75% neutrophils. A blood sample taken at that time was lost so that no baseline values are available. One week later, the investigator reported neutropenia (1.95 K/ml; normal is >2.2 K/ml). After 44 days in the study, the neutrophil count remained low (1.6 K/ml). Trandolapril treatment was discontinued at that time. A follow-up blood sample was taken 4 months later and showed an increased neutrophil count (3.55 K/ml).

Subject 207/6 in Study FF/88/570/29 was a 24 year old male who was entered into FF/87/570/21 (trandolapril 1 mg group). On entry to the long-term study on trandolapril 2 mg/day, his WBC count was 3.1 K/ml. After one-and-a-half months of trandolapril treatment, the subject was reported to have a low WBC count (2.3 K/ml). Persistently low values over one year led the investigator to withdraw the subject from the trial, when his WBC count was 3.6 K/ml.

7.3.4.9. Cerebrovascular disease

In the U.S. database, one subject on placebo experienced a fatal cerebrovascular accident, 2 subjects on long-term trandolapril experienced cerebrovascular accidents, and one subject on short-term trandolapril experienced a transient ischemic attack. It is difficult to associate trandolapril with cerebrovascular disease which more than likely was present in these subjects at the time of study initiation.

The trandolapril cases are summarized below:

Subject 3015-2113 in Study TR-30-HTN, a 65 year old obese Black female, experienced a cerebrovascular accident with decreased peripheral vision in the left eye after receiving trandolapril 1 mg/day for 157 days during the long-term open-label phase. The subject denied weakness, numbness, and paresthesia, or bulbar symptoms. She did not experience pain, other than a headache, on the night of admission. The loss of vision remained constant since onset. A head CT scan with contrast confirmed the presence of an infarction in both the right occipital lobe and right cerebellar hemisphere. The subject was terminated from the study by the investigator 15 days after the onset of the event. The subject was discharged from the hospital 5 days after admission. The adverse reactions were severe and were considered by the investigator to be "probably not" study drug related.

Subject 4013-4 in Study TV-40-HTN, a 70 year old Caucasian male, was receiving trandolapril 2 mg/day for 1 day and then 4 mg/day for 5 days during the open-label phase when he was hospitalized because of severe lumbar degenerative joint disease and bilateral cerebral infarctions on the day of onset. This subject was terminated from the study by the investigator on the day of onset of the adverse

reaction. This adverse reaction was considered by the investigator to be "severe" and "definitely not" study drug related.

Subject 2105-16 in Study TR-21-HTN, a 60 year old Black male with a 9-year history of hypertension and myocardial infarction, experienced a transient ischemic attack during the double-blind phase after receiving trandolapril 0.25 mg/day for 16 days. The subject was admitted to the hospital and started on intravenous heparin.

The European database (n=19,610) contained reports of two instances of transient ischemic attacks and 12 cerebrovascular accidents. Reports for only a few of these were available:

Subject 4937 in Study FFR/91/570/166 was a 63 year old man. Six weeks after starting on trandolapril 2 mg/day, the subject has a cardiac arrest. Reactive coma was diagnosed, with intrabronchial aspiration of gastric juice and status epilepticus. The subject was intubated and mechanically ventilated. The ECG showed no abnormality. The subject was admitted to Intensive Care. A brain scan was performed and showed no abnormality. Convulsions persisted despite intravenous antiepileptic therapy. Final diagnosis was deep cerebrovascular accident probably at truncus cerebri level and the subject died 2 months later.

Subject 11296 in Study FFR/91/570/166 was a 78 year old man with a previous history of hypertension who was began trandolapril 2 mg daily and died, apparently of a cerebrovascular accident, sometime in the next 30 days. No autopsy was performed.

Subject 7409 in Study FFR/91/570/166 was a 72 year old man with a history of recently-diagnosed hypertension and a past history of cerebral hemorrhage. He began on trandolapril 2 mg/day, and 30 days later, since hypertension was not well controlled (185/110 mmHg), furosemide 40 mg/day was added. Two weeks later, the subject was hospitalized because of cerebral ischemia secondary to embolism from cardiac origin (atrial fibrillation. This ischemia was in the same cerebral area as the previous one. Trandolapril was discontinued. After transient improvement, the subject died one month later. In the investigator opinion, cerebral hemorrhage was unrelated to study drug.

Subject 185/152 in Study FF/87/570/03 was a 56 year old man with a history of hypertension. On inclusion, blood pressure was 160/110 and EEG was normal. Trandolapril 4 mg/day was started. Sixty-seven days after trandolapril start, the subject had an acute onset of right hemiparesis and speaking problem. On clinical examination, the subject had aphasia, facial paralysis and right hemiparesis. Blood pressure was 230/130 mmHg. The subject was admitted to the hospital and trandolapril was stopped. Four days after trandolapril was stopped, the neurological symptoms had disappeared. Ultrasound imaging of carotid bifurcation was performed and revealed a 1 mm reduction in left carotid lumen diameter with no atheromatous lesions.

Subject 154/28 in Study FF/88/570/29 was a 56 year old female with no medical history of note. She had been enrolled in FF/87/570/21 (trandolapril 2 mg group) with a blood pressure on entry of 170/100 mmHg (supine). After having been enrolled in the long-term study on trandolapril 4 mg/day, she experienced a transient ischemic attack. EEG showed signs of vascular insufficiency. One month after trandolapril cessation, she still

complained of slight paresis of right arm.

Subject 72/225 in Study FF/87/570/22 was a 64 year old male with a history of coronary artery disease and a gastric ulcer. From the third day of treatment with trandolapril 2 mg/day, he complained of fatigue (intensity: moderate) lasting 4 days. After 2 months, he presented with a rash on his back (intensity: mild) which lasted for 2 weeks; the attending dermatologist concluded that this cutaneous event was unrelated to the test drug. Ten days after recovery, the subject developed shingles on his neck (intensity: severe), and was treated with acyclovir. Two months thereafter, he experienced a sudden severe rise in blood pressure which resulted in a left sided stroke (intensity: mild). He was admitted to hospital and withdrawn from the trandolapril trial. Complete recovery occurred within a few hours.

7.3.4.10. **Dizziness, light-headedness, and hypotension** In the U.S. database, 6 subjects were discontinued from short- and long-term treatment with trandolapril because of these symptoms. It is unclear if any events involved postural hypotension. Six subjects discontinued from the European studies because of dizziness, and five because of hypotension.

7.4. **Adverse events** The summary of adverse events was based upon information in the sponsor's integrated summary of safety.

7.4.1. **US studies**

7.4.1.1. **Controlled and uncontrolled studies** Data from all U.S. clinical trials except TR-22-HTN (n=1003) were analyzed. The 120-day Safety Update, which included the study report for TR-22-HTN, did not update the overall safety analysis to include that trial.

A total of 532 (53%) of subjects in U.S. clinical trials reported adverse events. Events regardless of attribution with an incidence >1% are shown in Table 20 below.

Table 20. Adverse events reported in controlled and uncontrolled U.S. clinical trials.

	n (%)		n (%)		n (%)
Any	532 (53)	Nausea	532 (53)	Abdom cramp/pain	13 (1.3)
Headache	119 (11.9)	Diarrhea	119 (11.9)	Arthritis	13 (1.3)
Upper resp infec	99 (9.9)	Dyspepsia	99 (9.9)	Impotence	13 (1.3)
Cough	79 (7.9)	Edema	79 (7.9)	Influenza	13 (1.3)
Upper resp inflam	42 (4.2)	Rash	42 (4.2)	Urinary tract infec	12 (1.2)
Upper resp cong	41 (4.1)	Throat inflam	41 (4.1)	Vomiting	12 (1.2)
Fatigue	40 (4.0)	Anxiety	40 (4.0)	Muscle cramps	11 (1.1)
Dizziness	39 (3.9)	Chest pain	39 (3.9)	Pain in extremity	11 (1.1)
Joint pain	30 (3.0)	Constipation	30 (3.0)	Trauma	11 (1.1)
Back pain	28 (2.8)	Bronchitis	28 (2.8)		

Adverse events appeared unevenly distributed between Caucasians and Blacks. By body system, the rates were generally higher in Caucasians. Specific events with apparent disparity in rates (no statistical comparison made) are listed in Table 21 below.

The distribution of adverse events between sexes was more even. The following were least evenly distributed (% in males, % in females): edema (1.2, 4.0), rash (1.2, 2.5), abdominal pain or cramps (0.2, 3.0), nausea (1.3, 4.8), bronchitis (0.8, 2.3), and cough (5.6, 11.3).

Table 21. Adverse events with uneven distribution by race (U.S. trials).

	Cauc n=670	Black n=333		Cauc n=670	Black n=333
Any	387 (57.8)	145 (43.5)	Headache	92 (13.7)	27 (8.1)
Angina	9 (1.3)	0 (0.0)	Dizziness	32 (4.8)	7 (2.1)
Palpitations	10 (1.5)	0 (0.0)	Fatigue	31 (4.6)	9 (2.7)

Adverse events were also quite evenly distributed between subjects age <65 and those ≥65.

Specific events with >1% incidence and possible, probable, or definite relationship to treatment are shown in Table 22 below.

Table 22. Adverse events with possible, probable, or definite relationship to treatment (U.S. trials).

	n (%)		n (%)
Any	372 (37.1)	Fatigue	23 (2.3)
Cough	51 (5.1)	Nausea	13 (1.3)
Headache	50 (5.0)	Edema	11 (1.1)
Dizziness	25 (2.5)	Constipation	11 (1.1)

7.4.1.2. Controlled studies

Eight hundred sixty-eight subjects were in U.S. controlled trials, of whom 168 were randomized to placebo and 700 were randomized to active drug 0.25 to 16 mg daily. One placebo and one active treatment subject actually received both treatments and were each counted twice.

Adverse events, regardless of attribution, with incidence >1% (in a trandolapril treatment group) are shown in Table 23 below. Among these events, the rate was higher for trandolapril group subjects for upper respiratory infections, cough, upper respiratory inflammation, and nausea.

Table 23. Adverse events in controlled U.S. clinical trials.

	Plcbo N=169 n (%)	Trand N=701 n (%)		Plcbo N=169 n (%)	Trand N=701 n (%)
Any	73 (43.2)	246 (35.1)	Nausea	1 (0.6)	12 (1.7)
Headache	18 (10.7)	42 (6.0)	Fatigue	4 (2.4)	12 (1.7)
Upper resp infec	7 (4.1)	39 (5.6)	Back pain	3 (1.8)	12 (1.7)
Cough	3 (1.8)	25 (3.6)	Edema	5 (3.0)	9 (1.3)
Upper resp cong	4 (2.4)	15 (2.1)	Abdom pain/cramps	2 (1.2)	8 (1.1)
Upper resp inflam	1 (0.6)	14 (2.0)	Dyspepsia	2 (1.2)	8 (1.1)
Diarrhea	4 (2.4)	14 (2.0)	Rash	2 (1.2)	7 (1.0)
Dizziness	3 (1.8)	12 (1.7)			

There were no marked differences in event rates between Blacks and Caucasians, or between subjects age <65 or ≥65. Headache was more common in females and among males, whether on placebo or active treatment.

Adverse events possibly, probably, or definitely related to treatment in controlled U.S. trials are shown in Table 24 below. For events attributed to treatment, cough, dizziness, and diarrhea were more common in the active treatment groups.

Table 24. Adverse events with possible, probable, or definite relationship to treatment (controlled U.S. trials).

	Plcbo N=169 n (%)	Trand N=701 n (%)		Plcbo N=169 n (%)	Trand N=701 n (%)
Any	40 (23.7)	131 (18.7)	Dizziness	1 (0.6)	10 (1.4)
Headache	9 (5.3)	19 (2.7)	Diarrhea	1 (0.6)	8 (1.1)
Cough	0 (0.0)	14 (2.0)	Fatigue	4 (2.4)	8 (1.1)

7.4.1.3. Studies in combination with verapamil and HCTZ

Two hundred and twenty-seven subjects received trandolapril plus verapamil in U.S. clinical trials. Seventy-three subjects received trandolapril plus verapamil and HCTZ.

Adverse events for which the incidence was higher on combination with verapamil than on trandolapril alone were dizziness (4.4% vs. 2.5%), upper respiratory infection (4.4% vs. 1.1%), constipation (3.1% vs. <1%), nausea (3.1% vs. 2.0%), impotence (1.8% vs. <1%), and first degree AV block (1.3% vs. <1%). Of these events, all but upper respiratory infections and impotence are recognized effects of verapamil.

Events with $n > 2$ and more common with trandolapril plus verapamil and HCTZ than with trandolapril alone were dizziness (8.2% vs. 2.5%), cough (6.8% vs. 6.6%), edema (5.5% vs. 1.7%), fatigue (5.5% vs. 3.2%), joint pain (5.5% vs. <1%), upper respiratory congestion (5.5% vs. 3.4%), impotence (4.4% vs. <1%), and influenza (4.4% vs. <1%).

7.4.1.4. Studies in normal volunteers

Ninety-three normal subjects received trandolapril in U.S. studies¹⁹. Adverse events reported by more than 2 subjects are shown in Table 25 below.

Table 25. Adverse events among normal volunteers in U.S. clinical trials.

	n (%)		n (%)
Any	37 (40)	Tiredness/fatigue	4 (4)
Headache	15 (16)	Backache	3 (3)
Light-headed/dizziness	6 (6)		

7.4.2. European studies

The data analyzed by the sponsor were derived from 27 clinical trials involving 407 subjects in the European Phase I and Phase II development program. Of these, 296 subjects were normal volunteers. Of the remaining 111 subjects, an unspecified number were from studies of renal or hepatic impaired patients.

Forty-eight subjects (12%) reported any adverse events. The only events reported by more than 2 subjects were headache (7%) and asymptomatic orthostatic hypotension (1%).

7.5. Special risk groups

7.5.1. Myocardial ischemia

Out of 1067 subjects enrolled in U.S. placebo-controlled trials, 16 (1.5%) had a history of coronary artery disease or myocardial ischemia and 12 (1.1%) had a history of myocardial infarction. The presence of ischemic heart disease was not an explicitly

¹⁹ Study TR-1-CP (Study report: vols 1.75; case report tabulations: vol 1.75; case report forms for deaths and discontinuations for adverse events: none) "Single dose, crossover bioavailability pilot study to evaluate two lots of trandolapril capsules". on page 206.

Study TR-2-CP (Study report: vols 1.76-1.85; case report tabulations: vols 1.77-1.78; case report forms for deaths and discontinuations for adverse events: none) "Single- and multiple-dose pharmacokinetic evaluation of 1 mg, 4 mg, and 16 mg trandolapril in healthy male subjects". on page 209, and

Study TR-4-CP (Study report: vols 1.62-1.74; case report tabulations: none; case report forms for deaths and discontinuations for adverse events: none) "Determination of the bioequivalence of trandolapril tablets vs. capsules in healthy male subjects". on page 214.

stated exclusion for participation in these trials. Similarly, the protocols had no exclusionary criteria based on nitrate usage. ECG exclusion criteria did not mention ischemic changes. Thus these studies probably included some subjects with silent ischemic heart disease, further bolstering the belief that myocardial ischemic events noted were more related to pre-existing disease induced by hypertension versus some property of trandolapril. Newly occurring or worsening angina was an infrequently reported adverse event. The following cases of myocardial ischemia (one suspected) were reported:

Subject 2013-8 in Study TR-20-HTN, a 59 year old Caucasian female, complained of neck, arm, and chest pain at day 17 of the double-blind phase while receiving trandolapril 4 mg/day. Her symptoms were thought to be related to a known back and neck problem; ECG was normal and unchanged from baseline and CPK enzymes were normal. Subject was found dead at home 2 days later. The subject's private physician stated that the cause of death was cardiorespiratory failure probably the result of myocardial infarction.

Subject 3007-2013 in Study TF 30-HTN, a 52 year old Caucasian male, experienced jaw pain, chest pain and diaphoresis after receiving trandolapril 1 mg/day for 26 days, 2 mg/day for 30 days and then 4 mg/day for 206 days, during the long-term open-label phase. He was admitted to an emergency room, where an EKG revealed an acute anterolateral myocardial infarction. He could not be resuscitated. An autopsy revealed occlusion of the left main coronary artery. This adverse reaction was considered by the investigator to be "definitely not" study drug related.

Subject 2008-14 in Study TR-20-HTN, a 51 year old Caucasian male had a myocardial infarction during the washout phase (day 51) after receiving 1 mg/day trandolapril for 42 days. The subject was hospitalized and discontinued from the study. This adverse reaction was considered by the investigator to be "severe" and "definitely not" related to study drug.

Subject 2126-3 in Study TR-21A-HTN, a 60 year old Black male experienced moderate to severe atypical chest pain after receiving 12 mg/day trandolapril during the double-blind phase for 12 days. The subject was discontinued from the study on the date of onset of the adverse reaction. He subsequently underwent cardiac catheterization and angioplasty.

Subject 4005-3 in Study TV-40-HTN, a 45 year old Caucasian male experienced chest pressure (angina) during the open-label phase after receiving trandolapril 2 mg/day for 2 days and 4 mg/day for 6 days. The subject was admitted to the hospital for a suspected myocardial infarction. The adverse event resolved on the same day as the onset, and the subject was discharged the next day after treadmill testing. The investigator concluded the subject experienced "chest pain secondary to supply/demand mismatch secondary to high afterload". The subject was discontinued from the study 4 days after the onset of the adverse event. This adverse event was considered by the investigator to be severe and "probably not" related to study drug.

Subject 3014-2010 in Study TR-30-HTN, a 62 year old Caucasian male experienced angina in between the short-term (double-blind) and the long-term (open-label) study period (while not receiving drug). The subject had received trandolapril 1 mg/day for 41 days followed by a 20 day washout period on placebo. The subject entered the long-term open-label study and received 1 mg/day of

trandolapril for 29 days, at which time trandolapril was stopped because of angina. The investigator noted that the subject was scheduled to have angioplasty.

Subject 3013-3003 in Study TR-30-HTN, a 68 year old Mexican-American male experienced angina pectoris after receiving trandolapril 1 mg/day for 21 days and then 2 mg/day for 4 days during the long-term open-label phase. His history included possible old myocardial infarction and carotid endarterectomy. The subject was discontinued from the study 17 days after the onset of the adverse reaction. ECG showed non-specific ST-T changes. Subject was admitted to a hospital and underwent subsequent 3-vessel CABG.

Subject 3001-2029 in Study TR-30-HTN, a 70 year old Caucasian male, experienced anginal pain after receiving 1 mg/day of trandolapril for 111 days during the long-term open-label phase. The subject was treated with nitroglycerin p.r.n. starting 73 days after the onset of the adverse event. This subject was terminated from the study by the investigator 104 days after the onset of the adverse reaction, when subject was hospitalized with acute myocardial infarction, confirmed with enzymes.

Subject 3006-3002 in Study TR-30-HTN, a 55 year old Caucasian male with an 11-year history of hypertension and diabetes mellitus, experienced new onset of chest tightness (angina) after receiving trandolapril that was titrated upward as follows: 1 mg/day for 28 days, 2 mg/day for 36 days, 4 mg/day for 135 days and then 2 mg/day of trandolapril with 180 mg/day of verapamil for 143 days during the long-term open-label phase. This subject was discontinued from the study on the day of onset of the adverse reaction. Subsequent to cardiac catheterization, he was recommended for CABG.

Subject 4003-6 in Study TV-40-HTN, a 51 year old Caucasian male experienced chest pain and left arm pain subsequent to positive exercise tolerance test after receiving trandolapril, verapamil, and HCTZ as follows: trandolapril 2 mg/day for 8 days then trandolapril 4 mg/day for 5 days then trandolapril 4 mg/day plus verapamil 180 mg/day for 7 days then trandolapril 4 mg/day plus verapamil 180 mg/day plus HCTZ 12.5 mg/day for 9 days and then trandolapril 4 mg/day plus verapamil 180 mg/day plus HCTZ 25 mg/day for 56 days during the open-label phase. This subject was terminated from the study 14 days after the onset of the adverse reactions, both of which resolved one day later. Subject was subsequently referred for cardiac catheterization.

Subject 2101- a 64 year old Black female, died during the placebo run-in phase of study TR-21-HTN. A few days into the run-in period, the subject experienced persistent chest discomfort, and was admitted with a diagnosis of acute myocardial infarction. The cause of death was listed as cerebral hypoperfusion and ventricular pump failure resulting from myocardial infarction. Although the study screening ECG suggested a possible old anteroseptal scar, there were no symptoms of ischemic heart disease.

Subject 2004-7 in Study TR-20-HTN, a 62 year old Caucasian male experienced ECG changes indicative of myocardial ischemia during the double-blind phase after receiving placebo for 42 days. The subject was discontinued from the study because of myocardial ischemia. Subsequent cardiac catheterization revealed no significant coronary artery lesions.

Subject 2029-3 in Study TR-20A-HTN, a 39 year old Caucasian male experienced an acute myocardial infarction during the double-blind phase after receiving placebo for 5 days. The subject was admitted into an acute coronary care unit and discontinued from the study because of myocardial infarction. The subject subsequently underwent a CABG.

From the European database (n=19,610), there were 15 myocardial infarctions, 4 of which were fatal. Detailed descriptions are available for 7 of these cases:

Subject 201/167 in Study FF/88/570/33 was a 47 year old obese male. Two months after entry into the long-term study, persistent high blood pressure led the investigator to add nifedipine to his treatment regimen. Two months later, hydrochlorothiazide was added because of high diastolic blood pressure. The next month, he had a myocardial infarction (intensity: severe) and was hospitalized. The test drug was stopped and cardiac surgery was recommended. Throughout the study ECGs had been judged normal.

Subject 279/465 in Study FF/87/570/05 was a 52 year old male who had been a smoker. On entry into the study, his ECG was normal. At the end of the second month of trandolapril, persistent high blood pressure led the investigator to double the dose of trandolapril as per the protocol. One-and-a-half months later, when the subject's blood pressure was still high (supine: 170/110 mmHg; standing: 160/110 mmHg), he had an antero-lateral myocardial infarction (intensity: severe). The investigator stopped the test drug.

Subject 258/672 in Study FF/87/570/08 was a 63 year old female who was in the FF/87/570/05 study (atenolol group). One day after starting trandolapril 2 mg/day, she awoke in the night with chest pain. The next night, she had the same symptoms and on the next day, she decided to stop trandolapril. Five days later, ECG displayed changes related to a recent myocardial infarction, with frequent unifocal ventricular ectopic beats. She refused hospitalization as well as blood sampling. Subsequent ECG displayed the normal evolution of an anterior myocardial infarction, and the subject made an uneventful recovery.

Subject 72 in study UK/89/570/05 was an overweight 69 year old man who upon entry into the placebo pre-selection period exhibited left ventricular hypertrophy with some unfolding of the aorta on chest X-Ray. ECG was normal. Trandolapril 1 mg daily was started but after 33 days of treatment, he suddenly collapsed and was dead on arrival at the hospital. Post mortem examination confirmed the cause of death as acute myocardial infarction.

Subject 257/766 in study FF/87/570/09 was a 44 year old smoking male who suffered a fatal myocardial infarction after 174 days of treatment with trandolapril. No additional information is available.

Subject 8972 in Study FFR/91/570/166 was a 77 year old man. One month after starting trandolapril 2 mg/day, HCTZ was added because of continued high blood pressure (190/90 mmHg). One month later, he had to be hospitalized for an antero-septal and lateral myocardial infarction (onset of chest pain after climbing 1 floor). He had several slight constricting chest pains while walking during the previous weeks. Two weeks later, the subject was discharged with acebutolol, nitroglycerin patch, and aspirin. One month after discharge, he experienced recurrence of myocardial infarction with circumferential necrosis which led to death 3 days

later. It is not known whether an autopsy was performed or not. In the investigator's opinion, myocardial infarctions were unlikely to be related to study drug.

Subject 16020 in Study FFR/91/570/166 was a 65 year old man with a history of hypertension and tobacco abuse. Two weeks after starting an unspecified dose of trandolapril, he was hospitalized for acute myocardial infarction. The following night, the subject experienced ventricular fibrillation and died. It is not known whether an autopsy was performed. In the investigator's opinion, myocardial infarction and consequent death are unrelated to study drug.

- 7.5.2. **Chronic respiratory disease** From the U.S. data, 30 subjects out of n=1134 were reported as having chronic obstructive pulmonary disease as a concurrent diagnosis; also, 30 subjects were reported as having upper respiratory tract inflammation/congestion as a concurrent diagnosis. Sixty-six percent of these subjects reported adverse reactions as opposed to 51% for the "any" trandolapril database. No specific studies were done in these populations.
- 7.5.3. **Diabetes mellitus** There were no specific studies included in the NDA done in these subjects. The number of subjects admitted to controlled trials with diabetes mellitus has not been tabulated. Serious increases or decreases in serum glucose were very infrequent.
- 7.5.4. **Renal impairment** A serum creatinine of over 2 mg/dl was an exclusion criterion in most U.S. hypertension studies. The NDA contains a single-dose study²⁰ and a multiple-dose study²¹ in subjects with chronic renal failure. Both studies demonstrated that declining creatinine clearance decreases renal and plasma clearance of trandolapril and increases plasma trandolapril C_{max} and AUC. However, dosage adjustments appear necessary primarily in subjects whose creatinine clearance is <30 ml/min.
- 7.5.5. **Hepatic impairment** Significant hepatic disease was an exclusion criterion in most studies. The NDA contains an uncontrolled single-dose study performed in 8 subjects with adult hepatic cirrhosis²². Although the lack of a control group impairs interpretation of these results, when the results are compared to those generated in normal subjects from other studies, cirrhosis causes an approximately two-fold increase in the trandolapril plasma C_{max} and AUC, and an approximately 9-fold increase in plasma trandolapril C_{max} and AUC. The sponsor recommends caution when using trandolapril in subjects with hepatic cirrhosis, especially in those subjects sodium- and water-depleted or in cases of hyperactive renin-angiotensin systems.

In the U.S., there were a number of cases of liver enzyme elevation among healthy adult male volunteers. In Study F/85/570/01, two subjects had isolated minimal elevations of SGOT.

Subject 9 in Study F/85/570/03 had a normal WBC count and SGOT at baseline as well as during the first (trandolapril) treatment period, but then manifested a modest leukopenia (total count 3.6 K/μl) and elevated SGOT (to 50 U/L) during the subsequent placebo period. No other liver function tests were abnormal. The report contained no description of the reproducibility, reversibility, clinical antecedents, or sequelae of these findings.

Subjects 2 and 7 in Study F/86/570/15 had isolated, transient, slight treatment-emergent elevations of SGPT (to 39 and 38 U/L, respectively). Subject 5 in study TR-1-CP had transient elevations of hepatocellular enzymes before and after trandolapril exposure.

²⁰. Study FF/87/570/25, reviewed on page 154.

²¹. Study FF/88/570/39, reviewed on page 173.

²². Study FF/88/570/38, reviewed on page 167.

Ten subjects in study TR-2-CP had elevations in SGPT, 7 of which were moderately high (66 to 165 U/L), 2 of which were >165 U/L. Five subjects had SGOT elevations, one of which was >150 U/L. All hepatic enzyme abnormalities were detected at the scheduled day 14 evaluation; all returned to normal over a 4-week or less follow-up. There appeared to be no relationship between the dose of trandolapril and the incidents of SGPT and SGOT increases.

Three subjects in TR-4-CP had liver function test elevations:

Subject 3 in Study TR-4-CP, a 29 year old Caucasian male, had elevations of 1% in SGPT and 48% in LDH but all subsequent tests were normal.

Subject 34 in Study TR-4-CP, a 22 year old Caucasian male, had an elevation of SGPT of 31% but all subsequent tests were normal.

Subject 51 in Study TR-4-CP, a 31 year old Caucasian male, experienced gradually increasing levels of SGPT and SGOT: SGPT went from 49 U/L to 63 U/L to 83 U/L on the last day of the study while SGOT rose from 30 U/L to 39 U/L to 116 U/L on the last day of the study. The investigator stated that these elevations may have been caused by recent ethanol ingestion. No follow-up was done.

Subject 10 in Study ZA/88/570/28, a single-dose warfarin drug interaction study in healthy males, had moderate elevation of SGPT (42 U/L) and total bilirubin (28 µM). There was no description of reversibility, clinical antecedents, or follow-up.

In Study ZA/89/570/43, a single-dose furosemide drug interaction study in healthy males, 3 subjects demonstrated liver function test elevations:

Subject 4 in Study ZA/89/570/43, a 21 year old male, manifested an isolated, further elevation of a baseline-elevated SGPT (initially 41 U/L). SGOT was higher than baseline levels throughout all treatment periods, peaking at 77 U/L with a last reported value of 55 U/L. There was no follow-up.

Subject 9 in Study ZA/89/570/43 had a baseline SGPT of 100 U/L which fluctuated (sometimes was even normal) throughout the treatment periods, with an end of trial value of 51 U/L (although gamma-GT was mildly elevated to 73 U/L at that time). No follow-up information is available.

Subject 11 in Study ZA/89/570/43, a 19 year old male, had maximum elevations of SGPT and SGOT of 100 and 121 U/L, respectively, with end of study SGOT and SGPT of 78 U/L and 116 U/L, respectively. These abnormalities were temporally associated with rigorous exercise, resolved 2 weeks later, and reappeared with exercise re-challenge.

There were also a number of cases of liver enzyme elevation among hypertensive subjects:

Subject 2121-14 in Study TR-21A-HTN, a 35-year-old Black female, had baseline SGOT of 24 U/L and SGPT of 30 U/L. During initial placebo run-in, SGOT rose to 52 U/L, then returned to normal range. After receiving trandolapril 16 mg/day for 42 days, SGPT increased to 58 U/L. Twenty days into the placebo wash-out period, the SGPT had increased to 74 U/L. Fourteen days after the end of the study, SGOT and SGPT were 27 U/L and 35 U/L, respectively.

Subject 2123-27 in Study TR-21A-HTN, a 41 year old Black male with a previous history of hepatic dysfunction (enzymes at baseline: SGOT 98 [normal 0-55] and SGPT 99 [normal 0-55]), experienced

increased SGOT and SGPT (3 times upper normal limit) and mild lymphocytosis after receiving 16 mg/day trandolapril during the double-blind phase for 7 days. The subject also admitted to drinking heavily. Enzymes were back to near baseline at 10-day follow-up.

Subject 2128-3 in Study TR-21A-HTN, a 42 year old Black male had a baseline SGOT level of 58 U/L. After 13 days of treatment, SGOT rose to 82 U/L. After 24 days, SGOT peaked at 275 U/L. On day 42 of treatment, the SGOT was 220 U/L. At the washout visit, SGOT was 69 U/L. This subject had a history of increased liver function tests secondary to sarcoidosis in 1978. The investigator commented that the SGOT elevation was attributable to sarcoidosis or ethanol use.

Subject 2202-12 in Study TR-22-HTN, a 45 year old Hispanic female, had a baseline SGPT of 17 U/L, but a value of 248 U/L at the last study visit, having taken trandolapril 4 mg/day for 28 days. The investigator stated that the subject had a "concurrent disease: cholelithiasis/cholecystitis". At two-day follow-up, SGPT was 118 U/L.

Subject 3019-3001 in Study TR-30-HTN, a 35 year old Caucasian male experienced headaches after receiving trandolapril 1 mg/day for 36 days, 2 mg/day for 35 days, and 4 mg/day for 2 days during the long-term open-label phase. The subject was discontinued from the study by the investigator 12 days after the onset of the adverse reaction and was also dropped because of mildly elevated SGPT and alkaline phosphatase (although both of these elevation were present at baseline and were considered to be related to alcohol abuse).

Subject 3011-3011 in Study TR-30-HTN, a 48 year old Caucasian female, developed elevated SGOT 102 U/L, (baseline was 25 U/L; normal range is 7 to 39 U/L), SGPT of 299 U/L, (baseline 31 U/L; normal range 2 to 54 U/L) and LDH 275 U/L, (baseline 180 U/L; normal range 88 to 230 U/L) after receiving trandolapril 1 mg/day for 26 days, 2 mg/day for 31 days, 4 mg/day for 107 days and then 2 mg/day trandolapril plus 180 mg/day verapamil for 28 days during the long-term open label phase. The subject was discontinued from the study by the investigator 7 days after the onset of the adverse reactions. The SGOT, SGPT and LDH elevations returned to normal ranges at 2-week posttreatment follow-up.

Subject 4201-22 in Study TR-42-CP, a 36 year old Caucasian male had baseline SGOT/SGPT of 31 U/L and 45 U/L, respectively. After receiving trandolapril 54 days, SGOT/SGPT levels were 73 U/L and 104 U/L. The washout SGPT level was 197 U/L. The subject was asymptomatic, hepatitis screen was negative, and there was no follow-up.

There was also one case of liver enzyme elevation in a study of chronic renal failure subjects: Subjects 11 and 12 in Study FF/87/570/26, a study in hemodialyzed subjects with chronic renal failure, had elevated values of liver function tests (serum SGOT and/or alkaline phosphatase) at baseline and these became slightly to moderately further elevated (3-12%) after 2 weeks of treatment. There was no other follow-up.

There was one case of liver enzyme elevation among hypertensive subjects on placebo:

Subject 2015-1 in Study TR-20-HTN, a 66 year old Caucasian female, had increased SGOT and SGPT during the double-blind phase after receiving placebo for 29 days. The subject was discontinued from the study because of elevated SGOT (188 U/L) and SGPT (173 U/L). One week earlier, the subject had received an

injection of triamcinolone and had been started on Clinoril 200 mg twice daily. Three days after drug discontinuation, the enzymes remained elevated: SGOT 174 U/L, and SGPT 171 U/L. This adverse reaction was considered by the investigator to be "probably not" related to study drug.

Four out of 831 subjects taking trandolapril in randomized controlled trials developed liver function test elevations of at least twice baseline (0.5%). One placebo subject out of 236 (0.4%) experienced the same. Out of the 1134 subjects taking any trandolapril, 6 subjects (1 taking verapamil concurrently; 0.5%) demonstrated liver function test elevations of at least twice baseline.

In comparing the incidence of hepatic enzyme elevation with trandolapril with that of other ACE inhibitors, reference is made to the Medical Officer's Review for NDA 20-240, spirapril, the section on "Safety", page 63. In the subsection on "Hepatic Impairment," spirapril's adverse effects on hepatic enzymes are compared to those reported for other ACE inhibitors. In well controlled trials, 1 of 921 subjects (0.1%) receiving spirapril had an elevation of SGPT and 1 of 331 (0.3%) placebo subjects had an elevated alkaline phosphatase and LDH. In U.S. trials of benazepril, 24/1859 (1.3%) and 14/1883 (0.7%) of subjects who were normal at baseline reported elevations during treatment to at least twice upper limit of normal of SGPT and SGOT, respectively. One out of 2338 (0.04%) quinapril (hypertensive) subjects were reported with elevated SGOT and SGPT. No ramipril (hypertensive) subjects were reported with elevation of liver enzymes.

Out of the European database of 19,610 subjects, there were only 6 cases of hepatic dysfunction, an incidence rate (numerically, if not statistically) lower than that seen in U.S. trandolapril or placebo data. Regardless, these are the cases available for review:

Subject 42/286 in Study FF/87/570/22 was a 51 year old male. After 3 months of treatment with trandolapril 2 mg/day, persistent high diastolic blood pressure (94 mmHg supine) led to the addition of nifedipine to the subject's treatment. Two weeks earlier, he had begun to experience a daytime cough (intensity: severe).

Symptomatic treatment with triprolidine, pseudoephedrine, and paracetamol was ineffective, and the subject was withdrawn after one month. He recovered within 2 weeks. Laboratory tests on withdrawal showed raised LDH (643 IU/L; normal is <480) and GGT (280 IU/L; normal is <43); these values normalized one month after he stopped treatment.

Subject 27 in Study F/86/570/10, a 25-year-old healthy male volunteer, was enrolled to in the 16 mg trandolapril group. Eight days before trandolapril was started, laboratory data were normal. Serologic tests for hepatitis B were negative. The volunteer complained of abdominal pain on day 2 and loose stools on day 3 of trandolapril and reported muscular pain on days 1, 4, and 5. On day 6, mild isolated increase of SGPT (to 2x normal) was noted and the subject was withdrawn from the study. Three days later, SGPT reverted to normal.

Subject 58 in Study FF/86/570/06, a 56 year old male not previously treated for hypertension, experienced elevation of liver enzymes. Enzymes were normal at baseline, but at the end of run-in, SGOT was 73, SGPT was 80, and AlkPhos was 108. At the end of double-blind treatment (day 8), SGOT was 479, SGPT was 828, and AlkPhos was 140. Enzymes climbed further during washout, then declined, but were still not normal range at 3 weeks. Subject tested positive for HBS antibody at 3 weeks.

Subject 267/719 in Study FF/87/570/05 was a 61 year old male who

complained of tiredness and lethargy (intensity: moderate) after one day of trandolapril. Laboratory results were within normal ranges. Tiredness persisted to a lesser extent throughout the study. After 12 weeks of active treatment, liver enzymes were raised ASAT 230 (normal is <41), ALAT 152 (normal is <41) and AlkPhos 264 (normal is <115). Despite the absence of symptoms of liver disease, the investigator decided to stop trandolapril and one week after drug withdrawal, enzymes were within the normal range.

7.6. Laboratory and physical abnormalities

- 7.6.1. **Physical examination** Summary data on physical abnormalities was abstracted from the sponsor's integrated summary of safety. The data for the US trials²³ are shown in Table 26 below (for incidence $\geq 1\%$). A listing which included combination treatment with verapamil and HCTZ was not materially different. No specific abnormality was sufficiently common to allow detection of dependence upon dose or time of exposure.

Table 26. Physical abnormalities ($\geq 1\%$) in US studies

	Trandolapril dose								
	0.25 n=76	0.5 n=75	1 n=449	2 n=472	4 n=477	8 n=103	12 n=74	16 n=74	Any n=986
Any	7.9	12	5.1	4.0	6.3	4.9	5.4	12	10.1
Any cardiovascular	2.6	4.0	1.6	0.8	1.5	1.0	0	1.4	2.4
Any dermatological	0	1.3	0.4	1.1	0.8		0	0	1.1
Any EENT	2.6	2.7	1.3	1.1	1.5	2.9	2.7	4.1	3.0
Upper respiratory congestion	0	1.3	0.4	0.2	0.6	0	0	4.1	1.0
Any general	0	1.3	1.3	0.2	1.7	1.0	4.1	2.7	2.2
Trauma	0	1.3	0.9	0.2	1.0	1.0	1.4	0	1.3
Any pulmonary	2.6	1.6	0.7	0.2	0.2	0	0	2.7	1.0

7.6.2. Clinical chemistry

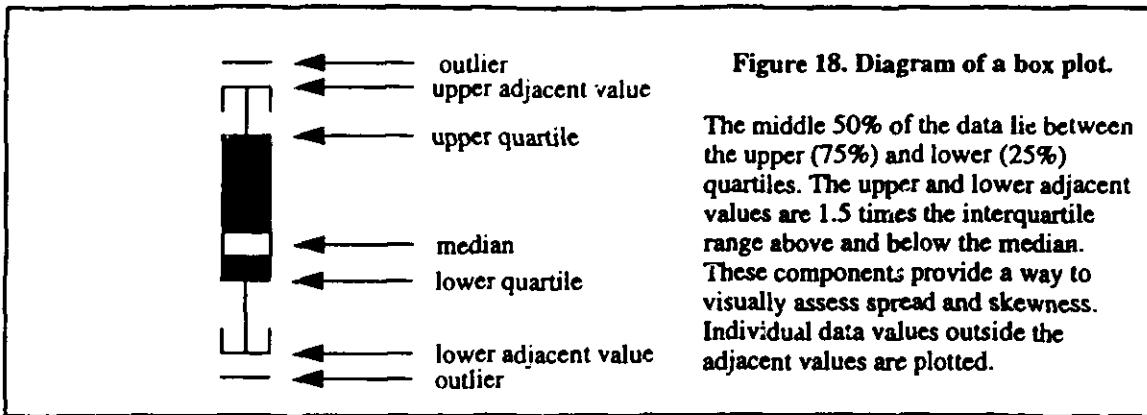
- 7.6.2.1. **Technical details of analyses** Clinical laboratory data were provided by the sponsor in machine-readable form for six major US clinical studies: TR-20-HTN, TR-20A-HTN, TR-21-HTN, TR-21A-HTN, TR-30-HTN, and TR-42-CP. Several graphical tools were employed to examine these data, on a study-by-study basis and across the set of studies.

For each laboratory test, a box plot was created with a box per dose. A simple box plot is shown in Figure 18 below to identify its components. In the figures of box plots by dose, the X-axis is labelled with the dose and a count of the number of measurements contributing to the bar. Sets of such plots are also incorporated in individual study reports.

A second style of plot was used to look at treatment effects on laboratory data from the six major US controlled clinical studies. Laboratory values at the final on-drug study visit were plotted as a function of the laboratory value at baseline. An example of such a figure is Figure 32. *Log₁₀(SGOT) on treatment vs. baseline (across studies)*, on page 63²⁴. A line of identity (no change on treatment) was added, and lines were fitted (loess curves) through the placebo and active treatment group data. In the margins were plotted normalized density functions showing the distributions of data values in the placebo and active treatment groups at baseline and on treatment. Medians are shown as vertical lines on these density plots.

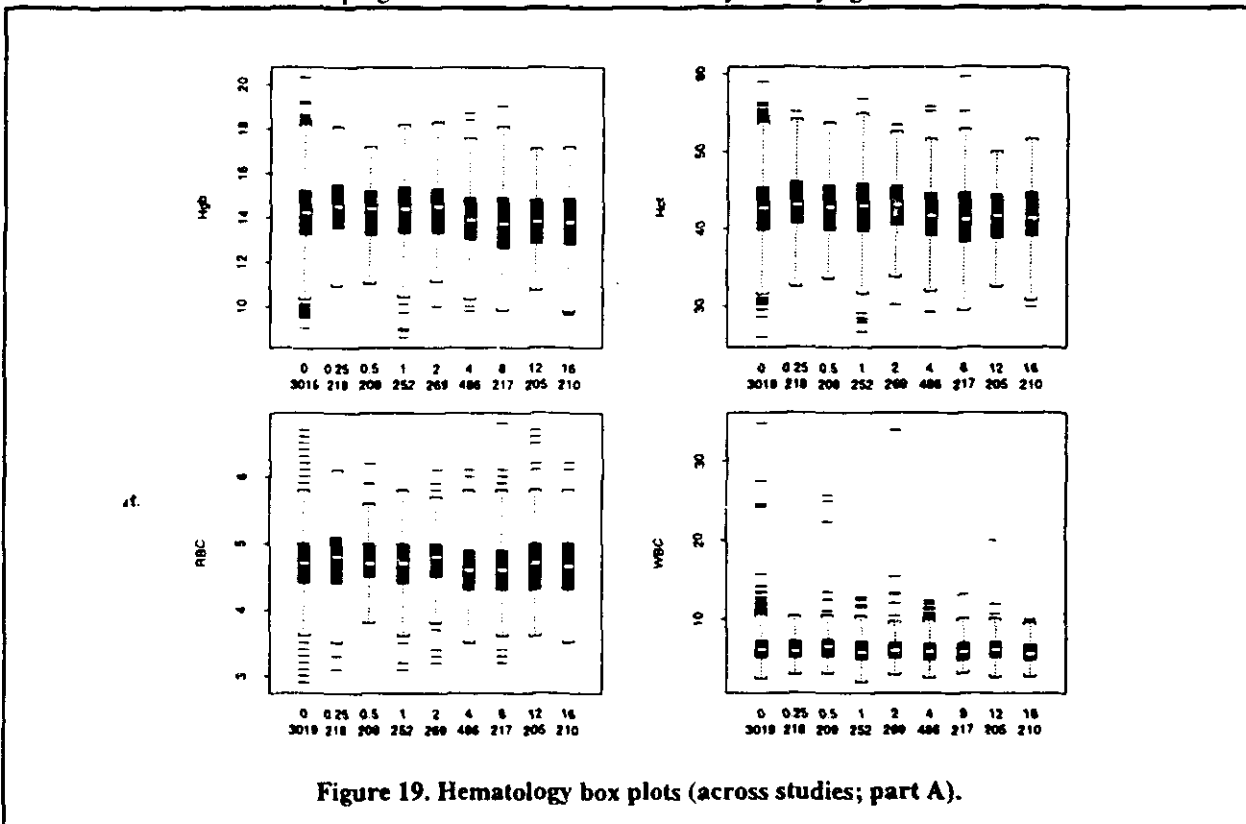
²³ Studies TR-20-HTN, TR-20A-HTN, TR-21-HTN, TR-21A-HTN, TR-30-HTN, TV-40-HTN, and TV-41-CP.

²⁴ In this case, the log₁₀ of the data were plotted.



7.6.2.2. Hematology

Figure 19 to Figure 21 below show box plots of hematology data by dose from all six major US clinical studies. For the twelve variables show in these figures, it is clear that there is no dose-dependence to the medians, to the distributions of data values, or progression in the number of severity of outlying observations.



Trends for changes from baseline were examined in on-treatment vs. baseline plots for the 4 pivotal US trials combined. There was a slight trend in evidence for a decrease in the slope in RBC, hematocrit (Figure 22 below), hemoglobin, and platelet count (Figure 23 below); i.e., subjects with low baseline values tended to increase on-treatment and those with high baseline values tended to decrease. The effect is, in each case, small (convincing only in its consistency among measures) and lacking in dose-dependence. The most likely explanation is regression to the mean.

Noteworthy outliers were culled from the sponsor's integrated summary of safety and study reports.

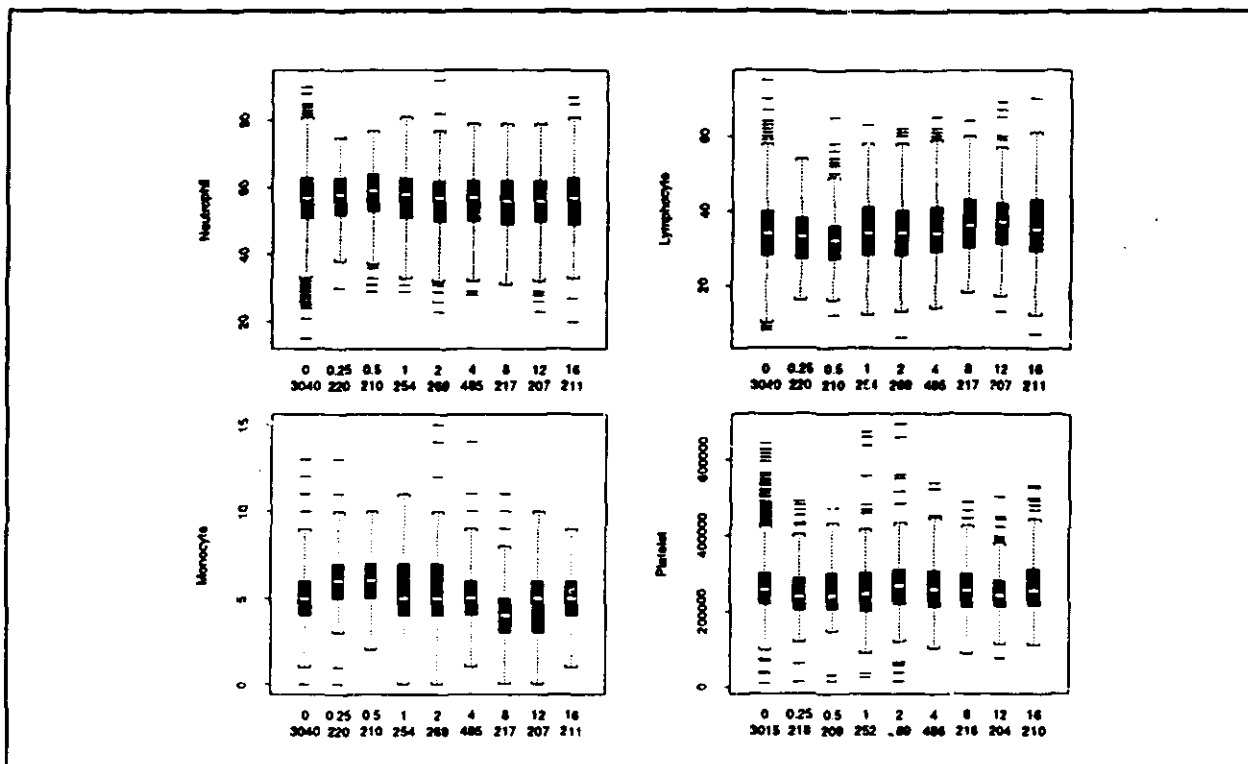


Figure 20. Hematology box plots (across studies; part B).

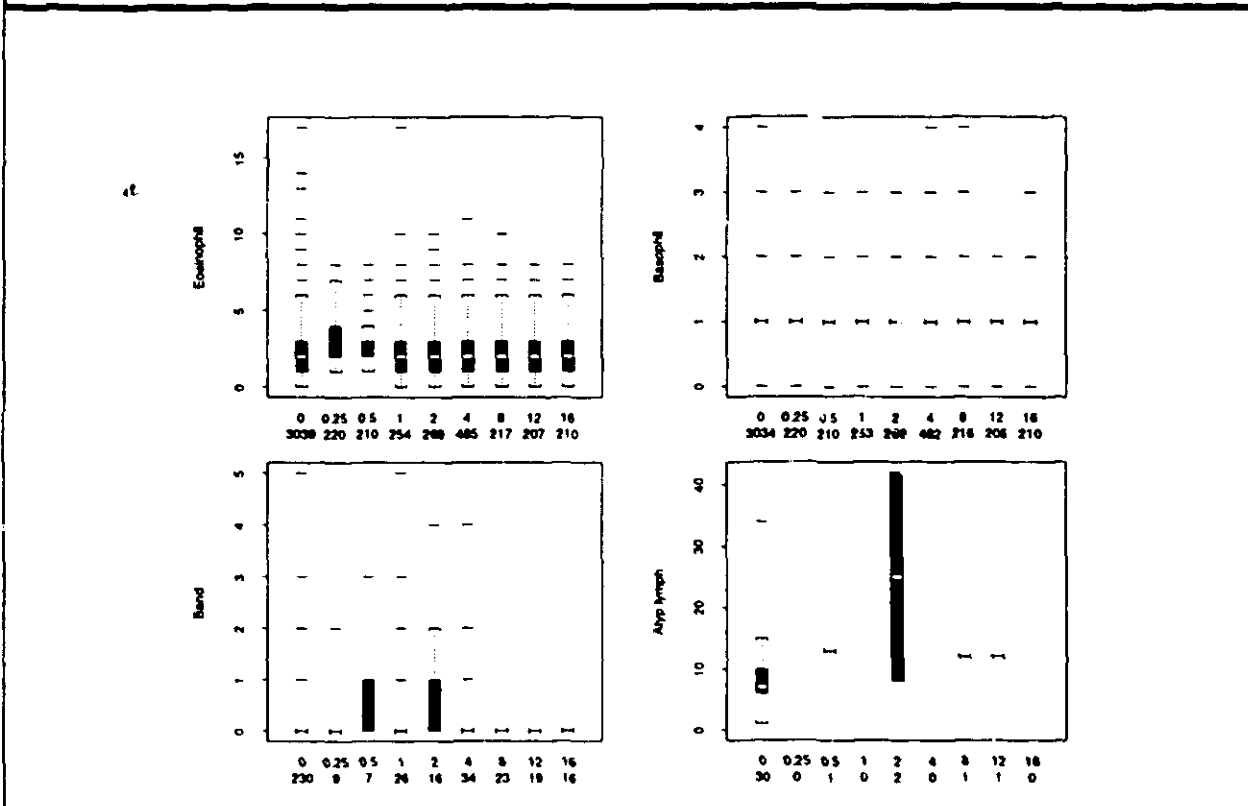


Figure 21. Hematology box plots (across studies; part C).

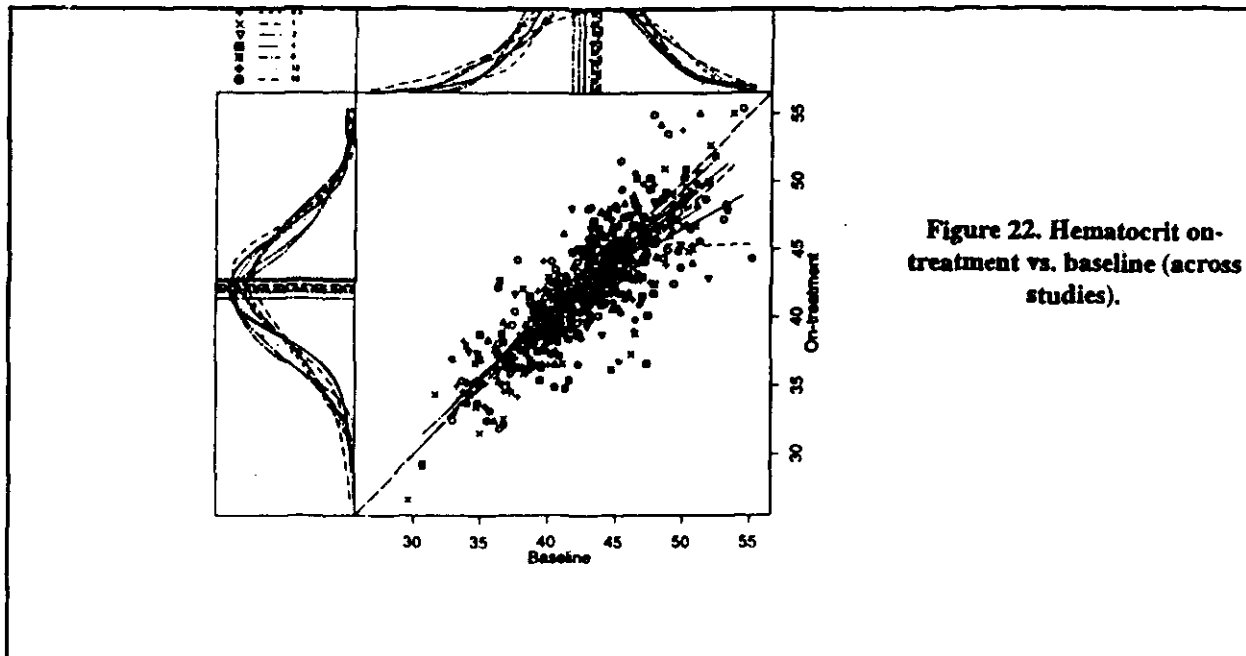


Figure 22. Hematocrit on-treatment vs. baseline (across studies).

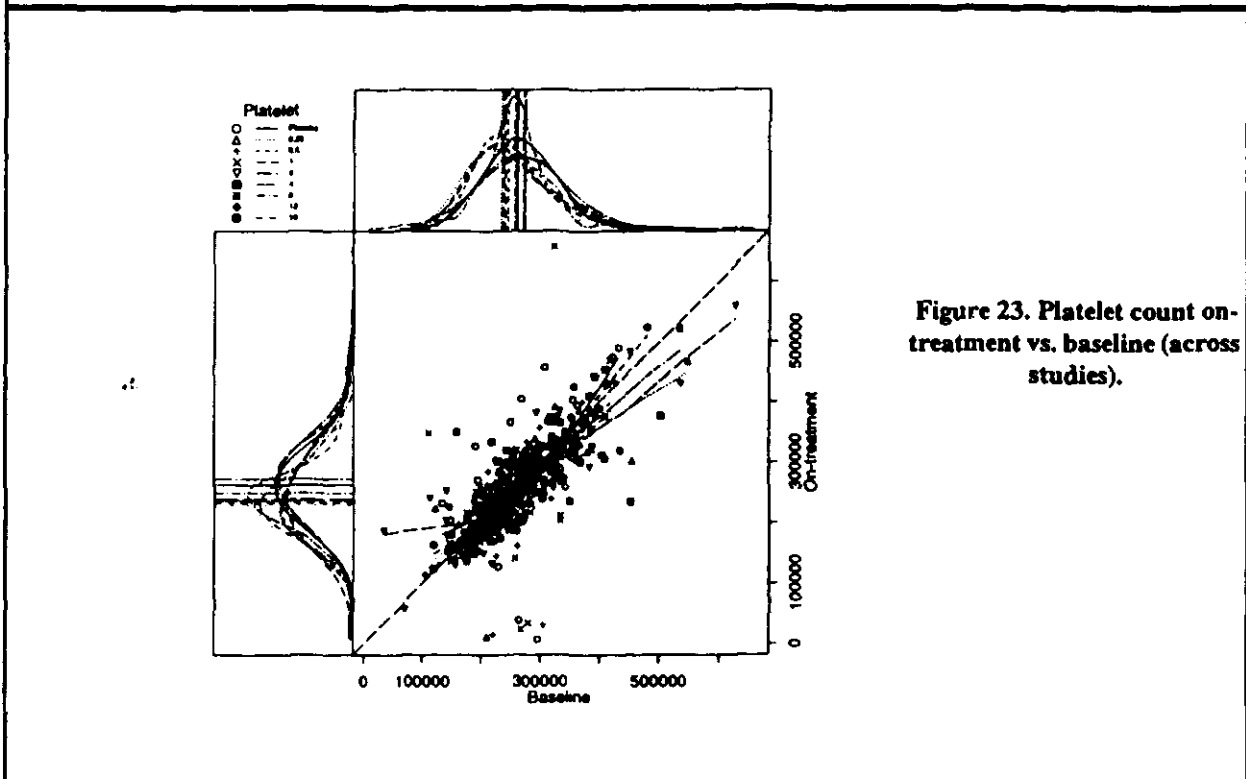


Figure 23. Platelet count on-treatment vs. baseline (across studies).

Neutropenia and agranulocytosis are suspected as rare complications of ACE inhibitors. The overall incidence of neutropenia was 24/972 (2.5%). "Moderate" or "severe" low neutrophil counts were reported for 11/682 (1.6%) of subjects ontrandolapril alone and 1/163 (0.6%) of subjects on placebo. Two subjects had neutropenia listed as adverse events, one of whom discontinued²⁵.

²⁵ Subject 3005-3010, described on page 273.

Thrombocytopenia was noted for 16/872 (1.6%) of subjects receiving trandolapril, one of whom discontinued with hypersplenism²⁶.

7.6.2.3. Urinalysis

Figure 24 to Figure 26 below show box plots of urinalysis data by dose from all six major US clinical studies. For most variables, the discrete nature of variables makes it impossible to judge the actual number of outliers. For the remaining parameters, it is clear that there is no dose-dependence to the medians, to the distributions of data values, or progression in the number of severity of outlying observations.

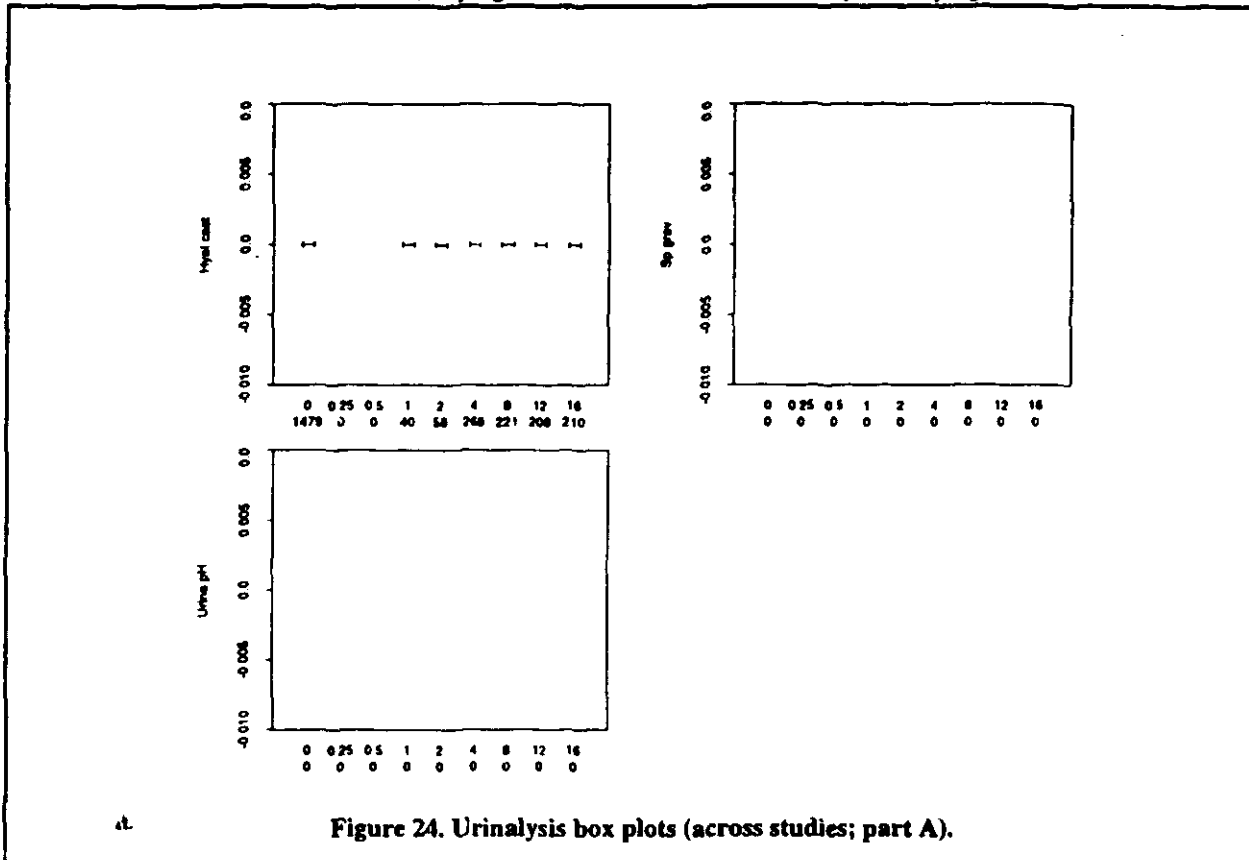


Figure 24. Urinalysis box plots (across studies; part A).

Trends for changes from baseline were inapparent in comparisons of on-treatment vs. baseline values.

7.6.2.4. Blood chemistry

Figure 27 to Figure 31 below show box plots of blood chemistry data by dose from all six major US clinical studies. For most variables, it is clear that there is no dose-dependence to the medians, to the distributions of data values, or progression in the number of severity of outlying observations. Notable exceptions are (1) ACE activity (Figure 31), which is clearly depressed at all dose levels and (2) SGOT (Figure 30). SGOT median levels do not appear to be dose-dependent, but the number of outliers and degree of departure from the normal range appears to increase with dose. The same characteristics are less well pronounced in SGOT and LDH (also Figure 30). Most of the outliers in SGOT are to values under 100 U/L, and so are of little clinical consequence. The concern would be in the nature of progression, since these data were obtained from relatively brief (6-week) controlled clinical trials.

Trends for changes from baseline were inapparent in on-treatment vs. baseline plots of SGOT, as shown in Figure 32 below. The base-10 logarithm of the data were plotted in this case because a few data points were far outliers. Trends to increases in SGOT with time and dose were also assessed by fitting least-squares lines to SGOT values as

²⁶ Subject 2014-4, described on page 225.

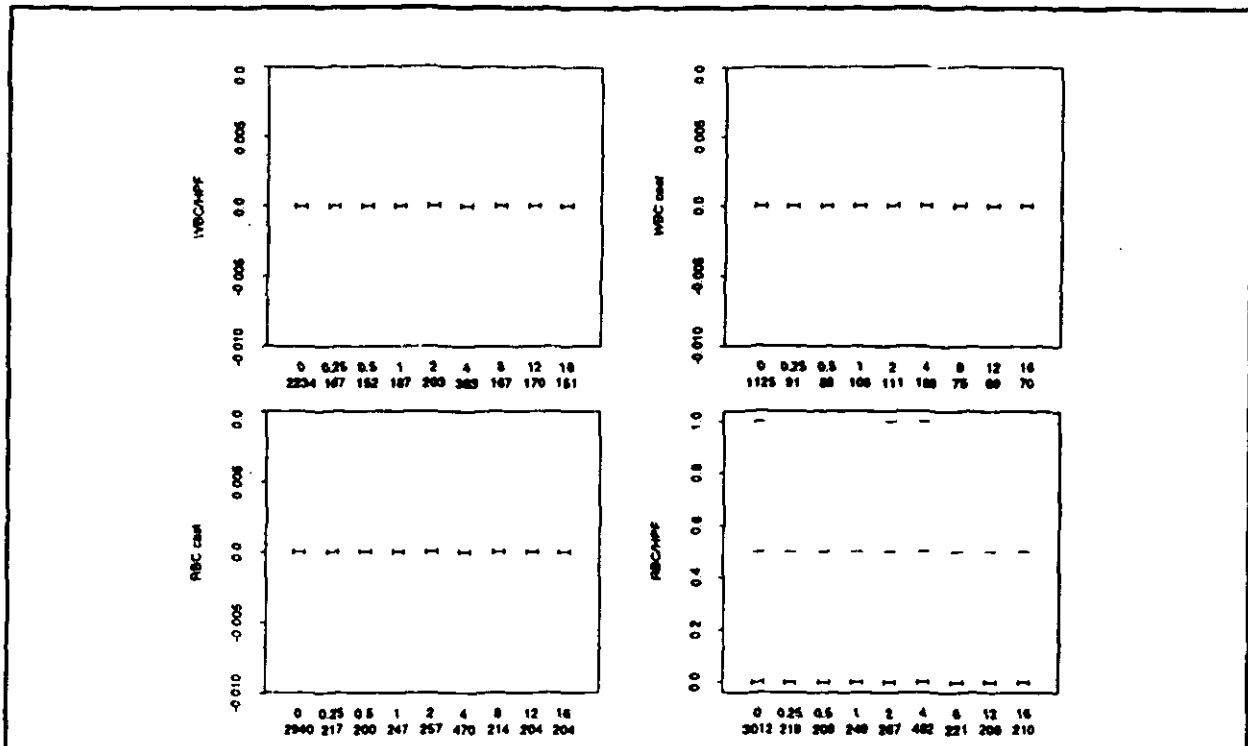


Figure 25. Urinalysis box plots (across studies; part B).

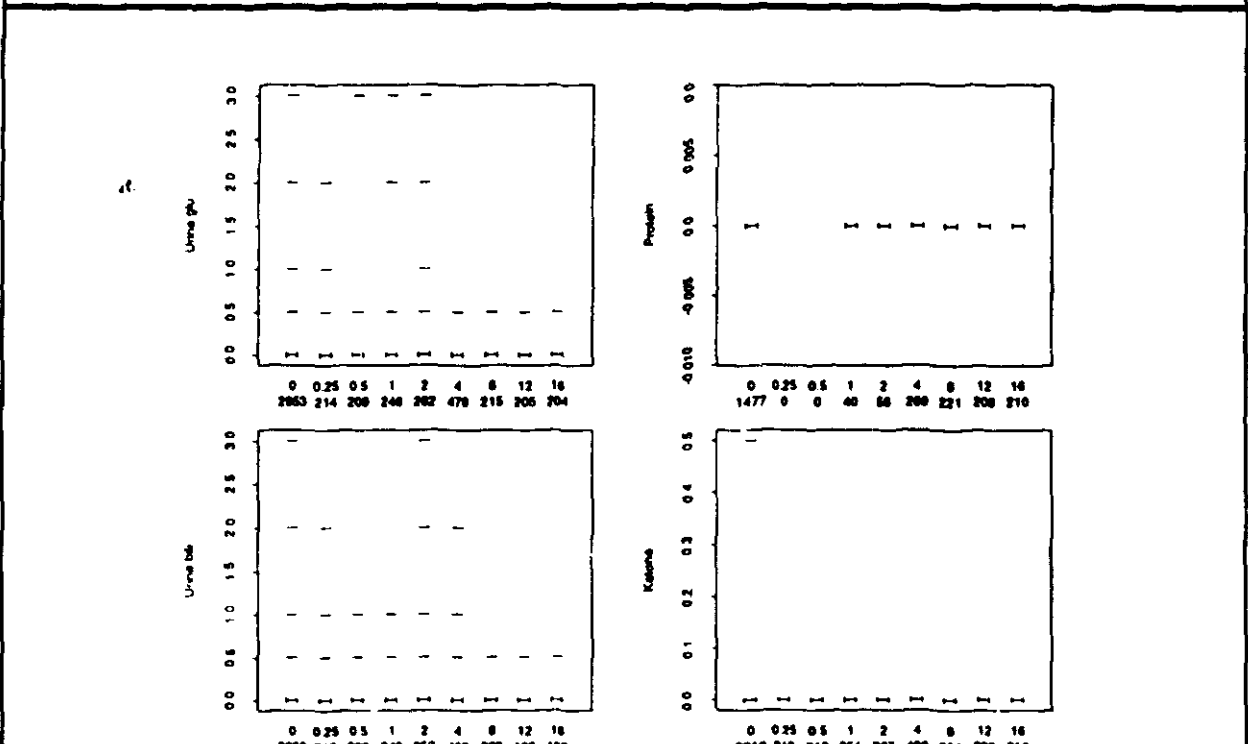


Figure 26. Urinalysis box plots (across studies; part C).

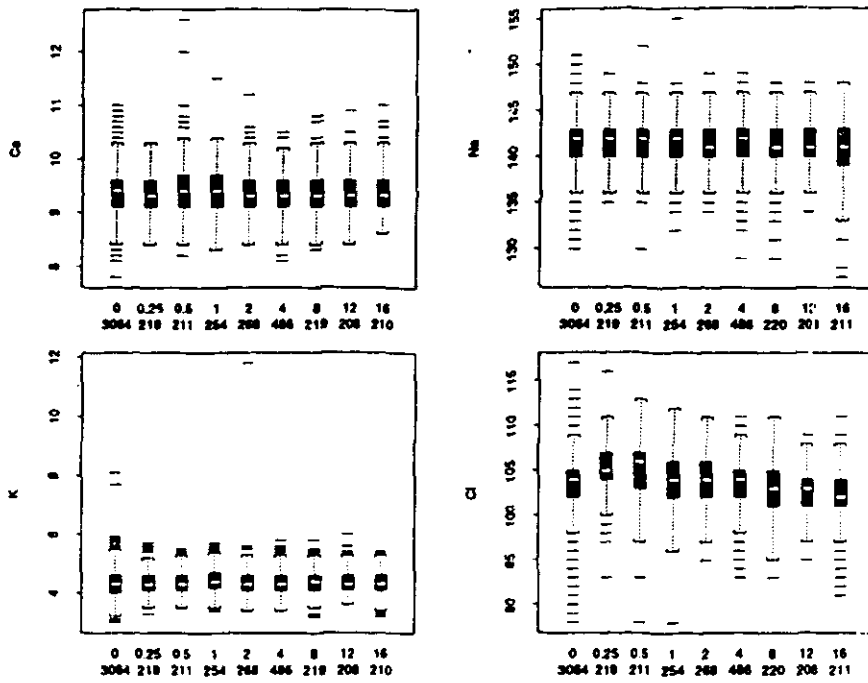


Figure 27. Blood chemistry box plots (across studies; part A).

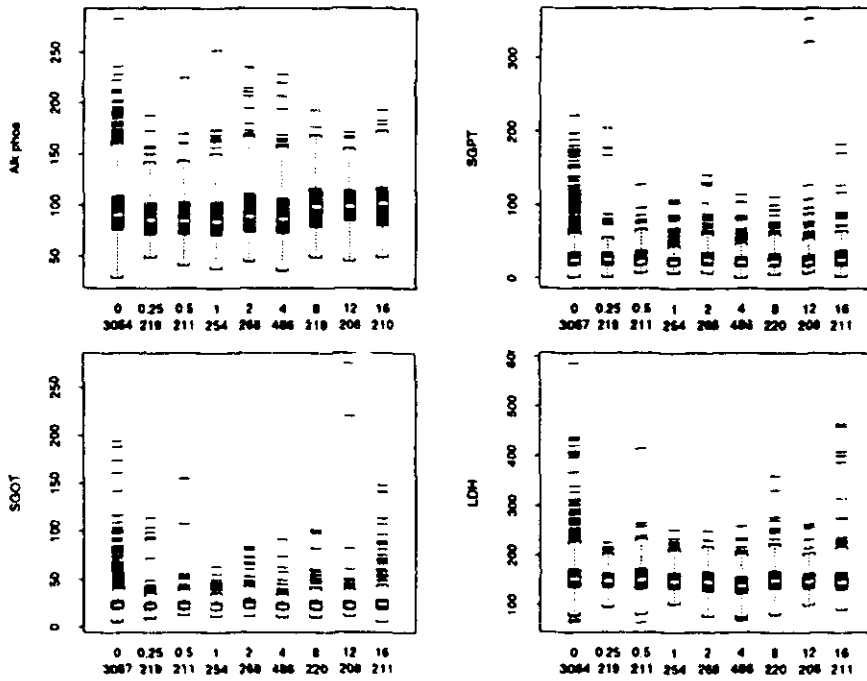


Figure 28. Blood chemistry box plots (across studies; part B).

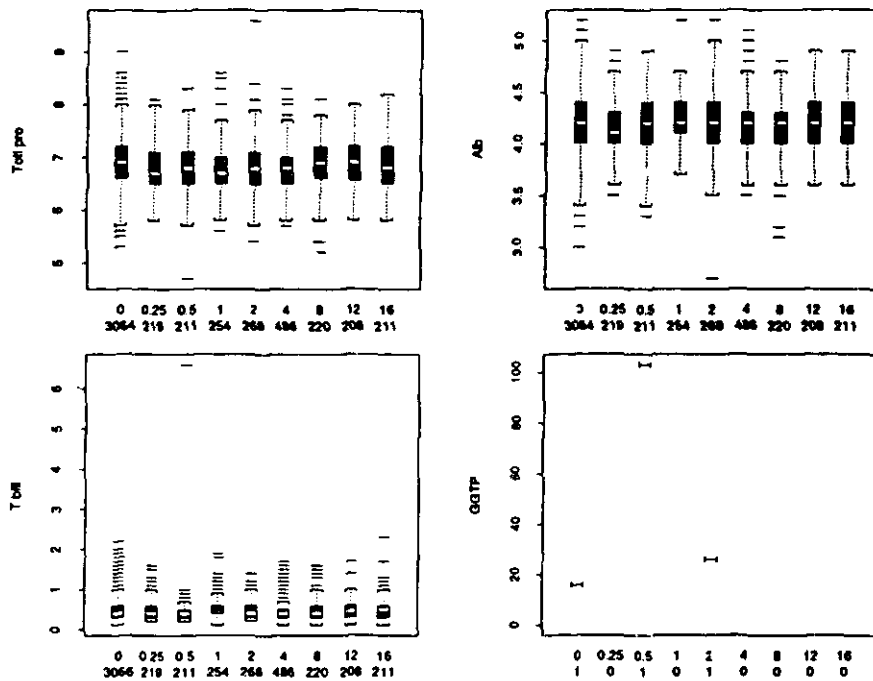


Figure 29. Blood chemistry box plots (across studies; part C).

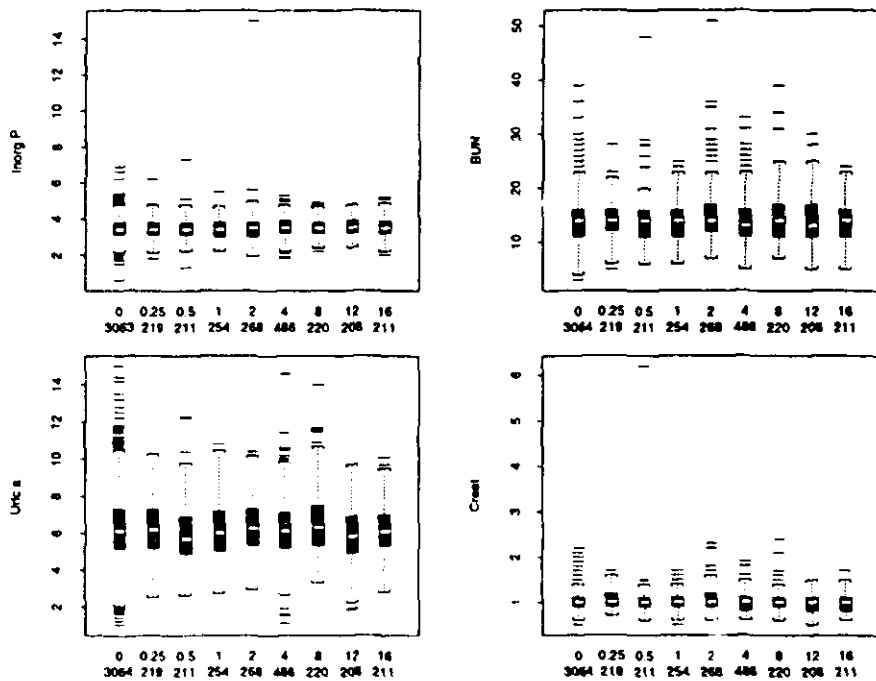


Figure 30. Blood chemistry box plots (across studies; part D).

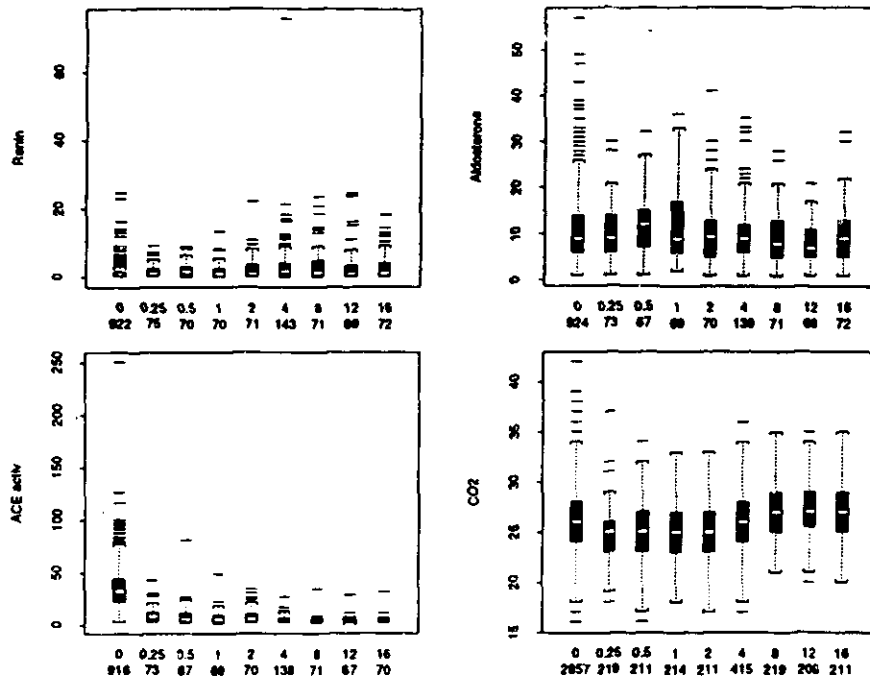


Figure 31. Blood chemistry box plots (across studies; part E).

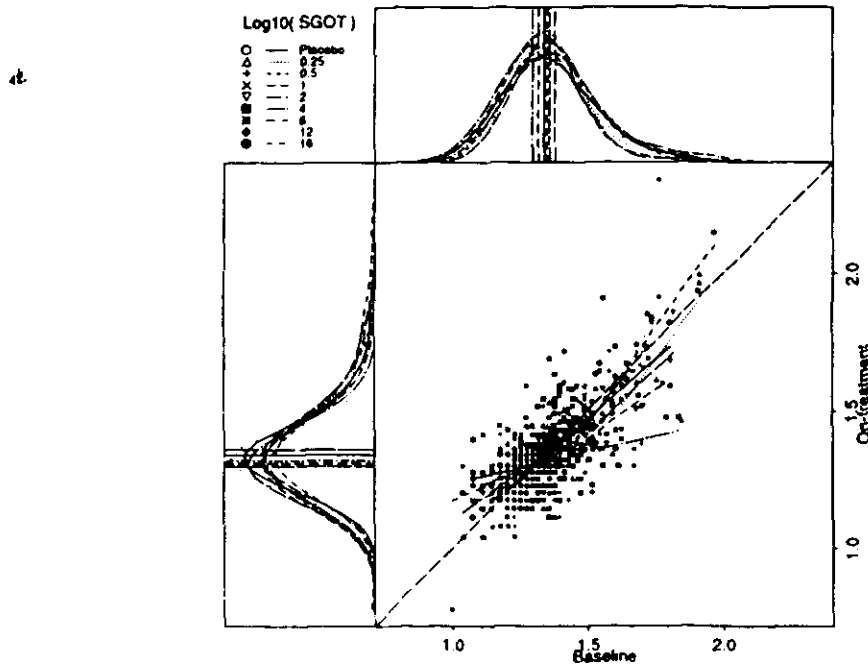


Figure 32. Log10(SGOT) on treatment vs. baseline (across studies).

a function of time in the double-blind treatment phase for each subject. The mean and standard deviation for the slopes are plotted as a function of dose in Figure 33 below. Again, no trend is evident.

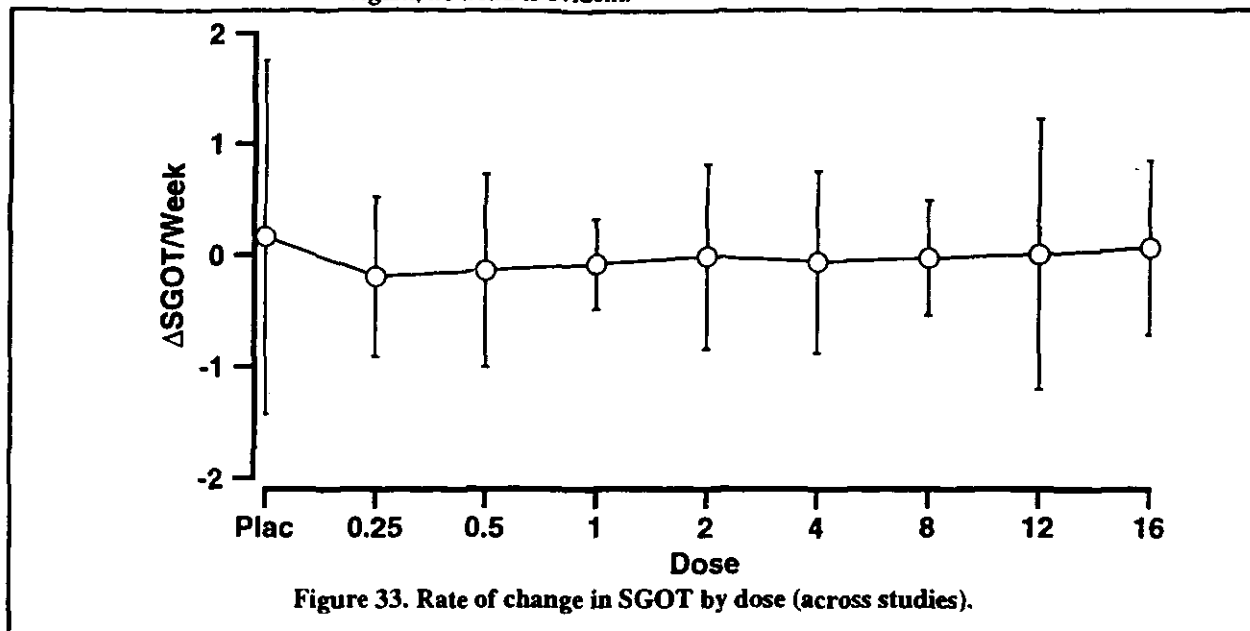


Figure 33. Rate of change in SGOT by dose (across studies).

Other on-treatment vs. baseline plots did demonstrate effects of treatment or study participation. ACE activity (Figure 34 below) was depressed in active treatment

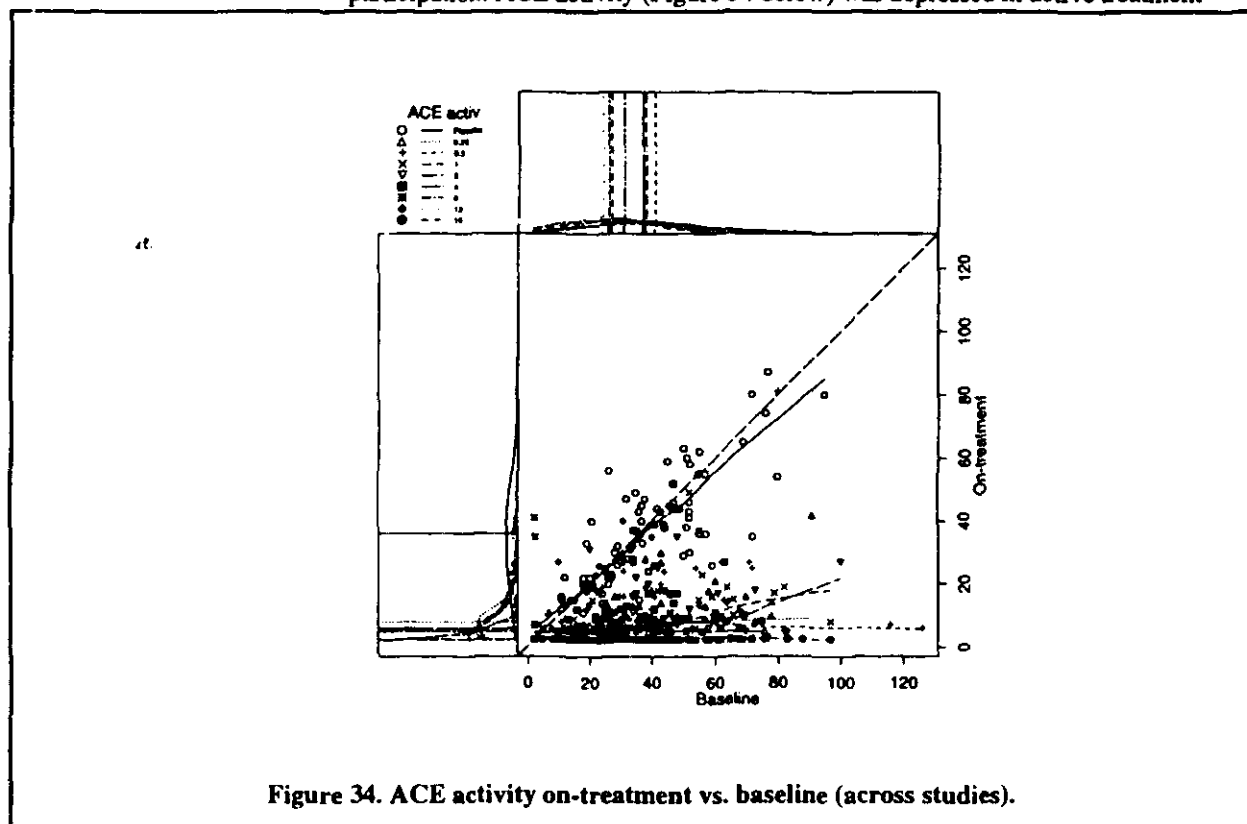


Figure 34. ACE activity on-treatment vs. baseline (across studies).

groups, although it is otherwise difficult to distinguish doses. Plasma renin activity (Figure 35 below) was increased, again with doses more easily distinguished from placebo than from one another. Many other clinical chemistry values tended to

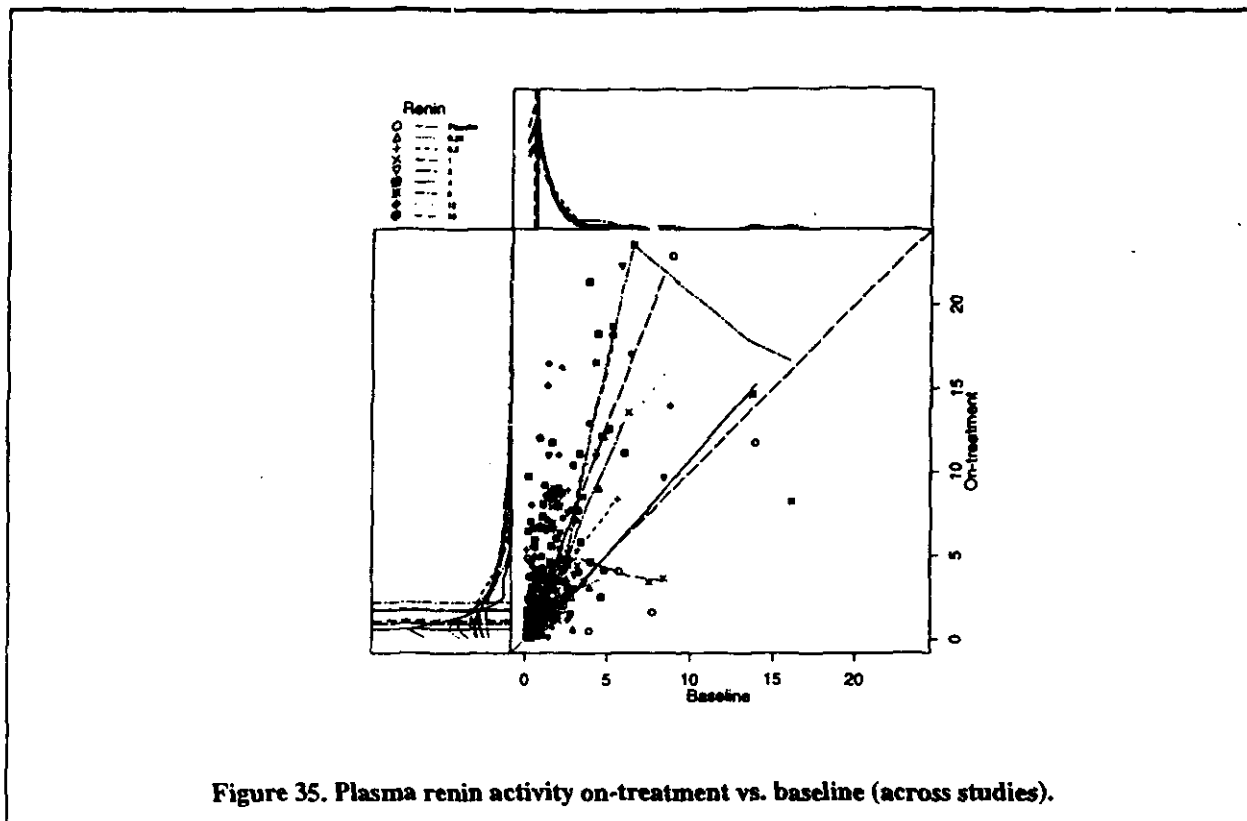


Figure 35. Plasma renin activity on-treatment vs. baseline (across studies).

decrease from high baseline or increase from low baseline values on treatment for both placebo and active treatment groups. A couple of examples of this are serum calcium (Figure 36 below) and total protein (Figure 37 below). The phenomenon appears to be similar to that described for some of the hematological parameters (see page 56).

Noteworthy outliers were culled from the sponsor's integrated summary of safety and study reports.

Seven trandolapril subjects had elevations in SGOT and SGPT reported as adverse events. Three placebo subjects had elevations reported as adverse events. Two trandolapril subjects²⁷ discontinued with elevations in SGOT and SGPT. One of these cases was associated with ethanol abuse. Both cases resolved within 2 weeks of discontinuation.

The sponsor's analysis of changes in clinical chemistry values from baseline to post-treatment by dose and time in study demonstrated no significant trends, except for alkaline phosphatase, the data for which is shown in Table 27 below. Baseline values were in the range of 90 to 100 U/L and the normal range runs to 140 U/L, so the clinical significance of this finding is probably minimal. The on-treatment vs. baseline plot for alkaline phosphatase, shown in Figure 38 below, also does not suggest a clinically significant trend.

7.6.3. Electrocardiography

Summary data on new ECG abnormalities was abstracted from the sponsor's integrated summary of safety. The data for the US trials²⁸ are shown in Table 28 below (for incidence $\geq 1\%$). A listing which included combination treatment with verapamil and HCTZ was not provided. No specific abnormality was sufficiently common to allow detection of dependence upon dose or time of exposure. Overall, 58% of observed abnormalities were detected in the first 2 weeks of exposure, but the bulk of

²⁷ Subject 3011-3011, described on page 273, and 2123-27, described on page 257.

²⁸ Studies TR-20-HTN, TR-20A-HTN, TR-21-HTN, TR-21A-HTN, TR-30-HTN, TV-40-HTN, and TV-41-CP.

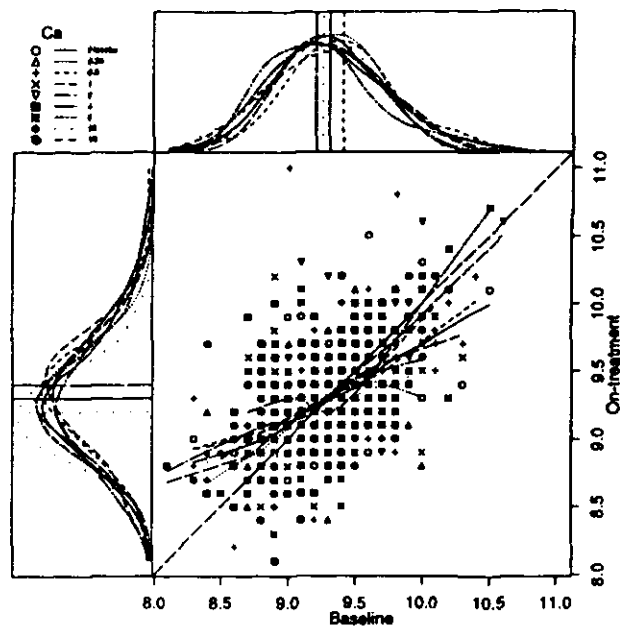


Figure 36. Serum calcium on-treatment vs. baseline (across studies).

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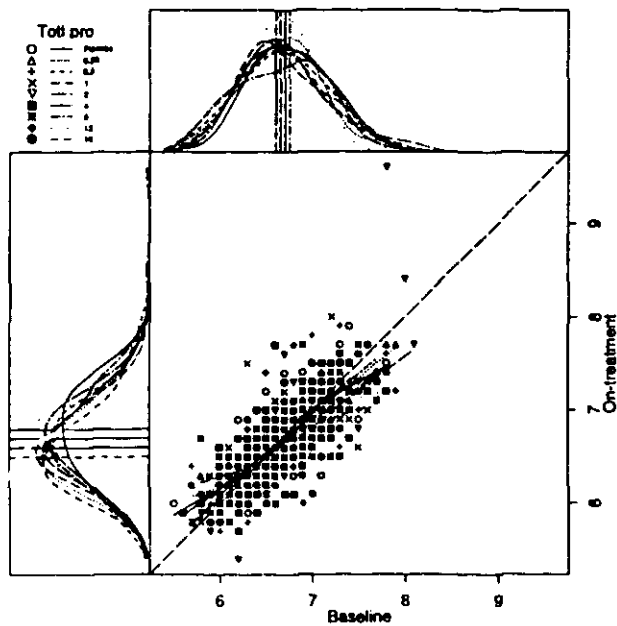


Figure 37. Total protein on-treatment vs. baseline (across studies).

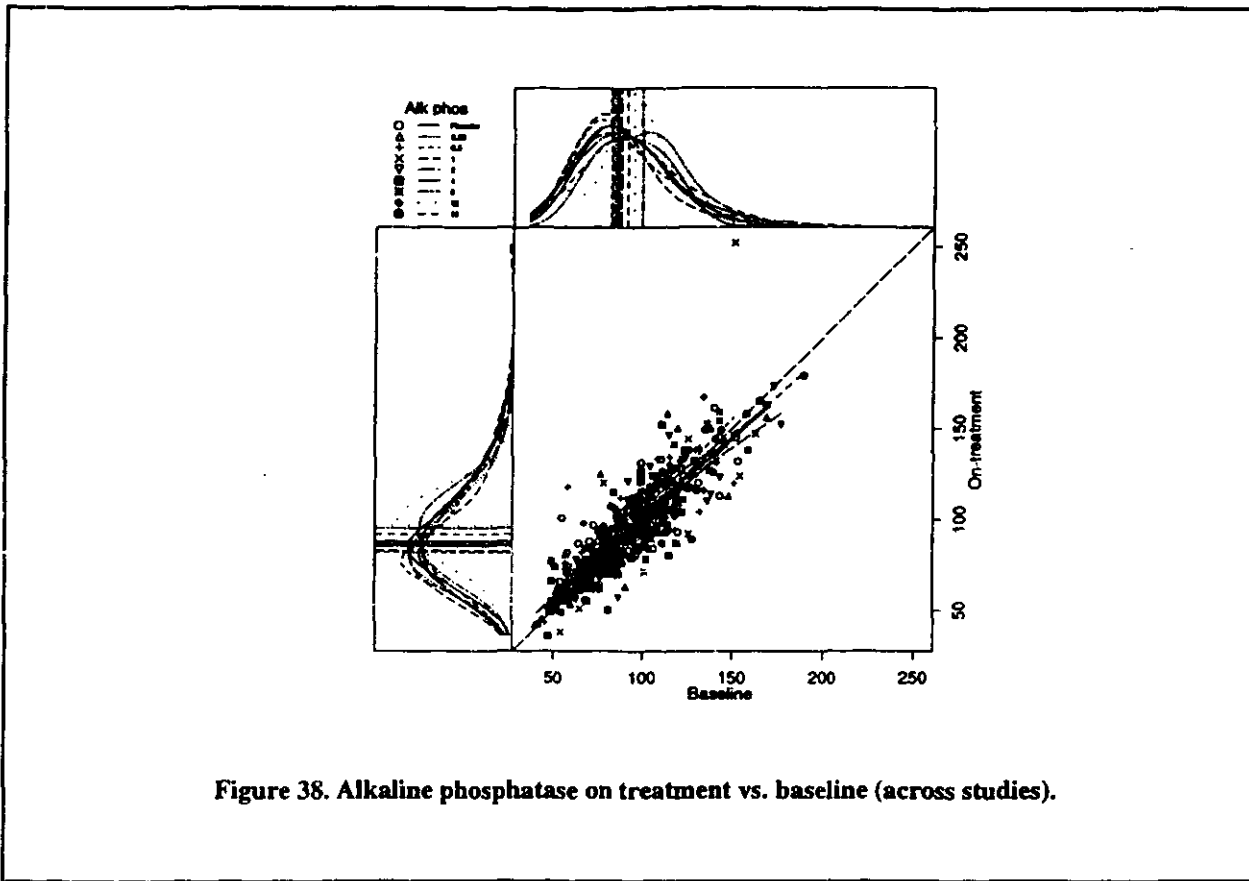


Figure 38. Alkaline phosphatase on treatment vs. baseline (across studies).

Table 27. Changes in alkaline phosphatase levels (U/L) in US studies.

	Dose (mg)																	
	0.25		0.5		1		2		4		8		12		16		Any	
	n	Δ	n	Δ	n	Δ	n	Δ	n	Δ	n	Δ	n	Δ	n	Δ	n	Δ
≤2 weeks	17	4.2	17	0.5	17	2.1	20	-0.3	75	1.9	53	3.5*	57	3.4*	55	1.0	311	2.2*
2 to 6 weeks	75	0.8	72	3.8*	252	3.3*	85	2.9*	161	1.5	78	0.5	71	1.2	70	-2.1	864	1.9*
6 weeks to 3 months	55	0.8	51	1.6	119	5.2*	155	2.0	140	3.4	22	-0.4	21	-0.9	15	-5.0	578	2.5*
3 to 6 months	—	—	—	—	78	7.9*	116	7.5*	131	8.5*	—	—	—	—	—	—	325	8.0*
6 to 9 months	—	—	—	—	59	7.6*	80	5.8*	102	3.1	—	—	—	—	—	—	241	5.1*
9 to 12 months	—	—	—	—	49	8.4*	70	6.0*	95	4.7*	—	—	—	—	—	—	214	6.0*

Sponsor's analysis; *p<0.05

observations were made over this time frame as well. For the one event most likely to have been treatment-related, sinus bradycardia, the rates were 3.9% at ≤2 weeks, 3.7% for 2 to 6 weeks, 3.2% for 6 weeks to 3 months, 3.8% for 3 to 6 months, 3.3% for 6 to 9 months, 4.8% for 9 to 12 months, and 2.7% for >12 months; these data are also difficult to interpret with irregular sampling.

Mean changes in atrial rate, ventricular rate, QT_c, QRS interval, and PR interval were tabulated by dose and time in study across US efficacy trials; none showed statistically significant changes or clinically significant trend.

Table 28. ECG abnormalities ($\geq 1\%$) in US studies

	Trandolapril-dose								
	0.25 n=76	0.5 n=75	1 n=152	2 n=215	4 n=418	8 n=398	12 n=74	16 n=74	Any n=939
Any	19.7	16.0	19.7	15.8	20.1	23.5	27.0	24.3	23.9
Rhythm or conduction	9.2	9.3	11.2	9.8	12.4	10.2	14.9	13.5	13.8
Sinus bradycardia	6.6	5.3	7.2	7.0	7.0	6.1	9.5	5.4	8.3
Premature ventricular contractions	0	1.3	1.3	1.9	1.9	1.0	4.1	2.7	2.1
Premature atrial contractions	0	0	0	0	1.7	1.0	2.7	0	1.1
Axis	2.6	1.3	1.3	0.5	2.7	8.2	1.4	5.4	3.2
Left axis deviation	2.6	1.3	0.7	0	1.2	5.1	1.4	4.1	1.9
Horizontal QRS	0	0	0.7	0.5	1.2	2.0	0	0	1.0
QRS-ST-T	7.9	6.7	7.9	6.5	9.0	10.2	13.5	6.8	10.0
Nonspecific ST-T wave changes	5.3	5.3	5.3	2.8	4.4	6.1	9.5	4.1	5.5
T wave changes	0	0	2.0	1.4	1.5	2.0	2.7	1.4	1.8
P wave	1.3	1.3	0	0.5	0.7	0	0	5.4	1.1

7.7. Summary

7.7.1. Exposure

Over 1000 subjects were exposed to trandolapril in U.S. clinical trials. Over 200 hypertensive subjects were exposed for more than one year. Forty percent of subjects were female, 33% were Black, and 20% were over age 65.

7.7.2. Deaths and serious adverse events

There were 3 deaths among actively treated subjects in U.S. clinical trials. Two of these were the result of myocardial infarction and the third was from pulmonary embolism. Two placebo subjects died from myocardial infarction and gastrointestinal hemorrhage subsequent to cerebrovascular accident.

There were 45 deaths in European studies. These events were related to cardio-respiratory arrest, myocardial infarction, or heart failure (11), bronchial or lung carcinoma (3), pancreatic carcinoma (1), cerebrovascular accidents (3), leiomyosarcoma (1), motor vehicle accident (1), myocarditis (1), pancreatitis (2), gastric carcinoma (1), pulmonary embolus (5), sepsis (6), suicide (1), and unknown causes (10).

For none of these deaths is a relationship to treatment considered likely.

Less than 2% of subjects withdrew from U.S. studies as a result of adverse events. Serious and potentially serious adverse events contributing to withdrawal, all rare, included cerebrovascular accident, myocardial infarction, ischemic heart disease, and pulmonary embolus. All of these events except pulmonary embolus were represented among withdrawals from the placebo group, are associated with hypertension, and are not likely attributable to the study drug.

Three subjects experienced angioedema in U.S. studies. There were 2 Caucasians and one Black, one male and 2 females. Two events resolved within 3 days; in the third, the subject was placed on enalapril with no further problem noted at a 2-week follow-up. Two additional cases of angioedema came from the European experience. Follow-up for one indicated resolution with 2 days. The time course for resolution was important because of concern regarding the long half-life of trandolapril.

7.7.3. Common adverse events

The more common events were less serious in nature. Events contributing to withdrawal from studies included cough and headache, the latter of which was more common in the placebo group. Other common events with at least possible attribution to trandolapril included dizziness, fatigue, nausea, edema, and constipation.

7.7.4. Laboratory and physical abnormalities

No meaningful trend was detected with physical examinations. New cardiovascular findings were seen in 2.4% of subjects in U.S. trials, with no single finding predominating.

On ECG, sinus bradycardia was the most common abnormality cited, but mean heart rate was not significantly affected.

Laboratory data from controlled U.S. trials showed expected dose-related effects in plasma ACE and renin activities. Comparisons of baseline and on-treatment values showed trends for many laboratory parameters; baseline values below the mean tended to increase and those above the mean tended to decrease, an effect most likely representing regression to the mean.

Noteworthy outliers were found for liver enzymes, although this happened as often among placebo subjects. Some elevations were attributed to ethanol abuse. No elevation was associated with clinical findings.

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9. Overall summary and proposed action

The U.S. studies formed most of the basis for evaluation of the safety and efficacy of trandolapril. Although the foreign database is much larger, the sponsor failed to provide study reports for many non-IND studies. The sponsor appears to have captured deaths and withdrawals from a database of 19,000 subjects. Other safety data come from a much smaller sample.

9.1. Preclinical findings

Trandolapril is an orally absorbable drug de-esterified by the liver to a more potent angiotensin converting enzyme inhibitor, trandolaprilat. There is no evidence to suggest a second mechanism contributes to effects of trandolapril on blood pressure.

Chronic toxicity with trandolapril was highlighted by glomerulonephritis, appearing at doses greater than 10-fold higher (on a weight-adjusted basis) than those producing maximal antihypertensive effects in man. The toxicity of trandolapril was similar to that seen with other ACE inhibitors.

Fetal loss in monkeys occurred at doses greater than 15-fold higher (on a weight-adjusted basis) than those producing maximal antihypertensive effects in man.

Carcinogenicity, mutagenicity, and clastogenicity screening were negative.

9.2. Pharmacokinetics

Upon oral administration under fasting conditions, plasma trandolapril levels rise to a peak at about 1 hour and decline to immeasurable levels within 8 hours. Trandolaprilat levels peak at about 8 hours and are about 50% of peak at 24 hours and about 10% of peak at 6 weeks.

Intravenous administration of trandolapril leads to 24% recovery of dose in urine as trandolaprilat, while intravenous administration of trandolaprilat leads to 70% recovery in urine. The difference supports hepatic first-pass metabolism as the predominant mechanism for conversion of trandolapril to trandolaprilat. However, in subjects with hepatic impairment, C_{max} and AUC for trandolaprilat were increased, suggesting that the role of the liver in metabolism is affected preferentially.

With oral administration of trandolapril, multiple conjugated and de-esterified metabolites are excreted in urine and feces, account for 33% and 66% of a dose, respectively.

Multiple dosing at a dose producing a maximum effect on blood pressure produces no detectable accumulation of trandolapril and about a 50% increase in the trough plasma concentration of trandolaprilat.

The effect of food is to delay the time to peak plasma concentration of trandolaprilat, but the effects on ACE inhibition were not clinically significant.

Drug interactions with digoxin, cimetidine, and furosemide were not clinically significant.

There is a need for downward adjustment of dose in patients with hepatic impairment or with renal impairment.

The to-be-marketed tablets were shown to be bioequivalent, with respect to trandolaprilat, to the capsules used in pivotal clinical trials.

9.3. Pharmacodynamics and efficacy

Once daily dosing with trandolapril was shown to be antihypertensive in separate adequate, placebo-controlled, U.S. dose-ranging studies in Caucasians and Blacks. The mean magnitude of the effect on supine diastolic pressure at trough was about

5 mmHg at doses from 4 to 16 mg once daily. The lowest dose with a statistically significant effect at trough is 0.5 mg.

Comparable effects on blood pressure were obtained in Caucasians and Blacks, in males and females, and in the young and elderly.

The peak antihypertensive effect occurs about 4 hours after dosing. The effect of a single dose lasts more than 24 hours. Twice-daily dosing produces somewhat greater effects at the inter-dosing interval than does once-daily dosing. With repetitive once-daily dosing, the antihypertensive effect reaches a plateau within a week. With continued once-daily dosing, the antihypertensive effects of trandolapril persist for at least 6 weeks. After 6 weeks of once-daily dosing, there are diminishing effects on blood pressure persisting for at least 3 weeks.

The combination of trandolapril plus HCTZ produces larger antihypertensive effects than does trandolapril alone.

The mechanism of action of trandolapril in man appears to be inhibition of angiotensin converting enzyme. There is little hysteresis in the relationship between plasma trandolaprilat concentration and plasma ACE inhibition.

9.4. Safety

Over 1000 subjects participated in U.S. clinical trials. Over 200 hypertensive subjects were treated for more than 1 year.

Three deaths occurred in the U.S. studies. There were 45 deaths in the European studies of around 19,000 subjects, which, assuming a similar distribution of exposure, is a similar death rate. Although the death rate is higher than expected in uncomplicated hypertension, it is not remarkably different than that found in other ACE inhibitor development programs, and may result from selection bias for clinical trial participation. For none of the deaths was the study drug thought a likely contributing factor.

Less than 2% of subjects in U.S. studies withdrew because of adverse events. Serious adverse events were rare and generally consistent in character and frequency with expectations for hypertension. Angioedema was rare.

Common adverse events were similar to those seen with other ACE inhibitors. Headache and cough were leading causes of withdrawal, but headache was more common in the placebo group than among subjects receiving trandolapril.

Because of the long plasma half-life of trandolaprilat, an effort was made to assess the time course for resolution of adverse events thought most likely drug-related. Cough and angioedema cases did not appear to be unusual in regard to severity, difficulty of treatment, or time course for resolution.

The European data, as submitted, were consistent with the U.S. experience.

9.5. Summary and recommendation

Trandolaprilat has a longer terminal half-life than does any approved ACE inhibitor. Plasma ACE inhibition is detectable, although not a controlling factor in blood pressure, for months following a single dose of trandolapril. There were no evident safety implications of this finding.

Trandolapril has demonstrated quite similar antihypertensive effects in Caucasians and in Blacks not receiving a diuretic.

In other respects, the overall risk-benefit assessment for trandolapril appears to be similar to that for previously approved drugs with a similar mechanism of action. A 1 mg starting dose is probably reasonable in most patients, with 0.5 mg to be used in

patients on a diuretic, with renal impairment, or with hepatic disease. These doses are half those proposed by the sponsor. Doses above 4 mg appear to offer no additional benefit. Twice daily dosing or addition of HCTZ are reasonable steps in patients not deriving an adequate response to 4 mg once daily dosing.

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A. Individual study reports

A1. Study 90/2183/CN (Study report: vol 1.123; case report tabulations: 1.123-1.124; case report forms for deaths and discontinuations for adverse events: none) "Investigation into a possible pharmacokinetic and pharmacodynamic interaction between nifedipine (sustained release form) and trandolapril administered in a single dose to healthy volunteers".

A1.1. Investigators The investigator was J Granier, Pontoise, France.

A1.2. Study dates September 1988 to November 1988.

A1.3. Drug formulation

Table 29. Drug substance used in Study 90/2183/CN.

Lot	Form	Type	Trandolapril	Source lot	Batch size
MB 21662-63	Cap	Tran	4.0 mg	17	40,000

Table 30. Capsule composition in Study 90/2183/CN.

	4 mg
Trandolapril	4.0
Corn starch	
Lactose, hydrous	
Povidone K 25	
Sodium stearyl fumarate	
Total	

A1.4. Study design This open-label, uncontrolled, 3-way crossover study assessed kinetic and dynamic drug-drug interactions under fasting conditions in 12 healthy adult males randomized (on 3 discrete treatment days separated by 15 day intervals) to a single dose of trandolapril (4 mg), a single dose of sustained release nifedipine (20 mg), or to their combination.

The exclusion criteria are shown in Table 31 below.

Table 31. Exclusion criteria in Study 90/2183/CN.

Weight not appropriate for height
Presence of hypersensitivity, malignancy, or any disease of the renal, hepatic, cardiovascular, neurological, endocrine, or gastrointestinal systems
Regular use of medication or ethanol
Use in the previous 3 months of a drug with the potential for major organ toxicity, or large quantities [undefined] of tea, coffee or tobacco
Inability to follow dietary restriction
Participation in another clinical trial in the previous 3 months

Supine and standing blood pressure, and heart rate were determined at baseline (pre-treatment), and at post-dose hours 1.25, 2.25, 3.25, 4.25, 6.25, 8.25, and 10.25. Blood samples for the determination of ACE activity, plasma renin activity, and aldosterone were collected at baseline, and at post-dose hours 2, 4, 6, 8, 12, and 24.

Student's *t* test for paired data was used to assess differences between the trandolapril monotherapy group and the trandolapril plus nifedipine combination group. Similarly, this test was applied to comparisons of nifedipine monotherapy to the combination.

A1.5. Results

The demographic characteristics of the subjects are presented in Table 32 below.

Table 32. Demographic and baseline characteristics of subjects in Study 90/2183/CN.

	n=19
Male	12
Female	0
Caucasian	12
Other	0
Age—mean	31.8
Weight—mean	74.6

There were reportedly no dropouts.

Changes from baseline blood pressure are shown in Figure 39 below. Conclusive

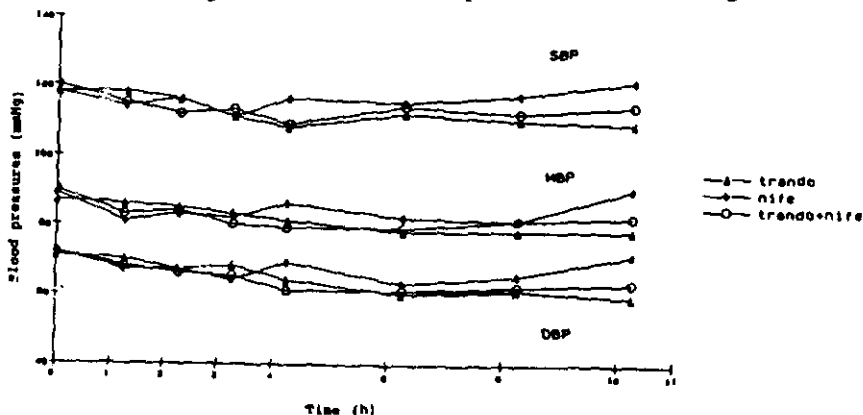


Figure 39. Blood pressure effects in Study 90/2183/CN.

inferences as to the additivity of drug effects are not obtainable here in the absence of placebo monotherapy control or "dummy pill" control. The average blood pressure lowering effect associated with either monotherapy appeared to be comparable to or slightly larger than the effect associated with the combination. Nifedipine monotherapy appeared to have a somewhat greater peak pressure lowering effect than did either trandolapril monotherapy or the combination.

Changes from baseline heart rate are shown in Figure 40 below. Roughly 10 bpm mean heart rate elevations were observed at 6 hours post-dose in all treatment periods, without production of absolute mean tachycardia.

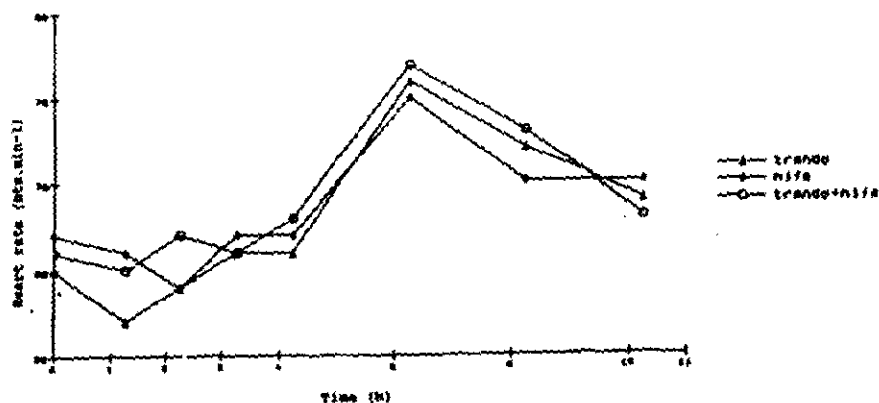


Figure 40. Mean heart rate in Study 90/2183/CN.

The extent of ACE inhibition is depicted in Table 33 below. Nifedipine had no

Table 33. ACE inhibition in Study 90/2183/CN.

	Trand alone	Nifed alone	Comb
ACE inhibition (%)			
Trough	83.9	-2.	78.2
Peak	94.8	-1.1	93.1

appreciable effect on the trandolapril-associated inhibition of this enzyme.

Plasma renin activity and plasma aldosterone concentration are depicted in Figure 41 below. Renin levels appeared to be comparably increased by trandolapril whether in the presence or absence of nifedipine. Aldosterone levels were increased in association with nifedipine monotherapy, but were decreased in the other treatment phases.

A1.6. Safety.

Case report forms were not submitted for evaluation. In the study report there is no explicit disclaimer that there were no deaths.

Only brief descriptions of adverse effects were provided. Two subjects experienced headache during trandolapril monotherapy, whereas 9 and 7 subjects had this complaint during combination and nifedipine monotherapy, respectively.

There were three notable treatment-emergent laboratory abnormalities, as follows:

Subject 1 manifested a moderate further elevation of baseline eosinophilia (from 7% to 9% at the end of the trial), although this was plausibly attributable to a nondrug factor (i.e. a recent parasitic infection).

Subject 8 had a small, isolated elevation of SGPT (38 IU/L), but this began after exposure to nifedipine monotherapy and was reportedly transient.

Subject 11 had a slight, isolated elevation of total serum bilirubin (19 µM) at the end of the trial. The report did not contain a description of the reversibility, or sequelae (if any) of this finding.

A1.7. Summary.

This open-label crossover study assessed the non-steady-state kinetic and dynamic interactions between single doses of trandolapril (4 mg) and nifedipine (20 mg) in 12 healthy males. These were not placebo-controlled data, and thus were not adequate:

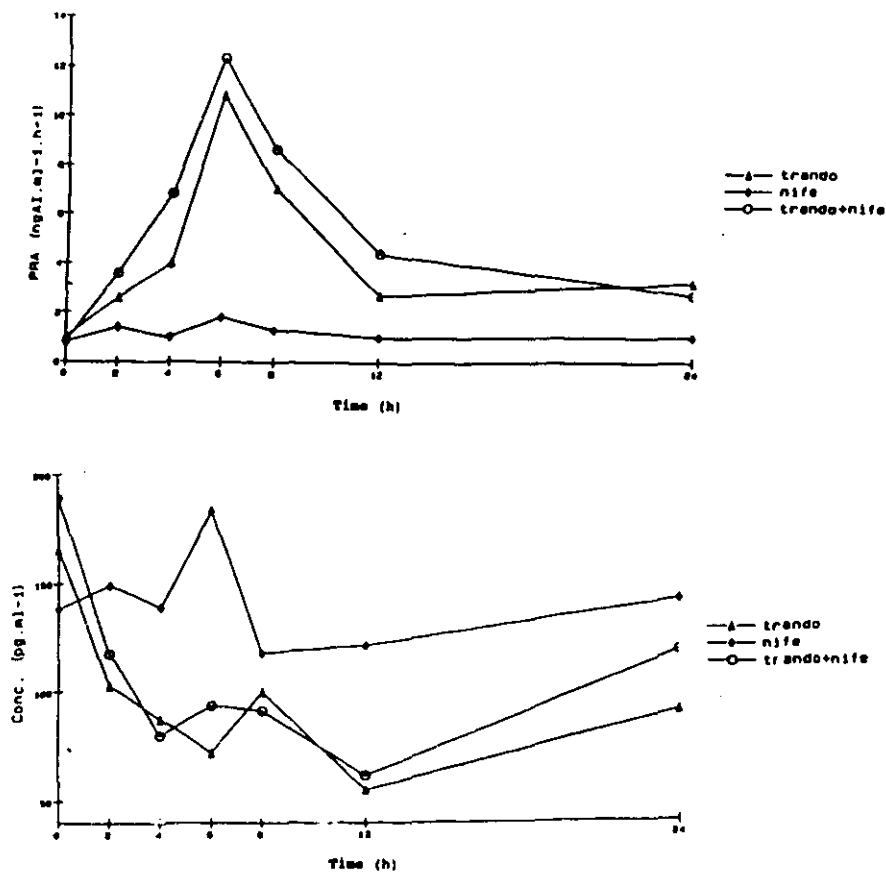


Figure 41. Plasma renin activity and aldosterone in Study 90/2183/CN.

for a conclusive assessment of effect additivity. Nifedipine had no apparent effect on either trandolapril-associated inhibition of ACE or trandolapril-associated peak heart rate elevation (which was moderate). The average blood pressure lowering effect associated with either monotherapy appeared to be similar or slightly larger than the effect associated with the combination. Nifedipine monotherapy appeared to have a somewhat greater peak pressure lowering effect than did either trandolapril monotherapy or the combination. There were no safety-related withdrawals, but there was one laboratory abnormality (a minimally elevated total serum bilirubin) for which a causal role of trandolapril cannot be excluded.

A2. Study CH/86/570/01 (Study report: vol 1.127; case report tabulations: vol 1.127; case report forms for deaths and discontinuations for adverse events: none) "Study of the neurohormonal and pharmacodynamic effects of trandolapril following a single dose administration and repeated doses over ten days in healthy volunteers".

A2.1. Investigators The clinical investigator was H Brunner, Lausanne, Switzerland.

A2.2. Study dates April 1986 to August 1986.

A2.3. Drug formulation

Table 34. Drug substance used in Study CH/86/570/01.

Lot	Form	Type	Trandolapril	Source	Batch size
MMG 20966-83	Cap	Tran	0.5 mg	11	450
LL 20898-59	Cap	Tran	2.0 mg	6	300
MMG 20966-77	Cap	Tran	4.0 mg	11	450

Table 35. Capsule composition in Study CH/86/570/01.

	0.5 mg	2 mg	4 mg
Trandolapril	0.5	2.0	4.0
Lactose, hydrous			
Total			

A2.4. Study design

This uncontrolled, single-blind, parallel-group study randomized 21 healthy adult male subjects (7 per group) to receive once-daily oral trandolapril (0.5, 2, or 8 mg/day) for 10 days. Drug was to be taken in the morning after an overnight fast. The groups were studied in a sequential fashion, starting with the 0.5 mg/day group. The 0.5 mg dose was administered as one 0.5 mg capsule, the 2 mg dose as one 2 mg capsule, and the 8 mg dose as two 4 mg capsules. Subjects were to refrain from the use of tobacco or ethanol (starting 48 hours before the study), and to maintain their usual dietary sodium intake. The objective was to assess pharmacodynamics and safety.

The exclusion criteria are shown in Table 36 below.

Table 36. Exclusion criteria in Study CH/86/570/01.

Weight 15% more or less than ideal
Presence of hypersensitivity, malignancy, or any disease of the cardiovascular, renal, hepatic, gastrointestinal, endocrine, or neurological system
Use in the previous 3 months of a drug with the potential for toxicity
Inability to follow treatment or dietary restriction
Participation in another clinical trial in the previous 3 months
Daily consumption of >10 cigarettes, >6 cups of coffee or tea, ethanol, or any pharmaceutical during the trial

Blood pressure and heart rate were determined at baseline; at 2, 4, 6, and 24 hours after the first dose; at the end of dosing days 3, 4, 5, 6, 7, 8, 9, and 10; at 2, 4, 6, and 24 hours after the final dose, and at 1, 2, 3, and 7 days following the final dose.

Blood samples for the determination of trandolaprilat levels were collected at baseline (pre-treatment); at 2, 4, 6, and 24 hours after the first dose; at the end of dosing days 3, 4, 5, 6, 7, 8, and 9; at 2, 4, and 6 hours after the final dose; and at 1, 2, 3, and 7 days following the final dose.

Blood samples for the determination of ACE activity were collected at baseline; at 2, 4, 6, and 24 hours after the first dose; at the end of dosing days 3, 4, 5, 6, 7, 8, and 9; and at 1, 2, 3, and 7 days following the final dose.

Blood samples for the determination of plasma renin activity and aldosterone were collected at baseline; and at 2, 4, 6, and 24 hours after the first and final doses.

Blood samples for the determination of atrial natriuretic factor were collected at baseline; at 4 hours after the first and final doses, and just prior to the final dose.

Blood samples for the determination of angiotensin I and II levels were collected at baseline; at 2, 4, 6, and 24 hours after the first dose; just prior to the final dose; at 4 hours after the final dose; and at 1, 2, 3, and 7 days following the final dose.

ANOVA was used for analyses, and Tukey's test was employed for pair-wise comparisons.

Hematology and clinical chemistry measures were obtained at baseline; during dosing days 4 and 7; and 24 hours after the final dose.

A2.5. Results

Demographic and baseline characteristics of subjects are shown in Table 37 below.

Table 37. Demographic and baseline characteristics of subjects in Study CH/86/570/01.

	0.5 mg n=7	2 mg n=7	8 mg n=7
Male	7	7	7
Female	0	0	0
Age mean	23.8	24.4	24.7
Weight mean	66.7	71.5	71.1
Renin (ng/ml/h)	1.2	0.7	1.6
ACE activity (nmol/ml/min)	112	92	77
Aldosterone (pg/ml)	88	51	66

There were imbalances in baseline neurohormonal status (renin activity being statistically significantly higher in the 8 mg/day group than in the 2 mg/day group, aldosterone levels being significantly higher in the 0.5 mg/day group than in the 2 mg/day group, and ACE activity tending to be higher in the 0.5 mg/day group than in the 8 mg/day group).

Sitting blood pressure and pulse data were analyzed for the first 24 hours post-dosing on days 1 and 11. Blood pressure reductions were generally dose-dependent.

There were reportedly no dropouts.

Blood pressures are shown in Figure 42 below. Apparent diastolic pressure reductions were generally more pronounced for the 8 mg/day dose than for the lower doses. For the 8 mg/d trandolapril dose the mean trough (24 hour post-dose) change from baseline ranged from approximately 2.5 to 10 mmHg over 10 days, and this apparent diastolic antihypertensive effect did not disappear 7 days after the drug's discontinuation. Apparent systolic pressure changes were also generally more pronounced for the 8 mg/day dose than for the lower doses. At the highest dose the mean trough change ranged from approximately 0 to 12 mmHg over the course of treatment. For the higher doses, some degree of systolic effect appeared to persist at 3 days post-withdrawal, but none was apparent at 7 days post-drug.

Heart rate changes were clinically nonsignificant.

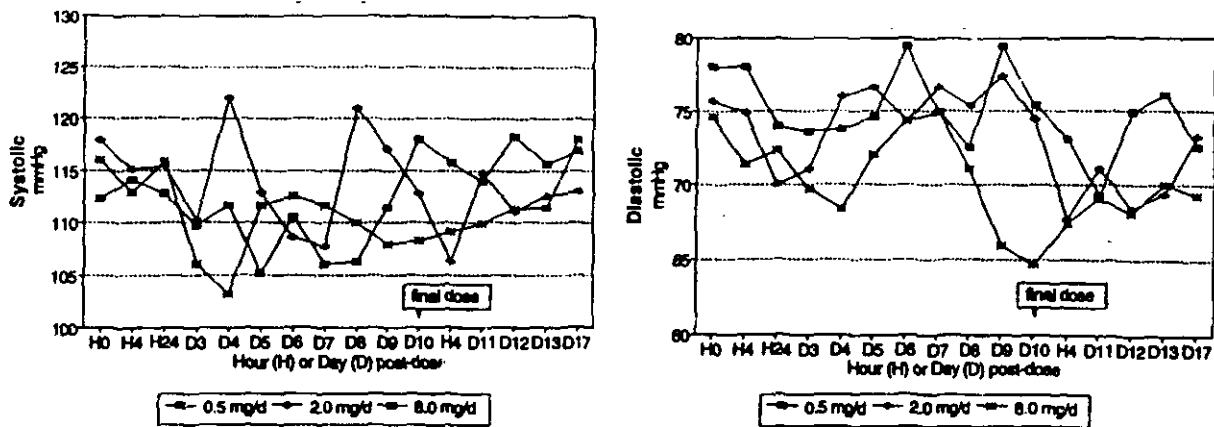


Figure 42. Blood pressures in Study CH/86/570/01.

Trandolaprilat levels were generally detectable at trough in the two higher dose groups, and tended to increase monotonically with dose.

Plasma ACE activity is depicted in Figure 43 below. A dose-related inhibition of ACE

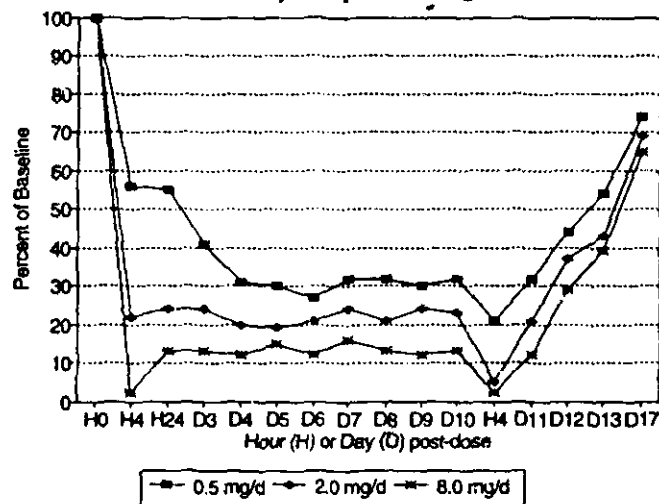


Figure 43. Plasma ACE inhibition in Study CH/86/570/01.

was observed, with 25 to 35% apparent inhibition still evident by 7 days after the drug's discontinuation.

Plasma renin activity and aldosterone are depicted in Figure 42 below. The higher doses produced transient, dose-related increases in renin which were apparent at 4 hours post-dose. Transient, dose-related decreases in aldosterone levels were apparent at 4 hours post-dose.

The ratio of angiotensin II to angiotensin I is shown in Figure 45 below. Decreases in angiotensin II/I ratios were apparent, and these were generally dose-related.

Atrial natriuretic factor levels were not appreciably affected by treatment.

A2.6. Safety.

Case report forms were not submitted for evaluation. There were reportedly no recorded adverse events, and no dropouts. Subject 19, a 22 year old male, had transient treatment-emergent leukopenia (with a WBC count nadir of 3.3 K/nl) after exposure to 8 mg/day trandolapril. The report did not contain a description of the clinical sequelae of this event (if any).

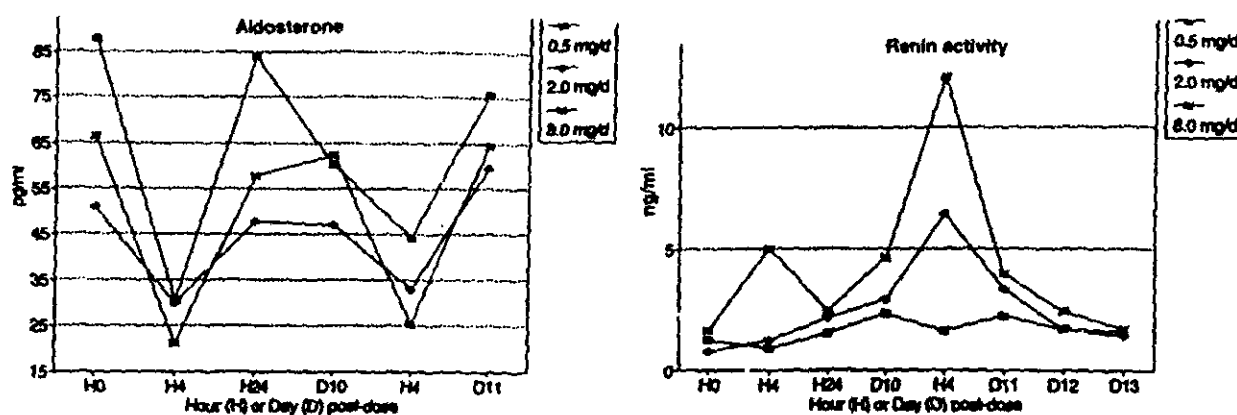


Figure 44. Plasma renin activity and aldosterone in Study CH/86/570/01.

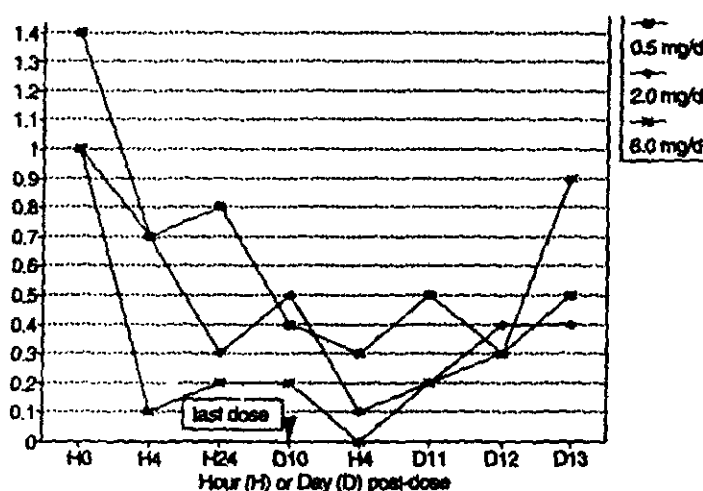


Figure 45. Ratio of angiotensin II to angiotensin I in Study CH/86/570/01.

d.

No other potentially clinically significant, treatment-emergent laboratory abnormalities were apparent.

A2.7. Summary.

This uncontrolled, single-blind, parallel-group study randomized 21 healthy adult male subjects to receive oral trandolapril (0.5, 2, or 8 mg/day) for 10 days. Three different formulations were used. Transient, dose-related increases in renin and aldosterone levels, and decreases in angiotensin II/I ratios were observed. A dose-related inhibition of ACE was also apparent, with 25-35% apparent inhibition still evident by 7 days after the drug's discontinuation. There is need for caution in taking this as evidence of long-persisting drug effect: in study F/91/570/133, the placebo group manifested this same extent of apparent inhibition of ACE activity. Apparent mean blood pressure reductions from baseline were small to modest, and generally more pronounced for the 8 mg/day dose than for the lower doses. The diastolic pressure lowering did not appear to fully dissipate by 7 days after the drug's discontinuation, and some degree of systolic effect appeared to persist at 3 days post-withdrawal. These apparently persistent blood pressure effects were no larger than those plausibly attributable to placebo. There was one case of transient leukopenia with no description of its clinical sequelae.

A3. Study F/85/570/01 (Study report: vols 1.125; case report tabulations: vol 1.125; case report forms for deaths and discontinuations for adverse events: none) "Oral tolerance study of in single, increasing doses in healthy volunteers".

A3.1. Investigators The investigator was J Granier, Pontoise, France.

A3.2. Study dates October 1985 to January 1986.

A3.3. Drug formulation

Table 38. Drug substance used in Study F/85/570/01.

Lot	Form	Type	Trandolapril	Samples	Batch size
LL 20532-157	Cap	Tran	0.125 mg	6	300
LL 20532-155	Cap	Tran	0.25 mg	6	220
LL 20532-153	Cap	Tran	1.0 mg	6	150
LL 20532-151	Cap	Tran	2.0 mg	6	150
LL 20989-21	Cap	Tran	2.0 mg	6	150
LL 20898-23	Cap	Tran	10.0 mg	6	250

Table 39. Capsule composition in Study F/85/570/01.

	0.125 mg	0.25 mg	0.5 mg	1 mg	2 mg	10 mg
Trandolapril	0.125	0.25	0.5	1.0	2.0	10
Lactose, hydrous						
Total						

A3.4. Study design This placebo-controlled, double-blind, parallel-group study randomized 90 healthy adult males to receive (under fasting conditions) either placebo (n=30), or a single oral trandolapril dose (0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 24, or 32 mg; n=6 per treated arm). The objectives were to assess pharmacodynamics, and safety.

Enrolled subjects were healthy adult males. The exclusion criteria are shown in Table 40 below.

Table 40. Exclusion criteria in Study F/85/570/01.

Weight 15% more or less than ideal
Presence of hypersensitivity, malignancy, or a disease of the cardiovascular, renal, hepatic, gastrointestinal, or neurological system
Daily consumption of > 10 cigarettes, > 6 cups of coffee or tea, ethanol or any pharmaceutical during the trial
Previous participation in a clinical trial within 3 months

Supine blood pressure, and heart rate were determined at baseline (pre-treatment), every 15 minutes during post-dose hours 0-4, every 30 minutes during post-dose hours 5-10, and on post-dose days 1, 2, 3, and 8.

Blood samples for the determination of ACE activity were collected at baseline, at post-dose hours 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 24, and on post-dose days 2, 3, and 8.

Hematology, and chemistry measures were obtained at baseline and during the final examination.

A two-way ANOVA was undertaken (with application of the least squares criterion to the general linear model) to assess differences before and after treatment.

A3.5. Results

Demographic and baseline characteristics of subjects are shown in Table 41 below.

Table 41. Demographic and baseline characteristics of subjects in Study F/85/570/01.

	Placebo (n=30)	Trandolapril									
		2.5 mg (n=6)	5 mg (n=6)	10 mg (n=6)	2 mg (n=6)	4 mg (n=6)	8 mg (n=6)	16 mg (n=6)	24 mg (n=6)	32 mg (n=6)	
Male	30	6	6	6	6	6	6	6	6	6	6
Female	0	0	0	0	0	0	0	0	0	0	0
Age mean	25	23	27	25	26	24	24	24	25	26	24
Weight mean	67	72	70	69	70	66	69	72	72	71	70
SysBP mean	118	123	125	118	119	118	127	122	117	122	124
DiasBP mean	66	70	76	64	65	70	71	67	63	66	66
HR mean	57	62	62	57	59	66	59	62	63	59	55

The treatment groups were reasonably well balanced with respect to demographic and pre-treatment characteristics.

Compliance was 100% and there were reportedly no dropouts. No protocol violations were described.

Changes from baseline blood pressures are shown in Figure 46 (diastolic) and Figure 47 below (systolic). Small to moderate peak blood pressure effect was observed with the lower doses (0.125 to 2 mg), and trough (24 hours post-dose) effects were small to none. With higher doses (4 to 32 mg), there was moderate to large peak effect, and small to no effect at trough. Mean peak effects were seen at 2 to 5 hours. Mean pressure-lowering effect generally disappeared by 24 to 36 hours after dosing, but appeared to have greater persistence in some groups (with this persistence bearing no apparent relationship to dose).

Heart rate changes from baseline were clinically nonsignificant.

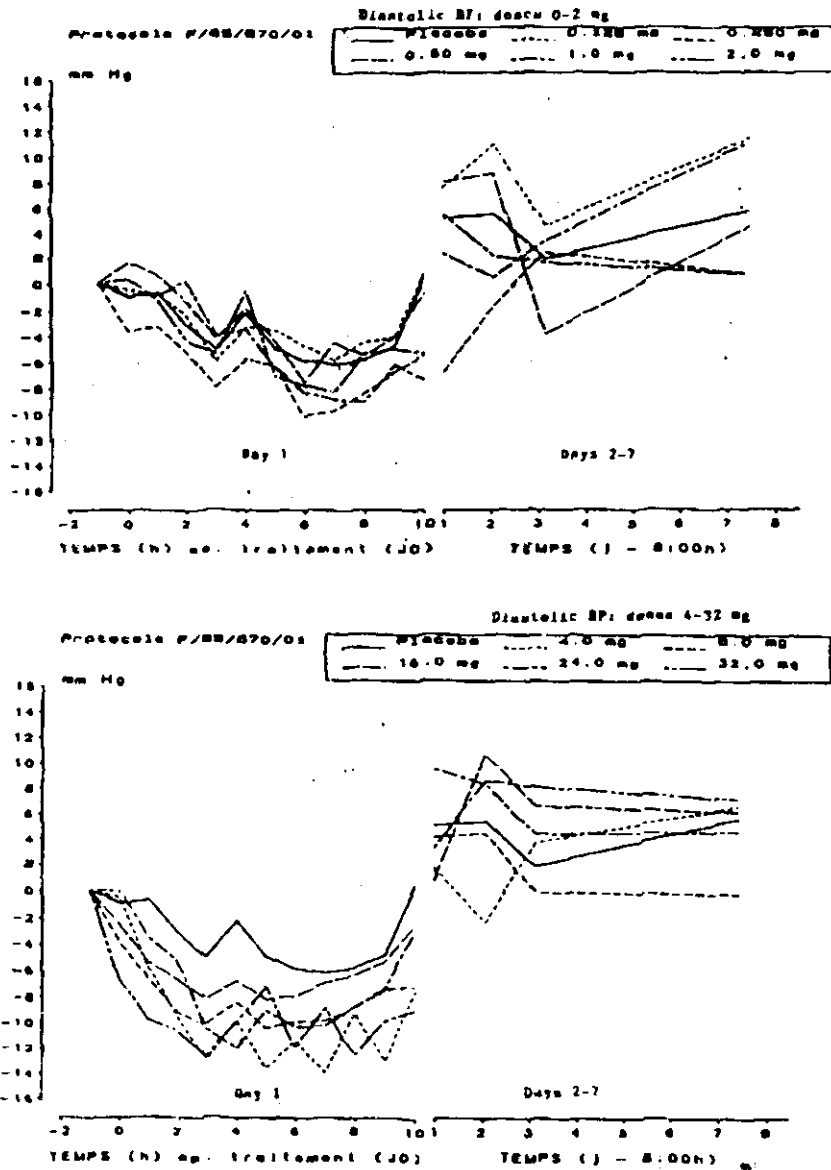


Figure 46. Diastolic pressure changes in Study F/85/570/01.

The extent of ACE inhibition is depicted in Figure 48 and Table 42 below. There is

Table 42. ACE inhibition in Study F/85/570/01.

	Plcbo n=30	Trandolapril									
		125 µg n=6	250 µg n=6	500 µg n=6	1 mg n=6	2 mg n=6	4 mg n=6	8 mg n=6	16 mg n=6	24 mg n=6	32 mg n=6
Baseline (U/L)	61	61	61	61	60	51	38	46	42	45	60
Inhibition Peak	14	33	53	78	88	95	97	99	99	98	98
Trough	1	22	44	60	75	81	95	89	88	92	87

some evidence of a dose-response apparent.

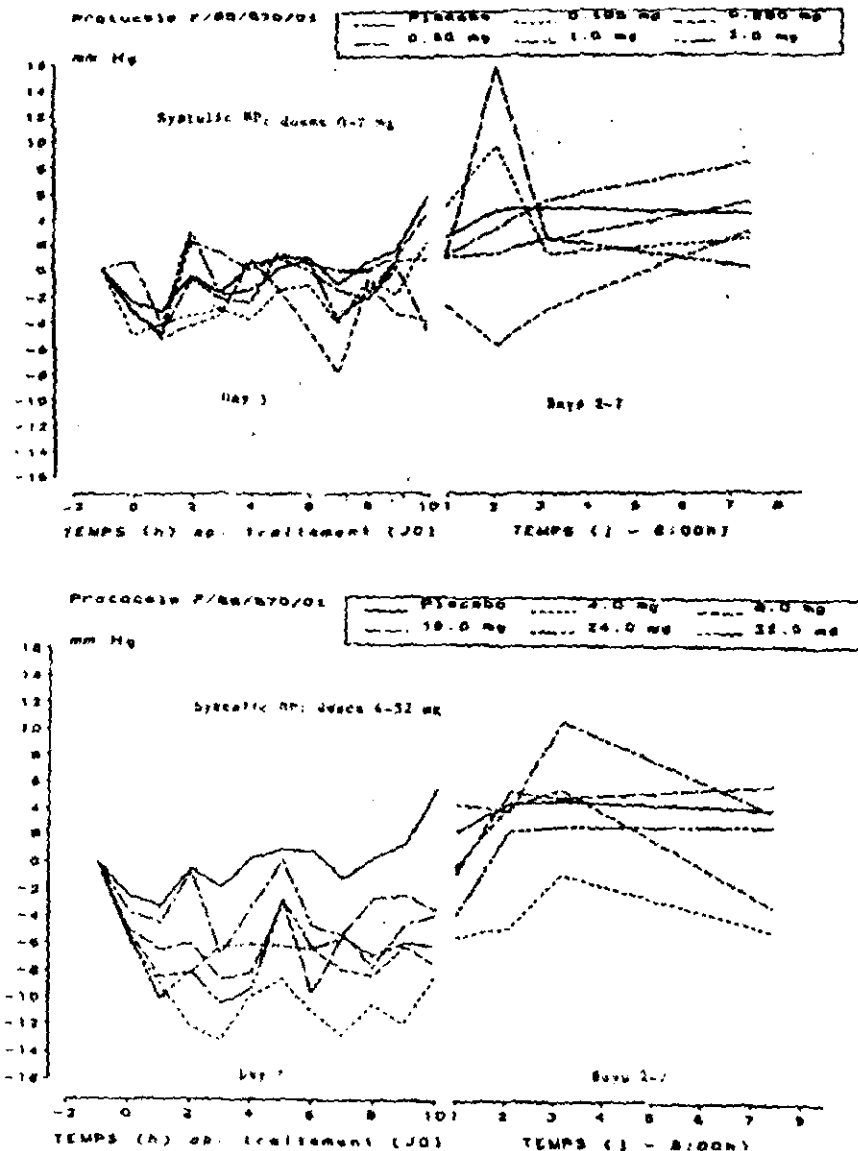


Figure 47. Systolic pressure changes in Study F/85/570/01.

A3.6. Safety.

Case report forms were not submitted for evaluation. In the study report there is no explicit disclaimer that there were no deaths, but it was reported that there were no dropouts. Only brief descriptions of adverse effects were provided. Among trandolapril-exposed subjects, 7 (12%) complained of headache (as did 6 placebo subjects (20%)) of mild to great severity but without apparent relationship to dose, 1 complained of weakness (as did 1 placebo subject), 1 had vomiting and diarrhea, and 1 experienced drowsiness.

Among trandolapril-exposed subjects, there were several treatment-emergent laboratory abnormalities: several had shifts in white cell differentials with only one demonstrating an abnormality (i.e., a minimal reduction) of total white cells, two subjects had isolated minimal elevations of SGOT (as did two placebo subjects), one had mild hyperuricemia, and one had minimal hypokalemia.

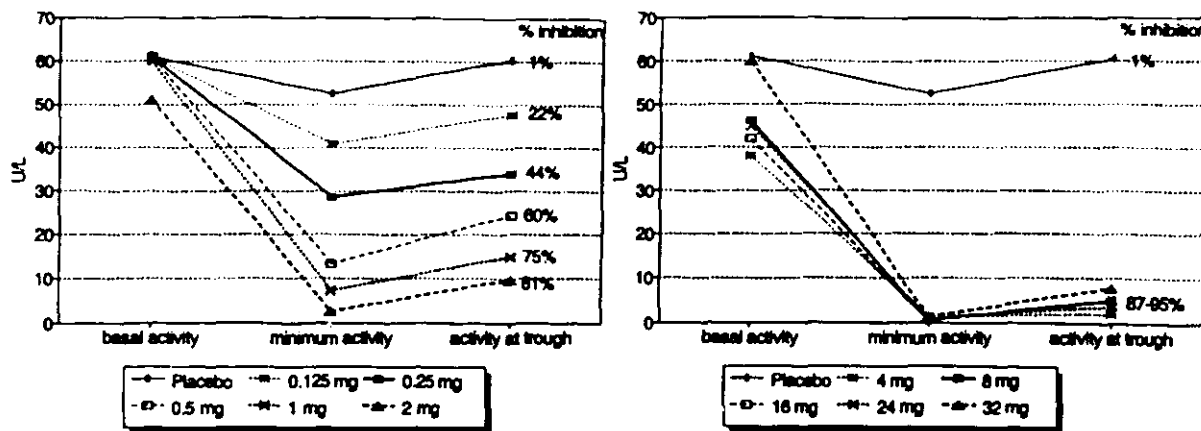


Figure 48. ACE inhibition in Study F/85/570/01.

A3.7. Summary.

This study randomized 90 healthy adult males to receive either placebo or a single oral trandolapril dose (0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 24, or 32 mg). The mean inhibition of ACE ranged from approximately 30 to 99% at peak and 20 to 95% at trough. Heart rate changes were clinically nonsignificant. With the high trandolapril doses (4 to 32 mg) there was moderate to large peak blood pressure effect, and small to no effect at trough. Small doses generally had less pressure-lowering effect. Mean peak effects were seen at 2 to 5 hours. Mean pressure-lowering effect generally disappeared by 24 to 36 hours after dosing, but appeared to have greater persistence in some groups (with this persistence bearing no apparent relationship to dose). There were reportedly several adverse events (including laboratory abnormalities), each of which was clinically nonserious. There were no reports of safety-related withdrawals.

A4. Study F/85/570/03 (Study report: vols 1.126; case report tabulations: vol 1.126; case report forms for deaths and discontinuations for adverse events: none) "Pharmacokinetics and pharmacodynamics of administered orally to healthy human subjects (single dose)".

A4.1. Investigators The investigator was J Granier, Pontoise, France.

A4.2. Study dates Unstated; report dated November 1987.

A4.3. Drug formulation

Table 43. Drug substance used in Study F/85/570/03.

Lot	Form	Type	Trandolapril	Source lot	Batch size
LL 20898-57	Cap	Plcbo	—	—	300
LL 20898-59	Cap	Tran	2.0 mg	6	300

Table 44. Capsule composition in Study F/85/570/03.

	2 mg
Trandolapril	2.0
Lactose, hydrous	
Total	

A4.4. Study design

This placebo-controlled, double-blind, crossover study sequence-randomized 12 healthy adult males to receive either placebo, or a single oral 4 mg trandolapril dose. Treatment periods were separated by a 3 week interval. The objectives were to assess pharmacodynamics, pharmacokinetics, and safety.

The exclusion criteria are shown in Table 45 below.

Table 45. Exclusion criteria in Study F/85/570/03.

Weight 15% more or less than ideal
Presence of hypersensitivity, malignancy, or a disease of the cardiovascular, renal, hepatic, gastrointestinal, or neurological system
Daily consumption of >10 cigarettes, >6 cups of coffee or tea, ethanol or any pharmaceutical during the trial
Previous participation in a clinical trial within 3 months

Blood pressure, and heart rate were determined via oscillometry (Dinamap 1846 device) at baseline (pre-treatment), and every 30 minutes during post-dose hours 0 to 4.5 and 6 to 10.

Blood samples for the determination of trandolapril and trandolaprilat levels were collected at baseline, at post-dose hours 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, and 34, and on post-dose days 2, 3, and 4. A value of zero was imputed for drug concentrations below the limit of detection.

Blood samples for the determination of ACE activity were collected at baseline, at post-dose hours 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24, and on post-dose days 2, 3, 4, 5, 8, 10, 12, 15 and 18.

Blood samples for the determination of plasma renin activity and aldosterone were collected at baseline, at post-dose hours 0.5, 1, 2, 4, 6, 8, 12, 16, and 24, and on post-dose days 2, 3, and 4.

Hematology, and chemistry measures were obtained at baseline and during the final examination.

Statistical analyses employed a 5-factor ANOVA model.

A4.5. Results

Demographic and baseline characteristics of subjects are shown in Table 46 below.

Table 46. Demographic and baseline characteristics of subjects in Study F/85/570/03.

	Placebo n=9	Trandolapril n=9
Male	6	6
Female	0	0
Age mean	25.7	27.3
Weight mean	64.7	72.3

Compliance was reported to be 100%, and there were reportedly no dropouts.

Changes from baseline blood pressures are shown in Figure 49 below. Moderate peak diastolic pressure reduction was observed, and small trough (24 hours post-dose) effect. Mean peak diastolic effect was seen at 6 hours. Mean diastolic pressure-lowering effect disappeared by 72 hours after dosing. There were generally lesser systolic blood pressure effects.

Heart rate changes from baseline were clinically nonsignificant.

Drug was present at low levels at post-dose hour 8, and metabolite was present at low levels at trough.

The extent of ACE inhibition, plasma renin activity, and plasma aldosterone concentrations are depicted in Table 47 below. Trandolapril increased renin levels

Table 47. Renin, aldosterone, and ACE activity in Study F/85/570/03.

	Plcbo n=12	Trand n=12
Renin		
Baseline	1.03	1.12
Trough	1.38	4.69
Aldosterone		
Baseline	219	231
Trough	261	271
ACE inhibition (%)		
Trough	0	77.5
Peak	5.7	95.5

more so than did placebo, but aldosterone changes were similar in the two groups.

A4.6. Safety.

Case report forms were not submitted for evaluation. In the study report there is no explicit disclaimer that there were no deaths or discontinuations for adverse events.

Only brief descriptions of adverse effects were provided. Two of the subjects had headache during trandolapril therapy and 3 had placebo-associated headache.

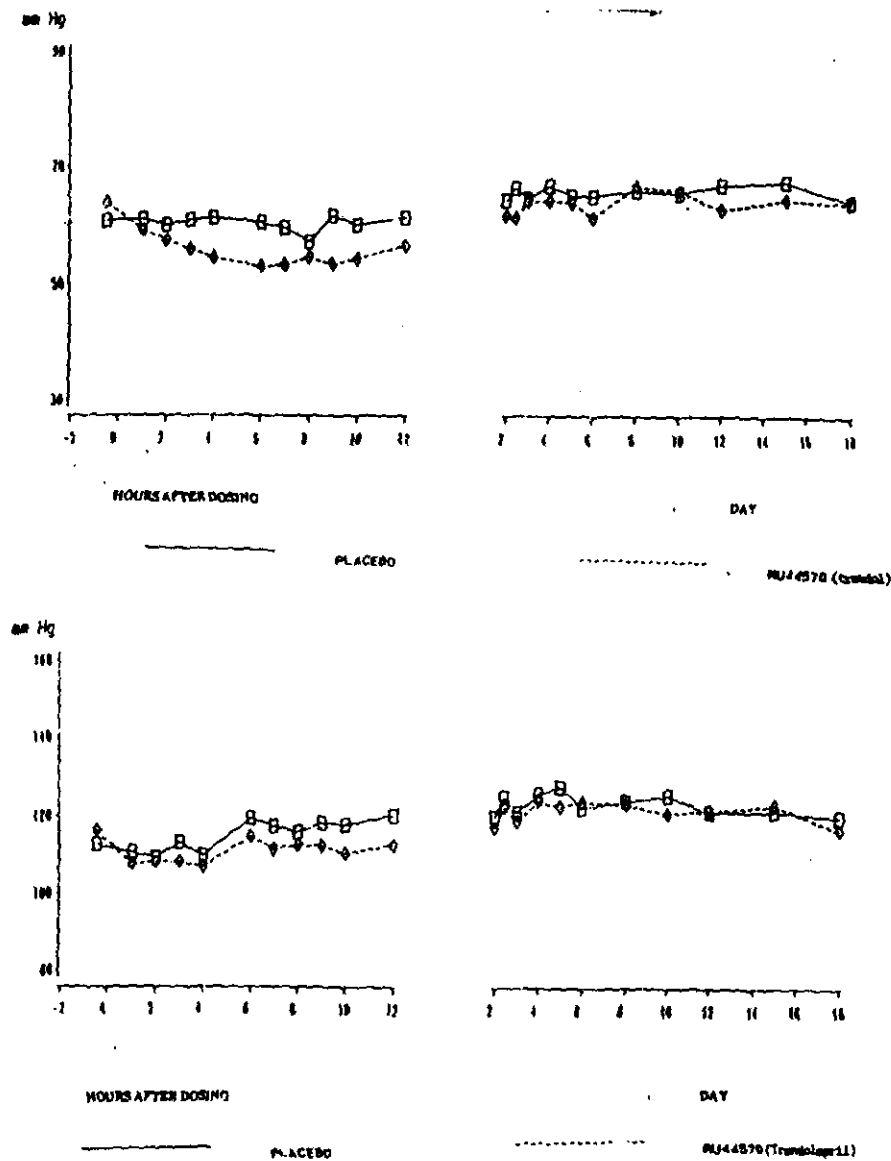


Figure 49. Diastolic (top) and systolic (bottom) pressure effects in Study F/85/570/03.

One subject, a 24 year old male (subject 9), had a normal white blood cell count and SGOT level at baseline as well as during the first (trandolapril) treatment period, but then manifested a modest leukopenia (total count 3.6 K/mm³) and elevated SGOT (to 50 U/L) during the subsequent placebo period. No other liver function tests were abnormal. The report contained no description of the reproducibility, reversibility, clinical antecedents, or sequelae of these findings.

A4.7. Summary.

Twelve healthy adult males randomly crossed-over between single doses of placebo and trandolapril 4 mg p.o. During trandolapril exposure, the mean inhibition of ACE was approximately 96% at peak, and 78% at trough. Moderate to small peak blood pressure reduction was observed (at 6 hours), and trough effect was small to nil. Mean diastolic pressure-lowering effect disappeared by 72 hours after dosing. There were no safety-related withdrawals, but one reported case of a subject in whom leukopenia and elevated SGOT developed during placebo treatment at least 3 weeks after trandolapril exposure.

A5. Study F/86/570/15 (Study report: vols 1.87; case report tabulations: vols 1.87-1.88; case report forms for deaths and discontinuations for adverse events: none) "Pharmacokinetics of trandolapril after the single oral administration of 2, 4, 12, and 24 mg".

A5.1. Investigators The investigator was G Strauch, Paris, France.

A5.2. Study dates November 1986 to February 1987.

A5.3. Drug formulation

Table 48. Drug substance used in Study F/86/570/15.

Lot	Form	Type	Trandolapril	Strength	Batch Size
LL 21353-33	Cap	Tran	2.0 mg	11	150
LL 21353-31	Cap	Tran	4.0 mg	11	150
LL 21353-29	Cap	Tran	10.0 mg	11	150

Table 49. Capsule composition in Study F/86/570/15.

	2 mg	4 mg	10 mg
Trandolapril	2.0	4.0	10.0
Unknown			
Total			

A5.4. Study design

This uncontrolled, open-label, 4-way crossover study randomized 12 healthy adult male subjects to one oral trandolapril dose (2, 4, 12, or 24 mg) on each of 4 treatment days separated by 5-week drug-free intervals. The 2 mg dose was administered as one 2 mg capsule, the 4 mg dose as one 4 mg capsule, the 12 mg dose as one 2 mg and one 10 mg capsule, and the 24 mg dose as one 4 mg and two 10 mg capsules. Doses were administered in the morning following an overnight fast. The objectives were to assess pharmacokinetics, pharmacodynamics, and safety.

The exclusion criteria are shown in Table 50 below.

Table 50. Exclusion criteria in Study F/86/570/15.

Weight 10% more or less than ideal
Diastolic blood pressure <55 or >90 mmHg or systolic blood pressure <100 or >150 mmHg
Daily consumption of >10 cigarettes, >6 cups of coffee or tea, ethanol, or any pharmaceutical during the trial
Presence of allergy, or any disease of the cardiovascular, renal, hepatic, gastrointestinal, endocrine, or neuropsychic system
Inability to follow treatment or dietary restriction
Participation in another clinical trial in the previous 3 months

Blood samples for the determination of parent drug and metabolite levels were collected at baseline (pre-treatment), at post-dose hours 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, and 24, and at post-dose day 1.5, 2, 5, 8, 10, 12, 14, and 16. Blood pressure and heart rate were determined at baseline (pre-treatment), at post-dose hours 4, 8, 12, and 24.

A 3-factor ANOVA was used for kinetic analyses.

A5.5. Results

Demographic and baseline characteristics of subjects are shown in Table 51 below.

Table 51. Demographic and baseline characteristics of subjects in Study F/86/570/15.

	n=12
Male	12
Female	0
Age mean	24.3
Weight mean	69.2

It is not clear whether there were any dropouts.

Changes from baseline blood pressure are shown in Figure 50 below and Table 52

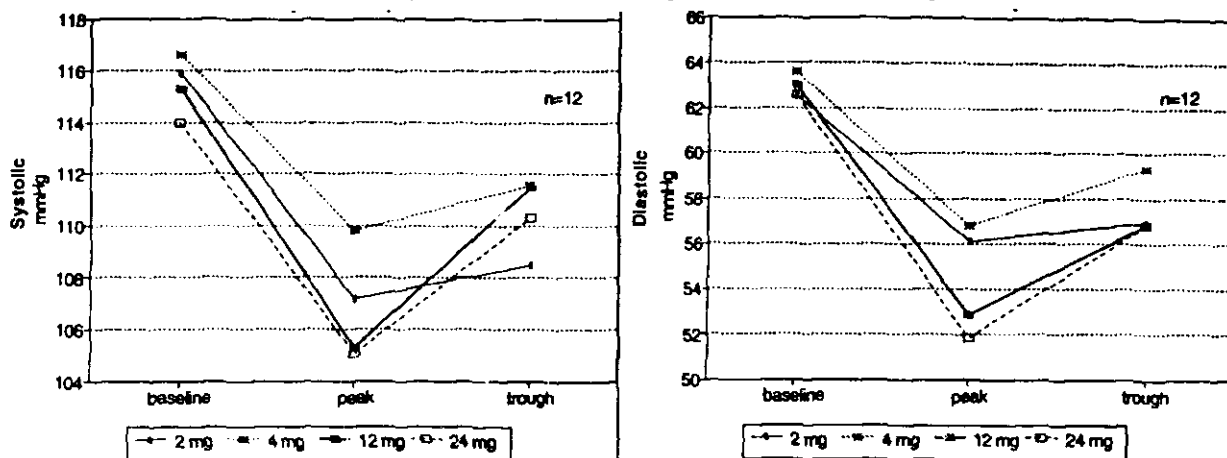


Figure 50. Blood pressure changes in Study F/86/570/15.

below. The drug-associated changes were modest, and lacked an apparent dose-

Table 52. Supine blood pressure effects in Study F/86/570/15.

	2 mg n=12	4 mg n=12	12 mg n=12	24 mg n=12		2 mg n=12	4 mg n=12	12 mg n=12	24 mg n=12
Diastolic					Systolic				
Baseline	63	64	63	63	Baseline	116	117	115	114
Peak	56	57	53	52	Peak	107	110	105	105
Trough	57	59	57	57	Trough	109	112	112	110

response.

Heart rate changes from baseline were clinically nonsignificant.

A5.6. Safety.

Case report forms were not submitted for evaluation. In the study report there is no explicit disclaimer that there were no deaths or discontinuations for adverse events.

Only brief descriptions of adverse events were provided.

Four subjects (6, 9, 10, and 12) experienced orthostatic hypotension. In these cases, systolic pressure reductions of 28 to 31 mmHg occurred up to 12 hours after trandolapril doses of 12 to 24 mg, and associated symptoms (if any) were not described.

Subjects 2 and 7 had isolated, transient, slight, treatment-emergent elevations of SGPT (to 39 and 38 U/L, respectively).

A5.7. Summary.

This uncontrolled, open-label, 4-way crossover study randomized 12 healthy adult male subjects to one oral trandolapril dose (2, 4, 12, or 24 mg) on each of 4 treatment days separated by 5-week drug-free intervals. Drug-associated hemodynamic changes were small to modest, and lacked an apparent dose-response. There were reportedly 4 cases of orthostatic hypotension, and 2 cases of transient mild hepatocellular enzyme elevation. It is not clear whether there were any safety-related withdrawals. This study did not undergo biopharmaceutical review because plasma trandolapril and trandolaprilat levels were not reported by the sponsor (assay problem), although urinary drug levels were reported.

d.

A6. Study F/87/570/24 (Study report: vols 1.92; case report tabulations: vol 1.92; case report forms for deaths and discontinuations for adverse events: none) "Study of the potential pharmacokinetic interaction of digoxin and trandolapril at steady-state".

A6.1. Investigators The investigator was J Granier, Pontoise, France.

A6.2. Study dates June 1988.

A6.3. Drug formulation

Table 53. Drug substance used in Study F/87/570/24.

Lot	Form	Type	Trandolapril	Source	Batch size
MB 21662-63	Cap	Tran	4.0 mg	17	40,000

Table 54. Capsule composition in Study F/87/570/24.

	4 mg
Trandolapril	4.0
Corn starch	
Lactose, hydrous	
Povidone K 25	
Sodium stearyl fumarate	
Total	g

A6.4. Study design

Open-label digoxin (one 0.5 mg loading dose followed by 0.25 mg/day daily maintenance therapy) was orally administered, as monotherapy, to 8 healthy adult males for one week, and subsequently given at the same maintenance dose in combination with oral trandolapril (4 mg/day) for a second week. The objective was to assess steady-state pharmacokinetics, pharmacodynamics, and safety. Drugs were administered one hour after breakfast. Subjects were to abstain from non-trial medication (beginning 8 days prior to the trial), as well as from the use of ethanol.

The exclusion criteria are shown in Table 55 below.

Table 55. Exclusion criteria in Study F/87/570/24.

Weight 15% more or less than ideal
Any acute disease in the previous 3 months, past or present hypersensitivity, malignancy or disease of the cardiac, hepatic, neurologic, renal, hepatic, gastrointestinal, or endocrine system
Use in the previous 3 months of a drug with the potential for toxicity
Consumption of large amounts [undefined] of coffee, tea, or ethanol; >10 cigarettes/day, or any pharmaceutical
Inability to follow dietary restriction
Participation in another clinical trial in the previous 3 months

Blood samples for drug assays were collected at baseline (pre-treatment), at post-dose hour 2 on day 1, and 24 hours after the final dose. Blood samples for the determination of ACE activity were collected at baseline, at 24 hours after each trandolapril treatment. Pharmacokinetic assays were assessed as shown in Table 56 below. The assays have been validated over the range of plasma and urine concentrations observed in the study.

C_{min} and C_{2h} were calculated for digoxin, trandolapril, and trandolaprilat.

Table 56. Pharmacokinetic assays in Study F/87/570/24.

Linearity	Accuracy		Precision			Sensitivity	Specificity
Plasma samples—digoxin							
Satisfactory.	Satisfactory.					LOQ - 0.063 ng/ml	—
Standard curves from 0.063 to 4 ng/ml.	ng/ml	Digoxin					
	0.53	92.5%					
	0.92	92.0%					
	2.10	81.9%					
Plasma samples—trandolapril and trandolaprilat—							
Satisfactory.	Satisfactory.		Satisfactory.			LOQ - 0.2 ng/ml	—
Standard curves from 0.019 to 10 ng/ml.	ng/ml	T' april	T' aprilat	ng/ml	T' april	T' aprilat	
	0.200	103.0%	112.0%	0.200	5.4%	7.5%	
	0.400	102.0%	110.5%	0.400	2.9%	3.6%	
	0.800	101.5%	107.5%	0.800	4.8%	2.7%	

Blood samples for plasma creatinine and potassium determinations were collected at baseline, and 24 hours post dose on days 5, 8, 12, and 15. Reportedly "complete" [undefined] clinical laboratory tests were also obtained at the end of the study.

Blood pressure, and heart rate were apparently determined at baseline (pre-treatment) and every 12 hours post-dose.

Analyses were undertaken by means of 2-factor ANOVA, and Tukey's method for pair-wise comparisons.

A6.5. Results

Demographic and baseline characteristics of subjects are shown in Table 57 below.

Table 57. Demographic and baseline characteristics of subjects in Study F/87/570/24.

	n=8
Male	8
Female	0
Age mean	30.9
Weight mean	72.1

Compliance was 100% and there were reportedly no dropouts.

Changes from baseline blood pressure are shown in Figure 51 below. Blood pressures tended to be lower after trandolapril exposure, but not to any clinically significant extent.

Changes from baseline heart rate pressure are shown in Figure 52 below. Mean heart rates fluctuated throughout the study, but there was no evidence of clinically significant changes.

Changes in electrocardiographic parameters are shown in Table 58 below. Only for QS21 (electromechanical systole corrected for heart rate) does there appear to be an effect of digoxin and a further effect of trandolapril.

Digoxin data are shown in Table 59 and Figure 53 below. Trandolaprilat levels are shown in Table 60.

Twenty-four hours after the first trandolapril dose (trough) ACE inhibition was approximately 87%, and it remained at this level throughout the week of observation.

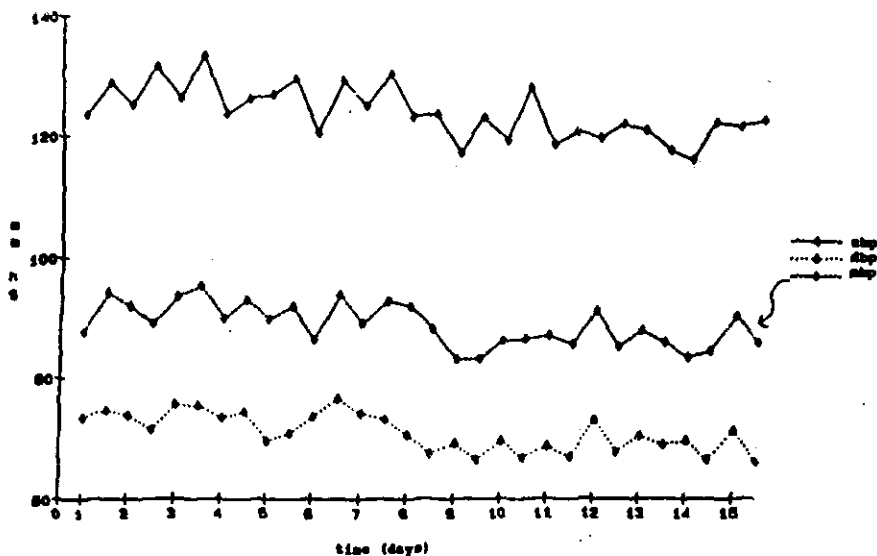


Figure 51. Mean blood pressure in Study F/87/570/24.

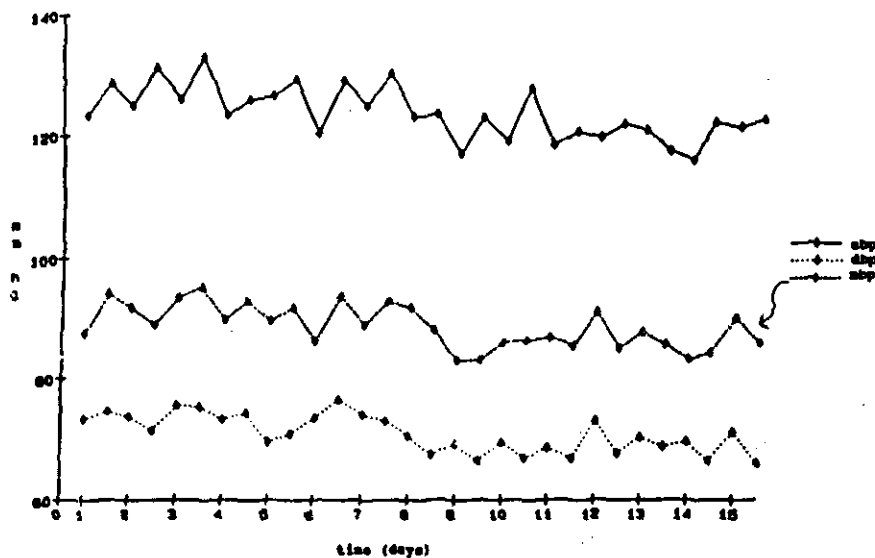


Figure 52. Mean heart rate in Study F/87/570/24.

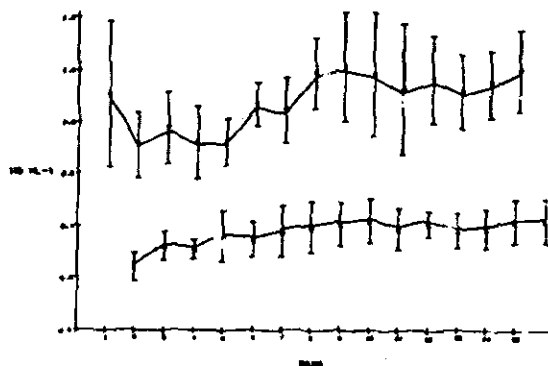


Figure 53. Digoxin levels at 2 hours and at trough in Study F/87/570/24.

A6.6. Safety.

Case report forms were not submitted for evaluation, and no case report tabulations of laboratory findings were submitted.

Table 58. Electrocardiographic data in Study F/87/570/24.

	Baseline	Digoxin	Trandolapril + Digoxin
QS2	0.41±0.01	0.40±0.01	0.39±0.01
QS2I	542±14	527±16	513±11
LVET	0.30±0.02	0.31±0.02	0.31±0.01
LVETI	414±14	417±17	410±14
PEP	0.10±0.0	0.09±0.01	0.08±0.01
PEP/LVET	0.34±0.03	0.28±0.05	0.26±0.05
HR	64±8	61±9	61±7

Table 59. Digoxin levels in Study F/87/570/24.

	Day 7 to 8	Day 8 to 15
C _{min} (ng/ml)	0.39±0.01	0.41±0.02
C _{2h} (ng/ml)	0.86±0.02	0.97±0.03

Table 60. Trandolaprilat levels in Study F/87/570/24.

	C _{min}	C _{2h}		C _{min}	C _{2h}		C _{min}	C _{2h}
Day 8	0.00±0.00	7.46±1.62	Day 11	2.39±0.31	11.15±1.37	Day 14	2.42±0.18	11.55±1.33
Day 9	1.87±0.17	9.46±0.90	Day 12	2.28±0.19	10.36±1.38	Day 15	2.40±0.16	11.70±1.30
Day 10	1.98±0.13	10.82±1.37	Day 13	2.45±0.22	11.12±1.33	Day 16	2.65±0.21	—

In the study report no adverse events were described, but there was no explicit disclaimer that there were no fatal or nonfatal events.

Serum levels of creatinine and potassium reportedly remained within normal limits throughout the study. The undefined clinical laboratory tests obtained at the end of the study reportedly revealed "no clinically significant variation attributable to the treatments".

A6.7. Summary.

In this steady-state kinetic interaction study open-label digoxin (one 0.5 mg loading dose followed by 0.25 mg/day daily maintenance therapy) was orally administered, as monotherapy, to 8 healthy adult males for one week, and subsequently given at the same maintenance dose in combination with oral trandolapril (4 mg/day) for a second week. Digoxin's C_{min} increased 5% and C_{2h} increased 12%. Trandolapril appeared to augment digoxin's effect on QS2I, possibly a result of undetected pharmacokinetic interaction. Trough ACE inhibition was approximately 87% throughout the trandolapril exposure. Blood pressures tended to be lower after trandolapril exposure, but not clinically significantly so. There were reportedly no safety-related withdrawals. No adverse events were described, but there was no explicit disclaimer that no such events occurred. Although laboratory data were not submitted for evaluation, the sponsor reports that there were no treatment-emergent laboratory abnormalities.

A7. Study F/89/570/47 (Study report: vols 1.86; case report tabulations: vol 1.86; case report forms for deaths and discontinuations for adverse events: none) "Effect of food on the bioavailability of trandolapril and trandolaprilat after oral administration of 1 mg of trandolapril to healthy volunteers".

A7.1. Investigators The investigator was J Granier, Pointoise, France.

A7.2. Study dates May 1989 to June 1989.

A7.3. Drug formulation

Table 61. Drug substance used in Study F/89/570/47.

Lot	Form	Type	Trandolapril	Source	Batch size
MB 23327-90	Cap	Tran	1.0 mg	18	2,000

Table 62. Capsule composition in Study F/89/570/47.

	1 mg _{ts}
Trandolapril	1.0
Lactose, hydrous	
Total	1g

A7.4. Study design

This open-label, 2-way crossover study randomized 12 healthy adult male subjects to 1 mg oral doses of trandolapril. Drug was administered once after an overnight fast and once 10 minutes after a standardized meal, with the 2 treatment days separated by a 3-week drug-free interval. In both study periods, there was a 4.5 hour fast after dosing. The objective was to assess food's effect on bioavailability and ACE inhibition. Subjects were to refrain from any tobacco or ethanol use starting the day before the study.

The exclusion criteria are shown in Table 63 below.

Table 63. Exclusion criteria in Study F/89/570/47.

Weight not appropriate for height
Presence of hypersensitivity, malignancy, HIV seropositivity, or any disease of the renal, hepatic, gastrointestinal, endocrine, or neurological system
Daily consumption of >10 cigarettes, >6 cups of coffee or tea, ethanol, or any pharmaceutical during the trial
Use in the previous 3 months of a drug with the potential for organ toxicity
Inability to follow treatment or dietary restriction
Participation in another clinical trial in the previous 3 months

Blood samples for the determination of ACE activity and parent drug and metabolite levels were collected at baseline (0.5 hours pre-treatment), at post-dose hours 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, and 96. Plasma samples were stored at -20°C until analyzed. Urine was collected for determination of trandolaprilat over intervals 0 to 12, 12 to 24, and 24 to 48 hours. Aliquots (10 ml) of urine samples were stored at -20°C until assayed. The standard meals consisted of ham (2 slices, 100 g), soft cheese (25 g), bread (100 g), butter (10 g), orange juice (150 ml), decaffeinated coffee (150 ml with 2 lumps of sugar and no milk), for a total caloric content of about 820 calories. Hematology, and clinical chemistry measures were obtained at baseline, prior to the second dose, and at the end of the study.

Pharmacokinetic assays were assessed as shown in Table 64 below. The assays have

Table 64. Pharmacokinetic assays in Study F/89/570/47.

Linearity	Accuracy		Precision		Sensitivity	Specificity		
Plasma samples—trandolapril and trandolaprilat—								
Satisfactory.	Satisfactory.		Satisfactory.		LOQ - 0.1 ng/ml.	Satisfactory.		
Standard curves from 0.078 to 2.5 ng/ml for trandolapril and for trandolaprilat	ng/ml	T'april	T'aprilat	ng/ml			T'april	T'aprilat
	0.2	94.5%		0.2			4.0%	
	0.4	97.5%		0.4			3.3%	
	0.8	101.6%		0.8			5.0%	
	1.0		97.0	1.0		6.6%		
Urine samples—trandolaprilat—								
Satisfactory.	Satisfactory.		Satisfactory.		LOQ - 10 ng/ml.	Satisfactory.		
Standard curves from 10 to 2500 ng/ml	ng/ml	T'aprilat	ng/ml	T'aprilat				
	111	99.1%	111	9.7%				
	554	93.3%	554	7.6%				
	1107	96.9%	1107	1.6%				
	1661	98.2%	1661	9.2%				
Plasma samples—ACE inhibition—								
	42.0 U/L for expected values of 29 to 53 U/L		5.0% CV at 42.0 U/L					

been validated over the range of plasma and urine concentrations observed in the study.

AUC, C_{max} , I_{max} , and T_{max} were calculated.

The data were analyzed using a 3-way ANOVA and the Milton-Freidmann test for T_{max} .

A7.5. Results

Demographic and baseline characteristics of subjects are shown in Table 65 below.

Table 65. Demographic and baseline characteristics of subjects in Study F/89/570/47.

	n=12
Male	12
Female	0
Age mean	28.4
Weight mean	70.0

There were reportedly no protocol violations. Changes from baseline blood pressure and heart rate were clinically nonsignificant.

Plasma trandolapril and trandolaprilat levels are shown in Figure 54 below. Pharmacokinetic data are shown in Table 66 below.

ACE inhibition is depicted in Figure 55 and Table 67 below

A7.6. Safety.

Case report forms were not submitted for evaluation. There were reportedly no dropouts. Only brief descriptions of adverse events were provided. Subject 11 had an asymptomatic orthostatic systolic blood pressure fall of greater than 25 mmHg.

The following treatment-emergent laboratory abnormalities were reported:

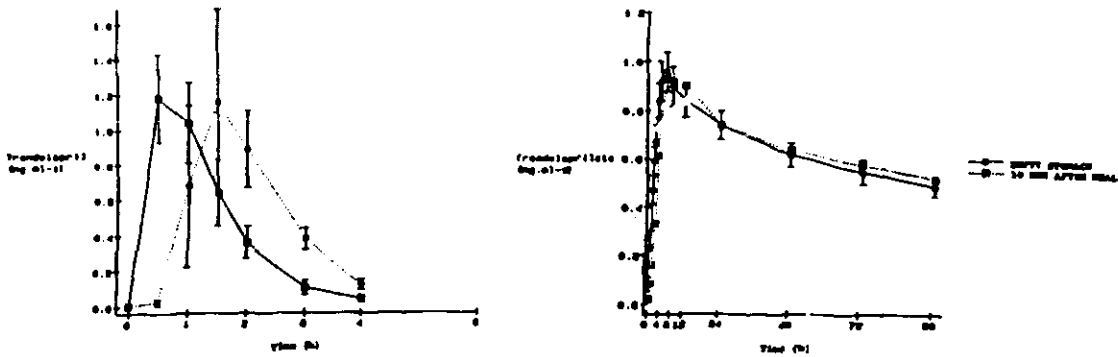


Figure 54. Plasma trandolapril and trandolaprilat levels in Study F/89/570/47.

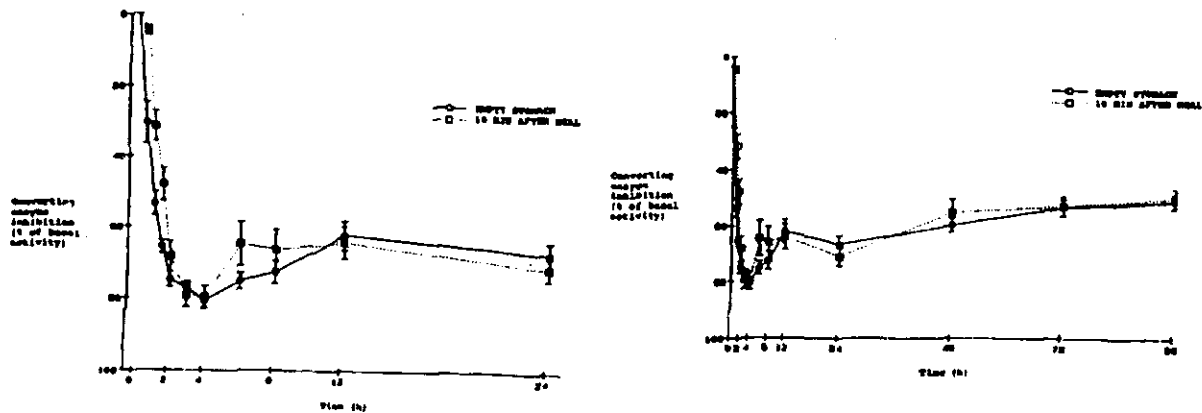


Figure 55. Plasma ACE inhibition in Study F/89/570/47.

Table 66. Pharmacokinetic data in Study F/89/570/47.

	Trandolapril				Trandolaprilat			
	Fasting n=12	Fed n=12	Westlake CI (%)	90% CI	Fasting n=12	Fed n=12	Westlake CI (%)	90% CI
AUC ₀₋₆ (ng/ml·h)	1.8±1.5	2.1±0.7	—	—	—	—	—	—
AUC ₀₋₂₄	—	—	—	—	19.1±5.4	18.4±5.7	12.7	—
AUC ₀₋₉₆	—	—	—	—	62.0±16.4	62.7±18.3	10.8	—
C _{max} (ng/ml)	1.3±0.8	1.3±0.5	—	—	0.99±0.3	0.97±0.3	15.2	—
T _{max} (h)	0.63±0.23	1.75±0.34	—	—	5.42±1.38	8.17±2.48	—	—
UE ₀₋₄₈ (µg)	—	—	—	—	40.3±43.1	42.9±42.4	—	—
% dose excreted	—	—	—	—	4.3±4.6	4.6±4.6	—	—

Subject 6 had a transient mild elevation of BUN.

Subject 8 had reportedly transient elevations of CPK to 1866 IU/L (in association with rigorous exercise) and SGOT (to 44 IU/L).

Subject 10 had a reportedly transient elevation of CPK (to 415 IU/L).

Subject 12 had a transient slight elevation of serum creatinine.

A7.7. Summary.

This open-label, 2-way crossover study randomized 12 healthy adult male subjects to 1 mg oral doses of trandolapril. Drug was administered once after an overnight fast and

Table 67. ACE inhibition (%) in Study F/89/570/47.

	Fasting n=12	Fed n=12
2 hours	74.7	48.1
4 hours	80.8	79.2
I_{max} (%)	83.5±5.7	79.2±3.2
I_{24}	66.8±11.4	71.1±3.1
AUC ₀₋₂₄ (%·h)	1594±163	1516±79
AUC ₀₋₉₆	5718±717	5556±307
T_{max} (h)	4.5±1.4	4.0±0.0

once 10 minutes after a standardized meal, with the 2 treatment days separated by a 3-week drug-free interval. The principal effect of food was to delay by about 2.75 hours the maximum plasma trandolaprilat level. There was reportedly one case of asymptomatic orthostatic systolic blood pressure reduction, and several transient treatment-emergent laboratory abnormalities, but reportedly no safety-related withdrawals.

A8. Study F/89/570/54 (Study report: vols 1.89; case report tabulations: vols 1.89-1.90; case report forms for deaths and discontinuations for adverse events: none) "Study of the pharmacokinetics and dose proportionality of trandolapril administered orally to healthy volunteers".

A8.1. Investigators The investigator was G Strauch, Paris, France.

A8.2. Study dates August 1989 to December 1989.

A8.3. Drug formulation

Table 68. Drug substance used in Study F/89/570/54.

Lot	Form	Type	Trandolapril	Source lot	Batch size
MB 22633-145	Cap	Tran	0.5 mg	MB22633-145	20,000
MB 22633-141	Cap	Tran	1.0 mg	18	60,000

Table 69. Capsule composition in Study F/89/570/54.

	0.5 mg	1 mg
Trandolapril	0.5	1.0
Corn starch		
Lactose, hydrous		
Povidone		
Sodium stearyl fumarate		
Total		

A8.4. Study design

Twelve healthy adult males were randomly crossed-over (latin square design) to open-label, oral trandolapril doses (0.5, 1, 2, or 4 mg) administered via a different single dose on each of 4 occasions. Drug was given after overnight fasting and subjects continued fasting for 4 hours after dosing. There were intervening 3-week drug-free periods, and there was no control arm. The objective was to assess pharmacokinetics, pharmacodynamics, and safety.

The 0.5 and 1 mg doses were given as single capsules, whereas the 2 and 4 mg doses were administered as 2 x 1 mg and 4 x 1 mg capsules, respectively.

The exclusion criteria are shown in Table 70 below.

Table 70. Exclusion criteria in Study F/89/570/54.

Weight 10% more or less than ideal
Daily consumption of ≥10 cigarettes or any number during a study day
Supine diastolic blood pressure <100 or >150 mmHg
Supine systolic blood pressure <55 or >90 mmHg
Presence of hypersensitivity, HIV seropositivity, or any disease of the cardiovascular, renal, hepatic, gastrointestinal, endocrine, or neuropsychiatric system
Daily consumption of "large quantities" [undefined] of coffee, tea, or ethanol
Use of any medication within 15 days prior to the trial
Inability to follow treatment or dietary restriction
Residence in a tropical region within 2 months before the study

Blood pressure, and heart rate were determined at baseline (pre-treatment), and at post-dose hours 4, 8, 12, and 24. Blood samples for drug levels were obtained at baseline

and at post-dose hours 0.5, 1, 1.5, 2, 3, 4, 6, 10, 12, 16, 24, 33, 48, 57, 72, and 96. Blood samples for the determination of ACE activity were collected at baseline, and at post-dose hours 1, 2, 3, 4, 8, 12, 16, and 24. Plasma samples for drug levels or ACE activity were stored at -20°C. Urine for drug levels was collected at baseline (emptied bladder), and over intervals 0 to 4, 4 to 12, 12 to 24, 24 to 48, 48 to 72, and 72 to 96 hours post-dosing. Aliquots of urine were stored at -20°C until assayed for trandolaprilat. Hematology and chemistry measures were reported at baseline, and at the end of the trial.

Pharmacokinetic assays were assessed as shown in Table 71 below. The assays have

Table 71. Pharmacokinetic assays in Study F/89/570/54.

Linearity	Accuracy		Precision		Sensitivity	Specificity
Plasma samples—trandolapril and trandolaprilat—						
Satisfactory.	Satisfactory.		Satisfactory.		LOQ - 0.1 ng/ml.	Satisfactory.
Standard curves from 0.078 to 1.25 ng/ml for trandolapril and 0.078 to 1.25 ng/ml for trandolaprilat	ng/ml	T'april	T'aprilat	ng/ml	T'april	T'aprilat
	0.2	89.5%	107.0%	0.2	7.2%	7.2%
	0.3	—	102.7%	0.3	—	7.7%
	0.4	89.0%	99.0%	0.4	4.9%	7.7%
	0.8	101.2%	—	0.8	4.6%	—%
Urine samples—trandolaprilat—						
Satisfactory.	Satisfactory.		Satisfactory.		LOQ - 10 ng/ml.	Satisfactory.
Standard curves from 10 to 2500 ng/ml	ng/ml	Trandolaprilat		ng/ml	Trandolaprilat	
	111	99.1%		111	9.7%	
	554	93.3%		554	7.6%	
	1107	96.9%		1107	7.6%	
	1661	98.2%		1661	9.2%	
Plasma samples—ACE inhibition—						
	54.0 U/L for expected values of 44 to 58 U/L		2.35% CV at 54.0 U/L			

been validated over the range of plasma and urine concentrations observed in the study.

AUC, C_{max} , $t_{1/2}$, CL_R , T_{max} , and $\lambda_{(n)}$ were calculated.

Only descriptive statistics appear to have been generated for the non-kinetic data.

A8.5. Results

Demographic and baseline characteristics of subjects are shown in Table 72 below.

Table 72. Demographic and baseline characteristics of subjects in Study F/89/570/54.

	n=12%
Male	12
Female	0
Age mean	23.8
Weight mean	72.2

Pertinent protocol violations were as follows: subject 9 was co-exposed to oxacillin between the second and third treatment periods; subject 10 was co-exposed to acetylsalicylate, amoxicillin, and paracetamol during the second treatment period; subject 8 was co-exposed to aluminium hydroxide on day 3.

The pertinent aspects of subject disposition were as follows: subject 7, a 20 year old and 65 kg male with no history of allergy, withdrew after developing urticaria starting the night after a morning trandolapril dose (0.5 mg) during the third treatment period. During the prior two treatment periods there was no allergic reaction to either 2 or 4 mg trandolapril doses. Urticaria resolved within 10 days. This subject's data were excluded from the analyses, and he was replaced by a new enrollee.

Supine blood pressure effects are shown in Table 73 below and Figure 56 below. These

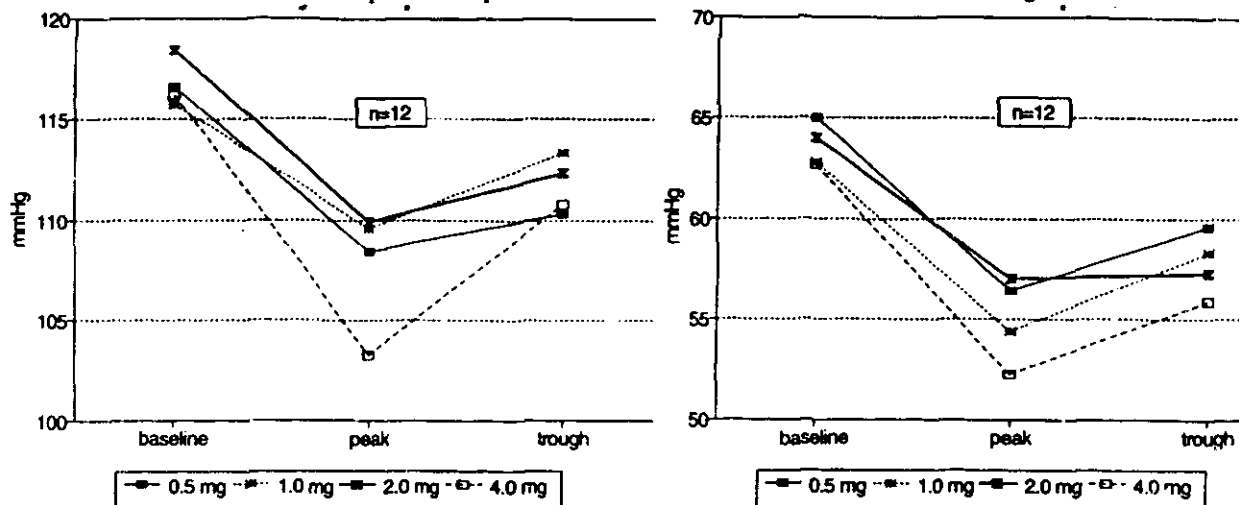


Figure 56. Blood pressure effects in Study F/89/570/54.

Table 73. Supine blood pressure effects in Study F/89/570/54.

	Trandolapril			
	0.5 mg n=12	1 mg n=12	2 mg n=12	4 mg n=12
Diastolic				
Baseline	65.0	62.8	64.0	62.7
Peak	56.4	54.4	57.0	52.3
Trough	59.6	58.3	57.3	55.9
Systolic				
Baseline	116.6	115.7	118.4	116.2
Peak	108.4	109.6	109.9	103.3
Trough	110.3	113.3	112.3	110.8

uncontrolled data suggested the mean trough (24 hours post-dose) supine diastolic pressure reductions from baseline (pre-treatment) to be small (5 to 7 mmHg, not accounting for placebo effect). The apparent maximal mean diastolic pressure reductions were moderate (7 to 11 mmHg). There was a weak relationship between dose and antihypertensive effect.

Heart rate changes from baseline were clinically nonsignificant.

Pharmacokinetic data are shown in Table 74 below.

Plasma drug levels are shown in Figure 57 below. Cumulative urinary excretion of trandolaprilat is shown in Figure 58 below.

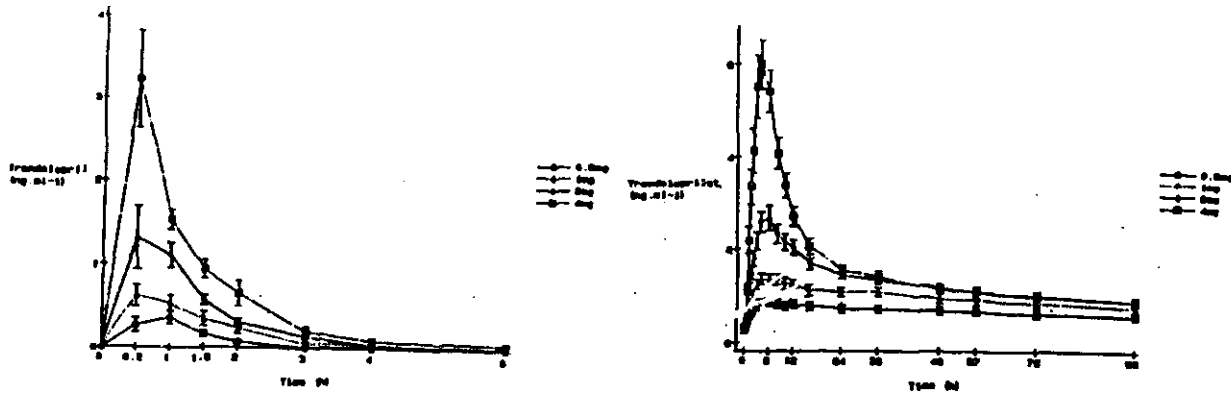


Figure 57. Plasma trandolapril and trandolaprilat levels in Study F/89/570/54.

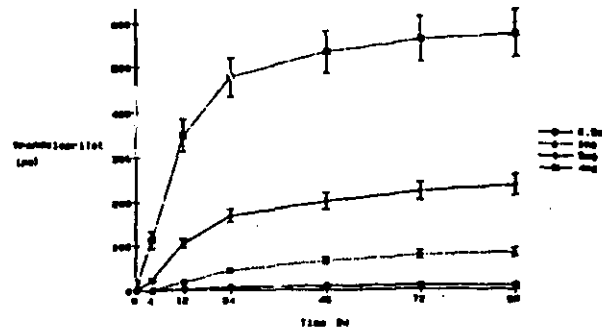


Figure 58. Cumulative urinary excretion of trandolaprilat in Study F/89/570/54.

Table 74. Pharmacokinetic data from Study F/89/570/54.

	Trandolapril				Trandolaprilat			
	0.5 mg	1 mg	2 mg	4 mg	0.5 mg	1 mg	2 mg	4 mg
C _{max} (ng/ml)	0.43±0.10	0.86±0.10	1.68±0.33	3.32±0.56	0.96±0.08	1.44±0.12	2.80±0.25	6.41±0.57
AUC (h·ng/ml)	0.40±0.11	0.95±0.15	1.86±0.30	3.64±0.44	64.4±7.08	92.8±9.12	124±7.89	155±11.3
AUC ₀₋₉₆					8.90±0.91	14.2±1.28	25.6±2.04	49.4±4.02
AUC ₀₋₁₂								
T _{max} (h)								
median	1.0	0.5	0.5	0.5	8	6	6	4
range	0.5-1.0	0.5-1.0	0.5-1.0	0.5-1.0	4-12	4-33	4-12	3-8
t _{1/2} (h)								
appearance	0.71±0.08 ^a	0.74±0.09	0.68±0.05	0.76±0.13	ND	1.28±0.13	1.44±0.10	1.49±0.08
rapid elim phase					ND	4.11±0.42 ^b	4.53±0.43	3.32±0.19
U ₀₋₉₆ (µg)	—	—	—	—	9.58±3.30	83.9±10.1	237±23.3	577±54.3
CL _{R 0-96} (L/h)	—	—	—	—	0.15±0.05	1.03±0.18	2.02±0.25	3.93±0.39

a. n=9

b. n=8

The extent of ACE inhibition observed within 8 hours post-dose is depicted in Table 75 below. There is an apparent dose-response.

A8.6. Safety.

Case report forms were not submitted for evaluation.

In the study report there is no explicit disclaimer that there were no deaths.

Table 75. Peak ACE inhibition in Study F/89/570/54.

	Trandolapril			
	0.5 mg	1 mg	2 mg	4 mg
I _{max} (%)	77.0 4.6	79.5 3.8	90.6 4.9	93.8 1.9
T _{max} (h)	6	3	2	3
median				
range	3-8	2-8	2-4	2-8

Subject 7 withdrew with urticaria (as described above). For the following treatment-emergent adverse effects only brief case descriptions were provided: subjects 5 and 6 experienced headache, and subject 13 reported drowsiness.

There were no clinically significant treatment-emergent laboratory abnormalities reported.

A8.7. Summary.

Twelve healthy adult males were randomly crossed-over to open-label, oral trandolapril doses (0.5, 1, 2, or 4 mg) administered in single doses on each of 4 occasions. There were intervening 3 week drug-free periods. The maximum mean inhibition of ACE ranged from approximately 69% to 92%; higher doses were associated with earlier onset of inhibition. These uncontrolled data suggested an apparent moderate maximal mean reduction in supine pressure, a small trough reduction, but no clear relationship of trandolapril dose to antihypertensive effect. Trandolapril displayed linear kinetics in the studied dose range. Trandolapril kinetics were non-linear. There was reportedly one case of urticaria requiring withdrawal from the study, 2 cases of headache, and one case of drowsiness.

A9. Study F/91/570/132 (Study report: vols 1.93; case report tabulations: vol 1.93; case report forms for deaths and discontinuations for adverse events: none) "Study of a possible pharmacokinetic/pharmacodynamic interaction between cimetidine (400 mg b.i.d.) and trandolapril (2 mg) administered orally to healthy volunteers".

A9.1. Investigators The investigator was JL Pinquier, Paris, France.

A9.2. Study dates September 1992 to December 1992.

A9.3. Drug formulation

Table 76. Drug substance used in Study F/91/570/132.

Lot	Form	Type	Trandolapril	Source lot	Batch size
L15B	Cap	Tran	2.0 mg	33	600,000

Table 77. Capsule composition in Study F/91/570/132.

	2 mg
Trandolapril	2.0
Corn starch	
Lactose, hydrous	
Povidone	
Sodium stearyl fumarate	
Total	

A9.4. Study design

In a preliminary phase, subjects received a single dose of trandolapril 2.0 mg, the purpose of which was to balance the residual trandolaprilat levels for the two cross-over arms. Four weeks later, subjects were randomized to a two-way, open-label, cross-over study in which subjects received a single dose of trandolapril alone (treatment A) and 5 days treatment with cimetidine with a single dose of trandolapril on day 4. The objectives were to assess pharmacokinetics, pharmacodynamics, and safety over a 2 month observation period.

Enrolled subjects were healthy, adult, male Caucasians, age 18 to 40 years, within 20% of ideal body weight, and with normal baseline laboratory values. The exclusion criteria are shown in Table 78 below.

Table 78. Exclusion criteria in Study F/91/570/132.

Cardiovascular, neurological, endocrine, gastrointestinal, hepatic, or renal disease or malignancy
Allergy or hypersensitivity to drugs
Any acute disease within 3 months
Regular use of any drugs
Alcohol abuse, use of more than 10 cigarettes/day, or consumption of large amounts of tea or coffee
Blood donation or study participation within 3 months

Blood pressure and heart rate were determined at baseline (pre-treatment), at post-dose hours 2, 4, 6, 8, 12, 24, 35, and 48.

Blood samples for the determination of plasma ACE activity were collected at baseline, at post-dose hours 2, 4, 6, 8, 12, 24, 35, and 48.

Blood samples for the assay of trandolapril and trandolaprilat were collected pre-dose and at post-dose hours 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 35, and 48. Urine was collected at 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 35, and 35 to 48 hours post-dose.

Pharmacokinetic assays were assessed as shown in Table 79 below. The assays have

Table 79. Pharmacokinetic assays in Study F/91/570/132.

Linearity	Accuracy			Precision			Sensitivity	Specificity
Plasma samples—trandolapril and trandolaprilat-								
Satisfactory.	Satisfactory.			Satisfactory.			LOQ - 0.1 ng/ml.	Satisfactory.
Standard curves from 0.078 to 2.5 ng/ml for trandolapril and 0.078 to 1.25 ng/ml for trandolaprilat	ng/ml	T' april	T' aprilat	ng/ml	T' april	T' aprilat		
	0.100	96.0%	95.0%	0.100	9.0%	5.6%		
	0.200	100.5%	96.5%	0.200	10.7%	4.8%		
	0.400	100.0%	98.2%	0.400	7.4%	3.8%		
	0.800	102.8%	98.4%	0.800	7.6%	3.5%		
			1.600	6.6%	—			
Plasma samples—ACE inhibition-								
	Satisfactory. 53.6 U/L for expected values of 43 to 59 U/L.			Satisfactory. 2.4% C.V. at 53.6 U/L.				

been validated over the range of plasma and urine concentrations observed in the study.

AUC, C_{max} , $t_{1/2}$, T_{max} , CL_r , I_{max} , and percentage of dose excreted in the urine were calculated.

Physical examinations, hematology, chemistry, and urinalysis measures were obtained on days 5 and 64.

Only descriptive statistics appear to have been generated.

A9.5. Results

Demographic and baseline characteristics of subjects are shown in Table 80 below.

Table 80. Demographic and baseline characteristics of subjects in Study F/91/570/132.

	n=10.
Male	10
Female	0
Caucasian	6
Other	0
Age mean	22.1
Weight mean	71.6

Pertinent protocol violations were as follows: urine collections were lost for 4 subjects at 8 to 12 hours and for one subject from 8 to 24 hours. Four subjects had drug use for acute illness prior to enrollment or during study.

There were reported to be no dropouts.

Pharmacokinetic parameters are shown in Table 81 below. Plasma trandolapril and

Table 81. Pharmacokinetic parameters in Study F/91/570/132.

	Trandolapril alone		Trandolapril + cimetidine	
	Trandolapril	Trandolaprilat	Trandolapril	Trandolaprilat
T_{max} (h) median range	0.75 0.5-1.5	6 3-8	0.625 0.5-1.5	6 5-8
C_{max} (ng/ml)	1.86±0.32	2.82±0.27	2.67±0.53	2.82±0.24
AUC (h·ng/ml) AUC ₀₋₈ AUC ₀₋₁₂ AUC ₀₋₂₄ AUC ₀₋₄₈	2.08±0.32	24.2±2.09 44.8±3.06 75.6±4.42	2.35±0.41	25.1±1.72 46.4±2.94 78.2±4.73
$t_{1/2}$ (h)	0.69±0.14 ^a	—	0.61±0.12	—
U_{0-12} (µg) U_{0-24} (µg) U_{0-48} (µg)	—	97±28 140±32 185±41	—	120±40 160±40 196±42
Cl_r 0-12 (L/h) Cl_r 0-24 (L/h) Cl_r 0-48 (L/h)	—	3.5±0.6 2.8±0.3 2.2±0.3	—	4.1±1.2 3.1±0.7 2.3±0.5

a. n=9

trandolaprilat levels are shown in Figure 59 below. Cumulative excretion of

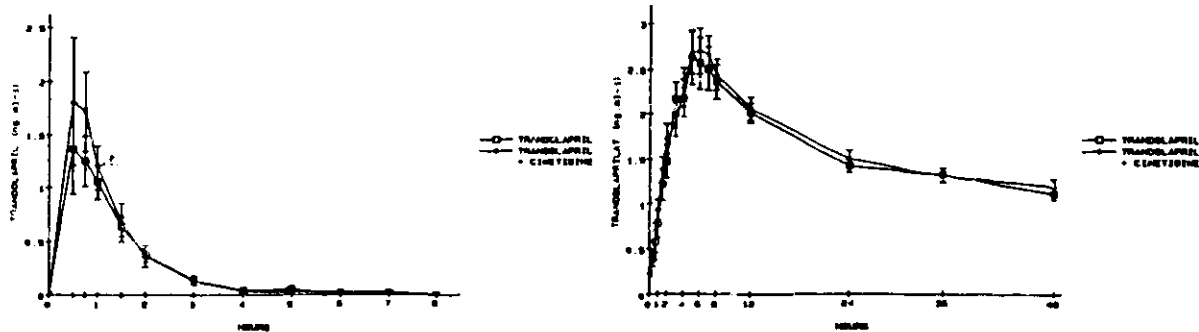


Figure 59. Plasma trandolapril and trandolaprilat levels in Study F/91/570/132.

trandolaprilat is shown in Figure 60 below.

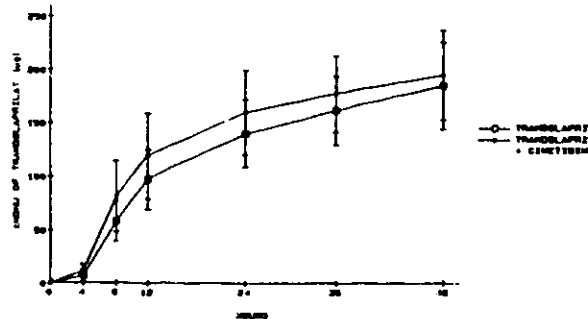


Figure 60. Cumulative trandolaprilat excretion in Study F/91/570/132.

ACE inhibition is described in Figure 61 and Table 82 below.

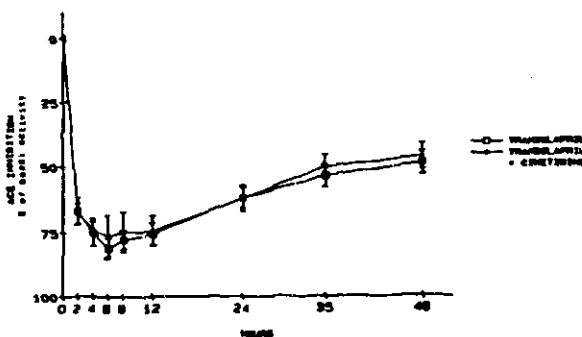


Figure 61. Plasma ACE inhibition in Study F/91/570/132.

Table 82. Plasma ACE inhibition in Study F/91/570/132.

	Trandolapril alone	Trandolapril +cimetidine
I_{max} (%)	83.4±3.9	84.5±3.0
$T_{1/2max}$ (h)	6	6
median	6	6
range	4-12	6-24

A9.6. Safety.

Case report forms were not submitted for evaluation. No subjects discontinued.

Two subjects had intercurrent upper respiratory track infections. There were no clinically significant changes in laboratory or ECG data.

A9.7. Summary.

Ten healthy male volunteers received single oral doses of trandolapril 2 mg alone or on day 4 of a 5-day regimen of cimetidine, a known inhibitor of cytochrome P₄₅₀. Cimetidine resulted in a 44% increase in the C_{max} for trandolapril, but no effect of cimetidine was demonstrated on trandolaprilat pharmacokinetics or plasma ACE inhibition.

4.

A10. Study F/91/570/133 (Study report: vols 1.91; case report tabulations: vol 1.91; case report forms for deaths and discontinuations for adverse events: none) "Pharmacokinetics and pharmacodynamics of trandolapril in healthy volunteers after a single oral dose of 2 mg".

A10.1. Investigators The investigator was JL Pinquier, Paris, France.

A10.2. Study dates October 1991 to March 1992.

A10.3. Drug formulation

Table 83. Drug substance used in Study F/91/570/133.

Lot	Form	Type	Trandolapril	Source lot	Batch size
Unkn	Cap	Plcbo	—		
L14	Cap	Tran	2.0 mg	33	600,000

Table 84. Capsule composition in Study F/91/570/133.

	2 mg
Trandolapril	2.0
Corn starch	
Lactose, hydrous	
Povidone	
Sodium stearyl fumarate	
Total	

A10.4. Study design

This open-label, placebo-controlled¹, parallel-group study randomized 12 healthy adult subjects to placebo or a single 2 mg oral trandolapril dose (under fasting conditions). The objectives were to assess pharmacokinetics, pharmacodynamics, and safety over a 2 month observation period.

Enrolled subjects were healthy, adult, male Caucasians. The exclusion criteria are shown in Table 85 below.

Table 85. Exclusion criteria in Study F/91/570/133.

Weight <90% or >110% of ideal
Daily consumption of > 10 cigarettes
Use of ethanol or any pharmaceutical during the trial
Blood donation within 3 months preceding the trial
History or presence of HIV seropositivity, or a disease of the cardiovascular, renal, hepatic, gastrointestinal, endocrine, or neuropsychiatric system

Supine and standing blood pressure, and heart rate were determined at baseline (pre-treatment), at post-dose hours 4, 8, 12, 24, and 34, and on post-dose days 3, 4, 5, 8, 15, 22, 29, 36, 43, 50 and 57.

Blood samples for the determination of plasma ACE activity were collected at baseline, at post-dose hours 1, 2, 3, 4, 8, 12, 16, and 24, and on post-dose days 3, 4, 5, 8, 15, 22, 29, 36, 43, 50 and 57.

¹ It is not completely clear from the study report whether this study had an untreated group or was placebo-controlled

Blood samples for the assay of trandolapril and trandolaprilat were collected pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 16, 24, 34, 48, 72, 86, 168, 336, 504, 672, 840, 1008, 1176, 1680, 2016, 2352, 2688, 3024, 3360, and 3696 hours post-dose in the treated group. Urine was collected pre-dose (bladder emptied) and at 0 to 4, 4 to 12, 12 to 24, 24 to 48, 48 to 72, and 72 to 96 hours post-dose, and 0 to 6 hours post-dose on study days 15, 29, 43, and 57.

Pharmacokinetic assays were assessed as shown in Table 86 below. The assays have

Table 86. Pharmacokinetic assays in Study F/91/570/133.

Linearity	Accuracy			Precision			Sensitivity	Specificity
Plasma samples—trandolapril and trandolaprilat—								
Satisfactory.	Satisfactory.			Satisfactory.			LOQ - 0.1 ng/ml.	Satisfactory.
Standard curves from 0.078 to 2.5 ng/ml for trandolapril and 0.078 to 1.25 ng/ml for trandolaprilat	ng/ml	T'april	T'aprilat	ng/ml	T'april	T'aprilat		
	0.1	102.0%	97.0%	0.1	4.2%	6.5%		
	0.4	101.0%	103.2%	0.4	5.9%	3.9%		
	1.6	102.5%	99.7%	1.6	5.7%	3.5%		
Urine samples—trandolaprilat—								
Satisfactory.	Satisfactory.			Satisfactory.			LOQ - 10 ng/ml.	Satisfactory.
Standard curves from 7.8 to 125 ng/ml	ng/ml	T'aprilat		ng/ml	T'aprilat			
	10	95.0%		10	4.2%			
	40	102.3%		40	4.9%			
	80	102.4%		80	3.4%			
Plasma samples—ACE inhibition—								
	47.6 U/L for expected values of 29 to 53 U/L			2.04% CV at 47.6 U/L				

been validated over the range of plasma and urine concentrations observed in the study.

f UC, C_{max} , $t_{1/2}$, T_{max} , CL_r , and percentage of dose excreted in the urine were calculated.

Physical examinations, hematology, chemistry, and urinalysis measures were obtained on days 5 and 64.

Only descriptive statistics appear to have been generated.

A10.5. Results

Demographic and baseline characteristics of subjects are shown in Table 87 below.

Table 87. Demographic and baseline characteristics of subjects in Study F/91/570/133.

	Placebo n=6	Trandol n=6
Male	6	6
Female	0	0
Caucasian	6	6
Other	0	0
Age mean	23.2	23.3
Weight mean	67.5	68.2

Pertinent protocol violations were as follows: subjects 4 and 5 received one paracetamol dose each on days 20 and 4, respectively; subject 7 received neomycin eye drops and an inhalational treatment² for sinusitis on days 43 to 50.

Compliance was 100% and there were reported to be no dropouts.

Blood pressure and heart rate effects are shown in Figure 62 below. Changes from

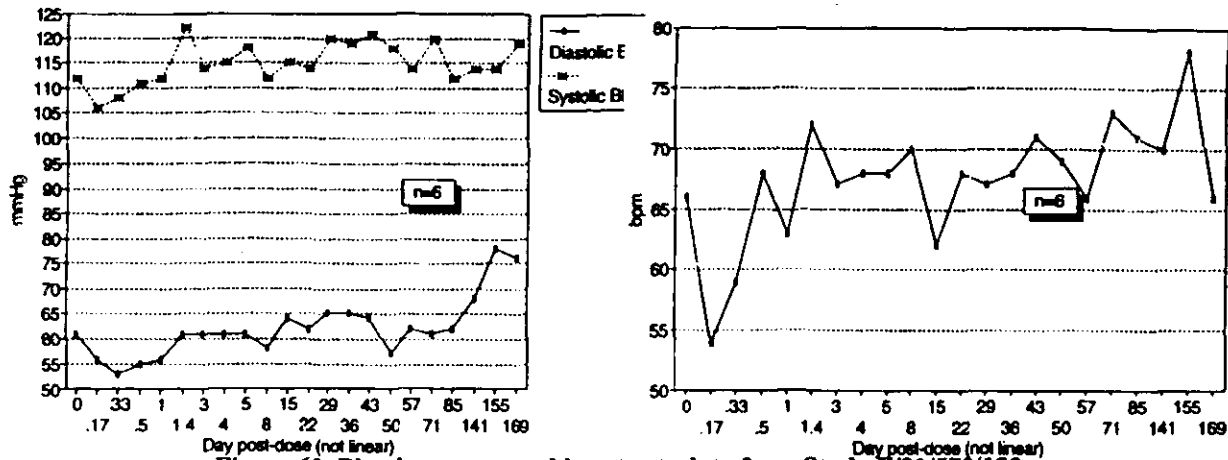


Figure 62. Blood pressure and heart rate data from Study F/91/570/133.

baseline mean supine blood pressure and heart rate were minimal in the placebo group. Hemodynamic data for actively treated subjects are shown in Table 88 below.

Table 88. Hemodynamic effects in the active treatment group of Study F/91/570/133.

	SysBP n=6	DiasBP n=6	HR n=6		SysBP n=6	DiasBP n=6	HR n=6
Baseline	112	61	66	21 days	114	62	68
4 hours	106	56	54	28 days	120	65	67
8 hours	108	53	59	35 days	119	65	68
12 hours	111	55	68	42 days	121	64	71
24 hours	112	56	63	49 days	118	57	69
34 hours	122	61	72	56 days	114	62	66
2 days	114	61	67	70 days	120	61	73
3 days	115	61	68	84 days	112	62	71
4 days	118	61	68	140 days	114	68	70
7 days	112	58	70	154 days	114	78	78
14 days	115	64	62	168 days	119	76	66

The mean peak reduction in supine diastolic pressure was 8 mmHg among trandolapril-treated subjects, and the mean trough (24 hours post-dose) reduction was 5 mmHg. A comparable finding was obtained for the peak systolic (supine) pressure reduction, and the trough systolic change was nil.

Heart rate changes from baseline were clinically nonsignificant.

Pharmacokinetic parameters are shown in Table 89 below. Subjects for whom plasma trandolapril levels were above limits for quantification are described in Table 90

² The drug, called balsalofumine®, is not described in the PDR

Table 89. Pharmacokinetic parameters in Study F/91/570/133.

	Trandolapril	Trandolaprilat
T _{max} (h) median range	0.5 0.5-1	5 4-8
C _{max} (ng/ml)	3.18±0.77	2.80±0.21
AUC (h·ng/ml)	3.08±0.54	—
t _{1/2} (h)	0.62±0.05	—
AUC ₀₋₂₄ (h·ng/ml)	—	49.3±3.7
AUC ₀₋₉₆ (h·ng/ml)	—	141±13.0
U ₀₋₂₄ (μg)	—	129±27
U ₀₋₉₆ μg % of dose	— —	196±29 ^a 10.5±1.5 ^a
Cl _{R 0-24} (L/h)	—	2.79±0.57 ^a
Cl _{R 0-96} (L/h)	—	1.55±0.30 ^a

a. n=5

below. In 6 subjects, plasma levels of trandolaprilat were detectable (mean of

Table 90. Subjects with plasma trandolaprilat levels above limit for quantification in Study F/91/570/133.

	Subject					
	2	4	6	7	9	12
Time (months)	5	3	3	3	2.5	3
Trandolaprilat (ng/ml)	0.10	0.13	0.13	0.10	0.14	0.11

0.19±0.04 ng/ml) for at least 2 months after oral administration of trandolapril 2 mg.

Plasma trandolapril levels (Figure 63 below) were well fit with a single exponential

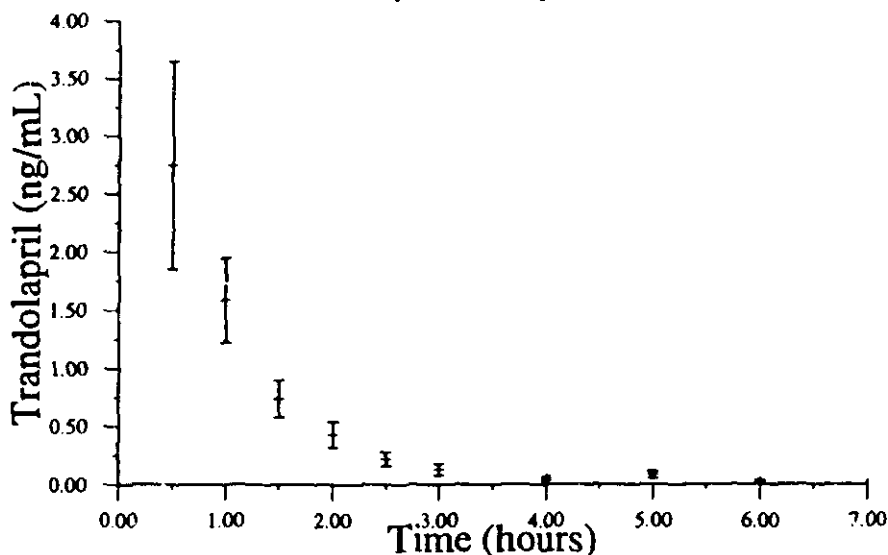


Figure 63. Trandolapril levels in Study F/91/570/133.

with a time constant of 0.8 hours, from which no accumulation would be expected with repeated dosing.

asma trandolaprilat levels (Figure 64 below) required 3 exponential terms to obtain

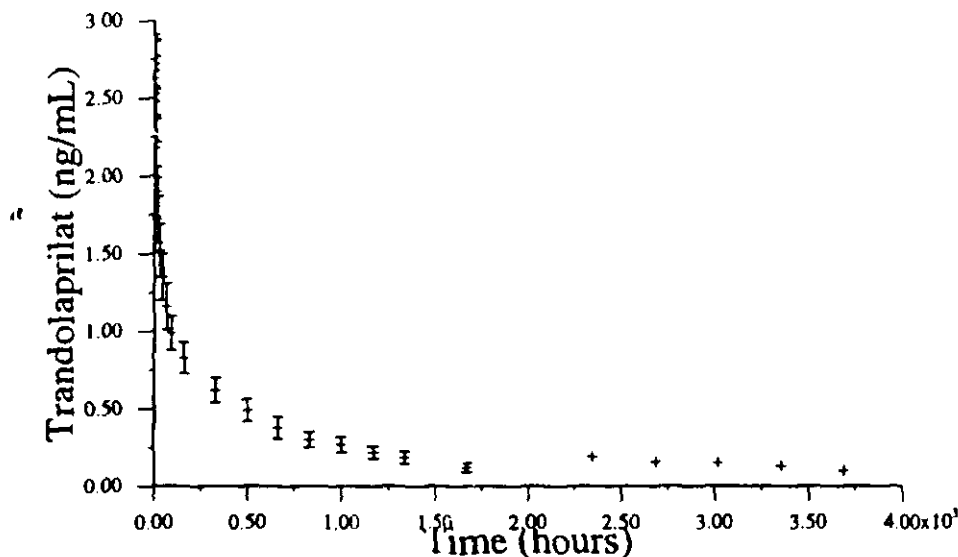


Figure 64. Trandolaprilat levels in Study F/91/570/133.

a good fit:

$$y = Ae^{-t/\tau_1} + Be^{-t/\tau_2} + Ce^{-t/\tau_3}$$

where A=1.46, $\tau_1=17$ hours, B=1.05, $\tau_2=14.4$ days, C=0.12, and $\tau_3=74$ days. Were superposition to be applicable, these data imply that repeated daily dosing would produce 90% of the quasi-steady-state trough level of 23.9 ng/mL after about 90 days³. Once at quasi-steady-state, plasma levels of trandolaprilat would be expected to remain >2 ng/mL (the level producing maximal plasma ACE inhibition) for 15 weeks.

Cumulative urinary excretion of trandolaprilat is shown in Figure 65 below.

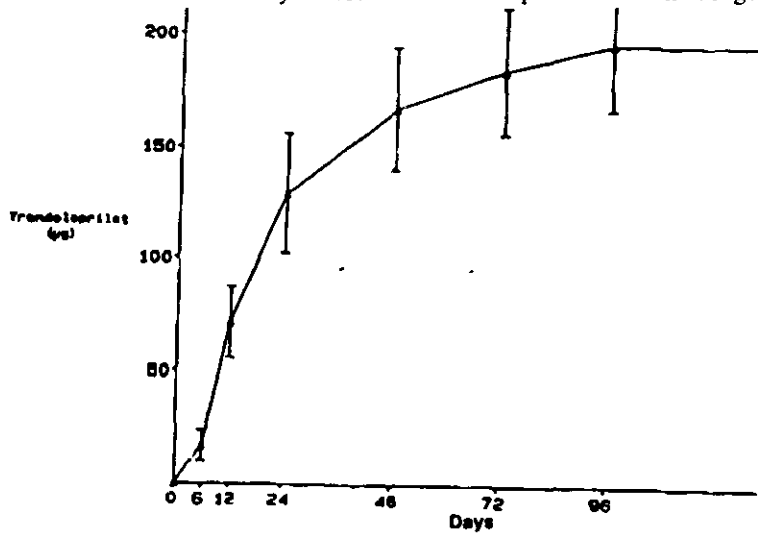


Figure 65. Cumulative urinary excretion of trandolaprilat in Study F/91/570/133.

ACE inhibition is described in Figure 66 and Table 91 below. Baseline ACE inhibition

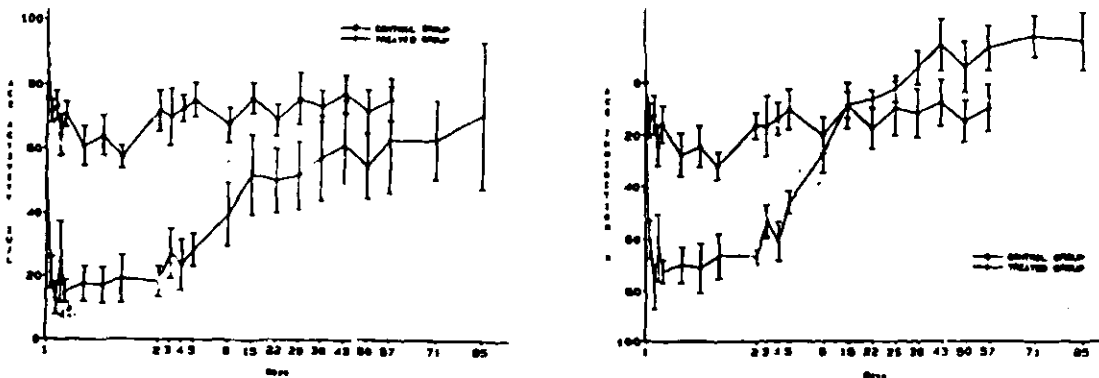


Figure 66. Plasma ACE activity and ACE inhibition in Study F/91/570/133.

Table 91. Plasma ACE inhibition in Study F/91/570/133.

	Control	Trandol	ANOVA
ACE activity at baseline (IU/L)	86.0 6.6	53.9 10.5	0.01 < p < 0.05
I _{max} (%)	—	81.2 7.1	—
T _{Imax} (h)	—	2	—
median range		2-24	

was higher in the placebo than in the treated group (86 vs 54 IU/L, respectively). ACE inhibition was maximal 2 hours after trandolapril dosing (with a mean value of 78.7%).

³ Weekly dosing was also modeled: With weekly dosing at 2 mg, trough plasma levels are >2 ng/mL (where plasma ACE inhibition is maximal) from week 4 onward. Quasi-steady-state trough levels are 2.9 ng/mL and peak levels are 5.5 ng/mL; one is within 90% of this quasi-steady-state after 15 weeks.

Hysteresis curves for ACE inhibition as a function of plasma levels of trandolapril and trandolaprilat are shown in Figure 67 below.

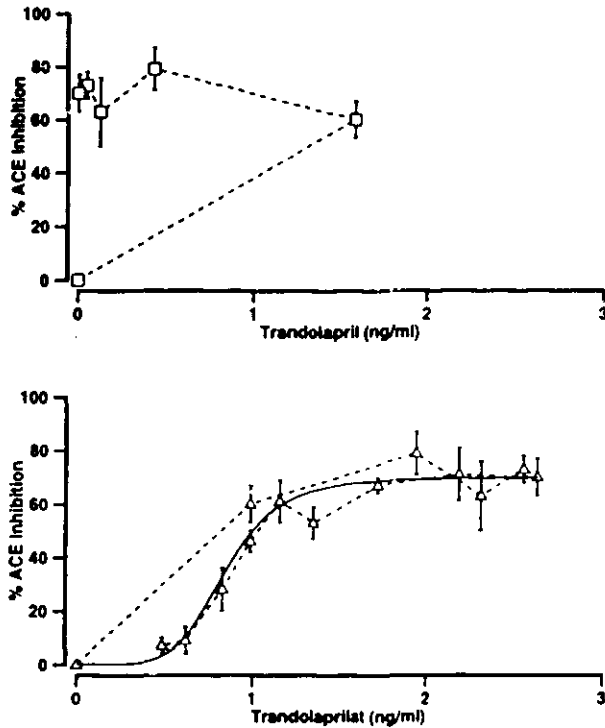


Figure 67. Plasma ACE inhibition vs. plasma drug levels in Study F/91/570/133.

The dashed lines connect data points in temporal order. The solid line in the trandolapril curve was fit using a simplex procedure to

$$y = \frac{A}{1 + \frac{B}{x^C}}$$

with A=69.8 (maximum % ACE inhibition), B=0.04 (implying half-maximal [trandolaprilat] of 0.85 ng/ml),

Although in the treated group the mean enzyme activity did not return to within 90% of baseline until 15 days after dosing, inferences about the persistence of drug activity need to account for the large (as much as 32%) placebo reductions in ACE activity. As early as day 8 post-dose the trandolapril-associated ACE inhibition was less than was the maximal placebo-associated inhibition.

A10.6. Safety.

Case report forms were not submitted for evaluation. In the study report there is no explicit disclaimer that there were no deaths or discontinuations for adverse events.

Four subjects in the trandolapril treatment group were reported to have had adverse events, as described in Table 92 below.

A10.7. Summary.

Healthy adult male subjects (6 per treatment group) were randomized to placebo or a single 2 mg oral trandolapril dose. Plasma trandolaprilat levels were detectable for 2 months after dosing. The trandolapril-associated inhibition of ACE activity appeared to be prolonged (15 days), however in the placebo group there were large (as much as 32%) reductions from baseline plasma ACE activity. As early as day 8, the trandolapril-associated ACE inhibition was less than was the maximal placebo-associated inhibition. In the trandolapril group the maximal mean inhibition of ACE

Table 92. Adverse events in Study F/91/570/133.

Subj	Event
4	25 year old male with mild headache on post-dose day 20. Responded to analgesic use.
7	25 year old male with sinusitis on post-dose day 42
9	20 year old male with asthenia and rhinorrhea starting on post-dose day 71 and resolving after 4 days without treatment. Neutropenia (1.52 K/mm^3) was reported for day 78. No other data described for this event.
12	25 year old male with headache and sinusitis beginning on day 1 and resolving after 2 days without treatment

was 78.7%, observed 2 hours after dosing. The trandolapril-associated reduction in mean supine blood pressure was small-to-modest, and the mean heart rate change from baseline was clinically insignificant. The only potentially serious adverse event among trandolapril-exposed subjects was a single case of late-occurring neutropenia (subject 9), for which the report lacks details necessary for judging its clinical significance.

A11. Study FF/86/570/05 (Study report: vols 1.128; case report tabulations: vols 1.128-1.131; case report forms for deaths and discontinuations for adverse events: none) "Randomized double-blind placebo controlled study of the effect of three single oral doses (1, 4, or 16 mg) of trandolapril on blood pressure in patients with mild to moderate essential hypertension".

A11.1. Investigators The investigators are listed in Table 93 below.

Table 93. Investigators in Study FF/86/570/05.

Investigator	Center	City
J Aldigier	2	Limoges, France
J Demange	1	Poitiers, France
Y Weiss	3	Meaux, France

A11.2. Study dates February 1987 to April 1989.

A11.3. Drug formulation

Table 94. Drug substance used in Study FF/86/570/05.

Lot	Form	Type	Trandolapril	Source lot	Batch size
MB 21092-122	Cap	Plcbo	—	—	84,176
JL 20985-93	Cap	Plcbo	—	—	5,300
MB 22327-90	Cap	Tran	1.0 mg	18	2,000
JL 20985-111	Cap	Tran	1.0 mg	11	1,500
MB 22327-55	Cap	Tran	4.0 mg	18	450
JL 20985-83	Cap	Tran	4.0 mg	11	2,500

Table 95. Capsule composition in Study FF/86/570/05.

	1 mg	4 mg
Trandolapril	1.0	4.0
Lactose, hydrous		
Total		

A11.4. Study design This placebo-controlled, double-blind, parallel-group study randomized 44 subjects (patients with mild-to-moderate arterial hypertension) to receive (after an overnight fast) single fixed doses of placebo or trandolapril (1, 4, or 16 mg) subsequent to a 4 week placebo run-in.

The objective was to assess safety and pharmacodynamic effects by observing changes from pre-treatment ACE inhibition, plasma renin activity, plasma aldosterone, plasma atrial natriuretic factor (ANF), and blood pressure (measured via cuff and ambulatory blood pressure monitor (ABPM)).

Enrolled subjects were adults of both sexes with uncomplicated essential hypertension, and supine diastolic BP ≥ 95 and < 120 mmHg. Exclusion criteria are listed in Table 96 below.

The primary end point was the change from baseline (pre-treatment) supine blood pressure, measured with a sphygmomanometer (cuff). Cuff blood pressures were to be determined pre-treatment, and at post-dose hours 1, 2, 3, 4, 9, 12, 24, 27, 33, 36, and

Table 96. Exclusion criteria in Study FF/86/570/05.

Cerebral hemorrhage or ischemia
Retinal hemorrhage or thrombosis
Coronary insufficiency
Heart failure
Mesenteric or lower extremity arteriopathy
Serum creatinine >130 μ M
Secondary hypertension
Serum potassium $\geq$ 5.5 mM
Body weight $\geq$ 130% of ideal
Use within 4 weeks of drugs known to induce or inhibit drug-metabolizing enzymes, cimetidine, ranitidine, chronic NSAIDs, glucocorticoids, digitalis, estrogen-progesterone combinations, or antihypertensive agents
Inability to follow treatment or dietary restriction

48. ABPM measurements were to be obtained every 15 minutes during post-dose hours 0 to 12 and 24 to 36 using an unspecified model :

Blood was to be sampled at post-dose hours 1, 2, 3, and 4 for routine chemistry and hematology measurements. Plasma was also to be assayed for ACE inhibition, renin activity, aldosterone, and ANF levels.

Changes from baseline blood pressure were compared using a two-way analysis of variance (ANOVA) with interaction (time, group). Where the interaction or group effect was significant ($p \leq 0.05$), then a one-way ANOVA was used to compare changes at each time-point. Each trandolapril group was compared with placebo using the Dunnett's test.

Where there were no missing or artifactual data, the hourly mean ABPM values were calculated as follows: baseline means were based on data acquired in the preceding hour, and on-treatment means were calculated from the data obtained within ± 30 minutes of the designated post-dose hour.

The following types of ABPM observations were assumed to be artifactual: diastolic BP values either <50 mmHg, >120 mmHg, or more than 20 mmHg different from the preceding or succeeding value; systolic BP values either <100 mmHg, >240 mmHg, or more than 30 mmHg different from the preceding or succeeding value; heart rate either <45 bpm, >120 bpm, or more than 20 bpm different from the preceding or succeeding value. Where baseline values were deemed artifactual the first values recorded in the next 15 minutes were used to impute the baseline. The mean value observed in the preceding hour was used to impute the hourly value when observed hourly values were deemed artifactual.

Pharmacokinetic assays were assessed as shown in Table 97 below.

Table 97. Pharmacokinetic assays in Study FF/86/570/05.

Linearity	Accuracy	Precision	Sensitivity	Specificity
Plasma samples—ACE inhibition—spectrophotometry				
	52.0 U/L for expected values of 44 to 58 U/L	4.52% CV at 52.0 U/L		
	43.8 U/L for expected values of 29 to 53 U/L	4.35% CV at 44.2 U/L		

A11.5. Results

Demographic and baseline characteristics of subjects are shown in Table 98 below. In

Table 98. Demographic and baseline characteristics of subjects in Study FF/86/570/05.

	Placebo n=10	Trandolapril		
		1 mg n=11	4 mg n=11	16 mg n=12
Male	4	10	6	6
Female	6	1	5	6
Age mean	52	49	47	45
Weight mean	67	80	69	69

the all-randomized dataset, baseline mean age was comparable among the treatment groups, however there was a statistically significantly higher proportion of males, and a higher mean weight among subjects randomized to 1 mg trandolapril, relative to the other groups (reportedly, block randomization was not correctly executed at investigator Weiss' center). Baseline blood pressures were approximately 10 mmHg higher in the 16 mg trandolapril group than in other groups (although not statistically significant).

Reportedly, there were no major protocol violations and no dropouts.

Baseline blood pressure values were imputed in the ABPM analyses of two placebo subjects (15 and 17), one subject randomized to 4 mg trandolapril (13), and one subject (16) receiving 16 mg. Among 1517 on-treatment ABPM values, 3.8% were imputed (the treatment distribution of these was not described).

ABPM data for placebo subjects 12 and 28, and for subject 44 (randomized to trandolapril 16 mg) were missing and excluded from the analyses.

Cuff blood pressure data are shown in Figure 68 below.

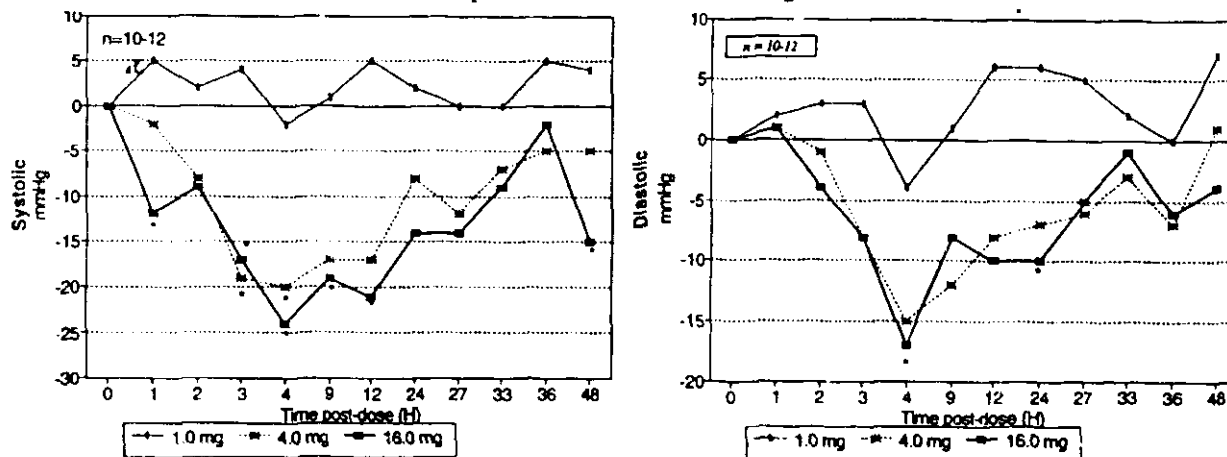


Figure 68. Cuff blood pressure changes in Study FF/86/570/05.

Placebo-corrected changes from baseline supine diastolic blood pressure are shown in Table 99 below. The sponsor's analysis showed the mean peak changes from baseline for the 4 and 16 mg trandolapril groups to be statistically distinguishable from placebo, and the mean trough (24 hour post-dose) diastolic change for 16 mg to be likewise differentiable. Beyond 24 hours no drug-induced diastolic change was distinguished with certainty from that of placebo. Maximal reductions in supine diastolic pressure (double differences from baseline and placebo) were substantial (means ranging from 15 to 17 mmHg), and the mean trough effect of the highest dose was moderately large

Table 99. Supine diastolic pressure data in Study FF/86/570/05.

	Placebo n=10	Trandolapril ^a				Placebo n=10	Trandolapril		
		1 mg n=11	4 mg n=11	16 mg n=12			1 mg n=11	4 mg n=11	16 mg n=12
Baseline	105	105	101	111	12 hours	-8	+6	-8	-10
1 hour	-2	+2	+1	+1	24 hours	-7	+6	-7	-10
2 hours	-2	+3	-1	-4	27 hours	-5	+5	-6	-5
3 hours	0	+3	-8	-8	33 hours	-7	+2	-3	-1
4 hours	+4	-4	-15*	-17*	36 hours	-3	0	-7	-6
9 hours	-5	+1	-12	-8	48 hours	-8	+7	+1	-4

a. Sponsor's analysis; trandolapril changes are double-differences from baseline and placebo. (* $p < 0.05$)

(10 mmHg). In the first day of therapy statistical significance was found for comparisons among all groups from 3 to 12 hours post-dose. The effects of 4 and 16 mg doses did not appear to be appreciably dose-ordered. Generally comparable findings were obtained for standing diastolic pressure.

Placebo-corrected changes from baseline supine systolic blood pressure are shown in Table 100 below. The sponsor's analysis showed changes from baseline for the 4 and

Table 100. Supine systolic pressure data in Study FF/86/570/05.

	Trandolapril ^a				Trandolapril		
	1 mg n=11	4 mg n=11	16 mg n=12		1 mg n=11	4 mg n=11	16 mg n=12
Baseline	164	158	175	12 hours	+5	-17	-21*
1 hour	+5	-2	-12*	24 hours	+2	-8	-14
2 hours	+2	-8	-9	27 hours	0	-12	-14
3 hours	+4	-19*	-17*	33 hours	0	-7	-9
4 hours	-2	-20*	-24*	36 hours	+5	-5	-2
9 hours	+1	-17	-19*	48 hours	+4	-5	-15*

a. Sponsor's analysis; trandolapril changes are double-differences from baseline and placebo. (* $p < 0.05$)

16 mg trandolapril groups to be statistically distinguishable from placebo at various times within the first 12 hours post-dose. Statistical significance was found for comparisons among all groups (generally from 3 to 12 hours post-dose), although once again the effects of 4 and 16 mg doses did not appear to be much related to dose. Maximal reductions in supine systolic pressure were substantial, ranging from 20 to 24 mmHg. Mean trough changes were not statistically significant. Beyond 24 hours, it was generally not possible to distinguish with certainty drug from placebo, with the exception of an apparently significant antihypertensive effect 48 hours after the 16 mg dose. Generally, the mean changes obtained for standing systolic pressure were slightly larger than these supine results.

Diastolic ambulatory blood pressure data are shown as changes from baseline on days 1 and 2 in Figure 69 below. ABI M data for 16 mg trandolapril appear to show a greater than placebo change from baseline during 36 hours post-dose.

Heart rate changes from baseline were clinically nonsignificant at peak, and minimal at trough.

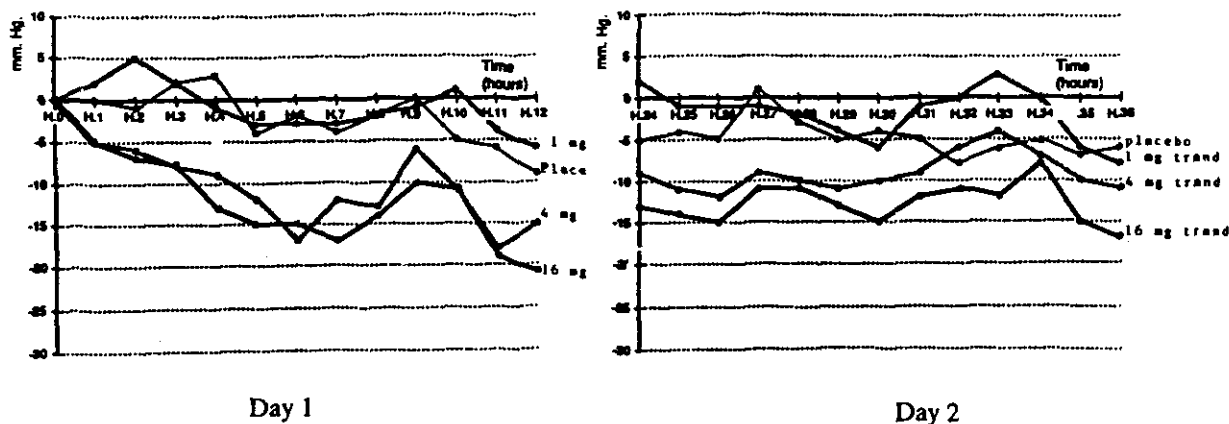


Figure 69. Changes from baseline diastolic ABPM in Study FF/86/570/05. (n=7 to 11 per group)

Trough trandolaprilat levels increased monotonically with dose.

ACE inhibition, plasma renin activity, aldosterone, and ANF are depicted in Table 101 below¹. There is a dose-response relationship evident for ACE inhibition, but not

Table 101. Renin, aldosterone, and ACE inhibition in Study FF/86/570/06.

	Plcbo	1 mg	4 mg	16 mg
ACE inhibition (%)	n=6	n=7	n=7	n=7
Peak	8	38	68	84
Trough	1	23	57	77
Renin activity	n=10	n=11	n=11	n=12
Baseline	0.79	1.02	1.15	1.15
Trough	0.87	1.80	1.91	1.88
Aldosterone	n=6	n=8	n=9	n=9
Baseline	141	162	239	141
Trough	148	135	182	104
ANF	n=6	n=6	n=6	n=6
Peak	123	78	102	87
Trough	88	101	82	62

much of one apparent for aldosterone or ANF. During active treatment renin levels were increased, aldosterone levels were decreased, and ANF levels were not changed in a consistent manner.

A11.6. Safety.

Case report forms were not submitted for evaluation. The report states that no adverse events were reported by investigators.

The interpretability of the submitted laboratory chemistry and hematology data presentations is limited by the absent description of baseline values and by uncertainty as to which sampling time was represented. The normal ranges for laboratory tests were not provided and so were assumed to be similar to those in Study FF/87/570/26².

It is not clear whether the following were pre-existing or treatment-emergent abnormalities: two subjects receiving 1 mg trandolapril (26 and 30) had mild-to-moderate elevations of erythrocyte sedimentation rate (as did two placebo subjects);

¹ Normal values were not provided.

one trandolapril subject had a persistent moderate elevation of total bilirubin, one had a moderate elevation of serum gamma GT, three had mild elevations of serum alkaline phosphatase, and several had mild elevations of serum creatinine.

A11.7. Summary.

A single oral dose of trandolapril (1 to 16 mg) was administered to 44 hypertensive subjects in this placebo-controlled study. The mean inhibition of ACE was approximately 25-80% at trough in the actively treated groups. During active treatment, renin levels were increased, aldosterone levels were decreased, and ANF levels were not consistently changed. The sponsor's statistical analysis of cuff data found a significant reduction in mean trough diastolic pressure in the trandolapril 16 mg group (with a similar trend for systolic pressure). Peak mean effects were seen at 4 hours, and the antihypertensive effect generally disappeared by 27 hours after dosing (with the exception of an apparent delayed systolic pressure lowering at 48 post-dose). Neither 1 mg or 4 mg trandolapril had statistically distinguishable antihypertensive effect 24 hours after a single administration. ABPM data for 16 mg trandolapril appeared to show a greater than placebo change from baseline during 36 hours post-dose. A number of laboratory abnormalities were noted, but the sponsor's presentation does not allow differentiation of treatment-emergent from pre-existing abnormalities.

² Review Study FF/86/570/05 (Study report: vols 1.128; case report tabulations: vols 1.128-1.131; case report forms for deaths and discontinuations for adverse events: none) "Randomized double-blind placebo controlled study of the effect of three single oral doses (1, 4, or 16 mg) of trandolapril on blood pressure in patients with mild to moderate essential hypertension". on page 130. The studies were conducted during the same period and involved a common site and investigator.

A12. Study FF/86/570/06 (Study report: vols 1.212-1.220; case report tabulations: vols 1.213-1.216; case report forms for deaths and discontinuations for adverse events: vols 1.383) "Evaluation of the effect of repeated doses of trandolapril (3 days) on blood pressure in patients with mild to moderate essential hypertension: double-blind, placebo-controlled, multicentre study evaluating doses of 2 mg, 4 mg, and 8 mg".

A12.1. Investigators The investigators are listed in Table 102 below.

Table 102. Investigators in Study FF/86/570/06.

Investigator	Center	City	Investigator	Center	City
L Boejinga	151	Amsterdam, Netherlands	M Lewis	251	Cardiff, UK
V Costigliola	101	Brussels, Belgium	B Meyer	201	Bloemfontein, South Africa
JH De Bruyn	153	Enschede, Netherlands	A Mimran	005	Montpellier, France
PW De Leeuw	152	Rotterdam, Netherlands	CAL Moon	254	Redruth, UK
J Duchier	007	Boulogne, France	L Poggi	006	Marseille, France
D Fraser	252	Dunfermline, UK	ME Safar	004	Paris, France
J Fritz	202	Sasolburg, South Africa	NN Stanley	255	Hitchin, UK

A12.2. Study dates November 1986 to (?) 1987.

A12.3. Drug formulation

Table 103. Drug substance used in Study FF/86/570/06.

Lot	Form	Type	Trandolapril	Source lot	Batch size
JL 20985-97	Cap	Tran	2.0 mg	11	1,500
MB 21092-146	Cap	Tran	2.0 mg	17	20,000
JL 20985-83	Cap	Tran	4.0 mg	11	2,500
JL 20985-95	Cap	Tran	4.0 mg	11	1,500
MB 21092-125	Cap	Tran	4.0 mg	17	20,000

Table 104. Capsule composition in Study FF/86/570/06.

	2 mg	4 mg
Trandolapril	2.0	4.0
Lactose, hydrous		
Total		

A12.4. Study design

The trial description is based upon the English version of the protocol. There was a 4-week single-blind, placebo lead-in phase, followed by a 8-day double blind treatment period, and a 7-day single-blind, placebo washout period. Forty subjects per treatment group were planned. Subjects were to be age 21 to 60 years, weight between 55 and 90 kg, with supine and standing diastolic pressure between 95 and 119 mmHg at the end of the placebo run-in phase. Women were permitted if post-menopausal or surgically sterile. Exclusion criteria are listed in Table 105 below.

Subjects who qualified at the end of the placebo run-in period were randomized to placebo or trandolapril 2, 4, or 8 mg q.d. and returned after 8 days. All subjects took the same number of capsules. Subjects were instructed to take their study drug in the morning on an empty stomach.

Table 105. Exclusion criteria in Study FF/86/570/06.

Cerebral hemorrhage or ischemia
Retinal hemorrhage or thrombosis
Coronary artery disease
Symptomatic left of global heart failure
Active arteritis of the lower limbs
Mesenteric arteritis
Renal failure as evidenced by creatinine >130 μ M
Severe chronic cardiac, pulmonary, hepatic, metabolic, or neurological disease
Chronic alcoholism
Identifiable secondary forms of hypertension
Hyperkalemia (>5.5 mM)
Weight >130% of ideal
Use within 30 days of antihypertensive drugs, enzyme-inducing drugs, cimetidine, ranitidine, chronic anti-inflammatory or analgesic drugs, glucocorticoids, digitalis, estrogen-progestogen combinations

Safety data included medical history, physical exam, 12-lead ECG, and routine hematology, clinical chemistry, and urinalysis. There were no explicit criteria for dropping a subject because of lack of efficacy.

Blood samples were obtained for ACE activity, aldosterone, and renin activity levels at baseline and at the final double-blind visit. Trandolapril and trandolaprilat levels were obtained at baseline and after the dose at the final double-blind treatment period visit.

Twenty-four hour urinary sodium excretion was measured at baseline, at the end of the double-blind treatment, and at the final follow-up visit.

The primary analysis was an intent-to-treat analysis of supine diastolic pressure at the final double-blind visit. Secondary end points were supine systolic and standing blood pressures. Blood pressure normalization was considered <160/90 mmHg. Responders were subjects with supine and standing diastolic pressure <90 mmHg or decreased by ≥ 10 mmHg. One-sided testing with $P=0.05$ considered statistically significant; is described in the final report.

A12.5. Results

Two hundred thirty-three subjects were enrolled, of whom 216 subjects were randomized and 209 completed the final washout period. Individual study centers enrolled 2 to 50 subjects. Demographic and baseline characteristics of subjects are shown in Table 106 below. The treatment groups were reasonably well matched for these factors, for general health, duration and severity of hypertension, and baseline drug use.

Seven subjects failed to complete the double-blind treatment period, 5 for protocol violations, 1 for adverse event, and 1 for intercurrent illness. Data from one center (n=6) were excluded because of questions regarding the randomization procedure. Data from the two South African centers were excluded from the secondary efficacy assessments because regulatory authorities initially refused to allow the 4 and 8 mg doses, and the blind was broken by the sponsor. Data from Black subjects (n=18) were excluded because of "well-recognized differences in response to ACE-inhibitors". Forty-eight subjects were improperly randomized. Nine subjects did not meet baseline blood pressure criteria, 7 subjects were excluded for use of concomitant drugs¹. In all, 80 subjects were excluded from secondary efficacy assessments (18 to 23 per group).

¹ Only 32 subjects had concomitant drug use recorded.

Table 106. Demographic and baseline characteristics of subjects in Study FF/86/570/06.

	Placebo n=54	Trandolapril		
		2 mg n=56	4 mg n=51	8 mg n=53
Male	1	37	25	32
Female	23	19	26	22
Age $\pm$ SEM	49 $\pm$ 1.1	48 $\pm$ 1.1	49 $\pm$ 1.4	49 $\pm$ 1.3
Range	24 to 60	28 to 60	23 to 64	26 to 76
Caucasian	51	47	45	49
Black ^a	3	7	4	6
Other	0	1	2	0
Weight $\pm$ SEM	76 $\pm$ 2.0	75 $\pm$ 1.7	71 $\pm$ 2.0	74 $\pm$ 1.8
Range	49 to 120	52 to 108	49 to 105	50 to 98

a. Black subjects were excluded by "verbal agreement" and were dropped from the efficacy assessment.

No subjects were excluded from primary efficacy or safety analyses because of protocol violations.

Diastolic blood pressure data are shown in Table 107 below. The sponsor's analysis

Table 107. Supine diastolic pressure data in Study FF/86/570/06.

	Placebo n=53	Trandolapril			Change from baseline and placebo		
		2 mg n=55	4 mg n=49	8 mg n=51	2 mg n=55	4 mg n=49	8 mg n=51
Baseline	102	102	103	102			
End point	99	94	95	94	-4.4	-5.2	-4.6
Peak (4 hours)	97	91	92	92	-6.1	-5.9	-5.1
Washout 3	98	98	97	98	-0.3	-2.2	-0.7

found statistical significance to changes in supine diastolic pressure comparing placebo with each of the treated groups². No statistical significance was found for comparisons among treated groups. Generally consistent findings were obtained for supine systolic pressure, and standing systolic and diastolic pressures, and for responder rates, but these results are not reproduced here, because of difficulties in interpretation caused by the high number of excluded subjects. Peak changes in supine pressure were about -13/-6 mmHg (double-differences from baseline and placebo).

Renin, aldosterone, and ACE activity are shown in Table 108 below. There is not much of a dose-response relationship evident. Renin levels increased and aldosterone levels decreased slightly in the active treatment groups. ACE inhibition was about 70 to 80% at trough in all actively treated groups.

A12.6. Safety.

A total of 25 adverse events were reported in 23 subjects. Of these, 19 events were described as treatment-emergent events. All adverse events are summarized in Table 109 below. Headache was the most common event, but it was reported more frequently in the placebo group. Subjects who discontinued because of adverse reactions are listed in Table 110 below.

² Overall one-sided $P=0.015$.

Table 108. Renin, aldosterone, and ACE inhibition in Study FF/86/570/06.

	Plcbo	2 mg	4 mg	8 mg
ACE inhibition (%)	n=47	n=48	n=44	n=45
Trough	-4	-71	-81	-59
Peak	-7	-75	-85	-78
Washout	-7	-41	-45	-39
Renin activity	n=49	n=53	n=49	n=52
Baseline	0.77	1.27	0.97	1.10
Trough	0.71	2.50	1.78	2.90
Peak	0.78	3.47	2.05	5.11
Washout	0.68	1.29	0.91	1.13
Aldosterone	n=51	n=53	n=47	n=52
Baseline	131	136	138	108
Trough	140	125	102	102
Peak	148	97	82	92
Washout	128	130	116	96

Table 109. Adverse events in Study FF/86/570/06.

Plcbo n=54	Trandolapril		
	2 mg n=56	4 mg n=51	8 mg n=55
Headache	Headache	Headache	Headache
Headache	Headache	Asthenia	Dizziness
Headache	Vertigo	Headache	Chest pain
Vertigo	Dizziness	Epistaxis	Diarrhea
Dyspnea	Malaise	Impotence	Hepatitis
Nervousness		Tachycardia	
		Constipation	
		Palpitations	
		Chest pain	

Table 110. Discontinuations for adverse events in Study FF/86/570/06.

Center	Subj	Group	Day	ADR
	229	4	1	48 year old Caucasian male with new hypertension diagnosis. Subject had onset of severe left-sided chest pain on day 7. ECG and enzymes were normal and he was treated with amoxicillin for a presumed chest infection with resolution of symptoms.
	41	4	7	60 year old Caucasian female with untreated hypertension. She discontinued with vertigo on the first day of double-blind treatment, with prompt resolution of symptoms.
	58 ^a	8	8	56 year old male not previously treated for hypertension. Enzymes normal at baseline, but at end of run-in, SGOT was 73, SGPT was 80, and AlkPhos was 108. At end of double-blind, SGOT was 479, SGPT was 828, and AlkPhos was 140. Enzymes climbed further during washout, then declined, but were still not normal range at 3 weeks. Subject + for HBS antibody at 3 weeks.

a. CRF not provided; description based upon sponsor's summary.

Orthostatic hypotension was not reported.

There were a variety of new findings on the final physical exam, in clinical laboratory data, and ECG; none were serious or had apparent relationship to the study drug.

A12.7. Summary.

This was the first placebo-controlled, multiple-dose study in man. Hypertensive subjects underwent an 8-day double-blind phase, with fixed doses, and a 7-day washout. Efficacy data are difficult to interpret because of defects in the conduct of the protocol affecting one-third of the subjects. Doses of 2 to 8 mg q.d. appeared to have antihypertensive effects distinguishable from placebo but not from one another. Safety data revealed no events with an apparent relationship to treatment.

A13. Study FF/86/570/14 (Study report: vols 1.221-1.230; case report tabulations: vols 1.223-1.227; case report forms for deaths and discontinuations for adverse events: vols 1.384) "Evaluation of the effect of trandolapril (RU 44570) in repeated doses (28 days) on blood pressure in patients with mild to moderate essential hypertension: double-blind, placebo-controlled, multicentre study to evaluate 2 mg, 4 mg, and 8 mg doses".

A13.1. Investigators The investigators are listed in Table 111 below.

Table 111. Investigators in Study FF/86/570/14.

Investigator	Center	City	Investigator	Center	City
C Aronzon	26	Marseille, France	Y Lassalle	24	Chapous, France
I Belleville	20	Gueret, France	B Meyer	201	Bloemfontein, South Africa
G Boxho	104	Verviers, Belgium	J Neiman	19	Metz, France
J Brun	11	Dijon, France	P Nel	203	Sunnyside, South Africa
C Crivelli	1302	Buenos Aires, Argentina	J Ribeyre		Le Chasnay, France
J Duchier		Boulogne, France	H Richter	401	Hamburg, West Germany
L Ferder	1301	Buenos Aires, Argentina	U Roeder	402	Henstedt, West Germany
J Fritz	202	Sasolburg, South Africa	J Ryckelynck	22	Caen, France
D Gautier	10	St. Mande, France	A Seigneuric	9	Versailles, France
F Giacardy	17	Tarbes, France	A Woittiez	154	Almelo, Netherlands
V Hamoir	105	Tournai, Belgium			

A13.2. Study dates March 1987 to March 1988.

A13.3. Drug formulation

Table 112. Drug substance used in Study FF/86/570/14.

Lot	Form	Type	Trandolapril	Source lot	Batch size
MB 21092-146	Cap	Tran	2.0 mg	17	20,000
MB 21092-125	Cap	Tran	4.0 mg	17	20,000
MB 21092-126	Cap	Tran	8.0 mg	17	20,000

Table 113. Capsule composition in FF/86/570/14.

	2 mg	4 mg	8 mg
Trandolapril	2.0	4.0	8.0
Unknown			
Total			

A13.4. Study design

The trial description is based upon the English version of the protocol. There was a 4-week single-blind, placebo lead-in phase, followed by a 28-day double-blind treatment period. There was no final washout period. Thirty-nine subjects per treatment group were planned. Subjects were to be age 21 to 60 years, weight between 55 and 90 kg and <130% of ideal body weight, with supine and standing diastolic pressure between 95 and 115 mmHg and systolic pressure $\leq$ 180 mmHg at the end of the placebo run-in phase. Women were permitted if post-menopausal or surgically sterile. Exclusion criteria are listed in Table 114 below.

Subjects who qualified at the end of the placebo run-in period were randomized to placebo or trandolapril 2, 4, or 8 mg q.d. and returned after 1 week, 2 weeks, and

Table 114. Exclusion criteria in Study FF/86/570/14.

Concurrent diseases related to hypertension (cerebral hemorrhage or ischemia, retinal hemorrhage or thrombosis, coronary insufficiency, heart failure, arteriopathy of the lower limbs, mesenteric arteriopathy, or renal failure).
Severe chronic cardiopulmonary, hepatic, metabolic, neurological, or psychological disease, allergy or drug hypersensitivity, collagen disease, dermatoses, or neutropenia.
Hypertension secondary to a known cause
Hyperkalemia (≥ 5.5 mM)
Use within 28 days prior to study of enzyme inhibitors, cimetidine, ranitidine, long-term anti-inflammatory or analgesic drugs, glucocorticoids, digitalis, or estrogen-progestogen.
Previous trial participation within 28 days

4 weeks. All subjects took one capsule per day. Subjects were instructed to take their study drug in the morning 15 minutes after breakfast.

Safety data included medical history, physical exam, 12-lead ECG, and routine hematology, clinical chemistry, and urinalysis. There were no explicit criteria for dropping a subject because of lack of efficacy.

Blood samples were obtained for ACE activity, aldosterone, and renin activity levels.

Trandolapril and trandolaprilat levels were obtained at baseline and after the dose at the final double-blind treatment period visit.

Twenty-four hour urines were collected for protein, glucose, sodium, and creatinine.

The primary end point was responder rate¹. The trial was sized to detect a 30% difference from a 25% placebo responder rate, with one-sided testing, $\alpha=0.05$, and $\beta=0.05$. Response was defined as having supine *and* standing diastolic pressure <90 mmHg or a reduction by ≥ 10 mmHg.

A13.5. Results

Two hundred twenty-three subjects were enrolled, of whom 208 subjects were randomized. Reasons for not randomizing enrolled subjects were placebo response (6), severe hypertension (4), adverse events (2), and loss to follow-up (3). Twenty subjects from one center were excluded because an internal audit indicated GCP guidelines were not followed. The analyses were based on the remaining 188 subjects, of whom and 180 completed the double-blind treatment period. Individual study centers enrolled 1 to 38 subjects. Demographic and baseline characteristics of subjects are shown in Table 115 below. Other than for gender, the treatment groups were reasonably well matched for these factors, for general health, duration and severity of hypertension, and baseline drug use.

Thirty-two subjects (6 to 10 per group) were excluded from a per-protocol-like analysis. This group excluded Black subjects² (n=11), subjects with treatment duration >40 days (n=5), subjects with supine diastolic pressure <95 mmHg on 2 of 3 measurements at baseline (n=10), subjects with improperly timed visits (<18 or >30 hours after dosing; n=11), or concomitant medication violations (anti-inflammatory, H₂ antagonist, other antihypertensive; n=2). Not excluded were subjects with age >60 (n=8), with weight outside limits (n=46), and other than surgical contraception (n=32).

Seven subjects failed to complete the double-blind treatment period, 5 for protocol violations, 1 for adverse event, and 1 for intercurrent illness. Data from one center

¹ The protocol clearly indicates responder rate was the primary end point. The sponsor's report on the trial indicates that the primary end point was supine diastolic pressure at trough at 4 weeks.

² The protocol did not exclude Black subjects.

Table 115. Demographic and baseline characteristics of subjects in Study FF/86/570/14.

	Plcbo n=46	Trandolapril		
		2 mg n=50	4 mg n=47	8 mg n=45
Male	23	28	18	25
Female	23	22	29	20
Age±SEM	48±1.3	50±1.4	49±1.2	46±1.7
Range	21 to 61	22 to 62	29 to 60	23 to 65
Caucasian	44	49	42	41
Black	4	0	4	3
Other	1	1	1	1
Weight±SEM	77±1.7	75±1.9	74±2.1	77±1.9
Range	54 to 110	50 to 103	52 to 120	54 to 107

(n=6) were excluded because of questions regarding the randomization procedure. Data from the two South African centers were excluded from the secondary efficacy assessments because regulatory authorities initially refused to allow the 4 and 8 mg doses, and the blind was broken by the sponsor. Data from Black subjects (n=18) were excluded because of "well-recognized differences in response to ACE-inhibitors". Forty-eight subjects were improperly randomized. Nine subjects did not meet baseline blood pressure criteria, 7 subjects were excluded for use of concomitant drugs³. In all, 80 subjects were excluded from secondary efficacy assessments (18 to 23 per group).

Response rate analysis was based upon diastolic pressure <90 or a reduction by ≥10 mmHg, not upon supine and standing diastolic pressure, as indicated in the protocol. The sponsor's analysis of response rate is shown in Table 116 below. No

Table 116. "Response rate" data (%) in Study FF/86/570/14.

	Plcbo n=46	Trandolapril		
		2 mg n=50	4 mg n=47	8 mg n=45
Week 1	23 (50)	28 (56)	25 (53)	20 (46)
Week 2	22 (50)	33 (66)	26 (57)	24 (53)
Week 4	28 (62)	29 (59)	31 (66)	27 (60)
End point	28 (61)	30 (60)	31 (66)	27 (60)

differences among groups were demonstrated.

A secondary analysis of rates of normalization of blood pressure (supine blood pressure <160/90 mmHg) likewise showed no differences among treatment groups.

Supine diastolic blood pressure data are shown in Table 117 below. The sponsor's analysis found statistical significance to changes from baseline for all treatment groups, but no significant differences among treatment groups. Other blood pressure data were not reviewed.

Renin, aldosterone, ACE activity, and plasma drug levels were not included in the submission.

³ Only 32 subjects had concomitant drug use recorded.

Table 117. Supine diastolic pressure data in Study FF/86/570/14.

	Plcbo n=46	Change from baseline			Change from baseline and placebo		
		2 mg n=50	4 mg n=47	8 mg n=45	2 mg n=50	4 mg n=47	8 mg n=45
Baseline	102	100	102	102			
Week 1	-7.2	-8.2	-8.5	-7.7	-1.0	-1.3	-0.5
Week 2	-7.9	-10.6	-10.9	-9.3	-2.7	-3.0	-1.4
Week 4	-9.9	-10.1	-12.0	-10.0	-0.2	-2.1	-0.1
End point	-9.8	-10.2	-12.0	-10.0	-0.4	-2.2	-0.2

A13.6. Safety.

Four to 9 subjects per group reported adverse events. All adverse events are summarized in Table 118 below. Subjects who discontinued because of adverse

Table 118. Adverse events in Study FF/86/570/14.

Plcbo n=46	Trandolapril		
	2 mg n=50	4 mg n=47	8 mg n=45
Headache	Headache	Headache	Vertigo
Headache	Vertigo	Vertigo	GI pain
Nausea	Malaise	Vertigo	Malaise
Nausea	Malaise	Nausea	
		Nausea	
		Nausea	
		GI pain	
		GI pain	
		Malaise	

reactions are listed in Table 119 below.

Table 119. Discontinuations for adverse events in Study FF/86/570/14.

Subj	Group	Day	ADR
47	Plcbo	5	51 year old Caucasian female with 1 year history of hypertension. Subject discontinued after 5 days because of nausea and vomiting, described as medium severity. Blind broken.
78	2 mg	19	Screening data forms missing. Subject discontinued after 19 days because of burning on urination.
58	2 mg	15	54 year old Caucasian male with newly diagnosed hypertension. Baseline supine BP was 120/50 mmHg. Unclear why he was randomized. Supine BP 130/60 at week 1. Severe a.m. vertigo after 2 weeks led to discontinuation. BP at discontinuation was 100/30 mmHg. Symptoms resolved 1 week after termination.
199	2 mg	25	60 year old Caucasian male with 3-year history of treated hypertension. Subject discontinued because of headache and dizziness. BP at visit 3 days later was 130/85 mmHg.
198	4 mg	13	51 year old Caucasian male with 10-year history of hypertension, untreated at time of enrollment. Baseline supine BP was 155/100 mmHg. Subject discontinued for severe dizziness; BP at last visit was 130/90 mmHg. Symptoms resolved after termination.

Three subjects (2 on placebo) had heart rate changes >25 bpm on moving from supine standing. Seven subjects had diastolic pressure changes ≥30 mmHg upon standing. Symptoms of orthostatic hypotension were not reported.

There were a variety of new findings on the final physical exam, in clinical laboratory data, and ECG; none were serious or had apparent relationship to the study drug. One

subject had serum sodium 105 mM at the final study visit; no follow-up data are reported.

A13.7. Summary.

Technical problems marred the conduct of this 4-week double-blind, placebo-controlled trial. For neither the primary end point of responder rate nor for supine diastolic pressure was there a significant difference between treated and placebo groups. This may have been a result of a large placebo effect. No truly serious adverse events were reported. There was no apparent relationship between adverse events or abnormalities in laboratory safety data and dosing group.

at

A14. Study FF/87/570/04 (Study report: vols 1.241-1.250; case report tabulations: vols 1.242-1.246; case report forms for deaths and discontinuations for adverse events: vols 1.386) "Comparison of the efficacy and safety of trandolapril and hydrochlorothiazide (HCTZ) in patients suffering from mild to moderate essential hypertension".

A14.1. Investigators The investigators are listed in Table 120 below.

Table 120. Investigators in Study FF/87/570/04.

Investigator	Gender	City	Investigator	Gender	City
H Bouzo	412	Augsburg, Germany	B Meyer	201	Bloemfontein, South Africa
C Crivelli	1302	Buenos Aires, Argentina	S Mouloupoulos	301	Athens, Greece
G Douret	110	Auderghes, Belgium	T Pasz	404	Nurnberg, Germany
C Geser	418	Garmisch-Partenkirchen, Germany	R Rau	416	Munchen, Germany
G Hitzenberger	705	Vienne, Austria	M Roex	111	Bruxelles, Belgium
G Jochems	112	Turnhout, Belgium	N Stanley	255	Hitchins, UK
M Lewis	251	Cardiff, UK	A Verhulst	116	Wilrijk, Belgium
FAT Lustermans	156	Heerlen, Netherlands	A Wester	155	Hengelo, Netherlands

A14.2. Study dates March 1988 to June 1989.

A14.3. Drug formulation

Table 121. Drug substance used in Study FF/87/570/04.

Lot	Form	Type	Trandolapril	Source lot	Batch size
MB 21662-94	Cap	Tran	2.0 mg	17	73,820

Table 122. Capsule composition in Study FF/87/570/04.

	2 mg
Trandolapril	2.0
Unknown	
Total	

A14.4. Study design

The trial description is based upon the English version of the protocol. There was a 4-week single-blind, placebo lead-in phase, followed by a 16-week double-blind treatment period. There was no final washout period. One-hundred twenty-four subjects per treatment group were planned. Subjects were to be of any age, weight <130% of ideal body weight, with supine and standing diastolic pressure between 95 and 115 mmHg and systolic pressure ≤180 mmHg at the end of the placebo run-in phase. Women were permitted if post-menopausal or surgically sterile. Exclusion criteria are listed in Table 123 below.

Subjects who qualified at the end of the placebo run-in period were randomized to trandolapril 2 mg q.d., HCTZ 25 mg q.d., or the combination, and returned after 1, 4, 8, 12, and 16 weeks. All subjects took 2 capsules per day. Subjects were instructed to take their study drug in the morning 15 minutes before breakfast.

Safety data included medical history, physical exam, 12-lead ECG, and routine hematology, clinical chemistry, and urinalysis. The criteria for dropping a subject because of lack of efficacy were diastolic pressure >115 or systolic pressure >180 mmHg.

Table 123. Exclusion criteria in Study FF/87/570/04.

Concurrent diseases related to hypertension (cerebral hemorrhage or ischemia, retinal hemorrhage or thrombosis, coronary insufficiency, heart failure, arteriopathy of the lower limbs, mesenteric arteriopathy, or renal failure).
Severe chronic cardiopulmonary, hepatic, metabolic, neurological, or psychological disease, allergy or drug hypersensitivity, collagen disease, dermatoses, or neutropenia.
Hypertension secondary to a known cause
Hyperkalemia (≥ 5.5 mM)
Use within 28 days prior to study of enzyme inhibitors, cimetidine, ranitidine, long-term anti-inflammatory or analgesic drugs, glucocorticoids, digitalis, or estrogen-progestogen.
Blacks
Previous trial participation with 28 days

The primary end point was responder rate¹. The trial was sized to detect a 10% difference from a 60% HCTZ responder rate, with $\alpha=0.05$ and $\beta=0.2$. Response was defined as having supine *and* standing diastolic pressure <90 mmHg or a reduction by ≥ 10 mmHg.

A14.5. Results

Two-hundred twenty-three subjects were enrolled, of whom 205 subjects were randomized. Reasons for not randomizing enrolled subjects were placebo response (3), severe hypertension or other failure to meet inclusion or exclusion criteria (9), loss to follow-up during run-in (6). Twenty subjects failed to complete the double-blind treatment period. Individual study centers enrolled 1 to 46 subjects. Demographic and baseline characteristics of subjects are shown in Table 124 below. The treatment

Table 124. Demographic and baseline characteristics of subjects in Study FF/87/570/04.

	HCTZ n=68	Tran n=68	Comb n=69
Male	42	40	47
Female	26	28	22
Age $\pm$ SEM	51 $\pm$ 1.3	50 $\pm$ 1.2	51 $\pm$ 1.2
Range	22 to 76	21 to 65	27 to 68
Caucasian	68	67	68
Black	0	0	0
Other	0	1	1
Weight $\pm$ SEM	77 $\pm$ 1.4	78 $\pm$ 1.5	78 $\pm$ 1.6
Range	53 to 120	50 to 106	53 to 104

groups were reasonably well matched for these factors, for general health, duration and severity of hypertension, and baseline drug use.

Significant protocol violations included clinic assessments >48 hours after dosing (9), failure to meet baseline blood pressure criteria (12), and a double-blind period which was too short (<100 days; $n=3$) or too long (>124 days; $n=12$).

Twenty subjects (6 to 8 per treatment group) failed to complete the double-blind treatment period, 5 for intolerance, 3 for lack of efficacy, 3 for intercurrent illness, 8 for loss to follow-up, and 1 voluntary withdrawal.

Response rate analysis was based upon supine diastolic pressure ≤ 90 or a reduction by ≥ 10 mmHg, not upon supine *and* standing diastolic pressure, as indicated in the

¹ The protocol clearly indicates responder rate was the primary end point. The sponsor's report on the trial indicates that the primary end point was supine diastolic pressure at trough at 4 weeks.

protocol. The sponsor's analysis of response rate is shown in Table 125 below. The

Table 125. "Response rate" data (%) in Study FF/87/570/04.

	HCTZ n=68	Tran n=68	Comb n=69
Week 8	38 (58)	40 (63)	51 (80)
Week 16	40 (67)	40 (62)	48 (76)
End point	41 (60)	43 (63)	53 (77)

sponsor's analysis demonstrated a statistical difference for the combination at end point, but no difference between the monotherapies.

The rates of normalization of blood pressure (supine blood pressure <160/90 mmHg) at end point were 32% for HCTZ, 40% for trandolapril, and 67% for the combination. The sponsor's statistical analysis found significance to the differences between either of the monotherapies and the combination, but not between the monotherapies.

Supine systolic and diastolic blood pressure data are shown in Figure 70 and Table 126

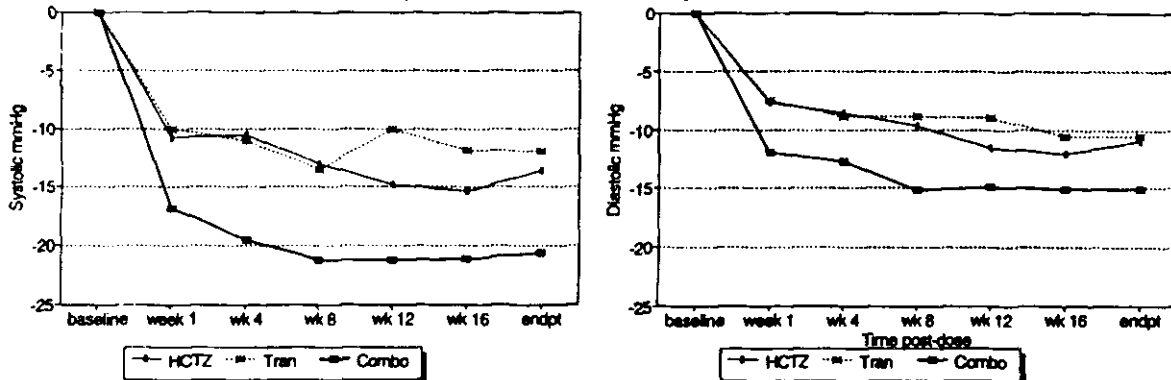


Figure 70. Change in supine systolic and diastolic pressure in Study FF/87/570/04.

below. Standing blood pressure data were similar. These data are all consistent with

Table 126. Change in supine diastolic and systolic pressure data in Study FF/87/570/04.

	Diastolic			Systolic		
	HCTZ n=68	Tran n=68	Comb n=69	HCTZ n=68	Tran n=68	Comb n=69
Baseline	104	102	103	160	157	160
Week 1	-7.7	-7.5	-11.9	-10.8	-10.1	-16.9
Week 4	-8.6	-8.9	-12.7	-10.6	-11.0	-19.5
Week 8	-9.7	-8.9	-15.2	-13.0	-13.5	-21.2
Week 12	-11.5	-9.0	-14.9	-14.8	-10.0	-21.2
Week 16	-12.0	-10.6	-15.1	-15.4	-11.8	-21.1
End point	-10.9	-10.6	-15.1	-13.6	-11.9	-20.6

the monotherapies being similar and the combination producing a greater effect on blood pressure than does either component.

A14.6. Safety.

Thirteen to 15 subjects per group reported adverse events. Non-unique adverse events are summarized in Table 127 below. Subjects who discontinued because of adverse

Table 127. Adverse events reported by >1 subject in Study FF/87/570/04.

	HCTZ n=68	Tran n=68	Comb n=69		HCTZ n=68	Tran n=68	Comb n=69
Any	15	13	13	Headache	5	1	1
Flu syndrome	2	1	0	Hot flushes	1	0	1
Infection	1	4	0	Nervousness	1	1	0
Back pain	1	1	1	Apathy	1	1	0
Asthenia	0	1	2	Cough	0	1	2
Constipation	2	0	0	Upper Resp Inf	1	2	2
Dizziness	1	0	1				

reactions are listed in Table 128 below.

Table 128. Discontinuations for adverse events in Study FF/87/570/04.

Subj	Group	Day	ADR
51	HCTZ	77	52 year old Caucasian male with peptic ulcer disease and 10-year history of treated hypertension. Discontinued with headache over 3 days. Event described as "moderate" severity, but resulted in hospitalization.
70	HCTZ	21	54 year old Caucasian female with 35-year history of treated hypertension. Discontinued because of hot flushes that began during placebo withdrawal. Event attributed to menopause; subject withdrew for medical treatment.
264	HCTZ	58	22 year old Caucasian male with 2-year history of hypertension. Discontinued with new onset of headache and nervousness.
53	Tran	46	55 year old Caucasian male with history of treated hypertension of unknown duration. Subject discontinued because of 2-week bout of flu.
83	Tran	3	27 year old Caucasian female with 5-year history of treated hypertension. Discontinued because of nausea and asthenia beginning with the first dose.
185	Tran	83	64 year old Caucasian female with 6-year history of hypertension. Discontinued because of cough beginning on day 31.
71	Comb	4	55 year old Caucasian female with recently diagnosed hypertension. Discontinued because of depression which predated study entry.
168	Comb et.	9	60 year old Caucasian male with 4-year history of hypertension. Discontinued because of digestive troubles, ants in arms ^a , hot flushes, nausea, and sweating, beginning during placebo withdrawal.

a. Sponsor's literal translation from French; "pins-and-needles" appears in a summary table.

Three subjects (1 on trandolapril, 2 on combination) had heart rate changes >25 bpm on moving from supine standing. Seventeen subjects had diastolic pressure changes ≥30 mmHg upon standing (6 on HCTZ, 4 on trandolapril, and 7 on combination). Symptoms of orthostatic hypotension were not reported.

There were a variety of new findings in clinical laboratory data; none were serious or had apparent relationship to the study drug. Two combination group subjects, one of whom discontinued (#168), had elevated serum potassiums of 5.3 and 5.8 mM. One HCTZ subject had 3 potassium measurements over 5.5 mM, the highest being 8.6 mM.

A14.7. Summary.

Differences were apparent in response rate, diastolic pressure, and systolic pressure, between the combination and the monotherapy groups. There was no significant difference between the two monotherapy groups. The combination group distinguished itself by the first week of double-blind treatment, and the differences were maintained for the full 12-week double-blind treatment period. Without a placebo group, it is not possible to determine if the effect of the combination was greater than the sum of the effects of the components. Adverse events were generally not serious and there was no convincing relationship between any event and a treatment group.

A15. Study FF/87/570/21 (Study report: vols 1.231-1.240; case report tabulations: vols 1.233-1.237; case report forms for deaths and discontinuations for adverse events: vols 1.385) "Evaluation of the effect of trandolapril in repeated doses (28 days) on blood pressure in patients with mild to moderate essential hypertension: double-blind, placebo-controlled, multicentre study to evaluate 0.5 mg, 1 mg, and 2 mg doses".

A15.1. Investigators The investigators are listed in Table 129 below.

Table 129. Investigators in Study FF/87/570/21.

Investigator	Center	Investigator	Center
N Alberts	207 Heidelberg, South Africa	A Van Veen	161 Harderwijk, Netherlands
J Boeiijnga	151 Amsterdam, Netherlands	P Van Wageningen	162 Harderwijk, Netherlands
JHB De Bruijn	153 Enschede, Netherlands	AJJ Woittiez	154 Almelo, Netherlands
J Duchier ^a	Boulogne, France	M Zwart	160 Harderwijk, Netherlands
ALCJ Van Den Hogen ^b	Amsterdam, Netherlands		

a. Nineteen investigators are named in association with Soci t  Innopharm, with separate centers identified only by unique numbers. Only three of the named investigators are shown as having enrolled subjects; J Duchier is not among them.

b. Data from this center were excluded from all analyses.

A15.2. Study dates March 1988 to December 1988.

A15.3. Drug formulation

Table 130. Drug substance used in Study FF/87/570/21.

Lot	Form	Type	Trandolapril	Source lot	Batch size
MB 21662-135	Cap	Tran	0.5 mg	17	12,000
MB 21662-138	Cap	Tran	1.0 mg	17	12,000
MB 21662-130A	Cap	Tran	2.0 mg	18	450,000
MB 21662-130B	Cap	Tran	2.0 mg	18	450,000

d.

Table 131. Capsule composition in Study FF/87/570/21.

	0.5 mg	1 mg	2 mg
Trandolapril	0.5	1.0	2.0
Corn starch			
Lactose, hydrous			
Povidone			
Sodium stearyl fumarate			
Total			

A15.4. Study design

The trial description is based upon the English version of the protocol. There was a 4-week single-blind, placebo lead-in phase, followed by a 28-day double-blind treatment period. There was no final washout period. Thirty-nine subjects per treatment group were planned. Subjects were to be age 21 to 60 years, weight between 55 and 90 kg and <130% of ideal body weight, with supine and standing diastolic pressure between 95 and 115 mmHg and systolic pressure $\leq$ 180 mmHg at the end of the placebo run-in phase. Women were permitted if post-menopausal or surgically sterile. Exclusion criteria are listed in Table 132 below.

Subjects who qualified at the end of the placebo run-in period were randomized to placebo or trandolapril 0.5, 1, or 2 mg q.d. and returned after 1 week, 2 weeks, and

Table 132. Exclusion criteria in Study FF/87/570/21.

Concurrent diseases related to hypertension (cerebral hemorrhage or ischemia, retinal hemorrhage or thrombosis, coronary insufficiency, heart failure, arteriopathy of the lower limbs, mesenteric arteriopathy, or renal failure).
Severe chronic cardiopulmonary, hepatic, metabolic, neurological, or psychological disease, allergy or drug hypersensitivity, collagen disease, dermatoses, or neutropenia.
Hypertension secondary to a known cause
Hyperkalemia (≥ 5.5 mM)
Use within 28 days prior to study of enzyme inhibitors, cimetidine, ranitidine, long-term anti-inflammatory or analgesic drugs, glucocorticoids, digitalis, or estrogen-progestogen.
Blacks
Previous trial participation with 28 days

4 weeks. All subjects took one capsule per day. Subjects were instructed to take their study drug in the morning 15 minutes after breakfast.

Safety data included medical history, physical exam, 12-lead ECG (within 30 days of baseline only), and routine hematology, clinical chemistry, and urinalysis. There were no explicit criteria for dropping a subject because of lack of efficacy.

Blood samples were obtained for ACE activity, aldosterone, and renin activity levels.

Twenty-four hour urines were collected for protein, glucose, sodium, and creatinine.

The primary end point was responder rate¹. The trial was sized to detect a 35% difference from a 25% placebo responder rate, with one-sided testing, $\alpha=0.05$, and $\beta=0.05$. Response was defined as having supine *and* standing diastolic pressure <90 mmHg or a reduction by ≥ 10 mmHg.

A15.5. Results

Two-hundred seven subjects were enrolled, of whom 191 subjects were randomized. Reasons for not randomizing enrolled subjects were placebo response (5), severe hypertension (4), adverse events or incompatible concomitant disease (4), uncooperativeness (2), and abnormal liver tests (1). Twenty-one subjects from one center were excluded from all analyses because of "major inconsistencies found during data verification". The analyses were based on the remaining 170 subjects, of whom 156 completed the double-blind treatment period. Individual study centers enrolled 2 to 64 subjects. Demographic and baseline characteristics of subjects are shown in Table 133 below. Other than for gender, the treatment groups were reasonably well matched for these factors, for general health, duration and severity of hypertension, and baseline drug use.

Twenty-seven subjects (3 to 11 per group) were excluded from a per-protocol-like analysis. This group excluded subjects with supine diastolic pressure <95 mmHg at baseline² (n=17), subjects with improperly timed visits (<18 hours after dosing; n=8), or concomitant medication violations (n=3). Not excluded were subjects with age >60 (n=17), with weight outside limits (n=39), baseline blood pressure below limits or inadequately documented (n=21), and subjects with a run-in period <25 days (n=41).

Fourteen subjects failed to complete the double-blind treatment period, 8 for protocol violations, 4 for adverse events or intolerance, 1 for intercurrent illness, 1 for lack of efficacy, 1 for being unreliable, and 4 because they did not begin double-blind treatment until 1 week after randomization.

¹ The protocol clearly indicates responder rate was the primary end point. The sponsor's report on the trial indicates that the primary end point was supine diastolic pressure at trough at 4 weeks.

² This despite retrospectively relaxing baseline hypertension criteria to retain subjects whose median supine diastolic pressure was ≥ 95 mmHg.

Table 133. Demographic and baseline characteristics of subjects in Study FF/87/570/21.

	Placebo n=44	Trandolapril		
		0.5 mg n=11	1 mg n=12	2 mg n=21
Male	18	17	8	23
Female	26	24	34	20
Age±SEM	50±1.3	49±1.7	48±1.8	46±1.9
Range	30 to 71	21 to 70	24 to 72	17 to 65
Caucasian	43	41	42	43
Black	0	0	0	0
Other	1	0	0	0
Weight±SEM	73±1.8	76±2.1	74±2.6	75±2.0
Range	41 to 105	54 to 107	41 to 131	50 to 100

Response rate analysis was based upon supine diastolic pressure <90 or a reduction by ≥10 mmHg, not upon supine *and* standing diastolic pressure, as indicated in the protocol. The sponsor's analysis of response rate is shown in Table 134 below. The

Table 134. "Response rate" data (%) in Study FF/87/570/21.

	Placebo n=43	Trandolapril		
		0.5 mg n=40	1 mg n=41	2 mg n=41
Week 1	21 (49)	18 (45)	26 (63)	29 (71)
Week 2	25 (58)	24 (62)	31 (76)	30 (75)
Week 4	23 (56)	27 (73)	30 (75)	28 (74)
End point	24 (56)	29 (72)	31 (76)	30 (73)

sponsor's analysis demonstrated an overall difference from placebo, but no treated group beat placebo or was distinguishable from another treated group at end point.

A secondary analysis of rates of normalization of blood pressure (supine blood pressure <160/90 mmHg) showed no statistically significant differences among treatment groups.

Supine diastolic blood pressure data are shown in Figure 71 and Table 135 below.

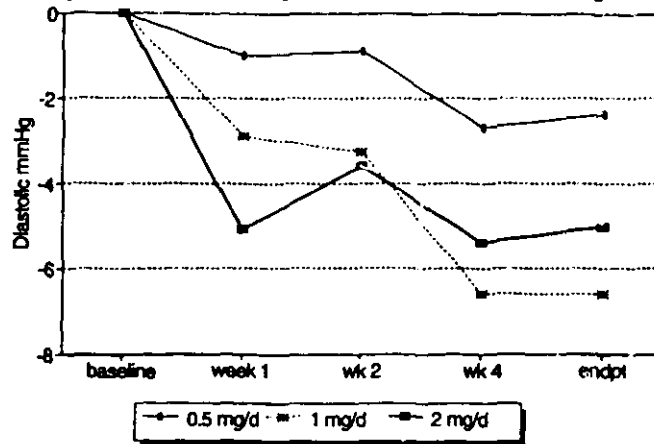


Figure 71. Change in supine diastolic pressure by time in Study FF/87/570/21.

Table 135. Supine diastolic pressure data in Study FF/87/570/21.

	Plcbo n=43	Change from baseline			Change from baseline and placebo		
		0.5 mg n=40	1 mg n=41	2 mg n=41	0.5 mg n=40	1 mg n=41	2 mg n=41
Baseline	99	100	100	100			
Week 1	-5.8	-6.8	-8.7	-10.9	-1.0	-2.9	-5.1
Week 2	-8.2	-9.1	-11.5	-11.8	-0.9	-3.3	-3.6
Week 4	-7.3	-10.0	-13.9	-12.7	-2.7	-6.6	-5.4
End point	-7.3	-9.7	-13.9	-12.3	-2.4	-6.6	-5.0

Other blood pressure data were not reviewed.

Renin, aldosterone, and ACE activity are shown in Table 136 below.

Table 136. ACE inhibition (%), renin activity (Δ), and aldosterone (Δ) in Study FF/87/570/21^a.

	Plcbo	Trandolapril		
		0.5 mg	1 mg	2 mg
ACE inhibition	13	58	58	68
Renin activity	-0.46	0.18	0.26	0.98
Aldosterone	-17	17	-10	12

a. 23 to 39 subjects per group contributed to measurements.

A15.6. Safety.

Eight to 15 subjects per group reported adverse events. All adverse events are summarized in Table 137 below. Subjects who discontinued because of adverse reactions are listed in Table 138 below.

Three subjects (2 on placebo) had heart rate changes >25 bpm on moving from supine standing. Seven subjects had diastolic pressure changes $\geq$ 30 mmHg upon standing. Symptoms of orthostatic hypotension were not reported.

Table 137. Adverse events in Study FF/87/570/21.

Placebo n=44	Trandolapril			
	0.5 mg n=11	1 mg n=42	2 mg n=43	
Any (8)	Any (9)	Any (9)	Any (15)	
Dry mouth (2)	Headache (2)	Headache (2)	Headache (4)	GI pain
Headache (2)	Asthenia	Hot flushes (2)	Insomnia (3)	Herpes
Dizziness	Decreased libido	Abnormal feeling	Palpitations (3)	Malaise
Duodenal ulcer	Dizziness	Dizziness	Asthenia (2)	Peripheral vasc dis
Palpitations	Hot flushes	Fall	Decreased libido (2)	Pruritus
Somnolence	Malaise	Increased appetite	Nausea (2)	Stomatitis
	Nausea	Joint disorder	Anxiety	Tinnitus
	Postural hypotension	Nausea	Dysuria	Vaginitis
	Rhinitis	Neoplasm	Edema	
	Sweating	Tinnitus		
	Tachycardia			
	Tremor			

Table 138. Discontinuations for adverse events in Study FF/87/570/21.

Subj	Group	Day	ADR
03	0.5 mg	17	48 year old Caucasian female with 10-year history of treated hypertension. Subject discontinued with headache, tremors, scotomas, malaise, and tachycardia from day 7. Lack of efficacy is also listed as cause for termination.
15	0.5 mg	11	42 year old Caucasian female with 8 year history of hypertension, untreated at time of enrollment. She discontinued because of acute urinary tract infection and orthostatic hypotension, necessitating hospitalization.
99	2 mg	7	34 year old Caucasian male with 1-year history of treated hypertension. He discontinued with facial edema of several days' duration.

There were a variety of new findings in clinical laboratory data; none were serious or had apparent relationship to the study drug. There is no description of findings from the final physical examination and there was no final ECG.

A15.7. Summary.

Technical problems marred the conduct of this 4-week double-blind, placebo-controlled trial. For the primary end point of responder rate and for supine diastolic pressure there was a significant treatment effect which was not accompanied by differences among the actively treated groups. No truly serious adverse events were reported. There was no apparent relationship between adverse events or abnormalities in laboratory safety data and dosing group.

A16. Study FF/87/570/25 (Study report: vol 1.104; case report tabulations: 1.105-1.106; case report forms for deaths and discontinuations for adverse events: none) "Trandolapril pharmacokinetics and pharmacodynamics after administration of a single oral dose of 4 mg to patients suffering from chronic renal failure".

A16.1. Investigators The investigators are listed in Table 139 below.

Table 139. Investigators in Study FF/87/570/25.

Investigator	Center	City	Investigator	Center	City
Aldigier	1	Limoges, France	McInnes	7	Glasgow, UK
Conte	3	Toulouse, France	Meyer	4	Bloemfontein, S. Africa
Feehally	5	Leicester, UK	Wilkinson	6	Newcastle, UK
Grunfeld	2	Paris, France			

A16.2. Study dates June 1988 to May 1990.

A16.3. Drug formulation

Table 140. Drug substance used in Study FF/87/570/25.

Lot	Form	Type	Trandolapril	Source lot	Batch size
MB 21662-88	Cap	Tran	4.0 mg	17	150,000
MB 21662-63	Cap	Tran	4.0 mg	17	40,000

Table 141. Capsule composition in Study FF/87/570/25.

	4 mg
Trandolapril	4.0
Corn starch	
Lactose, hydrous	
Povidone	
Sodium stearyl fumarate	
Total	g

A16.4. Study design

In this open-label, non-randomized study, a single 4 mg trandolapril dose was orally administered (under fasting conditions) to 27 adult chronic renal failure subjects (creatinine clearance <80 ml/min), and 4 subjects without renal insufficiency (creatinine clearance >80 ml/min). The intake of sodium chloride (4 g/day) and water (1500 to 2000 ml/day) was to be held constant, and the timing of meals was to be standardized. The objectives were to assess single dose pharmacokinetics, pharmacodynamics, and safety.

Enrolled subjects were adults of both sexes. The protocol allowed for the uninterrupted use of β -adrenoceptor antagonists, calcium channel influx inhibitors, centrally-acting antihypertensives, and potassium sparing or replenishing therapies.

The exclusion criteria are shown in Table 142 below.

Conventional plasma and urinary pharmacokinetic end points were assessed. Blood sampling for trandolapril and trandolaprilat was undertaken prior to treatment and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, and 96 hours post-dose. Blood samples for determination of ACE activity were taken pre-dose and 0.5, 1.2, 4, 8, 12, 24, 48,

Table 142. Exclusion criteria in Study FF/87/570/25.

Cerebral hemorrhage or ischemia
Retinal hemorrhage or thrombosis
Coronary insufficiency
Mesenteric arteriopathy
Malignant or secondary hypertension
Heart failure
Any severe chronic disease, in particular cardiac, pulmonary, hematologic, hepatic, metabolic, neurological, or psychiatric
Diabetes mellitus
History of hypersensitivity to drugs
Collagen diseases
Neutropenia ($<2500/\text{mm}^3$)
Proteinuria
Hemoglobin <7 g/dl
Severe renal insufficiency or history of renal transplantation
Major rhythm disorders
Use within one month of enzyme-inducing drugs, cimetidine, ranitidine, chronic NSAIDs, glucocorticoids, digitalis, or ACE inhibitors
Use within two weeks of diuretics, vasodilators, or anxiolytics
Inability to follow treatment or dietary restrictions
Participation in another clinical trial within 8 weeks

72, 96, 168, 336, and 672 hours post-dose. Blood sampling for renin and aldosterone were drawn pre-dose and at 4 and 24 hours post-dose. Urine collections for trandolaprilat were obtained at -24 to 0 (24-hour blank), 0 to 4, 4 to 12, 12 to 24, 24 to 48, 48 to 72, and 72 to 96 hours after dosing.

Pharmacodynamic end points included plasma converting enzyme activity and peripheral hemodynamics. Blood pressure determinations were made pre-treatment, as well as 0.5, 1, 1.5, 3, 4, 6, 8, 12, 24, and 48 hours post-dosing.

Pharmacokinetic assays were assessed as shown in Table 143 below. The assays have

Table 143. Pharmacokinetic assays in Study FF/87/570/25.

Linearity	Accuracy			Precision			Sensitivity	Specificity
Plasma samples—trandolapril and trandolaprilat								
Satisfactory.	Satisfactory.			Satisfactory.			LOQ - 0.2 ng/ml.	Satisfactory.
Standard curves from 0.078 to 2.50 ng/ml	ng/ml	T'april	T'aprilat	ng/ml	T'april	T'aprilat		
	0.155	100.0%	99.4%	0.155	3.1%	9.2%		
	0.314	100.0%	99.4%	0.314	6.2%	8.7%		
	0.857	100.0%	102.0%	0.857	3.2%	6.1%		
	1.970	—	94.3%	1.970	—	—		
2.327	92.1%	—	2.327	4.4%	7.6%			
Urine samples—trandolaprilat—								
Satisfactory.	Satisfactory.			Satisfactory.			LOQ - 10 ng/ml	Satisfactory.
Standard curves from 5.0 to 5000 ng/ml	ng/ml	T'aprilat		ng/ml	T'aprilat			
	111	99.1%		111	9.7%			
	554	93.3%		554	7.6%			
	1107	96.9%		1107	7.6%			
1661	98.2%		1661	9.2%				
Plasma samples—ACE inhibition—								
	Satisfactory. 44.0 U/L for expected values of 50 to 67 U/L.			Satisfactory. C.V. 8.5% at 44.0 U/L and 5.9% at 57.6 U/L.				

been validated over the range of plasma and urine concentrations observed in the study.

AUC, C_{max}, t_{1/2}, CL_R, T_{max}, MRT, I_{max}, AUCI, I_{24h} and the amount of trandolaprilat excreted in the urine were calculated.

Safety end points included physical assessments, hematology, and chemistry end points observed pre-treatment and 24 hours after the dose. Subjects were observed for 48 hours post-dose, and additional clinical follow-up was undertaken two and four weeks after dosing.

A 1-way analysis of variance (ANOVA) was used to analyze pharmacokinetic parameters across creatinine clearance strata.

A16.5. Results

The range of creatinine clearance among the renal failure subjects was 4 to 77 ml/min. The demographic characteristics of the subjects are presented in Table 144 below.

Table 144. Demographic and baseline characteristics of subjects in Study FF/87/570/25.

	Renal impaired n=14	Normal n=4
Male	13	3
Female	14	1
Age—mean	53	49
Weight—mean	64	71
Hypertension prevalence (%)	67	0
Creatinine clearance—mean	33	95

The pertinent aspects of subject disposition were as follows: 3 subjects (211, 506, and 105) with severe renal insufficiency (creatinine clearance <10) were arbitrarily excluded from the pharmacokinetic analyses; 4 subjects (401, 403, 405, 511) were co-exposed to diuretics (furosemide, hydrochlorothiazide, or bendroflouzide) in violation of the protocol¹.

Hemodynamic outcomes are shown in Table 145 below.

Table 145. Mean changes from baseline in hemodynamic data from Study FF/87/570/25.

	Renal impaired n=27			Normal n=4		
	Sys	Diast	HR	Sys	Diast	HR
At 24 hours	-15.1	-10.5	+3.5	-22.4	-10.1	-6.6
Peak	-20.1	-11.8	+5.0	-28.9	-17.1	-7.2

Pharmacokinetic data are summarized in Table 146 below. Plasma trandolapril and

Table 146. Pharmacokinetic data from Study FF/87/570/25.

	Trandolapril CL _{CR} (ml/min/1.73 m ²)				Trandolaprilat CL _{CR} (ml/min/1.73 m ²)			
	10-30 n=12	31-50 n=4	51-80 n=8	>80 n=4	10-30 n=12	31-50 n=4	51-80 n=8	>80 n=4
C _{max} (ng/ml)	10.5±1.21	22.7±11.3 ^a	9.59±2.08	6.64±1.56	16.6±1.30	10.0±1.98	8.72±0.90	9.56±2.33
T _{max} (h)	0.5	0.5	0.5	0.5	4.0	5.0	5.0	3.5
AUC (ng/ml·h)	14.0±1.80	38.9±19.4	15.5±2.42	11.1±5.2	211±15.3	128±23.1	109±8.31	98.7±16.9
AUC ₀₋₂₄					327±18.0	229±35.3	196±14.0	178±15.5
AUC ₀₋₉₆								
MRT (h)	1.87±0.15	3.31±0.92	2.10±0.17	1.53±0.37	—	—	—	—
t _{1/2} (h)	0.81±0.14	1.07 ^b	1.00±0.16	0.64±0.08	—	—	—	—
U ₀₋₂₄ (% dose)	—	—	—	—	4.4±1.6	8.2±1.6	10.7±0.8	13.3±4.1
U ₀₋₉₆	—	—	—	—	5.1±1.2	9.9±1.0	13.3±1.1	15.2±4.5
CL _{R 0-24} (l/h)	—	—	—	—	0.73±0.11	2.44±0.35	3.68±0.14	5.26±0.71
CL _{R 0-96}	—	—	—	—	0.55±0.11	1.69±0.24	2.62±0.29	3.19±0.68

a. One subject had a C_{max}=54.9 ng/ml and AUC=93.6 ng/ml·h

b. n=1

trandolaprilat levels are shown in Figure 72 below. Urinary excretion of trandolaprilat is shown in Figure 73 below. The correlation between creatinine clearance and urinary clearance of trandolaprilat is shown in Figure 74 below.

ACE inhibition, renin, and aldosterone levels are shown in Table 147 below. ACE inhibition by days is shown in Figure 75 below. ACE activity at 24 hours post-dose was inhibited 32 to 100% in renal failure subjects, and inhibited 46 to 74% in subjects with normal renal function. The change in diastolic pressure in the group with creatinine clearance >80 ml/min is shown as a function of the degree of ACE inhibition in Figure 76 below.

A16.6. Safety.

Case report forms were not submitted for evaluation. There were apparently no clinically significant changes from pre-treatment in chemistry or hematology results. There were reportedly cases of spontaneously resolving cutaneous rash (n=1).

¹ Additional details are on page 36 of vol 104.

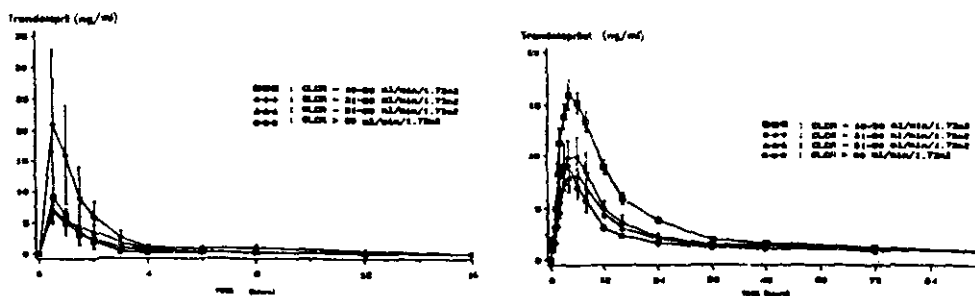


Figure 72. Plasma trandolapril and trandolaprilat levels in Study FF/87/570/25.

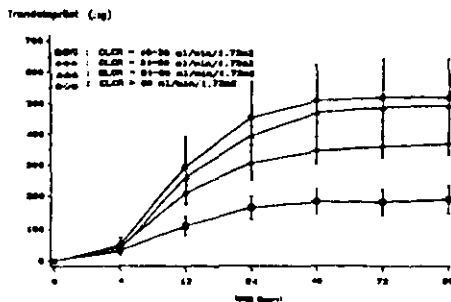


Figure 73. Cumulative urinary trandolaprilat in Study FF/87/570/25.

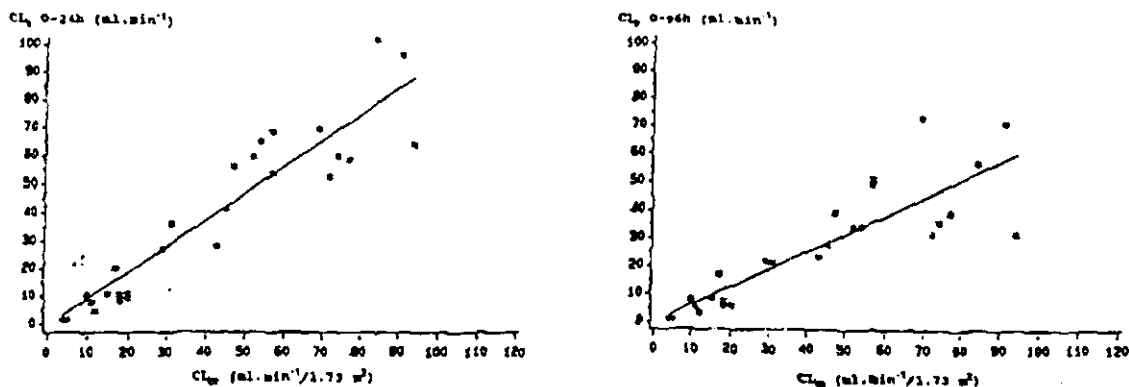


Figure 74. Correlation between urinary clearances of trandolaprilat and creatinine in Study FF/87/570/25.

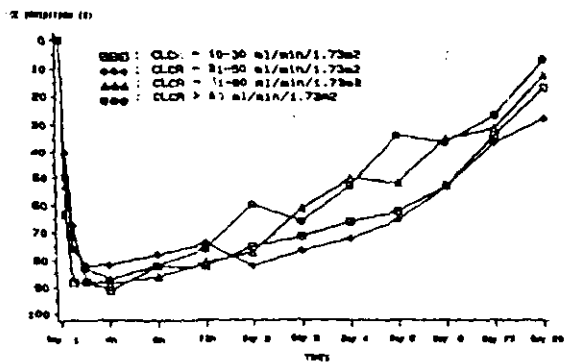


Figure 75. Plasma ACE inhibition in Study FF/87/570/25.

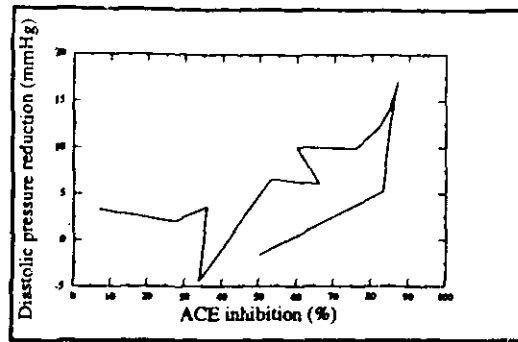


Figure 76. Diastolic pressure reduction vs. ACE inhibition in Study FF/87/570/25.

Table 147. ACE inhibition, renin, and aldosterone levels in Study FF/87/570/25.

	CL_{CR} (ml/min/1.73 m ²)			
	10-30 n=12	31-50 n=4	51-80 n=8	>80 n=4
ACE				
I_{max} (%)	93.8±2.8	91.1±5.3	92.1±3.0	88.9±4.8
T_{max} (h)	2.0	6.0	4.0	4.0
I_{24h} (%)	75.2±5.6	82.0±6.6	77.3±4.1	59.6±5.8
AUC_{0-336h} (%·h)	18294±1571	19026±1428	14615±2389	13548±1433
Renin activity (ng angiotensin I/ml/h)				
0 h	0.74±0.12	2.29±1.23	1.35±0.25	2.35±1.00
4 h	1.69±0.47	3.27±1.54	3.38±1.11	2.96±0.55
24 h	1.47±0.27	10.7±6.70	3.43±1.30	2.99±0.60
Aldosterone (pg/ml)				
0 h	331±58.2	322±94.9	105.3±24.9	88.5±10.4
4 h	237±24.0	229±70.7	90.2±41.7	69.0±8.9
24 h	187±25.4	164±25.8	96.0±18.0	89.0±30.1

headache during treatment (n=1), and postural dizziness without evidence of appreciable orthostatic hypotension (n=2; subjects 211 & 702).

Large blood pressure reductions occurred (with onset ranging from 3 to 12 hours post-dose) in an appreciable fraction of subjects (19% with renal failure and 50% with

normal creatinine clearance). These cases are described in Table 148 below. Several

Table 148. Hypotensive events in Study FF/87/570/25.

Subj	Sex	Age	CL _{cr}	Cofactors	Adverse event
Renal failure subjects					
102	M	65	31	Elevated baseline renin	Asymptomatic, non-orthostatic blood pressure reduction from 195/111 mmHg at baseline to 129/21
105	F	58	5	Verapamil	Symptomatic ("fainting") orthostasis; systolic pressure decreased by 31 mmHg
505	F	61	10	Atenolol	Asymptomatic blood pressure reduction from 130/60 mmHg at baseline to 88/32
701	F	70	77	Diltiazem	Symptomatic ("fainting") orthostasis; systolic pressure decreased by ≥ 30 mmHg
704	M	60	57	None apparent	Asymptomatic, non-orthostatic blood pressure reduction from 132/82 mmHg at baseline to 81/60
Normal subjects					
702	M	64	91	None apparent	Asymptomatic, non-orthostatic blood pressure reduction from 167/88 mmHg at baseline to 116/68
706	M	50	84	Elevated baseline renin	Asymptomatic, non-orthostatic blood pressure reduction from 119/76 mmHg at baseline to 82/59

of the cited blood pressures are so low as to be questionable.

Baseline (pre-treatment) mean blood pressures and heart rates were 147/88 mmHg and 64 bpm in the renal failure subjects, respectively. In the subjects with normal creatinine clearance, these baseline values were 136/82 mmHg and 70 bpm, respectively. The above-depicted hypotensive events contributed to relatively sizable mean peak blood pressure reductions from baseline (shown in Table 145).

In response to an inquiry, the sponsor indicated that there were no deaths. Six subjects (101, 102, 103, 104, 105, and 106) withdrew on day 15; the reasons for withdrawal were not related to adverse events.

A16.7. Summary.

A single 4 mg oral trandolapril dose was administered to 27 chronic renal failure subjects (creatinine clearance 4 to 77 ml/min) and 4 subjects without renal insufficiency (creatinine clearance >80 ml/min). Although these were non-placebo-controlled data not subjected to scrutiny, the maximum and trough (24 hours post-dose) blood pressure changes from baseline (pre-treatment) were substantial. To some extent the large mean peak blood pressure reduction was attributable to pronounced hypotensive events in 7 subjects. Plasma ACE inhibition was unaffected by renal failure. The pharmacokinetics of trandolapril were unaffected by renal failure, but renal clearance of trandolaprilat was well-correlated with creatinine clearance, a fact which has implications in labelling. Among the chronic renal failure subjects, orthostatic hypotension occurred in 11% (each of whom was receiving concomitant antihypertensive therapy), and non-orthostatic hypotension occurred in 7% (half of whom had elevated pre-treatment plasma renin activities). Among 4 subjects without renal insufficiency, non-orthostatic hypotension occurred in 2 (one of whom had elevated pre-treatment plasma renin activity). Potential risk factors (specifically, co-exposure to an antihypertensive agent, and baseline plasma renin elevation) were identifiable in only half of the hypotensive events. A single case of rash, a single case of headache during treatment, and 2 cases of postural dizziness without orthostatic hypotension were also reported.

A17. Study FF/87/570/26 (Study report: vol 1.110; case report tabulations: 1.110; case report forms for deaths and discontinuations for adverse events: none) "Trandolapril pharmacokinetics and pharmacodynamics after administration of a single oral dose of 4 mg to hemodialyzed patients suffering from chronic renal failure".

A17.1. Investigators The investigators are listed in Table 149 below.

Table 149. Investigators in Study FF/87/570/26.

Investigator	Center	City	Investigator	Center	City
Aldigier	1	Limoges, France	Grunfeld	2	Paris, France

A17.2. Study dates February 1988 to July 1989.

A17.3. Drug formulation

Table 150. Drug substance used in Study FF/87/570/26.

Lot	Form	Type	Trandolapril	Source lot	Batch size
MB 21662-88	Cap	Tran	4.0 mg	17	150,000
MB 21662-63	Cap	Tran	4.0 mg	17	40,000

Table 151. Capsule composition in Study FF/87/570/26.

	4 mg
Trandolapril	4.0
Corn starch	
Lactose, hydrous	
Povidone	
Sodium stearyl fumarate	
Total	

A17.4. Study design

In this open-label, non-randomized study a single 4 mg trandolapril dose was orally administered (under fasting conditions) one day after hemodialysis to 6 adults with severe chronic renal failure (creatinine clearances <10 ml/min, normalized for a body surface area of 1.73 m²). The daily intake of sodium chloride (4 g) and water (500 ml above urine excretion), and the timing of meals was to be regimented. Trandolapril was administered following overnight fast. Subjects underwent a second hemodialysis (as per their usual care) the day after trandolapril treatment. The objectives were to assess pharmacokinetics, pharmacodynamics, and safety.

Enrolled subjects were hemodialysis-dependent, adult chronic renal failure patients of both sexes. The protocol allowed for the uninterrupted use of α -adrenoceptor antagonists, calcium influx inhibitors, centrally-acting antihypertensives, and potassium replenishing therapies.

The exclusion criteria are shown in Table 152 below.

Conventional plasma and urinary pharmacokinetic end points were assessed. Pharmacodynamic end points included plasma renin activity, inhibition of plasma angiotensin converting enzyme (ACE) activity, and peripheral hemodynamics. Blood sampling for trandolapril and trandolaprilat was undertaken prior to treatment (-15 min), and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, and 96 hours post-dose. Blood samples for ACE activity were collected at -0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 168, 336, and 672 hours post-dose. Blood samples for renin and aldosterone were

Table 152. Exclusion criteria in Study FF/87/570/26.

Premenopausal women who have not had tubal ligation
Body weight <85% or >115% of ideal
Cerebral hemorrhage or ischemia
Retinal hemorrhage or thrombosis
Coronary insufficiency
Mesenteric arteriopathy
Malignant or secondary hypertension
Heart failure
Major cardiac rhythm disorders
Any chronic severe disease, in particular cardiac, pulmonary, hematologic, hepatic, metabolic, neurologic, or psychiatric
History of hypersensitivity to drugs
Neutropenia (<2500/mm ³)
Hemoglobin <7 g/100 ml
Proteinuria
History of renal transplantation
Serum potassium ≥5.5 mM
Use within one month of drugs known to induce or inhibit drug-metabolizing enzymes, cimetidine, ranitidine, chronic NSAIDs, glucocorticoids, or digitalis
Use within 2 weeks of ACE inhibitors, vasodilators, or anxiolytics
Inability to follow treatment or dietary restriction
Participation in another clinical trial within 8 weeks

drawn at -0.25, 4, and 12 hours post-dose. Urine samples for trandolapril were collected for intervals -24 to 0 (24-hour blank), 0 to 4, 4 to 12, 12 to 24, 24 to 48, 48 to 72, and 72 to 96 hours post-dose.

Pharmacokinetic assays were assessed as shown in Table 153 below. The assays have

Table 153. Pharmacokinetic assays in Study FF/87/570/26.

Linearity	Accuracy		Precision			Sensitivity	Specificity
Plasma samples—trandolapril and trandolaprilat—							
Satisfactory.	Satisfactory.		Satisfactory.			LOQ - 0.2 ng/ml.	Satisfactory.
Standard curves from 0.02 to 10 ng/ml for trandolapril and for trandolaprilat	ng/ml	T'april	T'aprilat	ng/ml	T'april	T'aprilat	
	0.157		99.4%	0.157		9.2%	
	0.163	105.0%		0.163	7.9%		
	0.314		99.4%	0.314		8.7%	
	0.323	105.0%		0.323	7.8%		
	0.837		102.0%	0.837		6.1%	
	0.862	105.0%		0.862	7.7%		
2.09		94.3%	2.09		7.6%		
2.10	102.0%		2.10	7.7%			
Urine samples—trandolaprilat—							
Satisfactory.	Satisfactory.		Satisfactory.		LOQ - 10 ng/ml.	Satisfactory.	
Standard curves from 25 to 2500 ng/ml	ng/ml	T'aprilat	ng/ml	T'aprilat			
	111	99.1%	111	9.7%			
	554	93.3%	554	7.6%			
	1107	96.9%	1107	7.6%			
	1661	98.2%	1661	9.2%			
Plasma samples—ACE inhibition—							
	Satisfactory. 50.6 U/L for expected values of 44 to 58 U/L and 43.3 U/L for expected values of 29 to 53 U/L.		Satisfactory. C.V. 3.2% at 50.6 U/L and 2.5% at 43.3 U/L.				

been validated over the range of plasma and urine concentrations observed in the study.

AUC, C_{max} , $t_{1/2}$, CL_r , T_{max} , MRT, I_{max} , AUCI, I_{24h} , and fraction of trandolaprilat excreted in urine were calculated.

Blood pressure determinations were made at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours post-dose (as well as during post-dose days 3, 4, 5, 8, 15, and 29).

Safety end points included physical assessments, hematology, and chemistry end points (laboratory end points were to be observed pre-treatment, as well as 1 and 28 days following drug administration). Subjects remained in hospital during the day following drug discontinuation.

Only descriptive statistics were generated.

A17.5. Results

The demographic characteristics of the subjects are presented in Table 154 below.

Subject 23 violated the protocol by receiving ranitidine up to 8 days before the study.

The pertinent aspects of subject disposition were as follows: subject 11 was co-exposed to acebutolol and nifedipine, subject 21 was co-exposed to calcium heparinate, subject 24 was co-exposed to chlormadinone (reportedly a contraceptive), and subject 22 was co-exposed to naftidrofuryl oxalate (reportedly a vasodilator). There were no losses to follow-up.

Table 154. Demographic and baseline characteristics of subjects in Study FF/87/570/26.

	n=6
Male	4
Female	2
Caucasian	4
Black	2
Age-mean	35
Weight-mean	62
Hypertension prevalence (%)	17

Blood pressure and heart rate effects are shown in Figure 77 below. Baseline (pre-

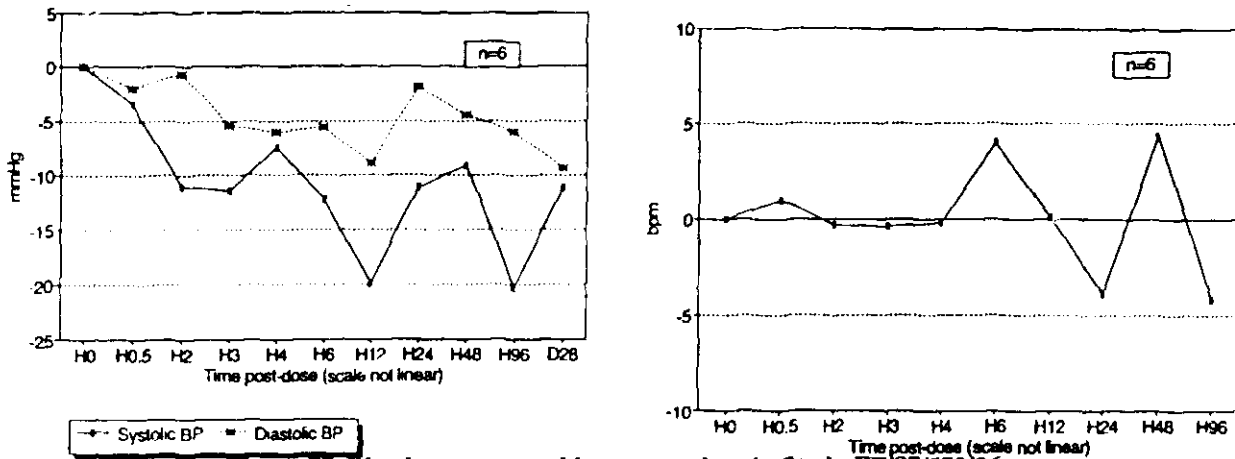


Figure 77. Blood pressure and heart rate data in Study FF/87/570/26.

treatment) mean supine blood pressures were 132.3/79.4 mmHg and mean heart rates were 76.7 bpm. Changes from baseline hemodynamics are shown in Table 155 below.

Table 155. Mean changes from baseline in supine blood pressure and heart rate in Study FF/87/570/26.

Time (hours)	n=6			Time (hours)	n=6		
	Sys	Dias	HR		Sys	Dias	HR
0.5	-3.4	-2.0	+1.0	12	-19.9	-8.9	+0.1
2	-11.0	-0.6	-0.3	24	-11.1	-1.8	-3.9
3	-11.5	-5.5	-0.4	48	-9.1	-4.4	+4.4
4	-7.5	-6.0	-0.2	96	-20.4	-6.0	-4.2
6	-12.2	-5.6	+4.0	28 days	-11.1	-9.3	-4.9

Plasma trandolapril and trandolaprilat levels are shown in Figure 78 below. Pharmacokinetic data are shown in Table 156 below.

ACE inhibition is depicted in Figure 79 and Table 157 below.

Plasma renin activity is depicted in Table 158 below.

A17.6. Safety.

Case report forms were not submitted for evaluation. In the study report there is no explicit disclaimer that there were no deaths or discontinuations for adverse events.

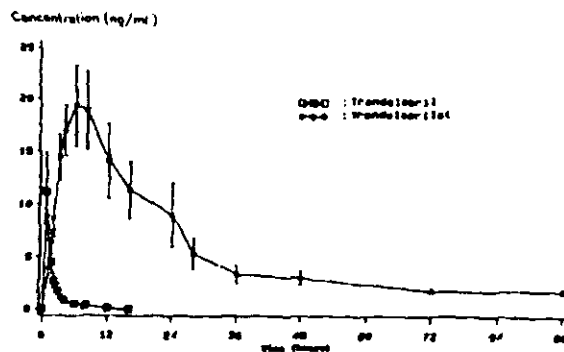


Figure 78. Plasma trandolapril and trandolaprilat levels in Study FF/87/570/26.

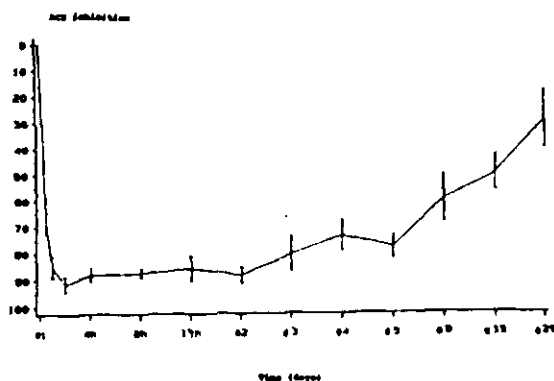


Figure 79. Plasma ACE inhibition in Study FF/87/570/26.

Table 156. Pharmacokinetic data in Studies FF/87/570/26 and FF/87/570/25.

	Trandolapril				Trandolaprilat			
	FF/87/570/26 Renal failure n=6	FF/87/570/25			FF/87/570/26 Renal failure n=6	FF/87/570/25		
		CL _{CR} <10 n=3	10 < CL _{CR} <30 n=12	Normal n=45		CL _{CR} <10 n=3	10 < CL _{CR} <30 n=12	Normal n=45
AUC (ng/ml-h)	17.1±0.2	29.8±12.1	14.0±1.8	11.1±5.2	—	—	—	—
AUC ₀₋₂₄	—	—	—	—	308±63.1	350±122	211±15.3	98.7±16.9
AUC ₀₋₉₆	—	—	—	—	551±105	570±198	327±17.2	178±15.5
C _{max} (ng/ml)	12.1±2.4	18.6±7.4	10.5±1.3	6.6±.6	19.8±3.6	24.4±8.0	16.6±1.3	9.6±2.3
T _{max} (median; h)	0.5	0.5	0.75	0.5	7	4	5	3.5
MRT (h)	2.1±0.3	2.4±0.8	1.9±0.2	1.5±0.4	—	—	—	—
CL _{R 0-24} (L/h)	—	—	—	—	0.03±0.01 ^a	0.12±0.12 ^b	0.73±0.11	5.26±0.71

a. n=4
b. n=2

Subjects 11 and 12 had elevated values of liver function tests (serum SGOT and/or alkaline phosphatase) at baseline and these became slightly to moderately further elevated (3-12%) after 2 weeks of treatment. The persistence of these elevations was not characterized.

A17.7. Summary.

A single 4 mg oral trandolapril dose was administered during the inter-dialysis interval to 6 subjects with hemodialysis-dependent, adult chronic renal failure. Although these were uncontrolled data from an exceedingly small sample of subjects, the mean trough

Table 157. ACE inhibition (%) in Study FF/87/570/26.

	FF/87/570/26	FF/87/570/25		
	Renal failure n=6	10 <math><GL_{CR}</math> ≤ 10 n=3	10 <math><GL_{CR}</math> <math>< 30</math> n=12	Normal n=45
I_{max} (%)	92.4 $\pm$ 2.4	92.3 $\pm$ 4.5	93.8 $\pm$ 2.8	88.9 $\pm$ 4.8
I_{24}	88.4 $\pm$ 2.9	92.1 $\pm$ 6.7	75.2 $\pm$ 2.6	59.6 $\pm$ 5.8
AUC I_{0-336} (%·h)	21883 $\pm$ 1903	23868 $\pm$ 1960	19026 $\pm$ 1428	13548 $\pm$ 1433
T_{max} (median; h)	2	4	2	4

Table 158. Renin activity in Study FF/87/570/26.

	n=6
Renin activity ^a	
baseline (mean $\pm$ SD)	1.02 $\pm$ 0.47
at 4 h (mean $\pm$ SD)	1.71 $\pm$ 0.65
at 24 h (mean $\pm$ SD)	1.67 $\pm$ 0.75

a. Normal range is 0.2 to 2.8 ng angiotensin I/ml-h.

(24 hours post-dose) blood pressure reductions from baseline appeared to be large; mean peak blood pressure reductions were seen at 12 hours, and blood pressures remained lower than baseline from 2 to 28 days post-dose. In comparison with less severe or normal renal function (Study FF/87/570/25), maximal ACE inhibition was comparable, but ACE inhibition lasts longer in subjects with severe renal failure. The pharmacokinetics of trandolapril and trandolaprilat are similarly affected. Renal clearance of trandolaprilat is insignificant in hemodialyzed subjects with terminal renal failure, resulting in large increases in plasma concentrations and AUC. Dosage of trandolapril will need to be adjusted in such patients. Trandolaprilat is dialyzable, an observation which should be included in labeling. No clinically significant adverse events were reported.

A18. Study FF/88/570/38 (Study report: vol 1.111; case report tabulations: 1.111; case report forms for deaths and discontinuations for adverse events: none) "Pharmacokinetics and pharmacodynamics of trandolapril after a single oral administration of 2 mg to patients with hepatic cirrhosis".

A18.1. Investigators The investigators were J Pascal and J Vinel at one center in Toulouse, France.

A18.2. Study dates October 1989 to June 1990.

A18.3. Drug formulation

Table 159. Drug substance used in Study FF/88/570/38.

Lot	Form	Type	Trandolapril	Source lot	Batch size
MB 22327-163	Cap	Tran	2.0 mg	MB22327-163	600,000

Table 160. Capsule composition in Study FF/88/570/38.

	2 mg
Trandolapril	2.0
Corn starch	
Lactose, hydrous	
Povidone	
Sodium stearyl fumarate	
Total	

A18.4. Study design In this open-label, non-randomized study a single 2 mg trandolapril dose was orally administered (under fasting conditions) to 8 adults with hepatic cirrhosis. The timing of meals was to be regimented. The objectives were to assess pharmacokinetics, pharmacodynamics, and safety.

Enrolled subjects were adult hepatic cirrhosis patients of both sexes who did not have marked ascites.

The exclusion criteria are shown in Table 161 below.

Table 161. Exclusion criteria in Study FF/88/570/38.

Pregnant women or women of child-bearing potential
Weight <50 kg
Hemodynamic instability
Abnormal ECG
Systolic blood pressure <110 mmHg
Acute myocardial infarction
Urinary retention
Plasma creatinine >150 µM
Hemoglobin ≤8 g/100 ml
Recent gastrointestinal hemorrhage
Greater than 5 L of ultrasonographically-detected ascites
Elevation of serum hepatocellular enzymes greater than >5 times normal
Hepatitis
Biliary tract infection

Table 161. Exclusion criteria in Study FF/88/570/38. (Continued)

Severe encephalopathy
Portacaval shunt
Hepatorenal syndrome
Use within 10 days of drugs known to induce or inhibit drug-metabolizing enzymes
Use within 5 days of diuretics, antacids, or ethanol
Use within 2 days of a chelating agent or ion exchange resin
Study-concurrent use of another ACE inhibitor
Participation in another clinical trial within 4 weeks

Conventional plasma and urinary pharmacokinetic end points were assessed. Pharmacodynamic end points included plasma renin activity, plasma aldosterone concentration, inhibition of plasma ACE activity, and peripheral hemodynamics.

Blood samples for trandolapril and trandolaprilat were drawn pre-dose, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, and 96 hours post-dose. Blood samples for ACE activity were drawn pre-dose, and at 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 168, 336, and 672 hours post-dose. Blood samples for renin and aldosterone were drawn pre-dose (-15 min) and at 2, 4, 8, and 12 hours post-dose. Urine collections for trandolaprilat covered intervals -24 to 0 (24-hour blank), 0 to 4, 4 to 12, 12 to 24, 24 to 48, 48 to 72, and 72 to 96 hours post-dose.

Pharmacokinetic assays were assessed as shown in Table 162 below. The assays have

Table 162. Pharmacokinetic assays in Study FF/88/570/38.

Linearity	Accuracy		Precision		Sensitivity	Specificity
Plasma samples—trandolapril and trandolaprilat—						
Satisfactory.	Satisfactory.		Satisfactory.		LOQ - 0.1 ng/ml.	Satisfactory.
Standard curves from 0.02 to 2.5 ng/ml for trandolapril and for trandolaprilat	ng/ml	T'aprilat	ng/ml	T'aprilat		
	0.100	93.0%	0.100	6.8%		
	0.200	106.0%	0.200	6.9%		
	0.400	103.3%	0.400	9.0%		
	0.800	102.6%	0.800	9.1%		
Urine samples—trandolaprilat—						
Satisfactory.	Satisfactory.		Satisfactory.		LOQ - 10 ng/ml.	Satisfactory.
Standard curves from 5 to 5000 ng/ml	ng/ml	T'aprilat	ng/ml	T'aprilat		
	111	99.1%	111	9.7%		
	554	93.3%	554	7.6%		
	1107	96.9%	1107	7.6%		
	1661	98.2%	1661	9.2%		
Plasma samples—ACE inhibition—						
	Satisfactory. 76.0 U/L for expected values of 64 to 86 U/L.		Satisfactory. 4.0% C.V. at 76.8 U/L.			

been validated over the range of plasma and urine concentrations observed in the study.

AUC, C_{max} , $t_{1/2}$, CL_R , T_{max} , MRT, and percentage of dose excreted in urine were calculated.

Blood pressure and heart rate determinations were made at -30, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours post-dose (as well as during post-dose days 3, 4, 5, 8, 15, and 29).

Safety end points included physical assessments, hematology, and chemistry endpoints. Laboratory end points were to be observed pre-treatment, as well as 1 and 29 days following drug administration. Subjects remained in hospital for 48 hours following drug administration.

Generally only descriptive statistics were generated. The differences from baseline (pre-treatment) values of plasma renin activity and aldosterone were evaluated using a t test.

A18.5. Results

The demographic characteristics of the subjects are presented in Table 163 below.

Table 163. Demographic and baseline characteristics of subjects in Study FF/88/570/38.

	n=8
Male	6
Female	2
Age-mean	54
Weight-mean	75
Incidence of elevated renin activity (%)	63
Incidence of elevated aldosterone (%)	88

Pertinent protocol violations were as follows: subject 1 received aluminum hydroxide (albeit 7 days after administration of trandolapril), and subject 3 was co-exposed to meprobamate.

There were reportedly no dropouts.

Blood pressure and heart rate effects are shown in Figure 80 below. Baseline (pre-

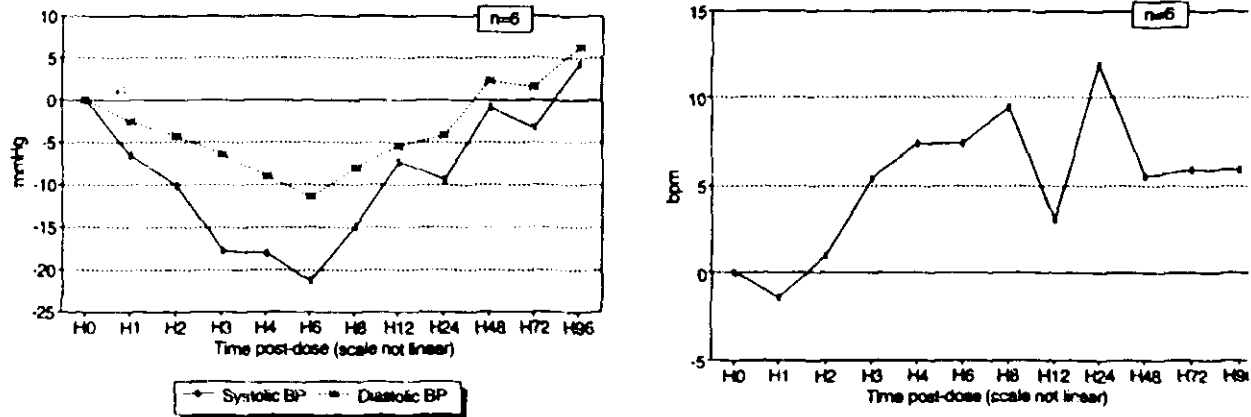


Figure 80. Blood pressure and heart rate data in Study FF/88/570/38.

treatment) mean supine blood pressures were 116.3/64.1 mmHg and mean heart rates were 73.3 bpm. Changes from baseline hemodynamics are shown in Table 164 below.

Plasma trandolapril and trandolaprilat levels are shown in Figure 81 below. Pharmacokinetic data are shown in Table 165 below.

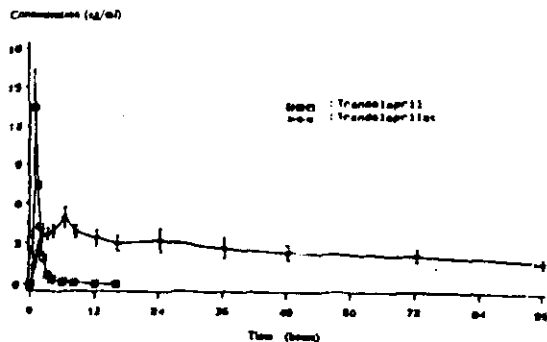


Figure 81. Plasma trandolapril and trandolaprilat levels in Study FF/88/570/38.

Table 164. Mean changes from baseline in supine blood pressure and heart rate in Study FF/88/570/38.

Time (hours)	n=6			Time (hours)	n=6		
	Sys	Dias	HR		Sys	Dias	HR
1	-6.6	-2.6	-1.4	12	-7.3	-5.4	+3.1
2	-10.1	-4.3	+1.0	24	-9.5	-4.1	+11.9
3	-17.8	-6.4	+5.4	48	-0.9	+2.3	+5.5
4	-18.0	-9.1	+7.4	72	-3.3	+1.5	+5.9
6	-21.4	-11.4	+7.4	96	+4.4	+6.4	+5.9
8	-14.8	-8.0	+9.4				

Table 165. Pharmacokinetic data in Studies FF/88/570/38 and F/89/570/54.

	FF/88/570/38 (cirrhosis)		F/89/570/54 (normal)	
	Trandolapril n=8	Trandolaprilat n=8	Trandolapril	Trandolaprilat
AUC (ng/ml·h)	16.3±2.8	—	1.9±0.3	—
AUC ₀₋₂₄	—	82.3±12.7	—	45.7±3.2
AUC ₀₋₉₆	—	252±51.7	—	—
C _{max} (ng/ml)	14.5±2.8	5.6±1.0	1.7±0.3	2.8±0.3
T _{max} (median; h)	0.5	7	0.5	6
Range	0.5 to 1.5	2 to 24	—	—
MRT (h)	1.5±0.2	—	—	—
U ₀₋₂₄ (µg)	—	238±73	—	—
% of dose	—	12.8±3.9	—	—
U ₀₋₉₆	—	365±83	—	—
% of dose	—	19.6±4.5 ^a	—	—
CL _{R 0-24} (L/h)	—	3.1±1.0	—	—

a. n=7

ACE inhibition is depicted in Figure 82 and Table 166 below. The relationship between plasma ACE inhibition and diastolic pressure reduction is shown (as a hysteresis curve) in Figure 83 below

Plasma renin activities, and the plasma aldosterone concentrations are depicted in Table 167 below.

A18 6. Safety.

Case report forms were not submitted for evaluation.

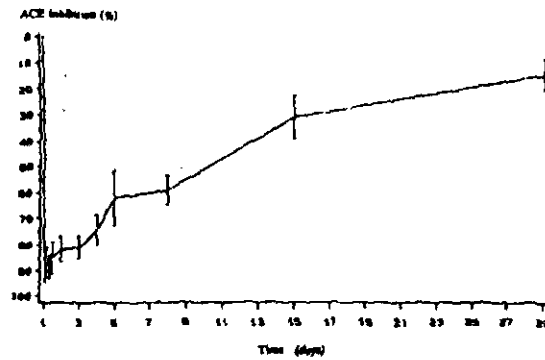


Figure 82. Plasma ACE inhibition in Study FF/88/570/38.

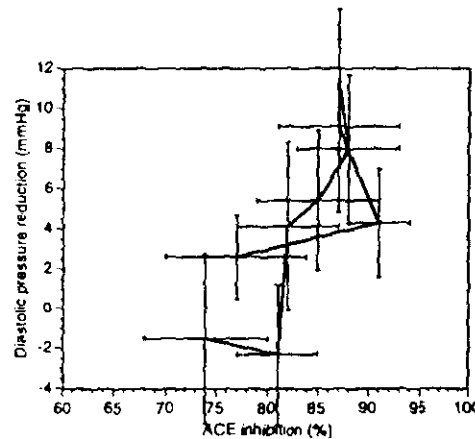


Figure 83. Diastolic pressure reduction vs. ACE inhibition in Study FF/88/570/38.

Table 166. ACE inhibition in Studies FF/88/570/38 and F/89/570/54.

	FF/88/570/38 (cirrhosis) n=8	F/89/570/54 (normal)
I_{max} (%)	94.3±2.8	95.6±1.0
I_{24}	81.5±4.9	83.9±1.7

Table 167. Renin activity, and aldosterone in Study FF/88/570/38.

	n=8		n=8
Renin activity ^a		Aldosterone ^b	
baseline (mean±SD)	7.6±2.6	baseline (mean±SD)	776±230
at 2 h (mean±SD)	14.5±4.3	at 2 h (mean±SD)	677±231
at 4 h (mean±SD)	19.0±4.1	at 4 h (mean±SD)	694±225
at 8 h (mean±SD)	17.2±2.6	at 8 h (mean±SD)	571±155
at 12h (mean±SD)	15.8±2.6	at 12h (mean±SD)	678±179

a. Normal range is 0.2 to 2.8 ng angiotensin U/ml-h.

b. Normal range is 604 to 2248 pg/ml.

There were reportedly no discontinuations, but the study report includes no explicit disclaimer that there were no deaths.

There was reportedly no orthostatic hypotension, but 2 subjects experienced severe non-orthostatic hypotension. These cases are described in Table 168 below.

Table 168. Hypotensive adverse events in Study FF/88/570/38.

Center	Subj	ADR
1	3	63 year old male with elevated baseline plasma renin activity and concomitant exposure to the vasodilator molsidomine. Beginning 3 hours post-dose, supine pressure fell from baseline 123/60 to a minimum of 88/44 mmHg at 6 hours. Treated with albumin and withdrawal of molsidomine. Urine output and plasma creatinine were unaffected.
1	7	57 year old female with ascites and elevated baseline plasma renin activity. Beginning 2 hours post-dose, supine pressure fell from baseline 97/53 to a minimum of 52/31 mmHg at 6 hours. Treated with albumin and pressors. Experienced transient oliguria and elevated creatinine.

Baseline abnormalities of liver function tests were common, and there were two cases of treatment-emergent further elevations of these test values (subject 1 had 12-15% elevations of baseline serum alkaline phosphatase and gamma-GT (glutamyltranspeptidase), and subject 6 had 230% (and later 117%) elevations of baseline SGOT which were, for the most part, isolated.

A18.7. Summary.

A single 2 mg trandolapril dose was orally administered to 8 adult hepatic cirrhosis patients. The mean inhibition of ACE was 85.1% at 12 hours, and 81.6% at 24 hours. Although these uncontrolled data were not subjected to scrutiny, the mean trough (24 hours post-dose) blood pressure changes from baseline appeared to be moderate to large. Mean peak effects were seen at 6 hours and the apparent antihypertensive effect disappeared by approximately 48 to 96 hours after dosing. C_{max} and AUC for trandolapril were 9-fold higher and C_{max} and AUC for trandolaprilat were about 2-fold higher in these subjects than in normal subjects. These differences should be included in labeling. Two subjects experienced plausibly drug-attributable severe hypotension, in association with known risk factors (baseline elevation of plasma renin activity, and/or ascites or co-exposure to a vasodilator). There were two cases of treatment-emergent further elevations of abnormal baseline liver function tests.

A19. Study FF/88/570/39 (Study report: vol 1.107; case report tabulations: 1.108-1.109; case report forms for deaths and discontinuations for adverse events: none) "Pharmacokinetics and pharmacodynamics of trandolapril after repeated administration of 2 mg to patients with chronic renal failure and healthy subjects".

A19.1. Investigators The investigators are listed in Table 169 below.

Table 169. Investigators in Study FF/88/570/39.

Investigator	Gender	City	Investigator	Gender	City
B Danielson	2	Uppsala, Sweden	P Larochelle	1	Montreal, Canada

A19.2. Study dates October 1989 to March 1990.

A19.3. Drug formulation

Table 170. Drug substance used in Study FF/88/570/39.

Lot	Form	Type	Trandolapril	Source lot	Batch size
MB 22327-149	Cap	Tran	2.0 mg	18	25,000
MB 22327-163	Cap	Tran	2.0 mg	MB22327-163	600,000

Table 171. Capsule composition in Study FF/88/570/39.

	2 mg
Trandolapril	2.0
Corn starch	
Lactose, hydrous	
Povidone	
Sodium stearyl fumarate	
Total	

A19.4. Study design

In this open-label, non-randomized study 2 mg trandolapril dose was orally administered once daily for 10 days (under fasting conditions) to 13 adult chronic renal failure patients (creatinine clearances $<60 \text{ ml/min}^1$), and 8 healthy subjects without renal insufficiency (creatinine clearance $>90 \text{ ml/min}$). The intake of sodium chloride and water was to be held constant, and the timing of meals was to be standardized. The objectives were to assess pharmacokinetics, pharmacodynamics, and safety.

Enrolled subjects were adults of both sexes. The protocol allowed for the uninterrupted use of diuretics, α -adrenoceptor antagonists, centrally-acting antihypertensives, and potassium sparing or replenishing therapies.

The exclusion criteria are shown in Table 172 below.

Conventional plasma and urinary pharmacokinetic end points were assessed. On days 1 and 10, blood sampling for trandolapril and trandolaprilat was undertaken prior to treatment, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 14 hours post-dose. Blood was also sampled just before drug administration on days 2, 3, 7, 9, and 10, and 24, 36, and 48 hours after the final dose.

¹ Normalized for a body surface area of 1.73 m^2 .

Table 172. Exclusion criteria in Study FF/88/570/39.

Women who are premenopausal or not using effective contraception
Body weight exceeding 130% of ideal
Cerebral hemorrhage or ischemia
Retinal hemorrhage or thrombosis
Coronary insufficiency
Mesenteric arteriopathy
Malignant or secondary hypertension
Heart failure
Major cardiac rhythm disorders
Any chronic severe disease, in particular cardiac, pulmonary, hematologic, hepatic, metabolic, neurologic, or psychiatric
History of hypersensitivity to drugs
Collagen disease
Neutropenia (<2500/mm ³)
Hemoglobin <7 g/100 ml
Proteinuria or hypoalbuminemia
Acute renal failure, current hemodialysis, or history of renal transplantation
Serum potassium ≥5.5 mM
Use within one month of enzyme-inducing drugs, cimetidine, ranitidine, chronic NSAIDs, glucocorticoids, digitals, or ACE inhibitors
Use within 2 weeks of vasodilators or anxiolytics
Inability to follow treatment or dietary restriction
Participation in another clinical trial within 8 weeks

Blood samples for ACE activity were obtained pre-dose (-15 min), and at 2, 4, and 6 hours on day 1, pre-dose on days 2, 3, 7, and 9, and on day 10, pre-dose, and at 2, 4, 6, 24, 48, 336, and 672 hours post-dose. Urine samples for trandolaprilat were collected over intervals -24 to 0 (24-hour blank), 0 to 10, 10 to 24 on day 1, 0 to 24 on day 9, and 0 to 10 and 10 to 24 on day 10.

Pharmacokinetic assays were assessed as shown in Table 173 below. The assays have

Table 173. Pharmacokinetic assays in Study FF/88/570/39.

Linearity	Accuracy	Precision	Sensitivity	Specificity				
Plasma samples—trandolapril and trandolaprilat								
Satisfactory.	Satisfactory.		Satisfactory.	LOQ - 0.1 ng/ml.				
Standard curves from 0.078 to 2.5 ng/ml for trandolapril and for trandolaprilat	ng/ml	T'april	T'aprilat	ng/ml	T'april	T'aprilat		
	0.100		93.0%	0.100		6.8%		
	0.200	106.0%	93.5%	0.200	6.9%	6.6%		
	0.400	103.3%	97.5%	0.400	9.0%	6.3%		
	0.800	102.6%		0.800	9.1%			
Urine samples—trandolaprilat—								
Satisfactory.	Satisfactory.		Satisfactory.	LOQ - 10 ng/ml.	Satisfactory.			
Standard curves from 5 to 5000 ng/ml	ng/ml	T'aprilat		ng/ml	T'aprilat			
	111	99.1%		111	9.7%			
	554	93.3%		554	7.6%			
	1107	96.9%		1107	7.6%			
	1661	98.2%		1661	9.2%			
Plasma samples—ACE inhibition—								
	Satisfactory. 54.3 U/L for expected values of 50 to 67 U/L.		Satisfactory. 3.6% C.V. at 54.3 U/L.					

been validated over the range of plasma and urine concentrations observed in the study.

AUC, C_{max} , $t_{1/2}$, CL_R , T_{max} , MRT, I_{max} , AUCI, I_{24} , and percentage of dose excreted in urine were calculated.

Blood pressure determinations were made at 0, 0.5, 1, 2, 4, 8, and 10 hours post-dose on days 1 and 10; at 0 and 2 hours post-dose on days 2, 3, 7, and 9; and 24 and 48 hours after the final (day 10) dose.

Safety end points included physical assessments, hematology, and chemistry end points. Laboratory end points were observed pre-treatment and 24 hours following drug discontinuation. Subject remained in hospital during treatment days 1, 9, 10, and during the day following drug discontinuation.

Subjects were stratified into 3 groups, according to creatinine clearance (with clearances of 7 to 30, 31 to 80, and >80 ml/min defining each respective subgroup). A one-way ANOVA was used to analyze differences among groups for ACE inhibition and pharmacokinetic parameters (other than T_{max}) on day 1, and to assess group differences on day 10. A 2-way ANOVA was used to compare these parameters between day 1 and day 10. Wilcoxon's nonparametric test was used to compare T_{max} between groups on discrete days, and the rank test was used to compare T_{max} on day 1 vs. day 10.

A19.5. Results

The demographic characteristics of the subjects are presented in Table 174 below. Subjects in the healthy group were all male, and significantly younger than those in the renal failure group.

The pertinent aspects of subject disposition were as follows: subject 104 was excluded from the analyses after discontinuing the study on day 8 for adverse events attributed to viral gastroenteritis; 9 renal failure subjects (101, 104, 107, 205, 207, 208, 209, 210,

Table 174. Demographic and baseline characteristics of subjects in Study FF/88/570/39.

	Renal impaired n=13	Normal n=8
Male	11	8
Female	2	0
Age-mean	50	34
Weight-mean	73	74
Incidence of hypertension (%)	85	0
Creatinine clearance-mean (ml/min)	25	108

and 212) were co-exposed to furosemide, nifedipine, hydralazine, or α -adrenoceptor antagonists (pindolol, metoprolol, atenolol, or betaxolol).

Blood pressure and heart rate effects are shown in Figure 84 below. Baseline mean

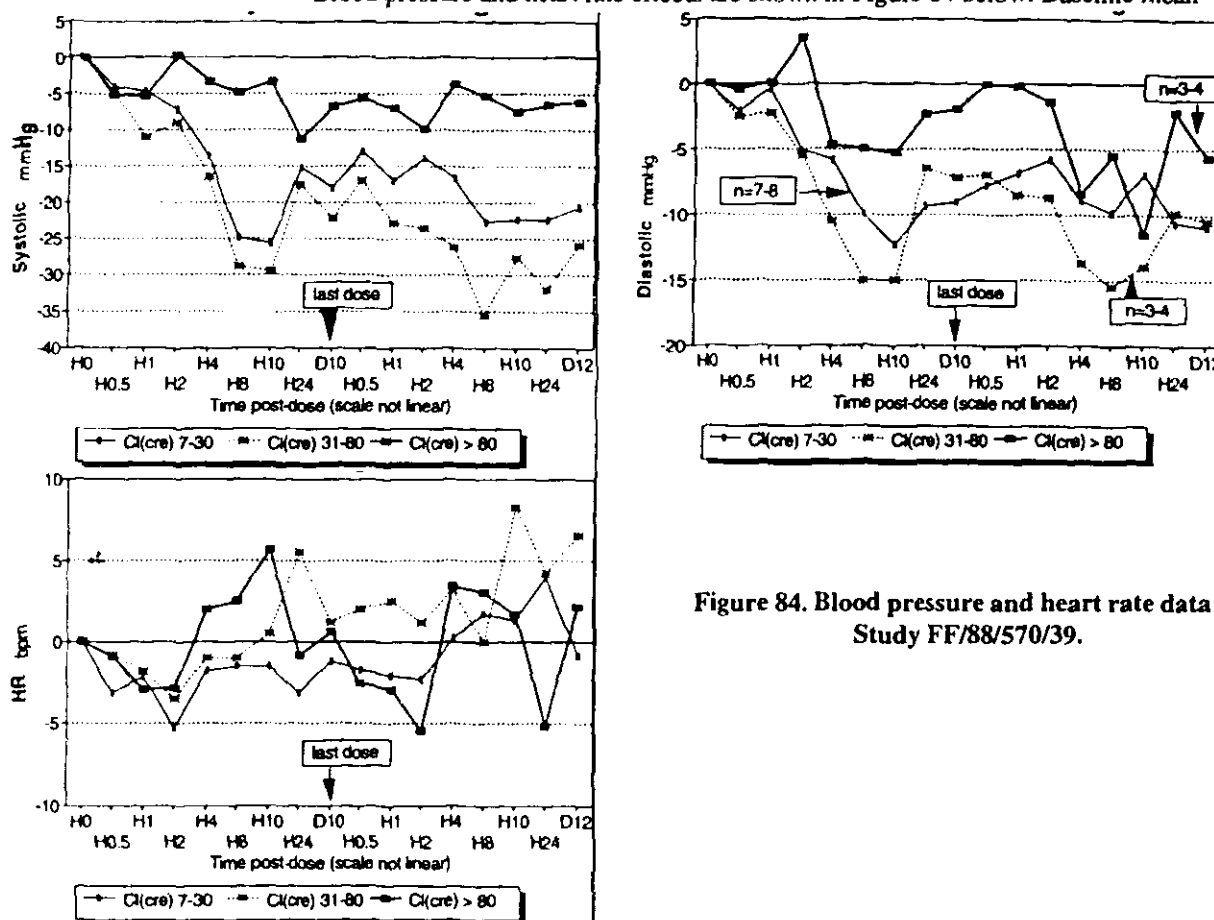


Figure 84. Blood pressure and heart rate data i Study FF/88/570/39.

supine blood pressures was 180.0/103.9 mmHg and mean heart rates was 65.6 bpm in the renal failure group with creatinine clearances of 7 to 30 ml/min. In the group with creatinine clearances of 31 to 80 ml/min, baseline mean supine blood pressures was 162.8/104.5 mmHg and mean heart rates was 64.8 bpm. Among healthy subjects (creatinine clearances >80 ml/min), baseline mean supine blood pressures was 117.6/73.4 mmHg and mean heart rates was 61.4 bpm. Changes from baseline hemodynamics are shown in Table 175 below.

Plasma trandolapril and trandolaprilat levels are shown in Figure 85 below. Pharmacokinetic data are shown in Table 176 below.

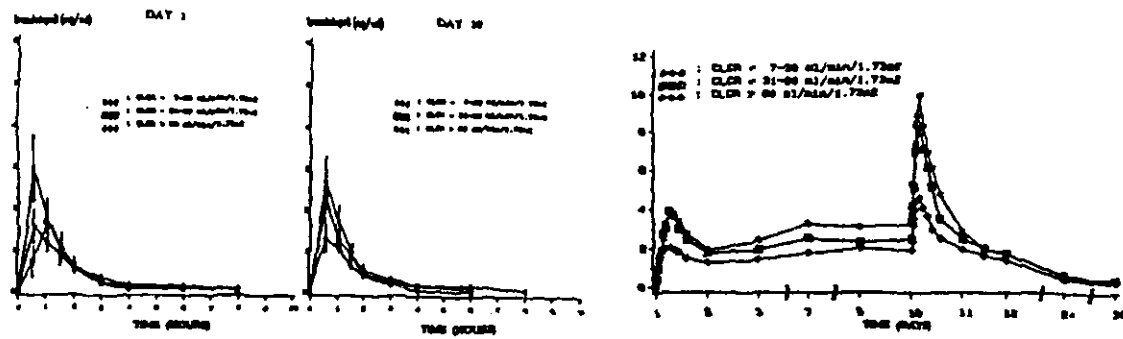


Figure 85. Plasma trandolapril and trandolaprilat levels in Study FF/88/570/39.

Table 175. Mean changes from baseline in supine blood pressure and heart rate in Study FF/88/570/39.

Day	Time after dosing (hours)	7 < CL _{CR} < 30 n=7/8			31 < CL _{CR} < 80 n=3/4			CL _{CR} > 80 n=3/4		
		Sys	Dias	HR	Sys	Dias	HR	Sys	Dias	HR
1	0.5	-3.9	-2.0	-3.1	-4.8	-2.5	-0.8	-5.1	-0.3	-0.9
	1	-4.6	-0.3	-2.2	-11.0	-2.2	-1.8	-5.3	+0.1	-2.9
	2	-7.1	-5.1	-5.3	-9.0	-5.5	-3.5	+0.2	+3.5	-2.8
	4	-13.6	-5.9	-1.8	-16.5	-10.5	-1.0	-3.3	-4.6	+2.0
	8	-24.9	-10.0	-1.5	-28.8	-15.0	-1.0	-4.8	-5.0	+2.5
	10	-25.6	-12.4	-1.5	-29.5	-15.0	+0.5	-3.3	-5.3	+5.6
2	pre	-15.2	-9.4	-3.1	-17.5	-6.5	+5.5	-11.2	-2.3	-0.8
10	pre	-18.1	-9.1	-1.2	-22.1	-7.2	+1.2	-6.8	-1.9	+0.6
	0.5	-13.1	-7.8	-1.7	-17.0	-7.0	+2.0	-5.7	0	-2.5
	1	-16.9	-6.8	-2.1	-22.8	-8.5	+2.5	-6.9	-0.2	-2.9
	2	-13.9	-5.8	-2.3	-23.5	-8.7	+1.2	-9.9	-1.4	-5.4
	4	-16.7	-8.9	+0.2	-26.3	-13.7	+3.2	-3.7	-8.4	+3.4
	8	-22.7	-9.8	+1.7	-35.5	-15.5	0	-5.2	-5.5	+3.0
	10	-22.4	-6.9	+1.3	-27.8	-14.0	+8.2	-7.5	-11.5	+1.7
	-24	-22.4	-10.6	+4.0	-32.1	-9.8	+4.2	-6.6	-2.1	-5.1
-48	-20.6	-10.9	-0.8	-25.8	-10.5	+6.5	-6.2	-5.6	+2.2	

ACE inhibition is depicted in Figure 86 and Table 176.

A19.6. Safety.

Case report forms were not submitted for evaluation. In the study report there is no explicit disclaimer that there were no deaths. One subject discontinued for an adverse event (subject 104), and several other treatment-emergent, non-laboratory adverse events were reported. These cases are described in Table 177 below.

Among renal failure subjects, there were apparently no clinically significant changes from pre-treatment chemistry or hematology results (per the submitted data tabulations). Several renal failure subjects (104, 208, 209) had mild treatment-emergent hyperkalemia (a finding plausibly confounded by instabilities in renal filtration function). Subject 213 had abnormally elevated hepatic enzymes prior to treatment and most values were slightly further elevated at the end of the study. Among healthy subjects, there were apparently no clinically significant changes from pre-treatment chemistry or hematology results. Healthy subject 105 had a serum

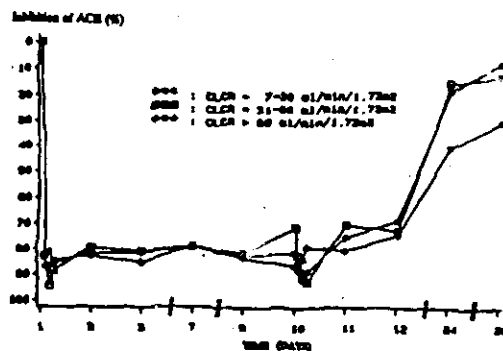


Figure 86. Plasma ACE inhibition in Study FF/88/570/39.

Table 176. Pharmacokinetic data and ACE inhibition in Study FF/88/570/39^a.

	Day 1			Day 10		
	7<CL _{CR} <30 n=8	31<CL _{CR} <80 n=4	CL _{CR} >80 n=8	7<CL _{CR} <30 n=8	31<CL _{CR} <80 n=4	CL _{CR} >80 n=8
Trandolapril						
AUC (ng/ml-h)	4.11±0.9	2.63±0.64	2.48±0.46	3.82±0.88	3.03±0.51	1.96±0.21
C _{max} (ng/ml)	3.12±0.76	1.92±0.45	1.88±0.27	2.70±0.57	2.48±0.79	1.62±0.20
T _{max} (median; h)	0.5	0.75	0.5	0.5	0.75	0.5
t _{1/2} (h)	0.77±0.14	0.71±0.13	0.67±0.09	0.68±0.05	0.82±0.02	0.59±0.04
Acc ratio	—	—	—	0.86	1.22	0.87
Trandolaprilat						
AUC ₀₋₂₄ (ng/ml-h)	63.4±8.1	59.5±4.5*	36.9±3.3†	133±12.5	110±13.0*	69.7±4.5
AUC ₀₋₄₈	—	—	—	184±15.8	158±18.5*	108±7.9†
C _{max} (ng/ml)	4.00±0.62	3.95±0.33*	2.09±0.22	9.90±0.85	8.38±0.68*	4.71±0.32
T _{max} (median; h)	6	6	6	4	3.5	4
U ₀₋₂₄ (% of dose)	1.0±0.5	3.4±0.7*	4.8±1.1†	4.1±1.0	9.3±1.0	15.2±1.6
CL _{R 0-24} (L/h)	0.3±0.1	1.1±0.3*	2.4±0.5†	0.7±0.2	1.6±0.3*	4.2±0.5
Acc ratio	—	—	—	2.29	1.82	1.90
ACE inhibition						
I _{max} (%)	94.0±3.2	94.2±3.7	92.9±0.7	90.5±2.7	94.6±2.9	91.7±2.0
I ₂₄ (%)	84.5±3.7	79.2±6.3	81.5±1.6	80.4±3.6	70.2±9.9	75.1±4.0
AUC ₁₀₋₆₇₂ (%·h)				30823±4408	20739±5047	16901±3148

^a *P<0.05 by ANOVA Tukey's test comparing lower creatinine clearance groups. †P<0.05 by ANOVA Tukey's test comparing upper creatinine clearance groups.

Table 177. Treatment-emergent, non-laboratory adverse events in Study FF/88/570/39.

Subj	ADR
101	65 year old male with baseline creatinine clearance of 12 ml/min and co-exposure to 4 non-ACE inhibitor antihypertensives. Marked asymptomatic systolic pressure fall from 207 to 147 starting on day 1, and stabilizing thereafter
104	71 year old female with baseline creatinine clearance of 8 ml/min. Discontinued with fever, diarrhea, atrial fibrillation and blood pressure reduction to 110/70 mmHg
107	43 year old male with baseline creatinine clearance of 44 ml/min. Developed spontaneously resolving abdominal pain, diplopia, and peribuccal paresthesias
201	32 year old healthy male subject. Developed mild postural vertigo without orthostatic hypotension
204	23 year old healthy male subject. Developed mild postural vertigo without orthostatic hypotension

alkaline phosphatase level near the upper limit of normal prior to treatment and this became slightly further elevated during treatment.

A19.7. Summary.

Trandolapril 2 mg/d was orally administered once daily for 10 days to 13 subjects with chronic renal failure (85% of whom were hypertensive), and 8 healthy subjects. ACE inhibition was 80-84% at approximately 24 hours post-dose (trough) on the first day, and 70-80% at trough on day 10. Although uncontrolled results from a small sample of renal failure patients, the mean trough blood pressure changes from baseline were substantial and larger after 10 days than after a single dose. With multiple dosing, the apparent mean peak effects were relatively late in the dosing interval (8 to 24 hours), and antihypertensive effects appeared to largely persist 48 hours after dosing. Multiple dosing had no effect on maximum ACE inhibition, but ACE inhibition was prolonged in subjects with renal impairment. C_{max} and AUC for trandolapril were both increased about 65% in subjects with $CL_{CR} \leq 30$ ml/min compared with normal subjects. The reduction in clearance of trandolapril supports a dosing adjustment in patients with $CL_{CR} \leq 30$ ml/min, an observation which should be included in labeling. One subject with renal failure experienced asymptomatic (albeit marked) blood pressure reduction. Healthy subjects (who, compared to the renal failure group, were younger and less represented by females) experienced small to moderate mean blood pressure reductions. Two normal subjects experienced mild postural vertigo, and one had clinically insignificant laboratory changes.

A20. Study FF/89/570/45 (Study report: vols 1.102; case report tabulations: 1.103; case report forms for deaths and discontinuations for adverse events: none) "Pharmacokinetics and pharmacodynamics of trandolapril after repeated doses of 2 mg in elderly patients with mild to moderate arterial hypertension".

A20.1. Investigators The investigators were A Wade (Glasgow, UK) and P Arner (Huddinge, Sweden).

A20.2. Study dates November 1989 to April 1990.

A20.3. Drug formulation

Table 178. Drug substance used in Study FF/89/570/45.

Lot	Form	Type	Trandolapril	Source lot	Batch size
JMP 22928-23	Cap	Plcbo	—		
MB 22327-163	Cap	Tran	2.0 mg	MB22327-163	600,000

Table 179. Capsule composition in Study FF/89/570/45.

	2 mg
Trandolapril	2.0
Corn starch	
Lactose, hydrous	
Povidone	
Sodium stearyl fumarate	
Total	

A20.4. Study design

This single-blind, non-randomized, baseline placebo-controlled, multi-center study orally administered trandolapril 2 mg once-daily for 10 days to 14 fasting elderly (mean age 69 years) subjects with mild-to-moderate arterial hypertension, subsequent to a 14 day placebo run-in. On days 1 and 10, the timing of post-drug meals was standardized. After discontinuation of drug treatment, subjects underwent placebo washout (one capsule once-daily) for two days.

Blood samples for the determination of trandolapril, trandolaprilat and ACE inhibition were drawn pre-dose on day 1 and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16 hours post-dose on day 1; pre-dose on days 2, 3, 5, 7, 9, 10; 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16 hours post-dose on day 10, and 24 and 48 hours after the dose on day 10.

Pharmacokinetic assays were assessed as shown in Table 180 below. The assays have been validated over the range of plasma and urine concentrations observed in the study.

AUC_{∞} , C_{max} , $t_{1/2}$, I_{max} , and I_{24} were calculated.

Enrolled subjects were adults of both sexes with age ≥ 65 years and uncomplicated mild-to-moderate arterial hypertension (diastolic blood pressure ≥ 95 mmHg and ≤ 115 mmHg). The exclusion criteria shown in Table 181 below, were conventional for a pharmacokinetic study of a cardiovascular agent.

The study objectives were to characterize pharmacokinetics, pharmacodynamics, and safety after single and multiple trandolapril doses in elderly subjects. Blood pressure determinations were made 15 minutes before dosing, and 1, 2, 3, 4, 6, 8, 12, and 48 hours post-dosing on days 1 and 10 as well as immediately before dosing on days 2, 3, 5, 7, and 9.

Table 180. Pharmacokinetic assays in Study FF/89/570/45.

Linearity	Accuracy	Precision	Sensitivity	Specificity
Plasma samples—trandolapril and trandolaprilat—				
Satisfactory. Standard curves from 0.078 to 2.50 ng/ml.	Satisfactory. 100.0% at 0.155 ng/ml, 100.0 at 0.314 ng/ml, 100.0% at 0.857 ng/ml, 92.1 at 2.237 ng/ml for trandolapril; 99.4% at 0.156 ng/ml, 99.4 at 0.312 ng/ml, 102.0% at 0.850 ng/ml, 94.3 at 1.970 ng/ml for trandolaprilat.	Satisfactory. %CV - 3.1% at 0.155 ng/ml, 6.2 at 0.314 ng/ml, 3.2% at 0.857 ng/ml, 4.4 at 2.237 ng/ml for trandolapril; 9.2% at 0.156 ng/ml, 8.7 at 0.312 ng/ml, 6.1% at 0.850 ng/ml, 7.6 at 1.970 ng/ml trandolaprilat.	LOQ - 0.1 ng/ml.	Satisfactory.
Plasma samples—ACE inhibition				
	Satisfactory. 76.8 U/L for expected values of 64 to 86 U/L.	Satisfactory. 2.3% C.V. at 76.8 U/L.		

Table 181. Exclusion criteria in Study FF/89/570/45.

Cerebral hemorrhage or ischemia
Retinal hemorrhage or thrombosis
Ischemic heart disease, angina, or myocardial infarction
Coronary insufficiency
Active arteriopathy
Mesenteric arteriopathy
Renal insufficiency (creatinine >150 μM)
Heart failure and cardiomegaly (cardiothoracic ratio >0.5)
Any severe chronic disease, especially cardiopulmonary, hepatic, metabolic, neurological, psychiatric, or alcoholism
Insulin-dependent diabetes mellitus
History of hypersensitivity to drugs, especially ACE inhibitors
Collagen diseases
Neutropenia ($\leq 2500/mm^3$)
Proteinuria
Hemoglobin <8 g/dl
Hypertension secondary to unilateral or bilateral renal artery stenosis, primary hyperaldosteronism, pheochromocytoma, acromegaly, Cushing's disease, or coarctation of the aorta
Major rhythm disorders
Hyperkalemia (>5.5 mM)
Overweight (>130% for height)
Use within one month of enzyme-inducing drugs, calcium blockers, vasodilators, cimetidine, ranitidine, chronic NSAIDs, glucocorticoids, digitalis, estrogen, progesterone, or ACE inhibitors
Use within two weeks of β-blockers or diuretics
Inability to follow treatment or dietary restrictions
Participation in another clinical trial within 8 weeks

Blood sampling was undertaken up to 16 hours post-dose on days 1 and 10. Trough plasma drug concentration was defined as the observed C_{min} . Drug accumulation

ratios were calculated as the ratio of AUC on day 10 to AUC on day 1¹.

Laboratory hematology, chemistry and urinalysis data were collected pre-treatment and 24 hours after the last drug dose. Adverse events were to have been recorded after spontaneous reporting by patients or in answer to non-leading questions. Clinical follow-up was undertaken one and two weeks after the final drug doses.

Statistical significance was defined at an α level of 0.05. A 2-way analysis of variance (ANOVA) was used to test for differences in pharmacokinetic parameters between treatment days (except for T_{max} , which was tested using the rank sign test). Changes from pre-treatment blood pressure were assessed using the t-test. Drug concentrations below the limit of quantification (0.1 ng/ml) were imputed to be zero.

A20.5. Results

The demographic characteristics of the subjects are presented in Table 182 below.

Table 182. Demographic and baseline characteristics of subjects in Study FF/89/570/45.

	All subjects n=14
Male	5
Female	9
Age—mean	69
Range	65 to 76
Caucasian	14
Black	0
Other	0
Weight—mean	70
Range	48 to 90
Baseline BP	168/101

Two subjects were excluded from the analyses because of noncompliance. Two subjects (202 and 207) violated the protocol by receiving concomitant β -blockers, but they were included in the analysis.

at.

Blood pressure and heart rate effects are shown in Figure 87 below. The mean

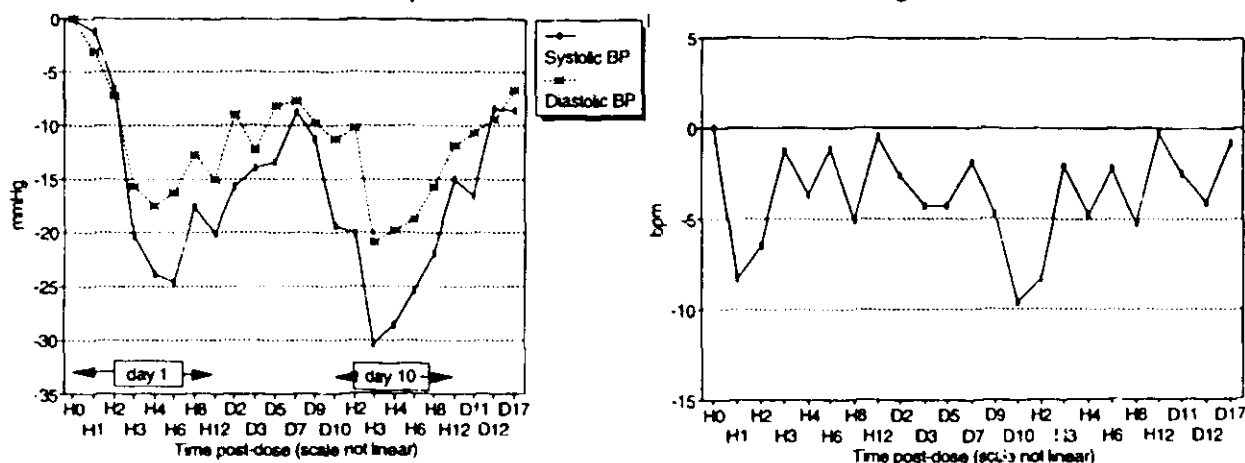


Figure 87. Blood pressure and heart rate data in Study FF/89/570/45.

hemodynamic changes from pre-treatment are shown in Table 183 below. The

¹ Unless otherwise noted, the AUC's were calculated up to the last time point where the plasma concentration was detectable

Table 183. Mean changes in blood pressure and heart rate in Study FF/89/570/45^a.

Day	Hours after dose	Sys	Dias	HR	Day	Hours after dose	Sys	Dias	HR
Baseline	0	0	0	0	10	1	-19.3	-11.3	-9.6
1	1	-1.4	-3.3	-8.3		2	-20.1	-10.3	-8.3
	2	-6.6	-7.3	-6.5		3	-30.3	-20.9	-2.1
	3	-20.3	-15.8	-1.3		4	-28.6	-19.8	-4.8
	4	-23.9	-17.5	-3.7		6	-25.3	-18.8	-2.2
	6	-24.6	-16.3	-1.1		8	-22.0	-15.8	-5.2
	8	-17.7	-12.8	-5.1		12	-15.0	-11.9	-0.3
	12	-20.1	-15.0	-0.5	11	none given	-16.5	-10.8	-2.5
2	pre	-15.7	-9.0	-2.6					
3	pre	-14.0	-12.3	-4.3	12	-48	-8.5	-9.5	-4.1
5	pre	-13.4	-8.3	-4.3	17		-8.7	-6.8	-0.8
7	pre	-8.8	-7.8	-1.9					
9	pre	-11.3	-9.8	-4.8					

a. N=12 except for day 17 (n=10).

pretreatment observations were at the end of a 2 week placebo run-in. There were no concurrent placebo observations with which to correct these data. The day 11 and 12 observations were during a placebo washout period.

Pharmacokinetic parameters are shown in Table 184 below. Trandolapril and

Table 184. Pharmacokinetic parameters in Study FF/89/570/45.

	All subjects		Males n=5		Females n=7-9	
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10
Trandolapril						
C _{max} (ng·ml ⁻¹)	4.05	4.57	4.38	5.18	4.28	4.14
T _{max} (h, median)	0.5	0.5	—	—	—	—
AUC (ng·ml ⁻¹ ·h)	5.29	6.05	6.22	7.10	6.37	5.31
t _{1/2} (h)	0.76	0.63	—	—	—	—
Accumulation ratio	1.16		—		—	
Trandolaprilat						
C _{max} (ng·ml ⁻¹)	4.70	8.35	5.35	8.88	3.90	7.97
T _{max} (h, median)	6	3	—	—	—	—
AUC _{0-24h} (ng·ml ⁻¹ ·h)	65.85	96.75	69.67	96.68	63.13	96.80
Accumulation ratio	1.49		—		—	
ACE inhibition						
IC _{max} (%)	84.4	89.1	86.98	—	91.10	—
T _{max} (h, median)	6	1.5	—	—	—	—
I ₂₄ (%)	63.7	58.9	—	—	—	—

trandolaprilat plasma levels are shown in Figure 88 below. Plasma ACE inhibition is shown in Figure 89 below.

A20.6. Safety.

Case report forms were not submitted for evaluation.

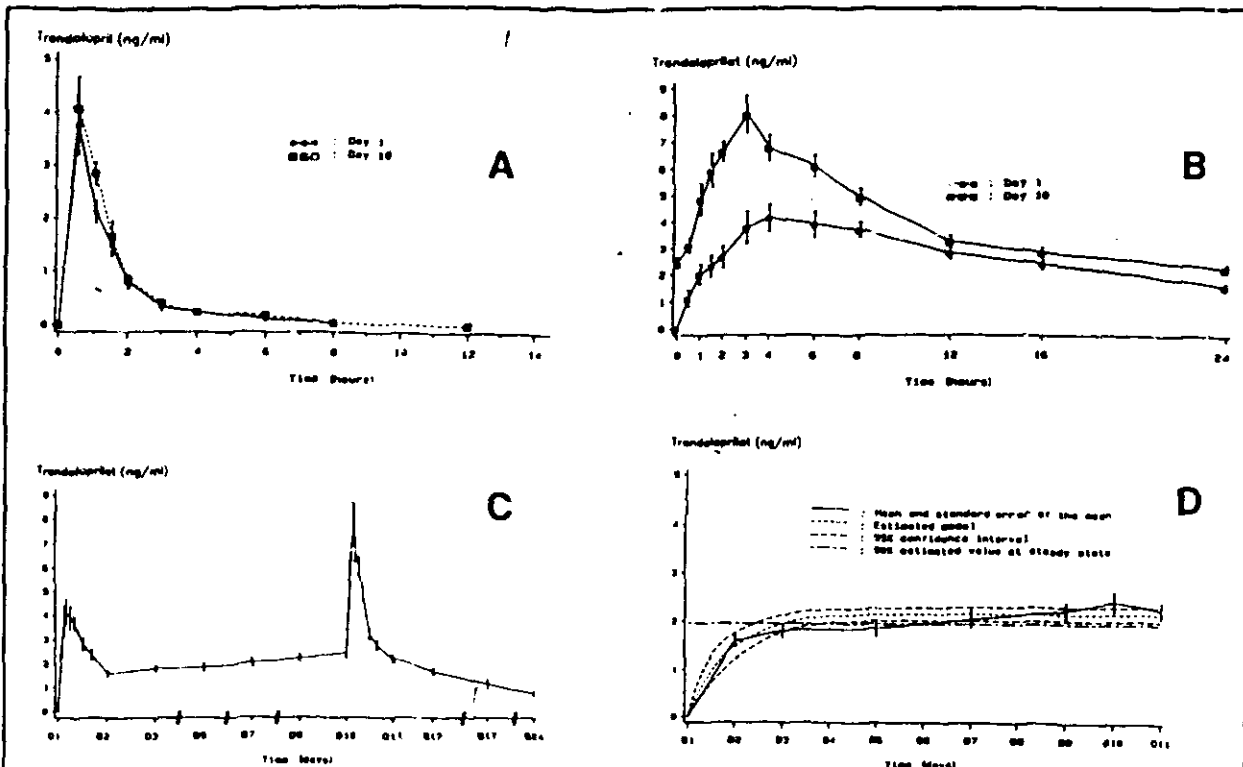


Figure 88. Plasma trandolapril and trandolaprilat levels in Study FF/89/570/45.

(A) Trandolapril as a function of hours after dosing on days 1 and 10. (B) Trandolaprilat as a function of hours after dosing on days 1 and 10. (C) Trandolaprilat as a function of time (days) during and after repetitive dosing. (D) Trough trandolaprilat levels during repetitive dosing.

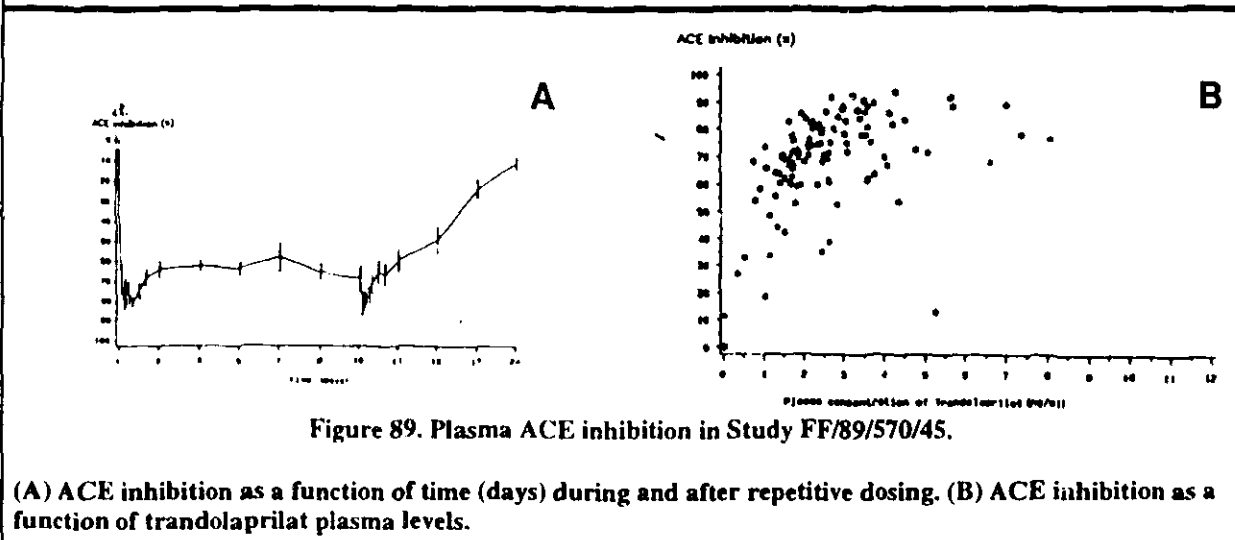


Figure 89. Plasma ACE inhibition in Study FF/89/570/45.

(A) ACE inhibition as a function of time (days) during and after repetitive dosing. (B) ACE inhibition as a function of trandolaprilat plasma levels.

There were no deaths or dropouts described in the submitted report. One subject (210) reportedly had several episodes of asymptomatic orthostatic hypotension (systolic BP difference of >25 mmHg between supine and standing positions) which resolved within 2 minutes of assuming the standing position. One subject (202) reportedly had mild palpitations 8 days after discontinuing trandolapril. A β -blocker was administered and the symptom did not recur.

There were no clinically significant changes from pre-treatment hematology and laboratory chemistry data². During trandolapril therapy, one subject had a small (2 µM) reduction from pre-treatment serum total bilirubin.

A20.7. Summary.

Trandolapril 2 mg p.o. was administered to 14 elderly hypertensive subjects. Although not subjected to scrutiny, the trough blood pressure changes from baseline were substantial and sustained over 10 days of treatment. Peak effects were seen at 3 to 6 hours and disappeared between 24 and 48 hours after dosing. Pharmacokinetics and pharmacodynamics in these elderly subjects were similar to those in younger subjects (32 to 57 years old; Study S/87/570/230) with mild to moderate hypertension. Pharmacokinetics and pharmacodynamics were similar in elderly male and female subjects. There was reportedly one case of asymptomatic orthostatic hypotension without other clinically significant adverse events.

4.

² As reported in appendix XI 2, pg. 333 of vol 1.103.

A21. Study GB/89/570/48 (Study report: vol 4.1; case report tabulations: vol 4.1; case report forms for deaths and discontinuations for adverse events: none) "Pharmacokinetics and pharmacodynamics of trandolapril and its active metabolite, trandolaprilat (), after intravenous administration of trandolapril and intravenous administration of trandolaprilat to healthy volunteers".

- A21.1. Investigators** The investigator was JA Sutton, Swindon, England.
- A21.2. Study dates** June 1989 to September 1989.
- A21.3. Drug formulation** Trandolapril 5 ml vials containing 5 mg trandolapril, lot MMG 22685-05. Trandolaprilat 5 ml vials containing 5 mg trandolaprilat, lot MMG 22685-09. Other ingredients are unknown.
- A21.4. Study design** Twelve healthy adult volunteers were to be randomly crossed-over (latin square design) to open-label, oral trandolapril 2 mg (2 ml in 48 ml mineral water), intravenous trandolapril (2 mg in 40 ml normal saline delivered over 2 hours), and intravenous trandolaprilat 1.87 mg (equimolar; similar delivery) on each of 3 occasions separated by 3 weeks. The objectives were to assess (1) the absolute bioavailability of trandolapril, (2) the fraction of absorbed trandolapril, F , (3) the fraction of trandolapril metabolized to trandolaprilat, fm , (4) the fraction of trandolapril absorbed and metabolized, $F' = F \times fm$, and (5) plasma ACE inhibition after intravenous and oral administration of trandolapril and after intravenous trandolaprilat. Hemodynamic measurements included standing and supine blood pressure and heart rate, 12-lead ECG, stroke volume, and cardiac output.

Subjects were to be males or females of low childbearing potential, age 20 to 55 year, weight between 60 and 90 kg (males) or 50 to 75 kg (females), with no abnormalities on baseline screening. The exclusion criteria are shown in Table 185 below.

Table 185. Exclusion criteria in Study GB/89/570/48.

Requirement for regular medication or occasional medication within 1 week of study
Significant illness within 3 months
Investigational drug exposure within 3 months
History of >10 cigarettes/day or equivalent
Alcohol abuse
History of cardiovascular disease, hypertension, allergy, hypersensitivity to drugs, diabetes, hyperlipidemia, or gout
Blacks

Blood samples for drug levels were obtained at baseline and at post-dose hours 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 6, 8, 10, 12, 14, 24, 32, 48, and 56. Blood samples for the determination of ACE activity were collected at baseline, and at post-dose hours 0.5, 1, 1.5, 2, 4, 8, 12, 24, 32, 48, and 56. Urine for drug levels was collected at baseline (blank), and over intervals 0 to 4, 4 to 8, 8 to 12, 12 to 24, and 24 to 56 hours post-dosing.

Pharmacokinetic assays were assessed as shown in Table 186 below. The assays have been validated over the range of plasma and urine concentrations observed in the study.

AUC, C_{max} , $t_{1/2}$, Cl, CL_R , T_{max} , vd_{ss} , percentage of dose excreted in the urine, and absolute bioavailability were calculated.

A21.5. Results Demographic and baseline characteristics of subjects are shown in Table 187 below.

There were minor deviations in sampling times. All subjects completed the study.

Table 186. Pharmacokinetic assays in Study GB/89/570/48.

Linearity	Accuracy			Precision			Sensitivity	Specificity
Plasma samples—trandolapril and trandolaprilat—								
Satisfactory.	Satisfactory.			Satisfactory.			LOQ - 0.2 ng/ml.	Satisfactory.
Standard curves from 0.078 to 2.5 ng/ml for trandolapril and 0.019 to 9.94 ng/ml for trandolaprilat	ng/ml 0.2 0.4 0.8	T' april 103.0% 100.8% 101.6%	T' aprilat 100.0 10% for 0.157 to 2.090 ng	ng/ml 0.2 0.4 0.8	T' april 6.1% 6.8% 7.7%	T' aprilat 4.2 to 6.6% for 0.157 to 2.090 ng		
Urine samples—trandolaprilat—								
Satisfactory.	Satisfactory.			Satisfactory.			LOQ - 25 ng/ml.	Satisfactory.
Standard curves from 5.0 to 5000 ng/ml	ng/ml 111 554 1107 1661	T' aprilat 99.1 93.3 96.9 98.2	ng/ml 111 554 1107 1661	T' aprilat 9.7 7.6 7.6 9.2				
Plasma samples—ACE inhibition—								

Table 187. Demographic and baseline characteristics of subjects in Study GB/89/570/48.

	=12
Male	10
Female	2
Caucasian	12
Other	0
Age mean	38.7
Weight mean	71.4

Hemodynamic data were only reported in line listings and summary statistics. The summary statistics were shown by study period rather than by treatment. Heart rate changes from baseline were clinically nonsignificant.

Pharmacokinetic data are shown in Table 188 below.

Table 188. Pharmacokinetic data (mean±SEM) from Study GB/89/570/48.

	Trandolapril		Trandolaprilat		
	Trandolapril 2 mg p.o.	Trandolapril 2 mg i.v.	Trandolapril 2 mg p.o.	Trandolapril 2 mg i.v.	Trandolaprilat 1.87 mg i.v.
C _{max} (ng/ml)	3.51±0.28	—	31.7±0.21	8.08±0.75	—
C _{end inf} ^a (ng/ml)	—	20.1±1.08	—	—	88.8±4.61
AUC (h·ng/ml)					
AUC ₀₋₆	4.22±0.38	39.2±1.88			
AUC ₀₋₁₂			29.7±1.7	60.0±4.4	216±9.2
AUC ₀₋₅₆			94.4±6.2	135±6.5	275±11.5
T _{max} (h)					
median	0.5	—	4.0	4.0	2.0 ^b
range	0.5-3	—			

Table 188. Pharmacokinetic data (mean±SEM) from Study GB/89/570/48. (Continued)

	Trandolapril		Trandolaprilat		
	Trandolapril 2 mg p.o.	Trandolapril 2 mg i.v.	Trandolaprilat 2 mg p.o.	Trandolaprilat 2 mg i.v.	Trandolaprilat 187 mg i.v. ^a
t _{1/2} (h)	0.67±0.08	0.26±0.02	—	—	—
Cl (l/h)	—	52.3±2.5	—	—	—
Cl _{R 0-56}	—	—	1.73±0.17	3.21±0.27	4.33±0.38
U ₀₋₅₆ μg %	—	—	161±14 8.6±0.8	441±36 23.6±1.9	1305±83 69.8±4.4
V _{ss} (l)	—	17.8±1.4	—	—	—
F (%)	10.7±0.7		—	—	—
F ^b	—		39±4	—	—
F ^b	—		13±1	—	—

a. Concentration at the end of the infusion

b. End of infusion

Plasma drug levels following oral administration are shown in Figure 90 below.

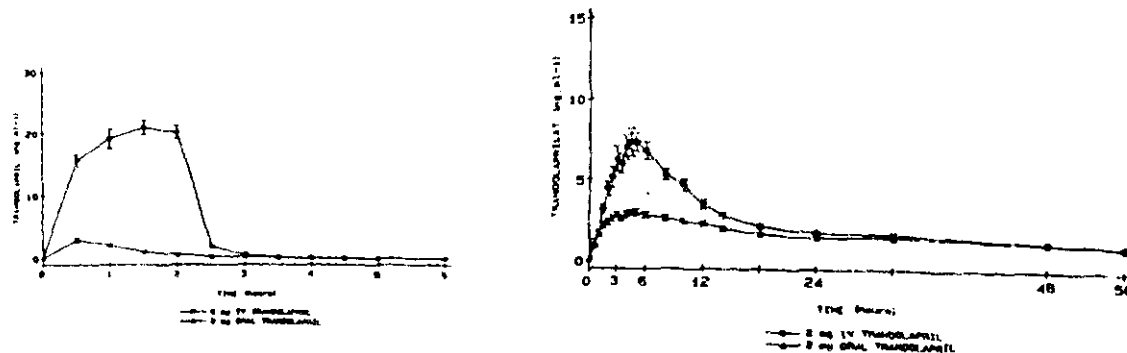


Figure 90: Plasma trandolapril and trandolaprilat levels after oral administration in Study GB/89/570/48.

Plasma trandolaprilat levels after intravenous trandolaprilat are shown in Figure 91 below. Cumulative urinary excretion of trandolaprilat is shown in Figure 92 below.

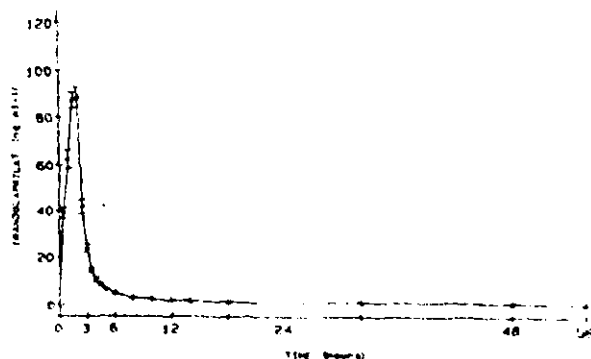


Figure 91. Plasma trandolaprilat after intravenous administration in Study GB/89/570/48.

The extent of ACE inhibition is depicted in Table 189 below and in Figure 93 below. There is an apparent dose-response.

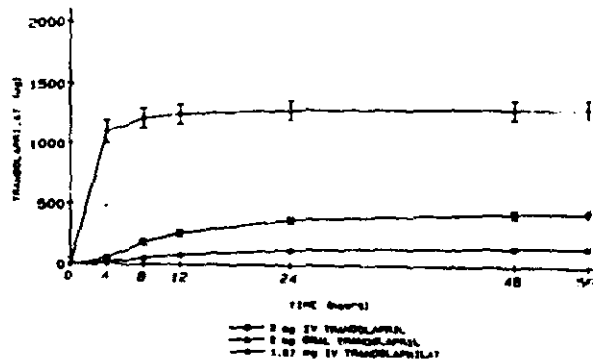


Figure 92. Cumulative urinary excretion of trandolaprilat in Study GB/89/570/48.

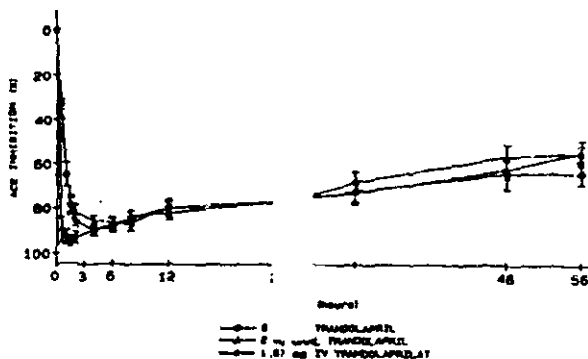


Figure 93. Plasma ACE inhibition in Study GB/89/570/48.

Table 189. ACE inhibition in Study GB/89/570/48.

	Trandolapril		
	Trandolapril 2 mg p.o.	Trandolapril 2 mg i.v.	Trandolaprilat 1.87 mg i.v.
I _{max} (%)	92.0±2.4	91.5±2.3	96.9±1.9
I _{24h} (%)	77.8±3.7	76.9±2.6	76.8±3.6
T _{max} (h) median range	3 1-8	4 1-12	1 0.5-24

A21.6. Safety.

Case report forms were not submitted for evaluation.

Five subjects reported faintness, 2 subjects reported dizziness, and 3 subjects reported light-headedness.

There were no clinically significant treatment emergent laboratory abnormalities reported.

A21.7. Summary.

Twelve healthy volunteers were randomly crossed-over to open-label, oral trandolapril and intravenous trandolapril and intravenous trandolaprilat administered in single doses on each of 3 occasions. Absolute bioavailability of trandolapril as trandolaprilat was about 50% using plasma data and 37% using urinary excretion data.

A22. Study GB-90-570-91 (Study report: vols 1.135-1.136; case report tabulations: none; case report forms for deaths and discontinuations for adverse events: none) "A within subject, placebo controlled, double blind study to investigate the ability of trandolapril 4 mg p.o. to protect against and antagonise scopolamine induced cognitive deficit in healthy volunteers".

A22.1. Investigators The investigator was K Wesnes, (Reading, UK).

A22.2. Study dates May 1990 to July 1990.

A22.3. Drug formulation

Table 190. Drug substance used in Study GB-90-570-91.

Lot	Form	Type	Trandolapril	Source lot	Batch size
CR-22-970-107	Cap	Tran	4.0 mg	18	32,300

Table 191. Capsule composition in Study GB-90-570-91.

	4 mg
Trandolapril	4.0
Corn starch	
Lactose, hydrous	
Povidone	
Magnesium stearyl fumarate	
Total	

A22.4. Study design

Scopolamine-induced amnesia is a model for age-induced cognitive impairment, thought to relate to angiotensin II effects on cholinergic function. This was a double-blind, placebo-controlled, cross-over study with a Latin Square design. The four periods involved single-dose administrations (a) placebo/placebo/placebo, (b) placebo/scopolamine/placebo, (c) trandolapril/scopolamine/placebo, and (d) placebo/scopolamine/trandolapril. The scopolamine dose was 0.5 mg s.c. and the trandolapril dose was 4 mg p.o. On study days, subjects took the first dose of trandolapril or placebo after overnight fast and baseline cognitive testing. A light breakfast is provided 30 minutes after dosing and cognitive testing is repeated at 2.5 hours after dosing. At 3 hours, the dose of scopolamine or placebo is administered. At 4 hours, there was repeat cognitive testing. At 4.5 hours, placebo or trandolapril are administered, followed by lunch at 5 hours, and repeat cognitive testing at 6, 8.5, and 9 hours. Blood samples were obtained for pharmacokinetic study.

Subjects were healthy males age 18 to 40, within 20% of ideal weight, meeting minimum criteria on the CDR Computerised Assessment System¹. The exclusion criteria are shown in Table 192 below. Twenty subjects were planned.

A22.5. Results

The demographic characteristics of the subjects are presented in Table 193 below.

All subjects completed all phases of the study.

Although not reviewed in detail, there were reductions in supine systolic and diastolic pressure and heart rate for treatment periods with scopolamine with or without trandolapril, compared with placebo.

¹ Elements of this test battery assess reaction time, choice reaction, 2-digit vigilance, rapid visual information processing, 5-digit short-term memory, immediate and delayed verbal recall, verbal recognition, and subjective state. Except for the latter, the procedures are briefly described in the protocol.

Table 192. Exclusion criteria in Study GB-90-570-91.

Significant abnormalities on hematology, biochemistry, or ECG
Any drug use other than paracetamol within 2 weeks of study
History of alcohol or drug abuse
Use of tobacco during the study period or regular use >10 cigarettes/day
Use of an investigational drug within 3 months or 3 investigational drugs within 12 months

Table 193. Demographic and baseline characteristics of subjects in Study GB-90-570-91.

	All subjects n=20
Male	20
Female	0
Age	27.7.1
Range	19 to 40
Caucasian	19
Black	1
Other	0
Weight—mean	76.8.2
Range	62 to 94

Scopolamine alone produced statistically significant deficits in cognitive measures at 1 to 5.5 hours following administration. The sponsor notes trends in improved scores when scopolamine followed pre-treatment with trandolapril, but none of the trends was statistically significant. A few of the cognitive measures were statistically significantly better when trandolapril was administered after scopolamine, but the analysis does not adjust for the multiplicity of comparisons.

Trandolapril levels attained in plasma were comparable when administered before or after scopolamine.

A22.6. Safety.

Case report forms were not submitted for evaluation.

There were no deaths or dropouts described in the submitted report. There were 130 adverse events reported; however, all subjects who received trandolapril also received scopolamine during the same study phase, and the adverse events case report form did not record the timing of events. The largest number of events occurred during the treatment phase in which scopolamine was given without trandolapril. Most of the observed events (xerostoma, 36; drowsiness, 34; accommodation defect, 15) are attributable to scopolamine. No other events raise novel safety concerns regarding trandolapril.

There were no clinically significant changes from pre-treatment hematology and laboratory chemistry data.

A22.7. Summary.

A dose of scopolamine was used to produce deficits in a battery of tests of cognitive function. Trandolapril had no significant effect on these deficits when administered before or after scopolamine. There was no significant effect of scopolamine on trandolapril or trandolaprilat levels.

A23. Study HAG 006472: In vitro protein binding.

A23.1. Investigators The investigator was C Johnson, U.K.

A23.2. Study design The objective of this study was to assess the extent of binding of ³H-trandolapril and ³H-trandolaprilat to human plasma proteins.

Methanolic toluene solutions of ³H-trandolapril and ³H-trandolaprilat were dried under nitrogen. Radiolabeled drugs were incubated in buffer or plasma at 37°C for 24 hours. Duplicate aliquots were removed for scintillation counting at time zero and aliquots for freeze drying were removed at 0, 2, 4, and 24 hours. Following freeze drying, the samples were reconstituted in distilled water and scintillation cocktail was added. The degree of tritium exchange was calculated by comparing the counts from freeze dried samples with counts made directly.

An equilibrium time study was undertaken at an initial concentration of 500 ng/ml for each compound using unlabeled material and the concentrations of the compounds were determined by radioimmunoassay.

For both radiolabeled compounds, the binding to human plasma proteins was determined by equilibrium dialysis over the nominal concentration range of 0.1 to 1000 ng/ml. The binding at each nominal concentration was examined at least in duplicate. All dialysis steps were performed at 37°C.

A23.3. Results

Based on the exchange studies carried out in potassium phosphate buffer and human plasma, there was no evidence of exchange of tritium with water at 37°C. Table 194 below shows results (mean of duplicate samples) of equilibrium studies and Table 195

Table 194. Equilibration (% by time) for trandolapril and trandolaprilat in Study HAG 006472.

Time (h)	Trandolapril		Trandolaprilat		Time (h)	Trandolapril		Trandolaprilat	
	Donor	Recipient	Donor	Recipient		Donor	Recipient	Donor	Recipient
0.5	60.4	39.6	59.5	40.5	3.0	49.1	50.9	52.8	47.2
1.0	51.6	48.4	51.9	48.1	3.5	49.5	50.5	47.9	52.1
1.5	53.2	46.8	49.2	50.8	4.0	46.6	53.4	51.1	48.9
2.0	49.1	50.9	53.2	46.8	5.0	52.6	47.4	48.6	51.4
2.5	51.9	48.1	50.9	49.1	6.0	49.8	50.2	50.9	49.1

below shows results of protein binding studies.

Table 195. Binding of trandolapril and trandolaprilat (%) to human plasma in Study HAG 006472.

Nominal conc (ng/ml)	Trandolapril	Trandolaprilat
0.1	83.7	94.2
1.0	84.1	89.3
10	84.3	71.5
100	80.6	65.6
1000	83.3	65.1

A23.4. Summary.

Binding of trandolapril to human plasma proteins is independent of concentration. Binding of trandolaprilat is concentration-dependent, indicating saturation of binding sites at low concentrations of trandolaprilat.

A24. Study RU44570/IGB-101 (Study report: vols 1.112; case report tabulations: vol 1.112; case report forms for deaths and discontinuations for adverse events: none) "Investigation of the pharmacokinetics and metabolism of [redacted] in healthy volunteers following oral administration of ^{14}C -labelled compound".

A24.1. Investigators The investigator was J Collins, Milton Keynes, England.

A24.2. Study dates May 1987.

A24.3. Drug formulation

Table 196. Drug substance used in Study [redacted] /IGB-101.

Lot	Form	Type	Trandolapril	Source lot	Batch size
MK87E008	Soln	Tran ^a	8.0 mg	MK003	500 ml

a ^{14}C -labelled trandolapril in 100 mL phosphate-citrate buffer

A24.4. Study design This open-label study administered single 8 mg doses of ^{14}C -labelled ($36 \mu\text{Ci} \equiv 1.33\text{MBq}$) trandolapril to 3 healthy adult male subjects. Measurements were made of plasma, urine and fecal recoveries of radioactivity (over 8 days), as well as parent drug and metabolite levels. Subjects were to refrain from the use of tobacco, ethanol, and caffeine for 1 to 4 days after dosing. Fluid and food intake was standardized for the first 12 hours post-dose.

The exclusion criteria are shown in Table 197 below.

Table 197. Exclusion criteria in Study [redacted] IGB-101.

Weight 15% more or less than ideal
Presence of hypersensitivity, or any disease of the renal, hepatic, or gastrointestinal system
Use in the previous 3 months of a drug with the potential for major organ toxicity
Regular use of ethanol, or any pharmaceutical
Participation in a radiolabelled-drug study in the previous 12 months

Blood samples for the determination of tracer recovery were collected at baseline, at post-dose hours 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, 72, 144, 168, 192, 216, 264, and 336. Plasma was harvested for cold determination, ^{14}C determination, and metabolic profiling. Plasma for metabolic patterns was collected from 20 ml blood at 1, 3, 5, 10, and 24 hours. Urine samples were collected pre-dose (blank), and at post-dose intervals 0 to 1, 1 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 12, 12 to 24, and as daily 24-hour collections until radioactivity was negligible. Urine and plasma samples were stored at -20°C until analyzed. Fecal collections for tracer measurements were made for 2 days pre-dose and during days 1 to 9 post-dose, until radioactivity was negligible. Fecal samples were analyzed immediately after collection.¹

Pharmacokinetic assays were assessed as shown in Table 198 below. The assays have been validated over the range of plasma and urine concentrations observed in the study.

AUC_{∞} , C_{max} , $t_{1/2}$, and percentage of dose excreted were calculated.

Hematology, and clinical chemistry measures were obtained at baseline, and at 72 hours post-dose.

¹ Trandolapril could not be measured directly. Urine and plasma samples were treated with porcine liver esterase to convert trandolapril to trandolaprilat and concentrations of trandolapril were obtained by subtraction.

Table 198. Pharmacokinetic assays in Study /IGB-101.

Linearity	Accuracy	Precision	Sensitivity	Specificity
Plasma samples—trandolaprilat—				
Satisfactory. Standard curves from 0.4 to 100 ng/ml.	Satisfactory. 111.7% at 2.5 ng/ml, 99.3% at 10 ng/ml, 103.1% at 25 ng/ml, 110.5% at 40 ng/ml.	Satisfactory. %CV — 19.5% at 2.5 ng/ml, 6.3% at 10 ng/ml, 3.0% at 25 ng/ml, 12.5% at 40 ng/ml.	LOQ—1.0 ng/ml.	Satisfactory.
Urine samples—trandolaprilat				
Satisfactory. Standard curves from 10 to 1500 ng/ml	Satisfactory. 90.9% at 78.5 ng/ml, 95.1% at 524 ng/ml, 95.4% at 1309 ng/ml	Satisfactory. %CV— 11.8% at 78.5 ng/ml, 7.3% at 524 ng/ml, 9.2% at 1309 ng/ml	LOQ—75 ng/ml	Satisfactory

Only descriptive statistics were generated.

A24.5. Results

Demographic and baseline characteristics of subjects are shown in Table 199 below.

Table 199. Demographic and baseline characteristics of subjects in Study /IGB-101.

	n=3
Male	3
Female	0
Age range	37-44
Weight range	73-86

The structures of trandolapril and some its metabolites are shown in Figure 94 below.

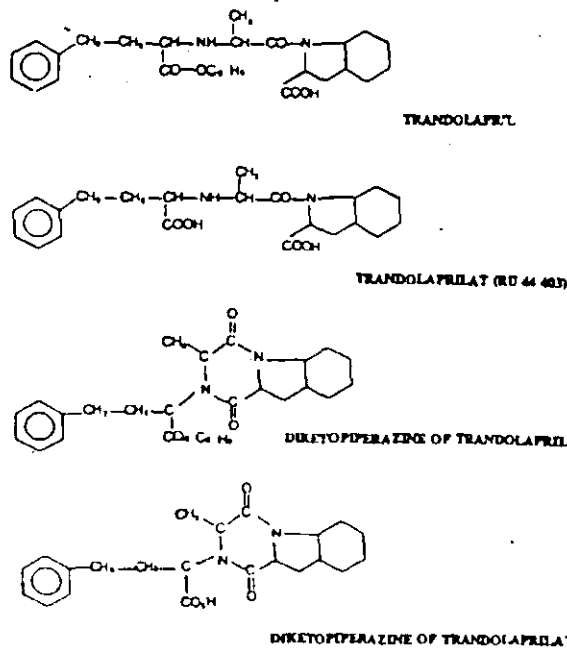


Figure 94. Structures of trandolapril and some metabolites found in Study /IGB-101.

The presumed metabolic pathway is shown in Figure 95 below. Pharmacokinetic parameters are summarized in Table 200 and Table 201 below. Plasma drug levels are shown in Figure 96 below. Recovery of radioactivity in urine and feces is shown in

Figure 95. Metabolic pathway for trandolapril in Study 1GB-101.

Table 200. Pharmacokinetic parameters in Study 1GB-101.

	Trandolapril				Trandolaprilat			
	Subjects			Mean±SD	Subjects			Mean±SD
	1	2	3		1	2	3	
C _{max} (ng/ml)	22.5	39.7	38.4	33.5±9.6	16.6	28.4	32.0	25.7±8.1
T _{max} (h)	0.75	0.50	0.50	0.58±0.14	1.25	1.5	1.5	1.42±0.14
t _{1/2, α} (h)	0.69	0.33	0.32	0.45±0.21	4.82	3.85	4.35	4.34±0.49
AUC ₀₋₈ (ng·h/ml)	50.7	36.9	31.3	39.6±10.0	93.7	150	112	117±23.9
AUC ₀₋₂₄	72.0	36.9	31.3	46.7±22.1	166	229	176	190±34.1
Ae ₀₋₁₆₈ (mg)	89.3	125	402	205±171	1126	1403	1068	1199±179
% excreted in urine	1.1	1.6	5.0	2.6±2.1	15.1	18.8	14.3	16.0±2.4

Table 201. Pharmacokinetic parameters for total ¹⁴C in Study 1GB-101.

	Trandolapril			
	Subjects			Mean±SD
	1	2	3	
C _{max} (ng/ml)	177	291	165	211±70
T _{max} (h)	1.00	0.5	0.5	0.67±0.29
t _{1/2, α} (h)	0.73	1.02	1.05	0.93±0.18
t _{1/2, β}	6.74	8.18	9.48	8.13±1.37
t _{h, 95%}	5.90	9.16	8.10	7.72±1.66
AUC ₀₋₈ (ng·h/ml)	530	740	440	570±150
AUC ₀₋₂₄	750	930	600	760±170
Apparent clearance (g/min)	178	143	222	181±39.5

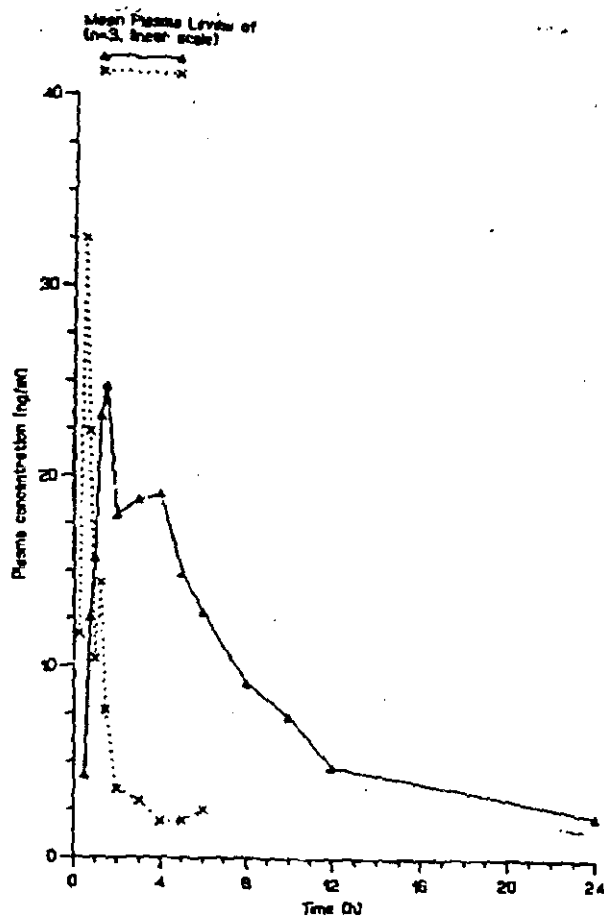


Figure 96. Plasma trandolapril and trandolaprilat levels in Study 1GB-101.

Table 202 below. Urinary excretion of trandolapril and trandolaprilat are shown in Figure 97 below.

A24.6. Safety.

Case report forms were not submitted for evaluation. There were apparently no safety-related discontinuations. Only brief descriptions of adverse events were provided. All 3 subjects experienced headache (at about 6 hours post-dose). Subject 2 had a mild, treatment-emergent reduction of hemoglobin (to 12.6 g/dL at 72 hours post-dose) for which the report did not contain a description of reproducibility, reversibility, or sequelae (if any).

A24.7. Summary.

Single 8 mg doses of radiolabelled trandolapril were administered to 3 healthy adult male subjects. This is the definitive study for metabolic profiling. More than 99% of total radioactivity was recovered over 168 hours, 33.3% in urine and 65.9% in feces. Headaches were experienced by each subject, and 1 developed mild anemia, but apparently there were no safety-related dropouts.

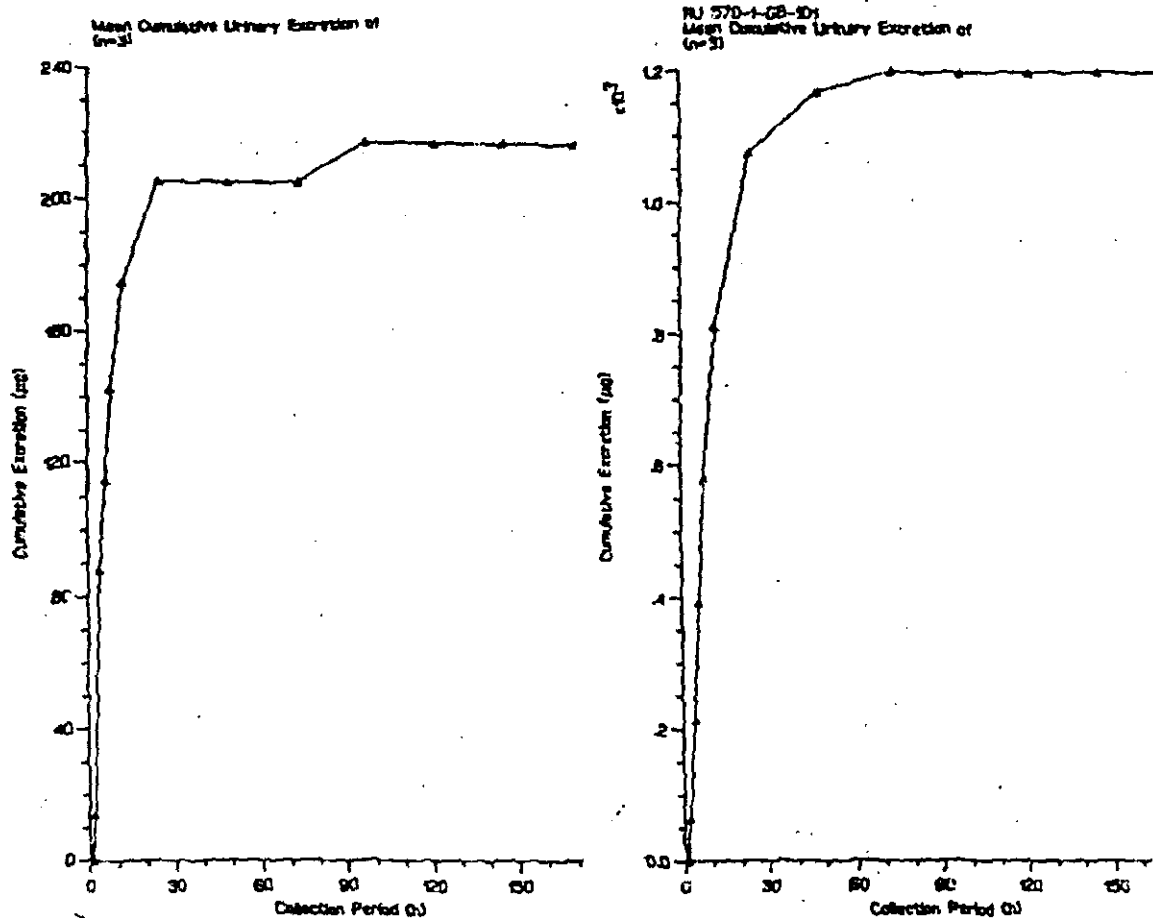


Figure 97. Urinary excretion of trandolapril and trandolaprilat in Study 1GB-101.

Table 202. Urinary and fecal radioactivity in Study IGB-101.

	% of dose recovered			
	Subj.1	Subj.2	Subj.3	Mean±SD
Urine				
0-1 h				1.930±0.519
1-2				3.516±1.662
2-4				5.685±1.216
4-6				4.120±0.444
6-8				4.517±0.290
8-12				4.843±1.563
12-24				4.899±1.319
24-48				2.522±0.378
48-72				0.706±0.270
72-96				0.276±0.024
96-120				0.222±0.052
120-144				0.102±0.092
144-168				0.000±0.000
Total				33.339±3.765
Feces				
1 day				20.097±31.945
2				29.840±25.417
3				10.657±10.242
4				3.540±4.398
5				2.580±0.778
6				0.850±0.683
7				0.350±0.252
Total				65.873±5.536
Total				99.212±3.433

A25. Study 'IGB-102(Study report: vols 1.113; case report tabulations: vol 1.113; case report forms for deaths and discontinuations for adverse events: none) "Investigation of the metabolism of in healthy volunteers following oral administration of ¹⁴C-labelled compound- a further analysis of plasma".

A25.1. Investigators The investigator was J Collins, Milton Keynes, England.

A25.2. Study dates November 1988.

A25.3. Drug formulation

Table 203. Drug substance used in Study 'IGB-102.

Lot	Form	Type	Trandolapril	Source lot	Batch size
MK88003	Soln	Tran ^a	32.0 mg	MK0C6	250 ml

a. 24.54 mg unlabeled and 7.46 mg ¹⁴C-labelled trandolapril in 100 mL water.

A25.4. Study design This open-label study administered single 32 mg doses of ¹⁴C-labelled (50 µCi) trandolapril to 2 healthy adult male subjects. Measurements were made of plasma, urine and fecal recoveries of radioactivity (over 2 days), as well as parent drug and metabolite levels. Subjects were to refrain from the use of tobacco, ethanol, and caffeine for 1 to 2 days after dosing. Fluid and food intake was standardized for the first 8 hours post-dose.

The exclusion criteria are shown in Table 204 below.

Table 204. Exclusion criteria in Study 'IGB-102.

Weight 15% more or less than ideal
Presence of hypersensitivity, or any disease of the renal, hepatic, or gastrointestinal system
Use in the previous 3 months of a drug with the potential for major organ toxicity
Regular use of ethanol, or any pharmaceutical
Participation in a radiolabelled-drug study in the previous 12 months

Blood samples for the determination of tracer recovery were collected at baseline, and at post-dose hours 0.25, 0.5, 0.75, 1, 1.5, 6, and 24. Feces and urine collections for tracer recoveries were made at baseline, and during the 48 hours following dosing. Blood samples for the determination of metabolite profiles were collected at baseline, and at 0.75 hours post-dose. Hematology, and clinical chemistry measures were obtained at baseline, and at 24 hours post-dose.

Only descriptive statistics were generated.

A25.5. Results Demographic and baseline characteristics of subjects are shown in Table 205 below.

Table 205. Demographic and baseline characteristics of subjects in Study 'IGB-102.

	n=2
Male	2
Female	0
Age range	46-47
Weight range	73-90

A25.6. Safety. Case report forms were not submitted for evaluation. Reportedly there were no adverse events. Subject 1 had a minimal further elevation of a baseline-elevated serum

potassium level, and a small reduction in erythrocyte count without an abnormally low hematocrit. Subject 2 had a minimal further elevation of a baseline-elevated serum urate level.

A25.7. Summary.

Single 32 mg doses of radiolabelled trandolapril were administered to 2 healthy adult male subjects. There were reportedly no adverse events and no clinically significant treatment-emergent laboratory abnormalities. This study did not undergo biopharmaceutical review because metabolic profiling was adequately described in Study 1GB-101 (page 193).

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A26. Study S/87/570/23 (Study report: vol 1.132; case report tabulations: vols 1.133-1.134; case report forms for deaths and discontinuations for adverse events: none) "Pharmacokinetics and pharmacodynamics of trandolapril (2 mg and 4 mg after repeated administration once a day for 10 days in patients with mild to moderate arterial hypertension".

A26.1. Investigators The investigators were P Arner and P Engfeldt, Huddinge, Sweden.

A26.2. Study dates August 1988 to February 1989.

A26.3. Drug formulation

Table 206. Drug substance used in Study S/87/570/23.

Lot	Form	Type	Trandolapril	Source lot	Batch size
MB 21662-130B	Cap	Tran	2.0 mg	18	450,000
MB 21662-88	Cap	Tran	4.0 mg	17	150,000

Table 207. Capsule composition in Study S/87/570/23.

	2 mg	4 mg
Trandolapril	2.0	4.0
Corn starch		
Lactose, hydrous		
Povidone		
Sodium stearyl fumarate		
Total		

A26.4. Study design

The trial description is based upon the protocol. This was an open-label, non-randomized study, with 10 subjects per group. There was a 14-day placebo-withdrawal period, followed by a 10-day treatment period with daily dosing at either 2 or 4 mg, and then a final 2-day placebo washout period. Subjects were to be age <65 years, with supine and standing diastolic pressure between 95 and 110 mmHg, and normal hepatic function tests. Women were permitted if post-menopausal or surgically sterile. Exclusion criteria are listed in Table 208 below.

Table 208. Exclusion criteria in Study S/87/570/23.

Concurrent diseases related to hypertension (cerebral hemorrhage or ischemia, retinal hemorrhage or thrombosis, coronary insufficiency, heart failure, arteriopathy of the lower limbs, mesenteric arteriopathy, or renal failure).
Severe chronic cardiopulmonary, hepatic, metabolic, neurological, or psychological disease, allergy or drug hypersensitivity, collagen disease, dermatoses, or neutropenia.
Hypertension secondary to a known cause
Hyperkalemia (≥ 5.5 mM)
Weight >130% of ULN
Use within 28 days prior to study of enzyme inhibitors, cimetidine, ranitidine, long-term anti-inflammatory or analgesic drugs, glucocorticoids, digitalis, or estrogen-progestogen.
Rhythm or conduction abnormalities
Previous trial participation within 3 months

Subjects were advised to take the drug with water following overnight fast.

Safety data included medical history, physical exam, 12-lead ECG (within 30 days of baseline only), and routine hematology, clinical chemistry, and urinalysis. There were no explicit criteria for dropping a subject because of lack of efficacy.

Blood samples were obtained for ACE activity and plasma drug levels. Drug levels were to be measured by specific radioimmunoassay with a detection limit of 2 ng/ml fortrandolapril and 1 ng/ml fortrandolaprilat.

Other pharmacokinetic assays were assessed as shown in Table 209 below.

Table 209. Pharmacokinetic assays in Study S/87/570/23.

Linearity	Accuracy	Precision	Sensitivity	Specificity
Plasma samples—ACE inhibition—				
	55.9 U/L for expected values of 44 to 58 U/L 43.8 U/L for expected values of 29 to 53 U/L	3.93% CV at 55.9 U/L 3.67% CV at 43.8 U/L		

A26.5. Results

Eleven subjects were enrolled, of whom 9 completed both treatments with an intervening 4-week washout. One subject completed only the 2-mg dosing and one completed only the 4-mg dosing. Demographic and baseline characteristics of subjects are shown in Table 210 below.

Table 210. Demographic and baseline characteristics of subjects in Study S/87/570/23.

	2 mg n=10	4 mg n=10
Male	8	8
Female	2	2
Age±SEM	44±2.3	45±2.2
Caucasian	10	10
Other	0	0
Weight±SEM	72±3.5	74±3.9

Plasma ACE inhibition is shown in Figure 98 below. The left panel shows the development of ACE inhibition during the 24 hours following the first dose. The right panel shows trough ACE inhibition for treatment days 2 through 10 and post-treatment day 11. Plasma ACE inhibition peaks at about 80% following the 2 mg dose, and about 90% following the 4 mg dose. Trough levels of plasma ACE inhibition were somewhat smaller following the 4 mg dose than following the 2 mg dose.

Plasma trandolapril levels rose and fell to undetectable levels within 6 hours of dosing; no accumulation was apparent with repetitive dosing. Plasma trandolaprilat levels are shown in Figure 99 below. The left panel shows the trandolaprilat levels following the 2 mg dose on days 1 and 10. The right panel shows the trandolaprilat levels resulting from the 4 mg dose. Trandolaprilat accumulated with repetitive dosing, but to a much lesser extent than would be predicted from the first day's pharmacokinetics. Peak levels of trandolaprilat appeared to be proportional to dose, but trough levels were much less than dose-proportional.

The relationship between plasma trandolaprilat levels and plasma ACE inhibition is shown in Figure 100 below.

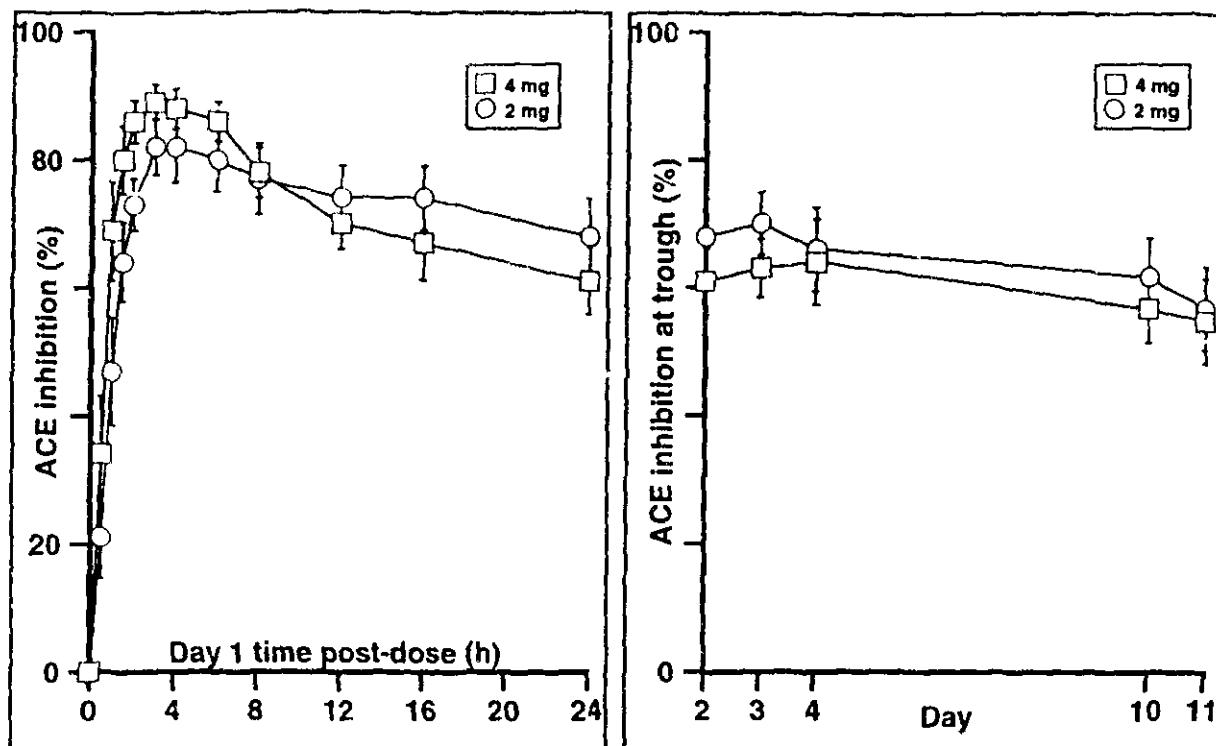


Figure 98. Plasma ACE inhibition in Study S/87/570/23.

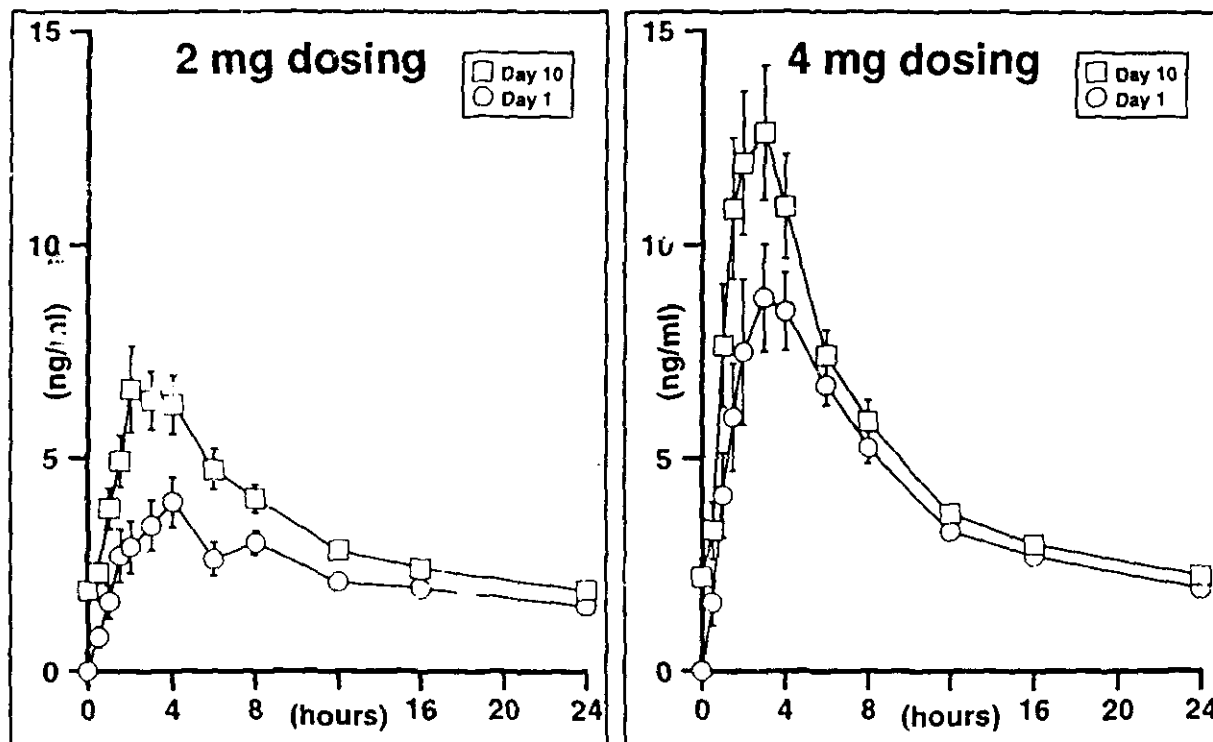


Figure 99. Plasma tranilolaprilat levels in Study S/87/570/23.

Supine blood pressure is displayed in Figure 101 below. There was only a baseline control, so there is a limited ability to discount a placebo effect, but the return toward baseline following the last active treatment dose is consistent with a treatment effect.

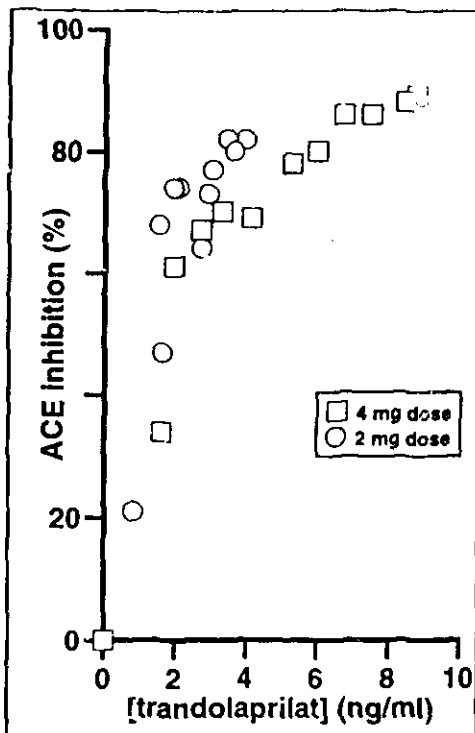


Figure 100. Plasma ACE inhibition vs. plasma trandolaprilat levels in Study S/87/570/23.

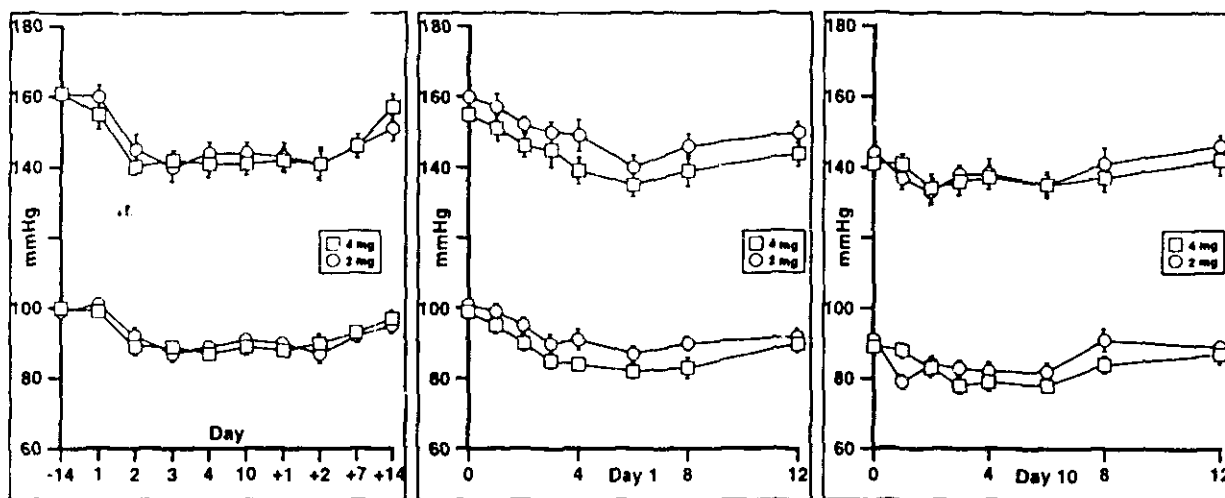


Figure 101. Blood pressure data in Study S/87/570/23.

The two doses are distinguishable on the first active treatment day, but not subsequently. The magnitude of effect appears to be a reduction of about 20/10 mmHg at trough; this effect is reached by the second day of treatment and was sustained for several days beyond the last active treatment dose.

Effects on heart rate were not demonstrated.

A26.6. Safety.

It is unclear why two subjects participated in only one arm of the trial. There were no adverse events reported. No clinically significant laboratory abnormalities were reported.

Safety data included history, physical exam, 12-lead ECG, and routine laboratory, all performed at baseline and at end point.

A36.5. Results

Eighty-five subjects enrolled in the trial, of whom 84 completed¹. Twelve subjects are listed with protocol violations; 11 subjects had baseline supine diastolic pressure ≥ 90 mmHg (of whom 8 were randomized to placebo) and 2 subjects received the wrong drug. One subject who was randomized in violation did not complete the study. Individual study centers enrolled 1 to 17 subjects. Demographic and baseline characteristics of subjects are shown in Table 313 below. The treatment groups were

Table 313. Demographic and baseline characteristics of subjects in Study TR-30-HTN-III.

	Plcbo n=38	Tran n=47
Male	23	31
Female	15	16
Age $\pm$ SD	54 $\pm$ 13	53 $\pm$ 11
Range	25 to 75	27 to 72
Caucasian	34	45
Black	4	2
Other	0	0
Weight $\pm$ SD	184 $\pm$ 43	193 $\pm$ 35
Range	113 to 286	105 to 324

reasonably well matched for these factors, for general health, duration and severity of hypertension, and baseline drug use.

No subjects were excluded from efficacy or safety analyses because of protocol violations.

The sponsor's analysis of blood pressure considered 'trough' as any measurement at least 10 hours after dosing.

Responder rate data are shown in Table 314 below. An intent-to-treat analysis was

Table 314. Responder rate data (%) in Study TR-30-HTN-III^a.

	Trandolapril						Pooled	
	Plcbo n=9	1mg n=15	Plcbo n=14	2mg n=19	Plcbo n=15	4mg n=18	Plcbo n=37	Any n=47
Week 2	5 (56)	11 (73)	8 (57)	13 (68)	8 (57)	8 (62)	21 (57)	32 (68)
Week 4	6 (67)	8 (53)	6 (43)	11 (58)	4 (27)	4 (33)	16 (42)	23 (49)

a. Sponsor's analysis.

performed by the sponsor; the overall difference (42% for placebo vs. 49% for trandolapril) was not statistically significant. This was consistent with the statistical reviewer's analysis with χ^2 ($p=0.53$) and Fisher's exact test ($p=0.66$), ignoring differences in dosing among trandolapril groups, and the Mantel-Haenszel test ($p=0.74$), controlling by dose level.

Changes in blood pressure are shown in Figure 135 below (supine only) and Table 315 below (again with the sponsor's definition of 'trough'). The sponsor's analysis of

¹ One subject in the 4 mg group was withdrawn because of compliance problems.

A26.7. Summary.

Pharmacokinetics and pharmacodynamics were characterized for subjects with mild to moderate hypertension in response to 10-day courses of 2 and 4 mg trandolapril. Plasma ACE inhibition plateaued at about 90% and closely tracked plasma trandolaprilat levels. Blood pressure changes were substantial, and, although not dose-dependent, were consistent during blinded administration, with a return toward baseline during washout. There were no adverse events or other safety concerns.

d.

A27. Study TR-1-CP (Study report: vols 1.75; case report tabulations: vol 1.75; case report forms for deaths and discontinuations for adverse events: none) "Single dose, crossover bioavailability pilot study to evaluate two lots of trandolapril capsules".

A27.1. Investigators The clinical investigator was K Lassiter, Miami, FL. Kinetic analyses were performed by R Kets, Sunnyvale, CA.

A27.2. Study dates June 1990.

A27.3. Drug formulation

Table 211. Drug substance used in Study TR-1-CP.

Lot	Form	Type	Trandolapril	Source lot	Batch size
MB22327-163	Cap	Tran	2.0 mg	MB22327-163	600,000
99-0190	Cap	Tran	4.0 mg	99-0190	31,595

Table 212. Capsule composition in Study TR-1-CP.

	2 mg	4 mg
Trandolapril	2.0	4.0
Corn starch		
Lactose, hydrous		
Povidone		
Sodium stearyl fumarate		
Total		

A27.4. Study design

This open-label, 2-way crossover study randomized 8 healthy adult male subjects to single 4 mg doses of trandolapril. Drug was administered as two 2 mg capsules vs. one 4 mg capsule on each of 2 treatment days separated by 6 day drug-free intervals. The objective was to assess the relative bioavailability of the two formulations. Doses were administered in the morning following an overnight fast. Water, but no food, was allowed for 4 hours post-dose.

The exclusion criteria are shown in Table 213 below.

Table 213. Exclusion criteria in Study TR-1-CP.

Weight 10% more or less than ideal
Diastolic blood pressure <60 mmHg or systolic blood pressure <100 mmHg
Heart failure
Major cardiac rhythm disorders
Cutaneous hypersensitivity, drug abuse, or any disease of the cardiovascular, renal, hepatic, or hematologic system
Use within one month of barbiturates, chronic salicylates, or NSAIDs
Participation in another clinical trial in the previous 45 days

Blood samples for the determination of parent drug and metabolite levels were collected at baseline (pre-treatment), and at post-dose hours 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, and 24. Plasma samples were stored at -20°C until analyzed. Pharmacokinetic assays were assessed as shown in Table 214 below. The assays have been validated over the range of plasma and urine concentrations observed in the study.

Table 214. Pharmacokinetic assays in Study TR-1-CP.

Linearity	Accuracy		Precision			Sensitivity	Specificity
Plasma samples—trandolapril and trandolaprilat—							
Satisfactory.	Satisfactory.		Satisfactory.			LOQ - 0.078 ng/ml.	—
Standard curves from 0.039 to 10.0 ng/ml.	ng/ml	T'april	T'aprilat	ng/ml	T'april	T'aprilat	
	0.15	102.8%	105.6%	0.15	10.4%	9.7%	
	1.50	102.6%	103.4%	1.50	7.8%	8.4%	

AUC_{0-24} , C_{max} , and T_{max} were calculated.

Blood pressure, and heart rate were determined at baseline, and at post-dose hours 5 and 24. Hematology and clinical chemistry evaluations were obtained at baseline, and again at 24 hours post-dose¹.

Paired *t* tests were used to compare kinetics parameters for the 2 formulations.

A27.5. Results

Demographic and baseline characteristics of subjects are shown in Table 215 below.

Table 215. Demographic and baseline characteristics of subjects in Study TR-1-CP.

	n=8
Male	8
Female	0
Caucasian	7
Hispanic	1
Other	0
Age mean	36
Weight mean	75

Pharmacokinetic data are shown in Table 216 below. Plasma drug levels are shown in

Table 216. Pharmacokinetic parameters in Study TR-1-CP.

	Trandolapril		Trandolaprilat	
	4 mg Knoll	2x2 mg	4 mg Knoll	2x2 mg
AUC_{0-24} (h-ng/ml)	6.92±3.60	6.02±2.45	73.6±28.4	70.9±25.0
C_{max} (ng/ml)	5.84±4.32	4.48±2.68	6.29±3.41	5.93±3.04
T_{max} (h)	0.6±0.2	0.8±0.3	3.6±1.1	3.9±1.1

Figure 102 below.

A27.6. Safety.

Case report forms were not submitted for evaluation. There were no clinically significant hemodynamic changes, and reportedly no "significant" [undefined] adverse events of any kind. It would logically follow that there were no safety-related withdrawals, but there was no explicit assertion made to this effect. Subject 5 had transient elevations of hepatocellular enzyme(s) before and after trandolapril exposure.

¹ This appears to refer to the final dose, but not to the first dose, since only one post-dose value was reported for each parameter

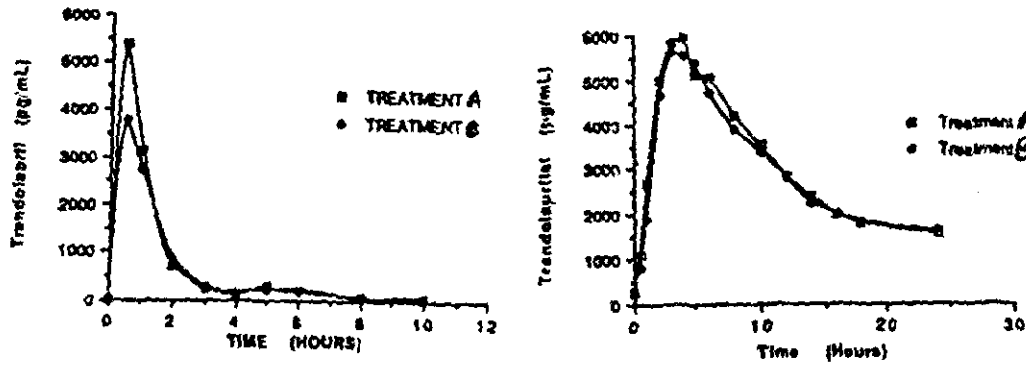


Figure 102. Plasma trandolapril and trandolaprilat levels in Study TR-1-CP.

A27.7. Summary.

This open-label, 2-way crossover study randomized 8 healthy adult male subjects to single 4 mg doses of trandolapril (administered as two 2 mg capsules vs. one 4 mg capsule) in order to assess the bioequivalence of the formulations. This pilot bioequivalence study suggests that the relative bioavailability of two 2-mg trandolapril capsules (manufactured by *[redacted]*) is comparable to one 4-mg trandolapril capsule (manufactured by Knoll Pharmaceuticals). There were no clinically significant hemodynamic changes, and reportedly no significant adverse events of any kind.

A28. Study TR-2-CP (Study report: vols 1.76-1.85; case report tabulations: vols 1.77-1.78; case report forms for deaths and discontinuations for adverse events: none) "Single- and multiple-dose pharmacokinetic evaluation of 1 mg, 4 mg, and 16 mg trandolapril in healthy male subjects".

A28.1. Investigators The clinical investigator was M Zinny, Boston, MA.

A28.2. Study dates December 1992 to February 1993.

A28.3. Drug formulation

Table 217. Drug substance used in Study TR-2-CP.

Code	Form	Type	Trandolapril	Sample	Batch
TRA-J140A	Cap	Tran	1.0 mg	L-12	546,100
99-0391	Cap	Tran	4.0 mg	99-0391	85,963

Table 218. Capsule composition in Study TR-2-CP.

	1 mg	4 mg
Trandolapril	1.0	4.0
Corn starch		
Lactose, hydrous		
Povidone		
Sodium stearyl fumarate		
Total		

A28.4. Study design

This was an open-label randomized, single- plus multiple-dose, parallel-group study. Subjects were randomized to trandolapril 1, 4, or 16 mg and received a single dose on study day 1, followed by 72 hours of pharmacokinetic sampling of blood and urine. Subjects then received their randomized dose once daily for 8 days, with pharmacokinetic blood and urine sampling for a total of 36 days. Subjects were healthy, normal, age 18 to 40 years, non-smoking males.

The exclusion criteria are shown in Table 219 below.

Table 219. Exclusion criteria in Study TR-2-CP.

Positive serology
Serious allergy, asthma, or hypersensitivity to any drug
History of heart failure, sinus bradycardia, sick sinus syndrome, A-V block, or more than 5 ectopic beats/minute
History of hepatic, hematopoietic, renal, or metabolic dysfunction
Significant gastrointestinal disorder or surgery
Sitting blood pressure outside 100/60 to 135/90 mmHg or pulse 50 to 85 bpm
Barbiturates within 4 weeks or other drugs within 1 week
Alcohol or drug abuse within 1 year
Investigational drug use within 60 days

Blood samples for the determination of parent drug and metabolite levels were collected at baseline (pre-treatment), and at post-dose hours 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 18, 24, 36, 48, 60, 72, then at 24 hour intervals for days 5 to 10. The last dose was administered on day 11; subsequent sampling was at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 18, 24, 36, 48, 60, 72, 96, 120, 336, 504, and 672. Urine samples were collected over intervals -8 to 0 (prior to dosing), and post-dosing intervals 0 to 4, 4 to

8, 8 to 12, 12 to 24, 24 to 36, 36 to 48, 48 to 60, and 60 to 72. Twenty-four hour urine collections were made for study days 4 through 10. Then, following the last dose on day 11, collections were made for intervals 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 36, 36 to 48, 48 to 60, and 60 to 72 hours. Pharmacokinetic assays were assessed as shown in Table 220 below. The assays have been validated over the range of plasma and urine

Table 220. Pharmacokinetic assays in Study TR-2-CP.

Linearity	Accuracy			Precision			Sensitivity	Specificity
Plasma samples—trandolapril and trandolaprilat—								
Satisfactory.	Satisfactory.			Satisfactory.			LOQ - 0.078 ng/ml for trandolapril; 0.078 or 0.78 ng/ml for trandolaprilat, depending upon dilution	—
Standard curves from 0.039 to 10 ng/ml.	ng/ml	T' april	T' aprilat	ng/ml	T' april	T' aprilat		
	0.150	102.3%	101.5%	0.150	14.4%	7.5%		
	1.500	104.7%	100.1%	1.500	5.8%	7.0%		
	4.000	105.3%	109.8%	4.000	9.0%	12.8%		
Urine samples—trandolapril and trandolaprilat—								
Satisfactory.	Satisfactory.			Satisfactory.			LOQ - 7.8 ng/ml	—
Standard curves from 3.9 to 1000 ng/ml.	ng/ml	T' april	T' aprilat	ng/ml	T' april	T' aprilat		
	15	96.0%	94.7%	15	14.1%	11.5%		
	150	98.2%	96.2%	150	7.3%	7.2%		
	300	97.5%	96.6%	300	6.2%	9.3%		

concentrations observed in the study.

AUC, $\lambda_{(n)}$, C_{max} , $t_{1/2}$, and T_{max} were calculated.

28.5. Results

Demographic and baseline characteristics of subjects are shown in Table 221 below.

Table 221. Demographic and baseline characteristics of subjects in Study TR-2-CP.

	1 mg n=10	4 mg n=10	16 mg n=10
Male	10	10	10
Female	0	0	0
Caucasian	10	10	10
Other	0	0	0
Age mean range	29.6±5.6 21-39	29.4±4.9 22-35	24.5±4.0 21-33
Weight mean range	172±29 129-223	179±23 135-209	176±26 131-214

All subjects completed the study. All subjects' data were analyzed.

Sitting blood pressure and pulse data were analyzed for the first 24 hours post-dosing on days 1 and 11. Blood pressure reductions were generally dose-dependent.

Single-dose pharmacokinetic data are shown in Table 222 below. Plasma drug levels following the single dose are shown in Figure 103 below. Multiple-dose pharmacokinetic data are shown in Table 223 below. Plasma drug levels following multiple dosing are shown in Figure 104 below. Cumulative urinary excretion of

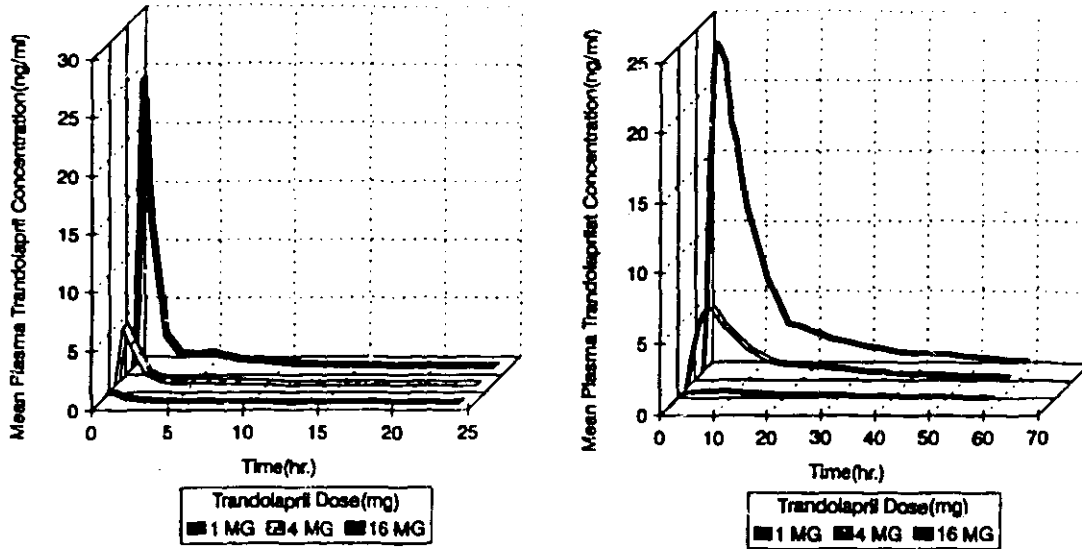


Figure 103. Plasma trandolapril and trandolaprilat levels following a single dose in Study TR-2-

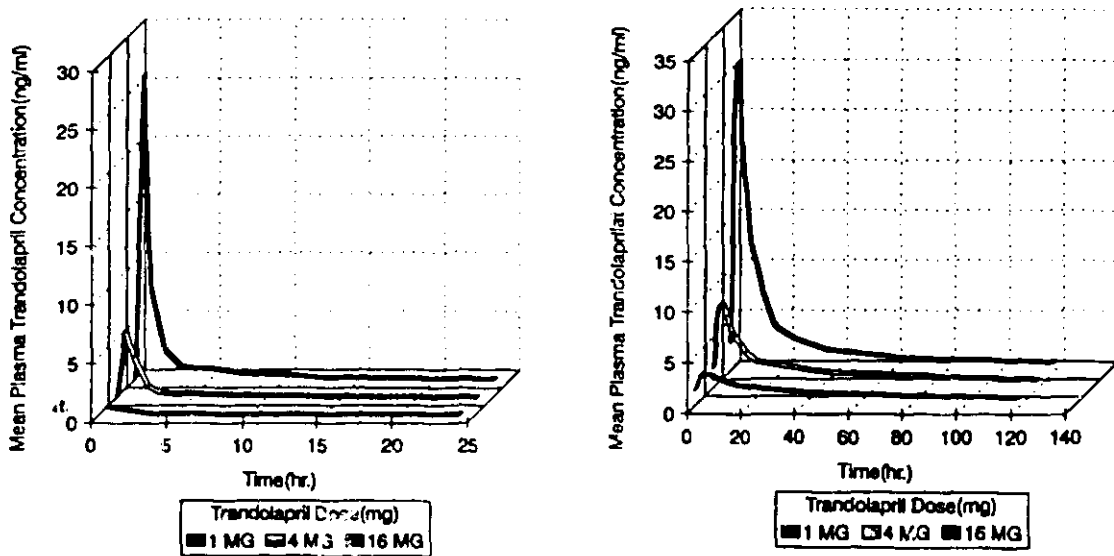


Figure 104. Plasma trandolapril and trandolaprilat levels following multiple dosing in Study TR-2-CP.

Table 222. Single-dose pharmacokinetic parameters in Study TR-2-CP.

	Trandolapril			Trandolaprilat		
	1 mg	4 mg	16 mg	1 mg	4 mg	16 mg
C _{max} (ng/ml)	1.07±0.63	6.11±3.14	26.2±14.6	1.22±0.34	5.97±2.41	25.7±9.81
T _{max} (h)	0.95±0.44	0.60±0.21	0.60±0.21	7.30±2.45	4.40±0.52	2.80±0.79
AUC ₀₋₂₄ (ng/ml-h)	1.65±0.87	7.67±3.80	30.7±16.3	22.8±6.13	72.0±24.1	214±58.6
AUC ₀₋₄₈	1.65±0.87	7.88±3.72	30.9±16.5	42.1±11.3	102±33.3	251±59.5
AUC ₀₋₇₂	1.65±0.87	7.88±3.72	30.2±16.9	58.8±15.9	124±41.6	270±65.5
AUC _{0-∞}	3.27±1.59 ^a	9.79±3.29	31.4±16.6	155±49.5	192±55.8	302±74.0
t _{1/2} (h)	7.56±3.98	6.62±6.54	3.92±2.00	98.1±24.7	47.4±24.3	22.1±7.46

Table 222. Single-dose pharmacokinetic parameters in Study TR-2-CP. (Continued)

	Trandolapril			Trandolaprilat		
	1 mg	4 mg	16 mg	1 mg	4 mg	16 mg
K_{el}	0.14±0.09	0.17±0.12	0.23±0.11	0.007±0.0002	0.019±0.011	0.036±0.014
K_a	—	—	—	0.49±0.34	0.53±0.41	0.50±0.12
Absorp $t_{1/2}$ (h)	—	—	—	1.80±0.80	1.84±0.93	1.45±0.30
CL (l/h)	384±214	546±501	634±296	7.10±2.33	22.7±7.86	55.7±12.6
U_{0-24} (ng)	0.24±0.49	4.79±3.10	26.1±19.9	10.4±14.7	358±90.5	1675±777
U_{0-48}	0.24±0.49	4.79±3.10	26.1±19.9	17.2±20.6	422±95.0	1802±794
U_{0-72}	0.24±0.49	4.79±3.10	26.1±19.9	22.2±19.8	448±96.7	1822±797

a. n=5

Table 223. Multiple-dose pharmacokinetic parameters in Study TR-2-CP.

	Trandolapril			Trandolaprilat		
	1 mg	4 mg	16 mg	1 mg	4 mg	16 mg
C_{max} (ng/ml)	0.81±0.36	6.18±4.31	26.5±15.8	3.81±1.54	9.00±3.16	32.8±11.5
C_{min}	—	16.1±33.4	42.8±70.2	1.69±0.32	2.25±0.62	2.95±0.96
C_{av} ^a	0.07±0.03	0.34±0.25	1.16±0.47	2.31±0.44	4.23±1.40	10.9±2.33
Acc ratio ^b	0.94±0.60	1.22±0.75	1.10±0.71	3.30±1.79	1.56±0.35	1.35±0.49
Fl ^c	13.1±5.22	19.1±5.41	21.6±8.11	0.97±0.88	1.60±0.32	2.66±0.60
T_{max} (h)	0.80±0.48	0.65±0.24	0.70±0.48	4.6±2.0	2.4±0.8	2.4±0.8
AUC_{0-24} (ng/ml·h)	1.56±0.68	8.18±6.00	27.9±11.2	55.3±10.6	101±33.6	262±56.0
AUC_{0-48}	1.56±0.68	8.28±6.16	28.2±11.6	89.7±16.8	145±43.5	315±54.9
AUC_{0-72}	1.56±0.68	8.31±6.13	28.2±11.6	117±22.2	177±52.0	346±55.2
$AUC_{0-\infty}$	2.46 ^a	12.3±6.03	29.2±11.5	569±230	351±162	507±102
$t_{1/2}$ (h)	1.10 ^a	8.42±5.94	8.18±5.81	311±109	115±116	76.0±42.1
t_{-el}	0.60 ^a	0.15±0.18	0.13±0.10	0.003±0.004	0.009±0.004	0.012±0.007
K_a	—	—	—	0.58±0.47	0.66±0.24	0.73±0.25
Absorp $t_{1/2}$ (h)	—	—	—	1.86±1.29	1.15±0.34	1.03±0.28
MRT (h)	2.97 ^d	5.88±4.20	4.53±3.77	424±153	153±174	73.2±43.2
CL (l/h)	759±305	751±525	664±266	18.7±3.33	46.8±27.6	63.6±13.2
U_{0-24} (ng)	0.088±0.25	10.3±9.35	18.2±11.4	141±37.0	670±138	2143±755
U_{0-48}	0.10±0.27	10.3±9.35	18.2±11.4	214±57.7	753±140	2328±719
U_{0-72}	0.12±0.29	10.3±9.35	18.2±11.4	203±26.7	780±140	2364±717

a. $C_{av} = AUC_{0-24}/24$ b. Accumulation ratio = $(C_{\infty})_{max}/(C_{n-1})_{max}$ c. Fluctuation index = $(C_{max} - C_{min})/C_{av}$

d. n=1

trandolapril after single and multiple doses is shown in Figure 105 below; similar data for trandolaprilat is shown in Figure 106 below.

A28.6. Safety.

Case report forms were not submitted for evaluation. Thirteen of 30 subjects reported adverse events. Events reported by more than one subject were headache (n=4), elevated SGPT (n=4), elevated SGOT (n=3), gastrointestinal upset (n=2), and viral syndrome (n=2). Twenty-two of 30 subjects had hypotension, defined as sitting systolic pressure <100 mmHg; none were reported as adverse events. One subject in the high-dose group reported being light-headed, the only associated symptom reported. Ten subjects had elevations in SGPT, two of which were >165 U/L. Five subjects had SGOT elevations, one of which was >150 U/L. All hepatic enzyme abnormalities were

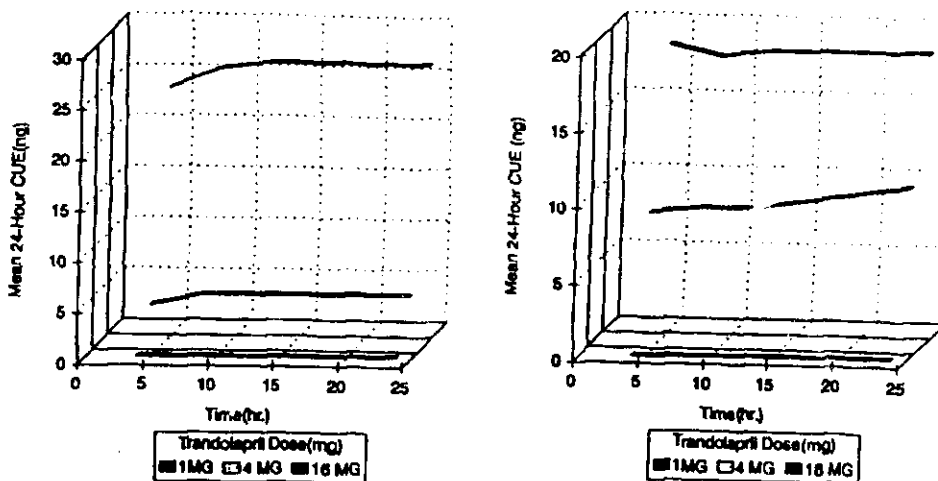


Figure 105. Cumulative urinary excretion of trandolapril following single and multiple doses in Study TR-2-CP.

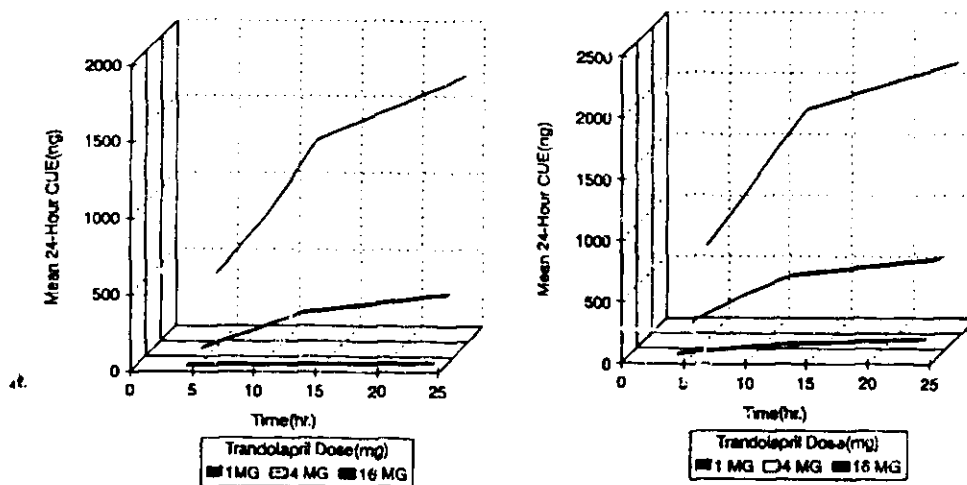


Figure 106. Cumulative urinary excretion of trandolaprilat following single and multiple doses in Study TR-2-CP.

detected at the scheduled day 14 evaluation; all returned to normal over a 4-week or less follow-up. A variety of other laboratory abnormalities were detected; none were clinically significant.

A28.7. Summary.

This parallel-group study in normal males evaluated pharmacokinetics following single and multiple doses of trandolapril 1, 4, and 16 mg. The disposition of trandolapril was nonlinear when administered as either single or multiple doses. Clearance for trandolapril increased with dose with single doses. Clearance for trandolaprilat increased with dose for both single and multiple doses. This increase in clearance resulted in a decreased $t_{1/2}$. Trandolaprilat accumulates with multiple dosing. There were numerous cases of mild to moderate increases in hepatic enzymes, not clearly dose-related; all of them resolved over a period of weeks following the end of treatment.

A29. Study TR-4-CP (Study report: vols 1.62-1.74; case report tabulations: none; case report forms for deaths and discontinuations for adverse events: none) "Determination of the bioequivalence of trandolapril tablets vs. capsules in healthy male subjects".

A29.1. Investigators The investigator was JC Kisicki, MD of Harris Laboratories, Lincoln, NE.

A29.2. Study dates March 1993 to July 1993.

A29.3. Drug formulation

Table 224. Drug substance used in Study TR-4-CP.

Lot	Form	Type	Trandolapril	Source lot	Batch size
TRA-0140A	Cap	Tran	1.0 mg	L-12	346,100
99-0391	Cap	Tran	4.0 mg	99-0391	85,963
131-0192	Tab	Tran	1.0 mg	131-0192	250,955
133-0192	Tab	Tran	4.0 mg	131-0192	140,361

Table 225. Tablet composition in Study TR-4-CP.

	1 mg	4 mg
Trandolapril	1.0	4.0
Iron oxide, yellow		
Iron oxide, red		
Corn starch		
Lactose, hydrous		
Hydroxypropyl methylcellulose		
Croscarmellose sodium		
Povidone		
Sodium stearyl fumarate		
Total		

Table 226. Capsule composition in Study TR-4-CP.

	1 mg	4 mg
Trandolapril	1.0	4.0
Corn starch		
Lactose, hydrous		
Povidone		
Sodium stearyl fumarate		
Total		

A29.4. Study design

This was an open-label, randomized, single-dose, crossover design to evaluate the bioequivalence of trandolapril tablets (potential future market formulations) vs. capsules. Following randomization, each subject received a single dose of either a 1 mg trandolapril tablet or capsule, or a 4 mg trandolapril tablet or capsule. Study drug administration was followed by a confined blood-sampling period of 72 hours (study days 1-3), then a 4-week out-of-house washout interval. On study day 28, subjects received the alternate trandolapril formulation at the same dose to which they were randomized. The 72-hour blood sampling period and 4-week washout was then repeated.

Subjects were to be healthy, normal, white adult volunteers who did not use tobacco products and were within 15% for height/weight range tables. Trandolapril was administered at 8:00 am in a fasted state.

Safety data collected included physical exam, blood pressure ("2-minute sitting"), heart rate (both determined at 0, 2, 4, 8, 12, and 24 hours post-dose), ECG, and a wide range of clinical laboratory tests performed on blood/urine.

Trandolapril and trandolaprilat levels were drawn at 0, 0.5, 1, 2, 3, 4, 6, 7, 8, 10, 12, 16, 18, 24, 36, 48, 60, 72, 96, and 120 hours post-dose and measured by RIA. Pharmacokinetic assays were assessed as shown in Table 227 below. The assays have

Table 227. Pharmacokinetic assays in Study TR-4-CP.

Linearity	Accuracy	Precision	Sensitivity	Specificity
Plasma samples—trandolapril and trandolaprilat—				
Satisfactory.	Satisfactory.		Satisfactory.	LOQ - 0.078 ng/ml.
Standard curves from 0.078 to 10.0 ng/ml.	ng/ml	T'april T'aprilat	ng/ml	T'april T'aprilat
	0.15	101.3% 100.7%	0.15	14.8% 7.1%
	1.50	104.4% 98.7%	1.50	6.0% 7.4%
	3.00	103.5% 102.0%	3.00	3.5% 8.1%

been validated over the range of plasma and urine concentrations observed in the study.

AUC₀₋₂₄, AUC_{0-t}, AUC_{0-∞}, C_{max}, t_{1/2}, and T_{max} were calculated.

Untransformed and log-transformed data will be employed as well as post-hoc power computations, two one-sided *t*-tests, and 90% confidence intervals.

A29.5. Results

Fifty-five healthy white male subjects enrolled in the trial, of whom 52 completed the final washout period. The demographic characteristics of the four groups are presented in Table 228 below and shows the groups to be relatively well-balanced.

a. Table 228. Demographic and baseline characteristics of subjects in Study TR-4-CP.

	1 mg		4 mg	
	tab⇒cap n=14	cap⇒tab n=14	tab⇒cap n=14	cap⇒tab n=13
Male	14	14	14	13
Female	0	0	0	0
Age±SD	25±6	23±5	25±6	28±6
Range	19 to 38	19 to 37	19 to 38	19 to 38
Caucasian	14	14	14	13
Black	0	0	0	0
Other	0	0	0	0
Weight±SD	179±18	178±17	165±17	170±25
Range	137 to 202	145 to 205	144 to 194	123 to 203

No subjects were excluded from efficacy or safety analyses because of protocol violations or lack of compliance.

Pharmacokinetic parameters are shown in Table 229 below. Trandolapril and trandolaprilat levels are shown in Figure 107 below.

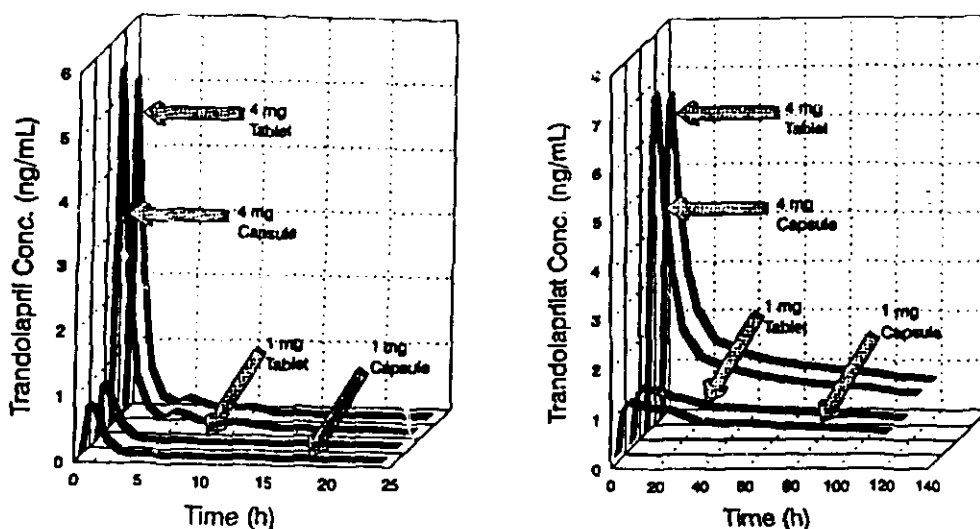


Figure 107. Plasma trandolapril and trandolaprilat levels in Study TR-4-CP.

Table 229. Pharmacokinetic parameters in Study TR-4-CP.

	Trandolapril			Trandolaprilat		
	Tablet	Capsule	90% CI	Tablet	Capsule	90% CI
1 mg						
AUC ₀₋₂₄ (h-ng/ml)	1.49 (0.93)	1.35 (0.80)	94-125	22.2 (6.71)	21.8 (7.31)	99-107
AUC _{0-∞} (h-ng/ml)	1.65 (0.83)	1.57 (0.99)	90-124	238 (161)	233 (138)	80-113
AUC _{0-t} (h-ng/ml)	1.49 (0.93)	1.35 (0.80)	94-125	82.0 (27.9)	79.9 (21.7)	97-104
C _{max} (ng/ml)	1.06 (0.56)	0.99 (0.52)	87-129	1.19 (0.34)	1.31 (0.58)	86-104
T _{max} (h)	0.70 (0.25)	0.83 (0.34)	—	10.4 (6.67)	9.52 (7.17)	—
t _{1/2} (h)	0.69 (0.19)	0.57 (0.08)	—	208 (220)	217 (170)	—
4 mg						
AUC ₀₋₂₄ (h-ng/ml)	6.78 (3.07)	7.15 (2.79)	84-102	78.8 (17.1)	80.0 (17.5)	96-102
AUC _{0-∞} (h-ng/ml)	9.08 (4.18)	9.57 (3.10)	79-104	345 (92.8)	364 (97.9)	87-104
AUC _{0-t} (h-ng/ml)	6.96 (3.65)	7.15 (2.80)	84-104	175 (37.6)	178 (29.3)	97-102
C _{max} (ng/ml)	5.95 (3.81)	8.17 (2.48)	76-108	6.80 (2.38)	7.52 (2.85)	87-97
T _{max} (h)	10.4 (6.67)	9.52 (7.17)	—	3.96 (0.94)	4.00 (1.49)	—
t _{1/2} (h)	3.96 (2.11)	3.62 (0.76)	—	147 (37.9)	162 (53.7)	—

A29.6. Safety.

Twenty-four of 55 subjects reported at least one adverse reaction. These events are summarized in Table 230 below. There did not appear to be a relationship between adverse events and dose. Most of the adverse reactions were considered mild according to the investigator. Two adverse events were rated severe by the investigator. One incident was a severe headache (3 days after the 1 mg dose, accompanied by chills, all lasting 3 days), the other was an ankle sprain (see below) that resulted in discontinuation.

No deaths were reported for any randomized subject. Of the three subjects who discontinued, one did so due to a construction accident, and another due to an ankle sprain as a result of a motor vehicle accident. The third admitted himself into an alcohol detoxification program. There appears to be no association between drug administration and these events.

Table 230. Adverse events (n≥2; %) by body system in Study TR-4-CP.

	1 mg		4 mg			1 mg		4 mg	
	tab n=27	cap n=28	tab n=27	cap n=27		tab n=27	cap n=28	tab n=27	cap n=27
Any	8 (30)	11 (39)	6 (22)	5 (19)	Headache	5 (19)	7 (25)	1 (4)	1 (4)
CNS	6 (22)	9 (32)	2 (7)	1 (4)	Lightheadedness	4 (15)	1 (4)	0	0
General body	2 (7)	3 (11)	0	1 (4)	Fatigue	2 (7)	0	0	1 (4)
Cardiovascular	1 (4)	1 (4)	0	2 (7)	Elevated SGPT	0	0	2 (7)	1 (4)
Metabolic/endocrine	0	0	2 (7)	2 (7)					
Gastrointestinal	0	2(7)	0	0					

Pulse rate changes were minimal and did not appear to differ between treatment groups or from baseline. Three subjects experienced symptomatic hypotensive episodes (all in the 1 mg group) one of which was accompanied by a 2-minute syncopal event. The following subjects experienced systolic blood pressures <100 mmHg: for the 1 mg group 15 subjects (54%) had 26 incidents; for the 4 mg group, 21 subjects (78%) had 77 incidents. Regarding subjects with a sitting blood pressure <90/60 with at least a 10 mmHg decrease from baseline in either diastolic or systolic pressures: for the 1 mg group, 7 (25%) subjects; for the 4 mg group, 15 (56%) subjects. Although not statistically analyzed, the 4 mg group did appear to have more of a problem with hypotension (as defined by the investigator). Orthostatic blood pressure data was not gathered by the investigator.

Although there were a variety of new finding on ECG, physical exam, and laboratory testing, none appeared serious or to be obviously related to the study drug. The vast majority of new ECG findings were mild episodes of sinus bradycardia (<50 bpm) in 11-32% of subjects (the highest percent seen with the 1 mg dose). The most common blood chemistry abnormalities were increases in triglyceride and cholesterol; decreased BUN/creatinine ratio; and increased or decreased inorganic phosphorous.

Three subjects, all in the 4 mg group, developed liver function test elevations that will be mentioned:

Subject 3, a 29 year old Caucasian male, had elevations of 21% in SGPT and 48% in LDH. All subsequent tests were normal.

Subject 34, a 22 year old Caucasian male, had an elevation of SGPT of 31%. All subsequent tests were normal.

Subject 51, a 31 year old Caucasian male, experienced gradually increasing levels of SGPT and SGOT: SGPT went from 49 U/L to 63 U/L to 83 U/L on the last day of the study; SGOT rose from 30 U/L to 39 U/L to 116 U/L on the last day of the study. The investigator stated that these elevations may have been caused by possible recent ethanol ingestion. Further follow-up was not done.

A29.7. Summary.

Fifty-five healthy white males were randomized to either a 1 mg or 4 mg arm to compare the bioequivalence of tablet versus capsule formulations. Trandolapril capsules (1 and 4 mg) used for clinical studies and the to-be-marketed tablet formulations are bioequivalent with respect to trandolaprilat, but bioinequivalent with respect to trandolapril. There were no serious events in the trials that could be directly associated with trandolapril. Adverse events with possible association with trandolapril include headache, lightheadedness, tiredness, bradycardia, and elevated SGPT

A30. Study TR-20-HTN (Study report: vols 1.138-1.154; case report tabulations: vols 1.284-1.298A; case report forms for deaths and discontinuations for adverse events: vols 1.377-1.378) "Double-blind, randomized, placebo-controlled, six-way parallel dose-ranging study to evaluate the safety and efficacy of oral trandolapril therapy (0.25, 0.5, 1, 2, and 4 mg/day) in White patients with mild to moderate essential hypertension".

A30.1. Investigators The investigators are listed in Table 231 below.

Table 231. Investigators in Study TR-20-HTN.

Investigator	Center	City	Investigator	Center	City
JW Allen, MD	2013	Los Angeles, CA	W Pleskow, MD	2008	Encinitas, CA
P Barden, MD	2002	Albuquerque, NM	R Reeves, MD	2006	Olympia, WA
JR Benz, MD	2010	Cedar Rapids, IA	S Savran, MD	2015	Las Vegas, NV
R Goldstein, MD	2011	Tampa, FL	D Shaw, MD	2009	San Diego, CA
J Gray, MD	2004	Houston, TX	H Silberman, MD	2007	Coral Gables, FL
D Kelley, MD	2014	Olympia, WA	LK Smith, MD	2012	Phoenix, AZ
JH Levine, MD	2005	Nashville, TN	WJ Stein, MD	2003	Rochester, NY
R Paster, MD	2001	Oregon, WI			

A30.2. Study dates October 1989 to October 1990.

A30.3. Drug formulation

Table 232. Drug substance used in Study TR-20-HTN.

Lot	Form	Type	Trandolapril	Source lot	Batch size
TRA-580	Cap	Plcbo	—	L-32	1,200,000
L-21	Cap	Plcbo	—	L-21	1,200,000
TRA-0150A	Cap	Plcbo	—	L-21	1,200,000
JMP-22928-23	Cap	Plcbo	—	JMP-22928-23	40,000
MB22633-172	Cap	Tran	0.25 mg	MB22633-172	20,000
MB22633-145	Cap	Tran	0.5 mg	MB22633-145	20,000
L-12	Cap	Tran	1.0 mg	L-12	546,100
MB22327-163	Cap	Tran	2.0 mg	MB22327-163	600,000

Table 233. Capsule composition in Study TR-20-HTN.

	0.25 mg	0.5 mg	1 mg	2 mg
Trandolapril				
Corn starch				
Lactose, hydrous				
Povidone				
Sodium stearyl fumarate				
Total				

A30.4. Study design

There was a 4-week single-blind, placebo lead-in phase, followed by a 6-week double blind treatment period, and a 3-week single-blind, placebo washout period. Fifty subjects per treatment group were planned. Subjects were to be age ≥ 21 years, $< 30\%$ above ideal body weight, with supine diastolic pressure between 95 and 114 mmHg at the end of the placebo run-in phase. Women were permitted if neither childbearing nor lactating, and if practicing recognized forms of contraception. Exclusion criteria are

listed in Table 234 below. Other excluded concomitant drugs were antiarrhythmics,

Table 234. Exclusion criteria in Study TR-20-HTN.

Hypertension other than essential and mild-to-moderate
History of evidence of malignant, accelerated, or severe hypertension
Cerebrovascular accident, convulsions, or hypertensive encephalopathy within 1 year
Clinically significant congestive heart failure or EF<40%
Use of antihypertensives within 4 weeks of double-blind period or spironolactone within 6 weeks
Heart rate <50 bpm, significant arrhythmia, sinus node dysfunction, or conduction abnormalities
Myocardial infarction within 3 months
Hypersensitivity or serious adverse reaction to any ACE inhibitor
Clinically significant renal failure, e.g., creatinine >2 mg/dl
Clinically significant hepatic failure, e.g., SGOT or SGPT >2 times ULN
Significant electrolyte imbalance
Significant hematologic abnormalities
Medical or surgical conditions affecting absorption, metabolism, or excretion
Previous clinical trial experience within 30 days or any previous exposure to trandolapril
History of chronic alcohol or drug abuse within 2 years
Terminal illness
Blood donation within 1 month

digitalis, MAO inhibitors, psychotropic drugs, chronic NSAIDs, phenylpropanolamine, and pseudoephedrine.

Subjects who qualified at the end of the placebo run-in period were randomized to placebo or trandolapril 0.25, 0.5, 1, 2, and 4 mg q.d. and returned after 1, 2, 4, and 6 weeks. All subjects took the same number of capsules. Subjects were instructed to take their study drug at 0900±1 hour, but the relationship to meals was not specified.

Safety data included medical history, physical exam, 12-lead ECG, and routine hematology, clinical chemistry, and urinalysis. There were no explicit criteria for dropping a subject because of lack of efficacy.

Blood samples were obtained for ACE activity, aldosterone, and renin activity levels at baseline and at the final double-blind visit. Trandolapril and trandolaprilat levels were obtained at two double-blind treatment period visits.

The primary analysis was an intent-to-treat analysis of supine diastolic pressure at the final double-blind visit. Secondary end points were sitting and standing diastolic pressure and percentage of responders (no criteria pre-specified). With the planned 50 subjects per arm, the study had 80% power to detect a 5 mmHg change from baseline with two-sided $\alpha=0.05$.

There were three amendments to the protocol, adding the 4 mg treatment group, changing the principal end point from sitting to supine diastolic pressure, calling for an interim analysis.

A30.5. Results

Three hundred thirteen subjects enrolled in the trial, of whom 287 completed the final washout period. Individual study centers enrolled 8 to 32 subjects. Demographic and baseline characteristics of subjects are shown in Table 235 below. The treatment groups were reasonably well matched for most factors, including general health, duration and severity of hypertension, and baseline drug use, but there were substantial

Table 235. Demographic and baseline characteristics of subjects in Study TR-20-HTN.

	Placebo n=50	Trandolapril				
		0.25 mg n=53	0.5 mg n=53	1 mg n=51	2 mg n=53	4 mg n=53
Male	35	35	36	33	36	28
Female	15	18	17	18	17	25
Age $\pm$ SD	61 $\pm$ 10	55 $\pm$ 11	53 $\pm$ 12	58 $\pm$ 12	57 $\pm$ 11	53 $\pm$ 12
Range	36 to 78	29 to 74	25 to 82	27 to 78	38 to 84	28 to 79
Caucasian	48	51	52	51	52	53
Black	0	0	0	0	0	0
Other ^a	2	2	1	0	1	0
Weight $\pm$ SD	85 $\pm$ 14	83 $\pm$ 14	84 $\pm$ 16	83 $\pm$ 17	86 $\pm$ 13	79 $\pm$ 15
Range	50 to 117	54 to 116	44 to 116	51 to 118	48 to 112	53 to 116

a. Total of 5 Orientals and 1 American Indian.

differences in age distribution among treatment groups. The mean age for the placebo group was significantly larger than that for the dosing groups.

The disposition of subjects is shown in Table 236 below.

Table 236. Disposition of subjects in Study TR-20-HTN.

	Randomized	Double-blind				Wash-out	
		Week 1	Week 2	Week 4	Week 6	Week 1	Week 3
Remaining in study	313	309	302	300	292	290	287
Dropouts		4	7	2	8	2	3
Adverse reaction		1	0	0	3	1	2
Lack of efficacy		1	4	1	4	1	0
Not meet entry criteria		1	—	—	—	—	—
Compliance		1	2	0	0	0	1
Other		0	1 ^a	1 ^b	1 ^c	0	0

a. Administrative problems

b. Death

c. Injury requiring surgery

No subjects were excluded from efficacy or safety analyses because of protocol violations. Violations of entry criteria included subjects >30% above ideal weight (n=13), conduction abnormalities (n=17), and failure to meet blood pressure criteria (n=14). Three subjects received treatments other than the ones to which they were randomized.

The sponsor's analysis of change in supine diastolic pressure at trough (change from baseline and placebo) as a function of time in trial is shown in Figure 108 below.

An intent-to-treat approach was used to analyze the efficacy variables. All subjects with data for at least one post-baseline visit were included and the last measurements for dropouts were carried forward in the analyses.

According to the sponsor, the supine diastolic blood pressure was analyzed using analysis of covariance (ANCOVA) with treatment and center as factors and baseline supine diastolic blood pressure as a covariate. Significant treatment difference was found based on this ANCOVA model ($p < 0.001$). Estimated mean supine diastolic blood pressure for each treatment group is given in Table 237 below.

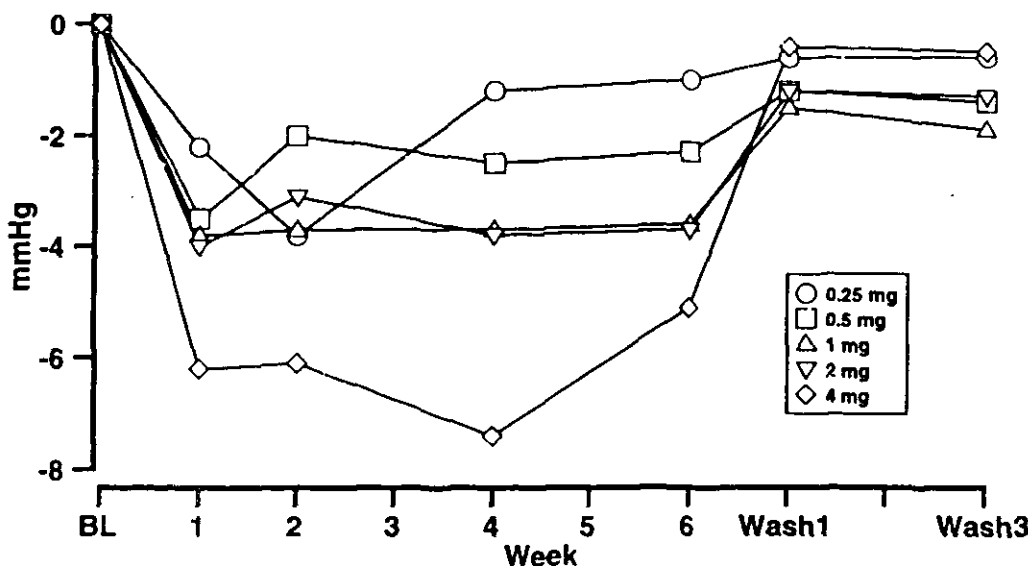


Figure 108. Change in supine diastolic pressure at trough by time in Study TR-20-HTN.

Table 237. Diastolic pressure data in Study TR-20-HTN^a.

	Plcbo n=49	Trandolapril				
		0.25 mg n=53	0.5 mg n=53	1 mg n=51	2 mg n=53	4 mg n=53
Mean supine diastolic pressure at trough and end point						
Baseline	100.0	99.7	99.8	100.2	100.2	99.4
End point	96.2	95.1	93.7	92.2	91.8	90.2
Change from baseline and placebo	—	-1.1	-2.5	-4.0	-4.4	-5.4
p-value		NS	NS	<0.01	<0.01	<0.01
Mean supine diastolic pressure at peak and end point						
Baseline	96.3	96.8	97.1	97.0	96.7	96.7
End point	92.5	91.3	88.3	88.6	87.5	84.5
Change from baseline and placebo	—	-1.2	-4.2	-3.9	-5.0	-8.0
p-value		NS	<0.01	<0.05	<0.01	<0.01
Mean sitting diastolic pressure at trough and end point						
Baseline	100.9	99.9	100.4	100.3	101.7	99.9
End point	97.7	97.1	97.2	95.0	93.2	92.2
Change from baseline and placebo	—	-0.6	-0.5	-2.7	-4.5	-5.5
p-value		NS	NS	<0.05	<0.01	<0.01

a. Statistical reviewer's analysis, with adjustment for baseline and investigator.

The sponsor's analysis of blood pressure considered 'trough' as any measurement at least 20 hours after dosing; 'peak' was considered 4 to 5 hours after dosing. Few trough measurements were actually outside the interval 24±2 hours, but this was the interval used in the analyses performed for this review.

No treatment-by-center or treatment-by-baseline interactions were found. Paired comparisons of adjusted mean supine diastolic blood pressures between each trandolapril group and the placebo group showed a significant decrease in supine diastolic blood pressure from baseline for the 1, 2, and 4 mg groups ($p=0.003$ for 1 mg,

$p=0.001$ for 2 mg, and $p<0.001$ for 4 mg, Table 237). Significant decreases in diastolic blood pressure from baseline/trough at end point were observed for all treatment groups.

ANCOVA (analysis of covariance) was used to analyze supine diastolic blood pressure at peak at end point. No treatment-by-center interaction was found here. However, there was a significant treatment-by-baseline interaction ($p=0.048$). In this case, the treatment difference was marginally significant ($p=0.067$), but the estimates of mean blood pressure were not much different from those based on the ANCOVA model without the interaction term. By regression of change in supine diastolic blood pressure from baseline at peak on the baseline, the estimated slopes for the placebo, 0.25, 1, 2, and 4 mg groups were -0.45, -0.20, -0.41, -0.72, and -0.85 indicating a larger decrease in supine diastolic pressure from baseline for a more hypertensive subject. Among these groups, the treatment effect was statistically significant ($p<0.001$) and no interactions were found. A reversed trend was found for the 0.5 mg group (slope=0.18). The estimated mean decrease for this group was larger than that for the placebo group (6.1 mmHg vs. 3.6 mmHg; $p=0.078$). No apparent difference in baseline characteristics between the 0.5 mg group and the other groups was found. Since the interaction does not appear crucial, the ANCOVA model with only baseline and center as terms was used to get the estimates of mean supine diastolic pressure (Table 237). In this case, p -value for testing treatment difference among the groups was less than 0.001.

Analyses of sitting and standing diastolic pressure were done using ANCOVA models. Significant treatment difference was found for trough standing diastolic pressure at end point ($p=0.001$) and there were no treatment-by-center or treatment-by-baseline interactions. For trough sitting diastolic pressure at end point, there was a significant treatment-by-center interaction ($p=0.045$) and mean supine diastolic pressure measurements averaging across the centers were significantly different among the treatment groups ($p<0.001$). The estimates of mean sitting diastolic blood pressure with the presence of the interaction are given in Table 237.

Based on the estimates of mean diastolic pressure at trough and peak (ANCOVA model without interaction terms), the trough-to-peak ratios were obtained as shown in Table 238 below.

Table 238. Supine diastolic pressure peak/trough ratio in Study TR-20-HTN^a.

Trandolapril				
0.25 mg n=53	0.5 mg n=53	1 mg n=51	2 mg n=53	4 mg n=53
0.79	0.61	0.93	0.87	0.65

a. Statistical reviewer's analysis.

The sponsor reasonably, but retrospectively, defined responders as subjects with supine diastolic pressure <90 mmHg or a ≥ 10 mmHg reduction at end point. The analysis of responders at end point using χ^2 test showed a significant difference in proportion of responders among the treatment groups ($p=0.020$). The proportion of responders for the 4 mg group was significantly higher than that in the placebo group (62% vs. 35%, $p=0.005$).

Quadratic regression on dose was done by the statistical reviewer. In the model, the dependent variable was change in supine diastolic pressure at trough at end point and the regression terms included the quadratic forms of dose and baseline. The estimated

response curve was

$$\Delta SupDP = -4.401 - 3.2633.187 \times Dose + 0.522 \times Dose^2$$

where the estimated coefficients for the linear and quadratic dose terms were significantly or marginally significantly different from zero ($p=0.003$ for dose and $p=0.043$ for dose²) indicating a quadratic dose-response relationship.

The duration of treatment effect after 6 weeks of dosing was determined by comparing trough blood pressure measurements obtained at washout weeks 1 and 3 to those at baseline and at the last double-blind visit (week 6). Significant decreases in supine diastolic pressure from baseline could still be seen for all treatment groups at washout week 1 and the dosing groups at washout week 3, as shown in Table 239 below.

Table 239. Duration of treatment effect in Study TR-20-HTN^a.

	Plcbo n=49	Trandolapril					
		0.25 mg n=53	0.5 mg n=53	1 mg n=51	2 mg n=53	4 mg n=53	
Mean change from baseline							
Was ¹	k 1	-4.2†	-4.8†	-5.4†	-5.7†	-5.4†	-4.6†
Wash.	.3	-1.3	-1.9*	-2.7†	-3.2†	-2.6†	-1.8*
Mean change from end of double-blind treatment period							
Washout week 1		0.1	0.5	1.2	2.2*	2.9†	4.8†
Washout week 3		3.0†	3.4†	4.1†	4.5†	5.6†	7.6†

^a Statistical reviewer's analysis. *Significantly different, $p < 0.05$.

†Significantly different, $p < 0.01$.

However, no difference in such decrease was found among treatment groups. Significant increases in supine diastolic pressure from those at week 6 were found for all treatment groups at washout week 3 and for the three higher dosing groups at washout week 1. Significant difference in such increase was found among the treatment groups at washout weeks 1 and 3 (ANOVA, Table 237).

Changes in sitting and standing diastolic pressure are shown in Table 240 below.

Table 240. Changes in trough sitting and standing diastolic pressure in Study TR-20-HTN^a.

	Sitting					Standing				
	0.25 mg n=53	0.5 mg n=53	1 mg n=51	2 mg n=53	4 mg n=53	0.25 mg n=53	0.5 mg n=53	1 mg n=51	2 mg n=53	4 mg n=53
Baseline	99.9	100.4	100.3	101.7	99.9	100.7	101.3	100.3	100.6	99.1
Week 1	-2.0	-2.1	-2.6	-4.4	-4.8	-3.4	-2.6	-3.4	-3.5	-4.7
Week 2	-2.5	-2.1	-2.5	-4.5	-5.9	-3.4	-2.7	-3.4	-2.8	-4.9
Week 4	-1.1	-1.2	-3.5	-4.7	-7.1	-1.4	-2.5	-3.3	-3.3	-6.4
Week 6	-0.8	-0.4	-2.4	-4.2	-5.3	-2.3	-1.4	-2.5	-4.3	-4.6
End point	-1.1	-0.7	-3.0	-5.0	-5.8	-2.4	-1.5	-3.0	-4.7	-4.5
Washout 1	0.3	0.3	-0.2	-1.6	-0.9	-0.9	-1.7	-0.8	-1.8	-1.1
Washout 3	1.3	-0.3	-1.3	-1.4	0.0	-0.6	-1.1	-0.7	-1.9	0.2

a. The sponsor's analysis, with adjustment for baseline and investigator.

Changes in sitting and standing diastolic pressure were somewhat smaller than changes in supine diastolic pressure, but there is pretty clear dose-dependence. Changes in systolic pressure (double-differences from baseline and placebo) are summarized in Table 241 below. Here, dose-ordering is not apparent.

Table 241. Changes in trough systolic pressure in Study TR-20-HTN^a.

	0.25 mg n=53	0.5 mg n=53	1 mg n=51	2 mg n=53	4 mg n=53
Supine	-3.5	-4.7	-9.1	-6.7	-9.1
Sitting	-3.4	-4.0	-10.0	-8.4	-8.7
Standing	-5.0	-4.8	-9.6	-8.8	-10.3

a. The sponsor's analysis, with adjustment for baseline and investigator.

Pulse rate changes from baseline to end point were not statistically significant for any treatment group.

Pharmacokinetic data were obtained only once at trough and 4 to 5 hours after dosing. Trandolapril and trandolaprilat are both present at low levels at trough. Post-dosing, trandolaprilat levels are some 9- to 10-fold higher than trandolapril levels.

Renin, aldosterone, and ACE activity are shown as changes from baseline to the end of the double-blind treatment period in Table 242 below. Renin levels increased in a

Table 242. Changes in renin, aldosterone, and ACE activity in Study TR-20-HTN.

	Plebo n=39	Trandolapril				
		0.25 mg n=46	0.5 mg n=49	1 mg n=46	2 mg n=47	4 mg n=44
Renin (ng/ml/h)	0.1	0.9	1.2	1.2	1.7	2.7
Aldosterone (ng/dl)	-0.1	1.1	1.5	1.0	0.6	-0.6
ACE activity (U/L)	-5.0	-29.2	-30.8	-30.5	-30.1	-30.9

dose-dependent manner. Aldosterone levels were not clearly affected. ACE inhibition was comparable in all actively treated groups, and clearly different from placebo.

A30.6. Safety.

Ninety-one of 263 actively treated subjects (35%) reported at least one adverse reaction, compared with 40% of placebo subjects. These adverse events are summarized in Table 243 below. Counts of events were too low to discern any

Table 243. Adverse events (%) in Study TR-20-HTN.

	Placebo n=50	Tran n=263	Events reported by ≥2 Trandolapril subjects			
Any	20 (40)	91 (35)	Bradycardia	2	Fatigue	4
Cardiovascular	3 (6)	13 (4.9)	Flushing	2	Trauma	3
CNS	9 (18)	25 (10)	Premat atrial beats	2	Back pain	6
Dermatologic	0	5 (1.9)	Headaches	19	Joint pain	2
EENT	0	5 (1.9)	Dizziness	4	Muscle pain	2
Emotional	0	2 (0.8)	Rash	2	Cough	4
Gastrointestinal	2 (4)	20 (7.6)	Anxiety	2		
General	1 (2)	10 (3.8)	URI symptoms ^a	28		
Genitourinary	2 (4)	3 (1.1)	Diarrhea	6		
Hematopoietic	0	1 (0.4)	Abdominal pain	3		
Metab/endoc	2 (4)	1 (0.4)	Constipation	2		
Musculoskeletal	5 (10)	13 (4.9)	Gastroenteritis	2		
Pulmonary	2 (4)	7 (2.7)	Nausea	4		

a. Sum of upper respiratory tract congestion, infection, and inflammation.

relationship to dose. Subjects who discontinued because of adverse reactions are listed in Table 244 below.

Table 244. Discontinuations for adverse events in Study TR-20-HTN.

Center	Subj	Group	Day	ADR
2002	7	Plcbo	15	59 year old Caucasian female with 19-year history of hypertension. Discontinued because of headaches, flushed feeling, and lack of efficacy
2004	7	Plcbo	42	52 year old Caucasian male with 20-month (year?) history of hypertension. Discontinued because of ECG changes indicative of myocardial ischemia. Blood pressure was not well-controlled. Subsequent cardiac catheterization revealed no significant coronary artery lesions
2011	17	Plcbo	35	37 year old Caucasian male with 3-month history of hypertension. Discontinued because of heel injury requiring surgery.
2013	11	Plcbo	7	76 year old Caucasian male with 25-year history of hypertension. Discontinued because of headache and lack of efficacy.
2015	1	Plcbo	29	66 year old Caucasian female; discontinued for elevated hepatic enzymes
2010	11	0.25 mg	46	55 year old Caucasian male with a 15-year history of hypertension. Discontinued because of hives over both forearms and edema of the face and lips. Hives began 1 week earlier, and all symptoms resolved after treatment with benadryl and prednisone, at 2 weeks follow-up
2005	15	1 mg	50	67 year old Caucasian male with 5-month history of hypertension. Discontinued because of migraine headaches. Blood pressure was not well-controlled. (The sponsor's narrative description of this case says he was discontinued during run-in, because of headaches.)
2008	14	1 mg	42	51 year old Caucasian male with 10-year history of hypertension. Discontinued because of myocardial infarction. Blood pressure was not well-controlled
2008	4	2 mg	2	67 year old Caucasian male with 12-year history of hypertension. Discontinued because of atrial flutter, described as severe, serious, and possibly drug-related
2012	8	2 mg	11	68 year old Caucasian female with 5-year history of hypertension. Discontinued because of blurred vision and lack of efficacy
2014	4	2 mg	28	83 year old Caucasian male with a 17-year history of hypertension and COPD. Discontinued with falling platelet count (69/nl at baseline to 57/nl), attributed to hypersplenism
2013	8	4 mg	20	59 year old Caucasian female with 5-year history of hypertension. At day 17, she complained of neck, arm, and chest pain, thought related to known neck problem, but was referred to cardiologist. ECG was WNL and unchanged from baseline. CPK was WNL. Found dead at home 2 days later

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Forty-four subjects were deemed to have had orthostatic hypotension, defined as a >20 mmHg reduction in systolic pressure in moving from a supine to standing position; the incidence of orthostatic hypotension was 14% on placebo, 21% on 0.25 mg, 13% on 0.5 mg, 14% on 1 mg, and 11% on 2 and 4 mg. The incidence was 13% during the washout phase.

There were a variety of new findings on the final physical exam and ECG; none were serious or had apparent relationship to the study drug.

Laboratory data are summarized in Figure 109 to Figure 112 below.

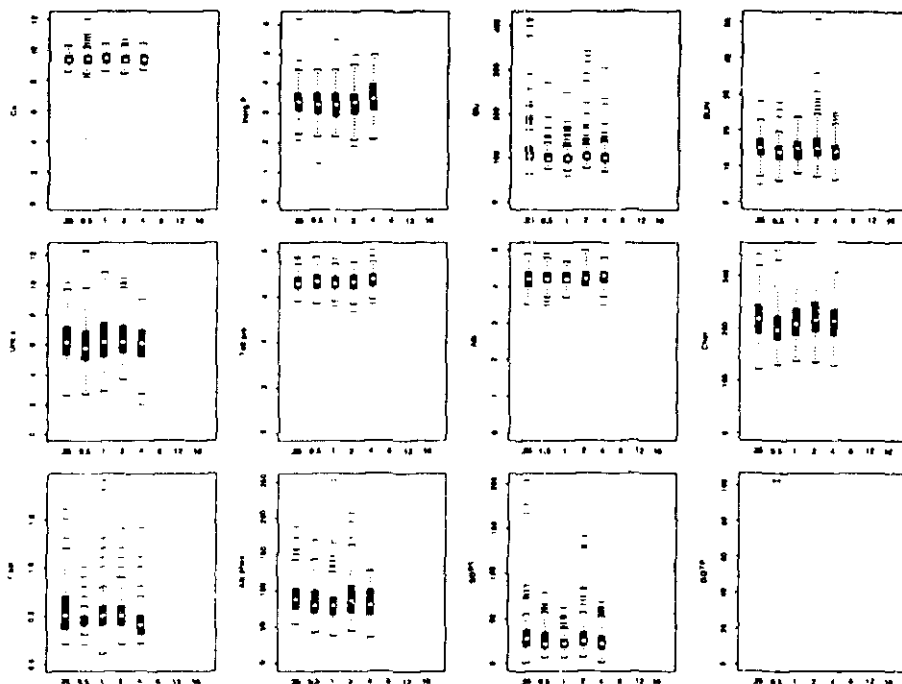


Figure 109. Clinical chemistry data (part A) in Study TR-20-HTN.

A30.7. Summary.

Caucasian subjects with mild-to-moderate hypertension were randomized to placebo or fixed doses of trandolapril 0.25, 0.5, 1, 2, or 4 mg q.d. for 6 weeks, followed by a 3-week single-blind washout period. Statistically significant differences between placebo and the active treatment groups in the primary end point—supine diastolic pressure—appeared at 1 week and were maintained to the final double-blind visit. Dose-dependence is evident in supine diastolic pressure. Consistent and statistically significant differences between placebo and active treatment groups were also found for secondary end points of sitting and standing diastolic pressure, systolic pressure, and response rate. There was one sudden death in a subject on 4 mg.

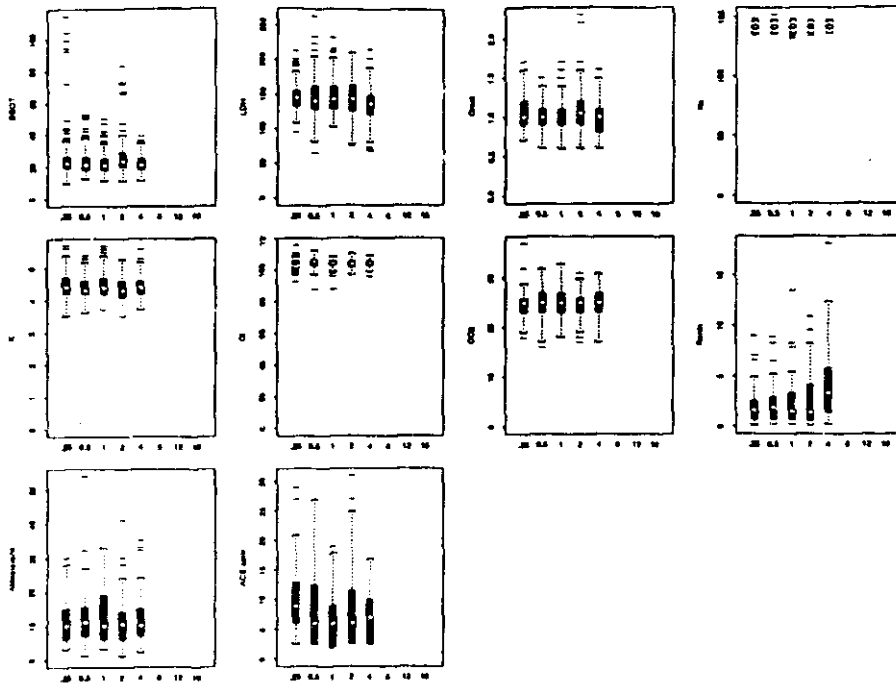


Figure 110. Clinical chemistry (part B) in Study TR-20-HTN.

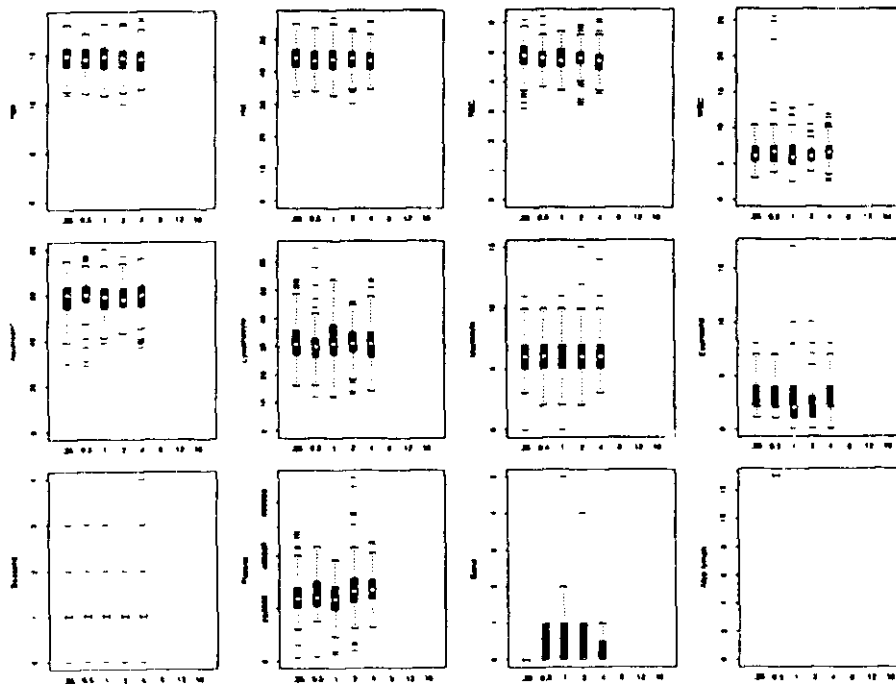


Figure 111. Hematology data in Study TR-20-HTN.

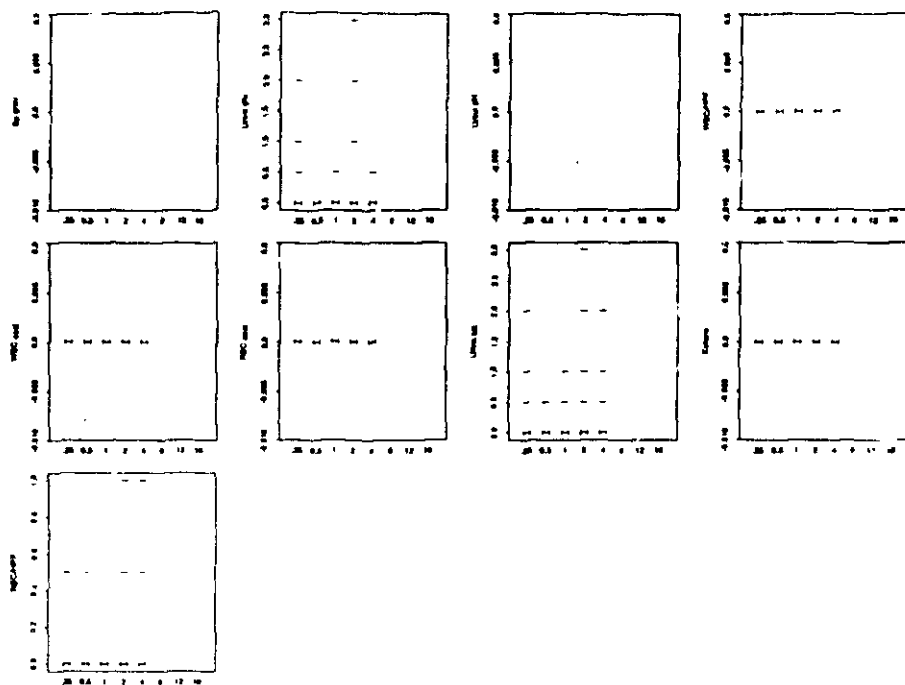


Figure 112. Urinalysis data in Study TR-20-HTN.

A31. Study TR-20A-HTN (Study report: vols 1.164-1.180; case report tabulations: vols 1.305-1.315; case report forms for deaths and discontinuations for adverse events: vol 1.380) "Double-blind, randomized, placebo-controlled, five-way parallel dose-ranging study to evaluate the safety and efficacy of oral trandolapril therapy (4, 8, 12, and 16 mg/day) in White patients with mild to moderate essential hypertension".

A31.1. Investigators The investigators are listed in Table 245 below.

Table 245. Investigators in Study TR-20A-HTN.

Investigator	Center	City	Investigator	Center	City
JW Allen, MD	2021	Los Angeles, CA	W Kirkpatrick, MD	2029	Mobile, AL
N Cutler, MD	2023	Beverly Hills, CA	WJ Mitchell, MD	2026	Albuquerque, NM
GW Dennish, MD	2021	Encinitas, CA	R Paster, MD	2030	Oregon, WI
LI Gilderman, DO	2024	Pembroke Pines, FL	HM Serfer, DO	2027	Hollywood, FL
PM Kaihlanen, MD	2025	San Antonio, TX	J Soler, MD	2031	Seffner, FL

A31.2. Study dates February 1991 to December 1991.

A31.3. Drug formulation:

Table 246. Drug substance used in Study TR-20A-HTN.

Lot	Form	Type	Trandolapril	Source lot	Batch size
TRA-580	Cap	Plcbo	—	L-32	1,200,000
TRA-0210	Cap	Plcbo	—	L-21	1,200,000
99-0190	Cap	Tran	4.0 mg	99-0190	31,595
99-0290	Cap	Tran	4.0 mg	99-0290	86,218
99-0391	Cap	Tran	4.0 mg	99-0391	85,963

Table 247. Capsule composition in Study TR-20A-HTN.

	4 mg
Trandolapril	4.0
Corn starch	
Lactose, hydrous	
Povidone	
Sodium stearyl fumarate	
Total	

A31.4. Study design

There was a 4-week single-blind, placebo lead-in phase, followed by a 6-week double blind treatment period, and a 3-week single-blind, placebo washout period. Fifty subjects per treatment group were planned. Subjects were to be age ≥ 21 years, $< 30\%$ above ideal body weight, with supine diastolic pressure between 95 and 114 mmHg at the end of the placebo run-in phase. Women were permitted if neither childbearing nor lactating, and if practicing recognized forms of contraception. Exclusion criteria are listed in Table 249 below. Other excluded concomitant drugs were antiarrhythmics, digitalis, MAO inhibitors, psychotropic drugs, chronic NSAIDs, phenylpropanolamine, and pseudoephedrine.

Subjects who qualified at the end of the placebo run-in period were randomized to placebo or trandolapril 4, 8, 12, and 16 mg q.d. and returned after 1, 2, 4, and 6 weeks.

Table 248. Design for Study TR-20A-HTN.

Visit #	Screen	Placebo run-in				Double-blind				Washout	
		1	2	3	4	5	6	7	8	9	10
Week of trial		1	2	3	4 ^a	1	2	4	6	1	3
Medical history	x										
Physical exam	x										x
Interim physical		x	x	x	x	x	x	x	x	x	
Vital signs & BP ^b	x	x	x	x	x	x	x	x	x	x	x
12 lead ECG	x				x	x			x		x
Lab evaluations ^c	x				x ^d		x	x	x		x
Plasma trandolapril ^d							x		x		
Compliance check		x	x	x	x	x	x	x	x	x	x
ADR assessment		x	x	x	x	x	x	x	x	x	x

a. Baseline measurements were made just prior to the first double-blind treatment dose.

b. Measurements were made at 'trough' and 'peak' at weeks 4, 6, and 10.

c. ACE activity, aldosterone, and renin activity were measured on weeks 4 and 10.

d. Samples were obtained at trough and 4-5 hours after dosing.

Table 249. Exclusion criteria in Study TR-20A-HTN.

Hypertension other than essential and mild-to-moderate
History of evidence of malignant, accelerated, or severe hypertension
Cerebrovascular accident, convulsions, or hypertensive encephalopathy within 1 year
Clinically significant congestive heart failure or EF<40%
Use of antihypertensives within 4 weeks of double-blind period or spironolactone within 6 weeks
Heart rate <50 bpm, significant arrhythmia, sinus node dysfunction, or conduction abnormalities
Myocardial infarction within 3 months
Hypersensitivity or serious adverse reaction to any ACE inhibitor
Clinically significant renal failure, e.g., creatinine >2 mg/dl
Clinically significant hepatic failure, e.g., SGOT or SGPT >2 times ULN
Significant electrolyte imbalance
Significant hematologic abnormalities
Medical or surgical conditions affecting absorption, metabolism, or excretion
Previous clinical trial experience within 30 days or any previous exposure to trandolapril
History of chronic alcohol or drug abuse within 2 years
Terminal illness
Blood donation within 1 month

All subjects took the same number of capsules. Subjects were instructed to take their study drug at 0900±1 hour, but the relationship to meals was not specified.

Safety data included medical history, physical exam, 12-lead ECG, and routine hematology, clinical chemistry, and urinalysis. There were no explicit criteria for dropping a subject because of lack of efficacy.

Blood samples were obtained for ACE activity, aldosterone, and renin activity levels at baseline and at the final double-blind visit. Trandolapril and trandolaprilat levels were obtained at two double-blind treatment period visits.

The primary analysis was an intent-to-treat analysis of supine diastolic pressure at the final double-blind visit. Secondary end points were sitting and standing diastolic pressure and percentage of responders (no criteria pre-specified). With 35 subjects per arm, the study had 80% power to detect a 5 mmHg change from baseline with two-sided $\alpha=0.05$.

There was one amendment to the protocol, calling for an interim analysis¹.

A31.5. Results

One hundred ninety-two subjects enrolled in the trial, of whom 171 completed the final washout period. Individual study centers enrolled 12 to 20 subjects. Demographic and baseline characteristics of subjects are shown in Table 250 below. The treatment

Table 250. Demographic and baseline characteristics of subjects in Study TR-20A-HTN.

	Placebo n=37	Trandolapril			
		4 mg n=38	8 mg n=40	12 mg n=39	16 mg n=38
Male	20	18	25	28	28
Female	17	20	15	11	10
Age $\pm$ SD	50 $\pm$ 13	56 $\pm$ 12	54 $\pm$ 11	55 $\pm$ 13	56 $\pm$ 11
Range	26 to 78	30 to 81	24 to 77	27 to 78	33 to 78
Caucasian	36	37	40	38	38
Black	0	0	0	0	0
Other ^a	1	1	0	1	0
Weight $\pm$ SD	185 $\pm$ 38	174 $\pm$ 34	187 $\pm$ 34	186 $\pm$ 40	183 $\pm$ 33
Range	132 to 299	112 to 250	111 to 256	98 to 270	116 to 256

a. Total of 2 Orientals and 1 American Indian.

groups were reasonably well matched for most factors, including general health, duration and severity of hypertension, and baseline drug use, but there were substantial differences in gender distribution among treatment groups. One hundred two of 155 actively treated subjects (66%) and 27 of 37 (73%) of placebo subjects received concomitant medications. Eight percent of subjects had cardiovascular diagnoses (coronary artery disease, myocardial infarction, aortic sclerosis, valvular disease) other than hypertension; these diagnoses were somewhat more common in actively-treated (9%) than placebo subjects (5%). Slightly more than half the subjects had noncardiovascular diagnoses common in this population (arthritis, hypercholesterolemia, hyperlipidemia, diabetes, hypothyroidism).

The disposition of subjects is shown in Table 251 below.

No subjects were excluded from efficacy or safety analyses because of protocol violations, although one placebo subject was excluded from efficacy assessments because there were no post-baseline blood pressure data. Violations of entry criteria included subjects >30% above ideal weight (n=14), chronic use of aspirin (n=7), and failure to meet blood pressure criteria (n=5).

An intent-to-treat approach was used to analyze the efficacy variables. All subjects with data for at least one post-baseline visit were included and the last measurements for dropouts were carried forward in the analyses.

Peak and trough supine diastolic pressure are shown graphically by dose in Figure 113 below and by time in Figure 114 below.

¹ The study report says that the 'interim' analysis was performed after all subjects were enrolled.

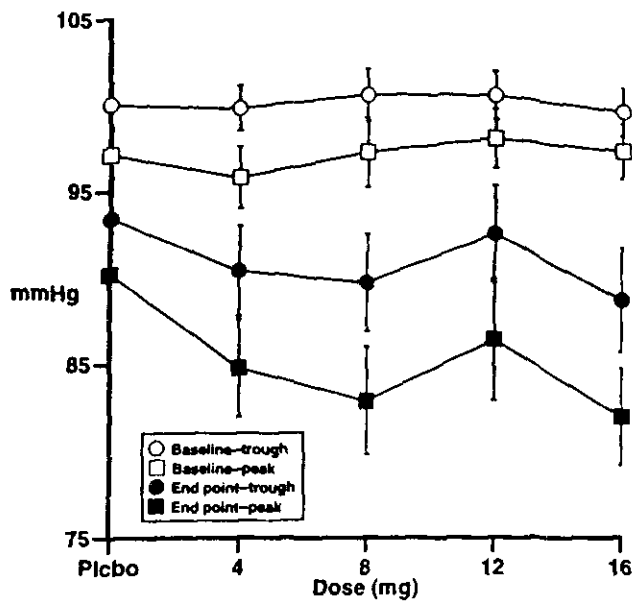


Figure 113. Supine diastolic pressure (2xSEM) at end point by dose in Study TR-20A-HTN.

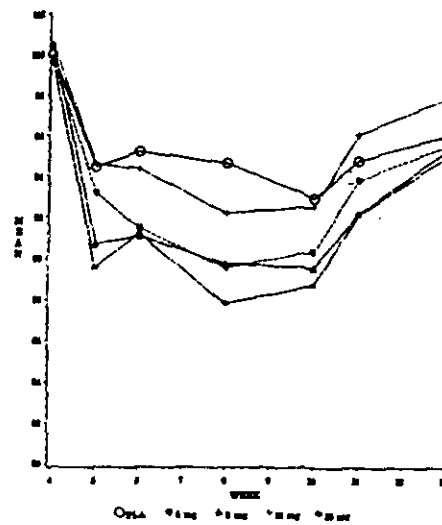


Figure 114. Supine diastolic pressure by dose and time in Study TR-20A-HTN.

Table 251. Disposition of subjects in Study TR-20A-HTN.

	Randomized	Double-blind				Wash-out	
		Week 1	Week 2	Week 4	Week 6	Week 1	Week 3
Remaining in study	192	187	184	181	177	177	171
Dropouts		5	3	3	3	0	6
Adverse reaction		2	1	1	1	0	0
Lack of efficacy		0	0	0	0	0	1
Not meet entry criteria		0	—	—	—	—	—
Compliance		2	1	2	3	0	2
Other ^a		1	1	0	0	0	3

a. Cited reasons were study stopped, SGPT 2xULN, elevated BP, subject traveling, and subject moved. It is unclear why some of these were not subsumed in listed categories.

The sponsor's analysis of blood pressure considered 'trough' as any measurement at least 10 hours after dosing; 'peak' was considered 4 to 5 hours after dosing. Few trough measurements were actually outside the interval 24±2 hours, but this was the interval used in the analyses performed for this review.

According to the sponsor, the supine diastolic pressure was analyzed using analysis of covariance (ANCOVA) with treatment and center as factors and baseline supine diastolic pressure as a covariate. The study report also gave estimated mean supine diastolic pressure for each treatment group, as shown in Table 252 below. Significant decreases in diastolic pressure from baseline at trough were observed for all treatment groups. Paired comparisons of adjusted mean supine diastolic blood pressures between a trandolapril group and the placebo group showed that the trandolapril therapy significantly lowered the supine diastolic pressure for the 8 mg ($p=0.020$) and 16 mg ($p=0.025$) groups. However, based on this ANCOVA model, no overall treatment difference was found ($p=0.08$).

Table 252. Diastolic pressure data in Study TR-20A-HTN^a.

	Placebo n=36	Trandolapril			
		4 mg n=38	8 mg n=40	12 mg n=39	16 mg n=38
Mean supine diastolic pressure at trough and end point (ANCOVA)					
Baseline	100.0	99.8	100.5	100.5	99.6
End point	93.1	90.4	89.2	92.0	89.0
Change from baseline and placebo p-value	—	-2.7 NS	-3.9 0.02	-1.1 NS	-4.1 .025
Mean supine diastolic pressure at trough and end point (statistical review)					
Baseline	100.0	99.8	100.5	100.5	99.6
End point	93.7	92.0	88.5	90.3	89.4
Change from baseline and placebo p-value	—	-1.7 NS	-5.2 0.011	-3.4 NS	-4.3 .003
Mean supine diastolic pressure at peak and end point					
Baseline	97.1	95.8	97.2	98.0	97.3
End point	89.7	85.0	82.6	85.8	81.6
Change from baseline and placebo p-value	—	-4.7 0.021	-7.1 <0.001	-3.9 0.063	-8.1 <0.001

a. Statistical reviewer's analysis, with adjustment for baseline and investigator.

The statistical reviewer found that there was a significant treatment-by-center interaction ($p=0.004$). Therefore, caution must be taken in interpreting the sponsor's results based on the above additive model. When the interaction term was included, significant difference in mean supine diastolic pressure at trough at end point was found ($p=0.020$). However, with the presence of the interaction, a mean blood pressure for a treatment group here should be interpreted as an average across the investigation centers. The results of the statistical reviewer's analysis are also given in Table 252. More detailed checks were done for the interaction. It was found that the study center #24 (Investigator Larry Gilderman, D.O.) had a great contribution to the interaction. In this center, the mean trough supine diastolic pressure at end point increased from baseline for the 12 mg group, while it decreased from baseline for all other treatment groups including the placebo one. Comparing with the other centers, this center had the largest mean decrease in supine diastolic pressure for the 8 mg group (18 mmHg) and, together with center #29, had the largest decrease in supine diastolic pressure for the 16 mg group (>22 mmHg). As compared to the other centers, this center had more protocol violations, shorter mean duration of hypertension (5.2 vs. 10.2 years), and greater mean weight (196.8 vs. 182.4 lb). To retain a simple interpretation, a procedure based on multiple testing was used by the statistical reviewer to compare mean supine diastolic blood pressure in each dosing group with that in the placebo group without adjustment for center effect. Hochberg's multiple comparison procedure was used to test the hypothesis that the mean supine diastolic blood pressures at trough at end point were same between a dosing group and the placebo group. A significant treatment difference was found at significant level $\alpha=0.05$. By this method, a significant difference exists between the 8 mg group and placebo group and between the 16 mg group and the placebo group. The hypothesis tested here is equivalent to the hypothesis of ANOVA.

A similar ANCOVA model was used to analyze supine diastolic pressure at peak at end point. The results are presented in Table 252. Again significant decreases from baseline in diastolic pressure at peak were found at end point for all treatment groups. The treatment difference was significant ($p=0.001$). No treatment-by-center or treatment-by-baseline interactions were found in this case. It was noticed that for both

trough and peak blood pressure, the smallest decrease from baseline in supine diastolic pressure among the dosing groups was in the 12 mg group and the two largest decreases were in the 8 mg and 16 mg groups. Similar patterns were found for the supine diastolic pressure at trough at double-blind treatment weeks 1, 2, 4, and 6.

Analyses on sitting and standing diastolic pressure were done using ANCOVA models. A significant treatment difference was found for sitting diastolic pressure at trough at end point with treatment and baseline in the ANCOVA model. In this case, treatment-by-center and treatment-by-baseline interactions were not significant. There was a mild treatment-by-center interaction for standing diastolic pressure at trough. No treatment difference was found by ANCOVA.

Based on the estimates of mean diastolic pressure at trough and peak, the trough to peak ratios were obtained, as shown in Table 253 below. The analysis of responders at

Table 253. Supine diastolic pressure peak/trough ratio in Study TR-20A-HTN^a.

Trandolapril			
4 mg n=38	8 mg n=40	12 mg n=39	16 mg n=38
0.68	0.56	0.28	0.50

a. Statistical reviewer's analysis.

end point failed to show a significant difference among the treatment groups ($p=0.374$, χ^2 test).

Regressions on dose was done by the statistical reviewer. In the final model, the dependent variable was change in supine diastolic pressure at trough at end point. The estimated response curve was

$$\Delta SupDP = -6.468 - 1.522 \times Dose + 0.156 \times Dose^2 - 0.0003 \times Dose^4$$

where the estimated coefficients for the 3 dose terms were at least marginally significantly different from zero at the $\alpha=0.05$ level. The fitted curve has a local peak (lesser reduction in blood pressure) at the 12 mg dose, the clinical significance of which cannot be excluded categorically.

The duration of treatment effect was determined by comparing trough blood pressure measurements obtained during the placebo washout weeks 1 and 3 to those at baseline and at the last double-blind visit (week 6), as shown in Table 254 below. Significant

Table 254. Duration of treatment effect in Study TR-20A-HTN^a.

	Plebo n=36	Trandolapril			
		4 mg n=38	8 mg n=40	12 mg n=39	16 mg n=38
Mean change from baseline					
Washout week 1	-5.3†	-5.9†	-8.3†	-5.0†	-7.4†
Washout week 3	-4.0*	-4.2†	-5.1†	-3.0	-4.5†
Mean change from end of double-blind treatment period					
Washout week 1	2.1	3.5†	2.8*	4.1†	3.4†
Washout week 3	3.4*	5.2†	5.9†	6.1†	6.3†

a. Statistical reviewer's analysis. *Significantly different, $p < 0.05$. †Significantly different, $p < 0.01$.

decreases in supine diastolic pressure from baseline were still seen for all treatment groups at washout weeks 1 and 3. Significant increases in supine diastolic pressure from the corresponding measurements at week 6 were found for all treatment groups at washout weeks 1 and 3, except for placebo group at washout week 1. However no significant differences in such changes were found among the treatment groups (ANOVA).

anges in sitting and standing diastolic pressure are shown in Table 255 below.

Table 255. Changes in trough sitting and standing diastolic pressure in Study TR-20A-HTN^a.

	Sitting				Standing			
	4 mg n=38	8 mg n=40	12 mg n=39	16 mg n=38	4 mg n=38	8 mg n=40	12 mg n=39	16 mg n=38
Baseline	99.6	100.3	98.7	99.2	97.3	98.5	98.5	99.8
Week 1	-2.2	-5.0	0.2	-4.6	-0.5	-2.9	-1.4	-6.3
Week 2	-3.1	-5.7	-1.4	-3.2	-2.3	-4.4	-2.3	-6.6
Week 4	-5.8	-6.3	-3.2	-7.2	-4.3	-4.1	-3.5	-7.8
Week 6	-3.9	-5.1	-1.2	-3.4	-0.8	-2.3	-2.1	-4.0
End point	-3.8	-5.2	-0	-3.3	-0.9	-2.2	-1.4	-4.1
Washout 1	-1.9	-3.8	1.4	-2.7	-0.9	-2.5	-0.6	-2.9
Washout 3	-0.7	-1.3	2.3	-0.5	2.1	-1.0	1.6	-1.1

a. The sponsor's analysis, with adjustment for baseline and investigator. Differences shown are based on group mean changes for the given time-point.

Changes in sitting and standing diastolic pressure were variable and lacked clear dose-dependence. Changes in systolic pressure (double-differences from baseline and placebo) are summarized in Table 256 below. Here as well, dose-ordering is not

Table 256. Changes in trough systolic pressure in Study TR-20A-HTN^a.

	4 mg n=38	8 mg n=40	12 mg n=39	16 mg n=38
Supine	-3.2	-9.6	-4.2	-6.2
Sitting	-1.9	-11.5	-4.3	-5.4
Standing	-4.1	-8.7	-5.4	-10.2

a. The sponsor's analysis, with adjustment for baseline and investigator.

apparent.

The sponsor reasonably, but retrospectively, defined responders as subjects with supine diastolic pressure <90 mmHg or a ≥10 mmHg reduction at end point. So defined, the rates were 39% on placebo, 53% on 4 mg, 58% on 8 mg, 46% on 12 mg, and 55% on 16 mg. Differences from placebo were not statistically significant, nor was there dose-dependence.

There was a small but statistically significant decrease in trough supine pulse rate for the 8 mg group compared with placebo. Peak sitting and standing pulse rates were

statistically significantly elevated in the 4 mg group. These effects are of doubtful clinical significance.

Pharmacokinetic data were obtained only once at trough and 4 to 5 hours after dosing. Trandolapril and trandolaprilat are both present at low levels at trough. Post-dosing at 16 mg, trandolaprilat levels are some 40-fold higher than trandolapril levels.

Renin, aldosterone, and ACE activity are shown as changes from baseline to the end of the double-blind treatment period in Table 257 below. Renin levels increased,

Table 257. Changes in renin, aldosterone, and ACE activity in Study TR-20A-HTN.

	Plicbo n=28-30	Trandolapril			
		4 mg n=30-36	8 mg n=34-35	12 mg n=32-33	16 mg n=34-36
Renin (ng/ml/h)	0.3	3.5	2.4	2.8	3.0
Aldosterone (ng/dl)	-0.7	-1.3	-2.2	-0.1	-0.3
ACE activity (U/L)	0.9	-22.5	-25.9	-22.9	-30.6

although not in a dose-dependent manner. Aldosterone levels were not clearly affected. ACE inhibition was comparable in all actively treated groups, and clearly different from placebo.

A31.6. Safety.

Sixty-five of 155 actively treated subjects (42%) reported at least one adverse reaction, compared with 51% of placebo subjects. These adverse events are summarized in Table 258 below. Counts of events were too low to discern any relationship to dose.

Table 258. Adverse events (n≥2) in Study TR-20A-HTN.

Events reported by ≥2 Trandolapril subjects			
Cough	10	Bradycardia	2
Headache	8	Drowsiness	2
URI symptoms ^a	14	Decreased libido	2
Dizziness	4	Allergy	2
Nausea	4	Ear inflammation	2
Fatigue	3	Abdominal pain/cramps	2
Epistaxis	3	Dyspepsia	2
Diarrhea	3	Chest pain	2
Muscle cramps	3	Bronchitis	2

a. Sum of upper respiratory tract congestion, infection, and inflammation.

Subjects who discontinued because of adverse reactions are listed in Table 259 below.

One subject in each of the 4, 8, and 16 mg groups had hypotension, defined as a supine systolic pressure <100 mmHg. Twenty-eight subjects were deemed to have had orthostatic hypotension, defined as a >20 mmHg reduction in systolic pressure in moving from a supine to standing position, during the double-blind treatment period; the incidence of orthostatic hypotension was 19% on placebo, 21% on 4 mg, 10% on 8 mg, 19% on 12 mg, and 24% on 16 mg. The incidence was 5% during the washout phase.

Table 259. Discontinuations for adverse events in Study TR-20A-HTN.

Center	Subj	Group	Day	ADR
2023	23	placebo	46	55 year old Caucasian female with 5-month history of hypertension, discontinued because of severe tingling and numbness in the right hand and arm. Events resolved a 3-week follow-up.
2024	16	placebo	45	54 year old Caucasian female with 4-year history of hypertension, hyperlipidemia, and COPD, discontinued because of periorbital edema and lethargy, both of which resolved at follow-up 4 weeks later.
2029	3	placebo	36	39 year old Caucasian male with unknown duration of hypertension, suffered acute myocardial infarction and subsequently underwent CABG.
2031	14	8 mg	12	66 year old Caucasian male with a 32-year history of hypertension, discontinued because of dizziness. Also complained of headaches. Both events resolved after discontinuation. Baseline BP was 136/95; last double-blind BP was 156/80.
2022	7	12 mg	3	54 year old Oriental male with a 4-year history of hypertension and diabetes mellitus, discontinued with a dry, nonproductive cough.

The most frequent new ECG findings were, in the active treatment groups, sinus bradycardia (6.6%), non-specific ST changes (3.9%), premature ventricular contractions (3.3%), and left axis deviation (2.6%). In the placebo group, the common findings were sinus bradycardia (17.1%), and left axis deviation (5.7%).

Laboratory data are summarized in Figure 115 to Figure 118 below.

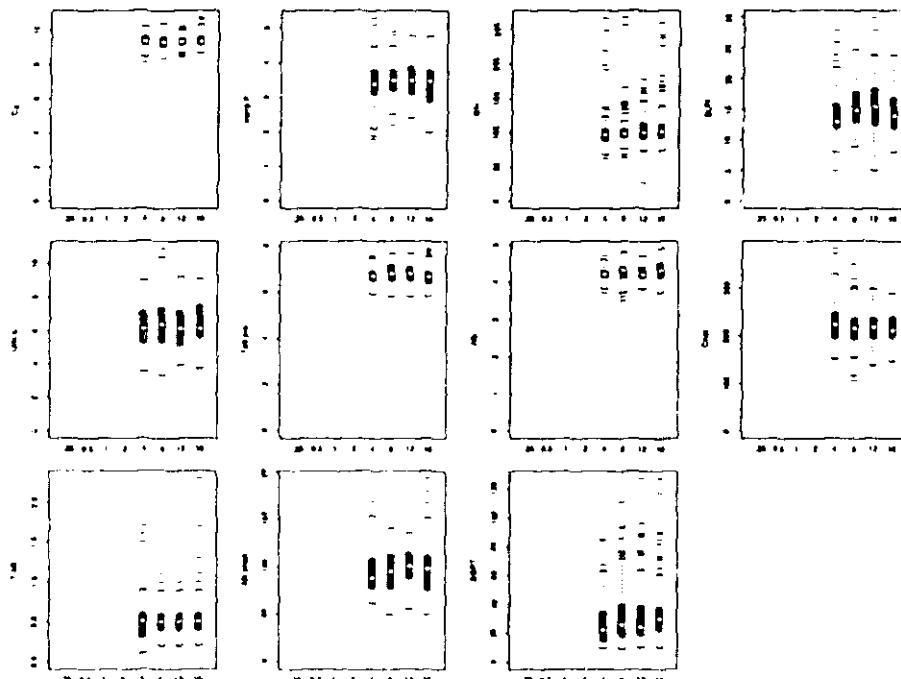


Figure 115. Clinical chemistry data (part A) in Study TR-20A-HTN.

Significant lab abnormalities in the active treatment groups were hematuria (n=1), microscopic hematuria (n=3), proteinuria (n=1), microscopic pyuria (n=1), hyaline casts (cylinduria; n=1), anemia (n=1), and thrombocytopenia (n=1; an isolated reading).

A31.7. Summary.

Caucasian subjects with mild-to-moderate hypertension were randomized to placebo or fixed doses of trandolapril 4, 8, 12, or 16 mg q.d. for 6 weeks, followed by a 3-week single-blind washout period. The sponsor's analysis was flawed by defining 'trough' as measurements made at least 10 hours after once-daily dosing. The lack of a good dose-response relationship was probably the result of poor efficacy in the 12 mg group.

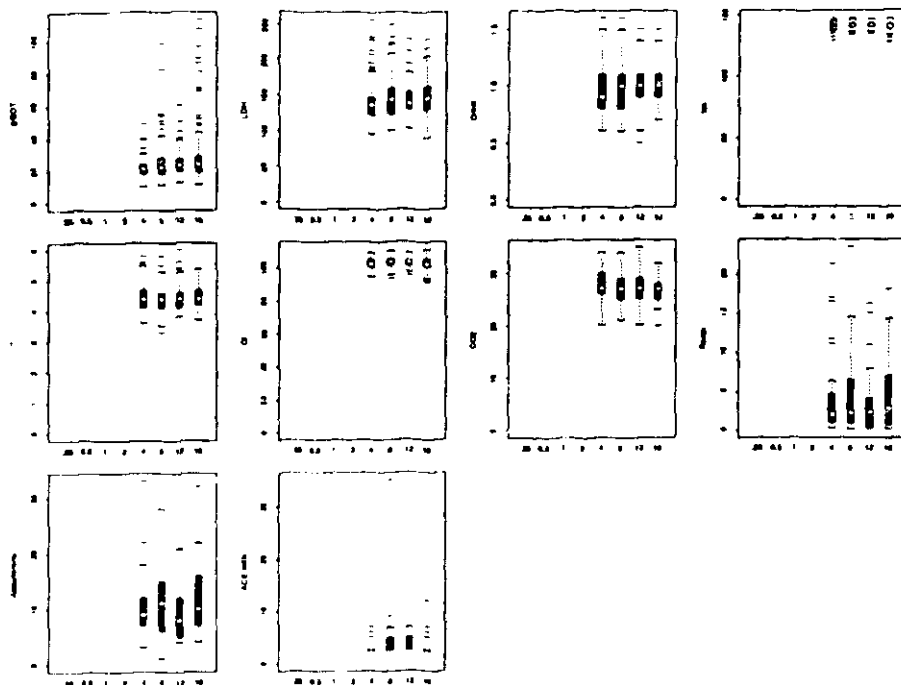


Figure 116. Clinical chemistry (part B) in Study TR-20A-HTN.

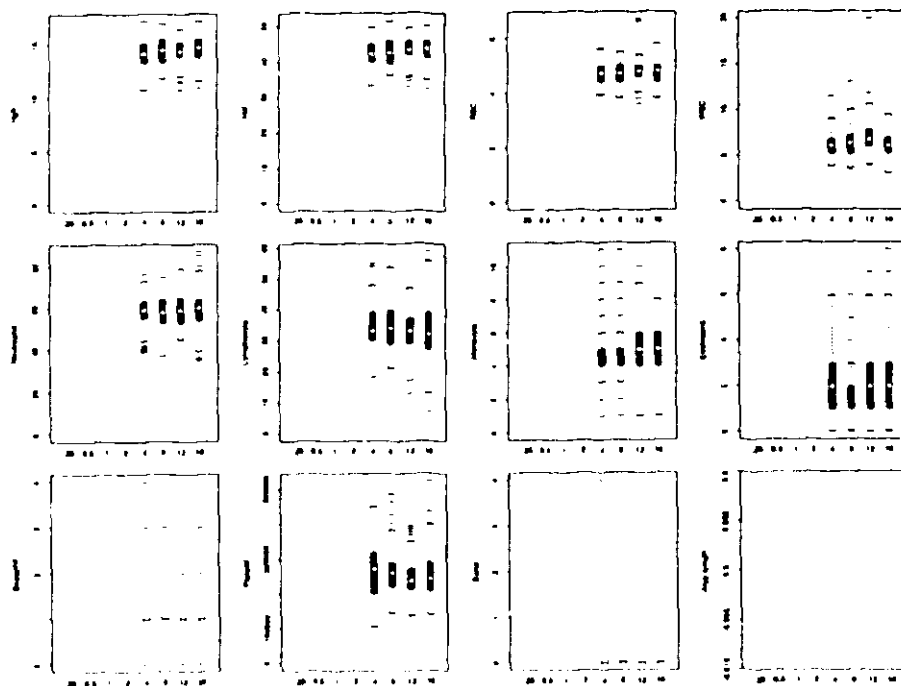


Figure 117. Hematology data in Study TR-20A-HTN.

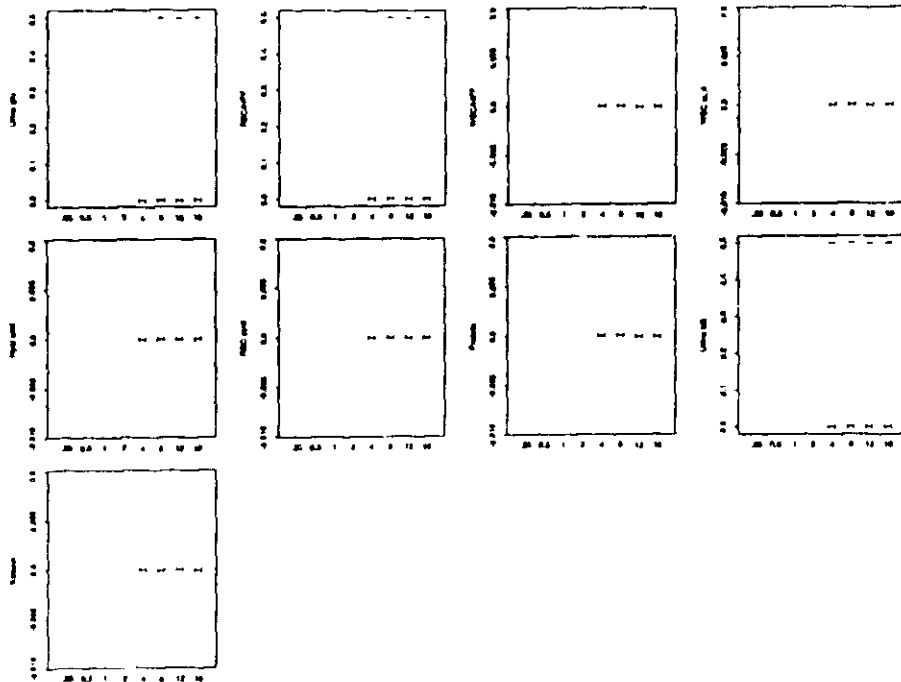


Figure 118. Urinalysis data in Study TR-20A-HTN.

Otherwise, the response seems to plateau with the 4 mg dose. There were no unusual adverse events in type or frequency.

A32. Study TR-21-HTN (Study report: vols 1.155-1.163; case report tabulations: vols 1.299-1.304; case report forms for deaths and discontinuations for adverse events: vol 1.379) "Double-blind, randomized, placebo-controlled, six-way parallel dose-ranging study to evaluate the safety and efficacy of oral trandolapril therapy (0.25, 0.5, 1, 2, and 4 mg/day) in Black patients with mild to moderate essential hypertension".

A32.1. Investigators The investigators are listed in Table 260 below.

Table 260. Investigators in Study TR-21-HTN.

Investigator	Center	City	Investigator	Center	City
J Gray, MD	2102	Houston, TX	CVS Ram, MD	2105	Dallas, TX
PM Kaihlanen, MD	2104	San Antonio, TX	HW Schraper, MD	2106	Birmingham, AL
FG McMahon, MD	2101	New Orleans, LA	M Weir, MD	2103	Baltimore, MD

A32.2. Study dates October 1989 to October 1990.

A32.3. Drug formulation

Table 261. Drug substance used in Study TR-21-HTN.

Lot	Form	Type	Trandolapril	Source lot	Batch size
L-21	Cap	Plcbo	—	L-21	1,200,000
TRA-0150A	Cap	Plcbo	—	L-21	1,200,000
JMP-22928-23	Cap	Plcbo	—	JMP-22928-23	40,000
MB22633-172	Cap	Tran	0.25 mg	MB22633-172	20,000
MB22633-145	Cap	Tran	0.5 mg	MB22633-145	20,000
L-12	Cap	Tran	1.0 mg	L-12	546,100
MB22327-163	Cap	Tran	2.0 mg	MB22327-163	600,000

Table 262. Capsule composition in Study TR-21-HTN.

	0.25 mg	0.5 mg	1 mg	2 mg
Trandolapril	0.25	0.5	1.0	2.0
Corn starch				
Lactose, hydrous				
Povidone				
Sodium stearyl fumarate				
Total				

A32.4. Study design

There was a 4-week single-blind, placebo lead-in phase, followed by a 6-week double blind treatment period, and a 3-week single-blind, placebo washout period. Twenty to thirty subjects per center were planned. Subjects were to be age ≥ 21 years, $< 30\%$ above ideal body weight, with supine diastolic pressure between 95 and 114 mmHg at the end of the placebo run-in phase. Women were permitted if neither childbearing nor lactating, and if practicing recognized forms of contraception. Exclusion criteria are listed in Table 263 below. Other excluded concomitant drugs were antiarrhythmics, digitalis, MAO inhibitors, psychotropic drugs, chronic NSAIDs, phenylpropanolamine, and pseudoephedrine.

Subjects who qualified at the end of the placebo run-in period were randomized to placebo or trandolapril 0.25, 0.5, 1, 2, or 4 mg q.d. and returned after 1, 2, 4, and 6 weeks of double-blind treatment. All subjects took the same number of capsules (2).

Table 263. Exclusion criteria in Study TR-21-HTN.

Hypertension other than essential and mild-to-moderate
History of evidence of malignant, accelerated, or severe hypertension
Cerebrovascular accident, convulsions, or hypertensive encephalopathy within 1 year
Clinically significant congestive heart failure or EF<40%
Use of antihypertensives within 4 weeks of double-blind period or spironolactone within 6 weeks
Heart rate <50 bpm, significant arrhythmia, sinus node dysfunction, or conduction abnormalities
Myocardial infarction within 3 months
Hypersensitivity or serious adverse reaction to any ACE inhibitor
Clinically significant renal failure, e.g., creatinine >2 mg/dl
Clinically significant hepatic failure, e.g., SGOT or SGPT >2 times ULN
Significant electrolyte imbalance
Significant hematologic abnormalities
Medical or surgical conditions affecting absorption, metabolism, or excretion
Previous clinical trial experience within 30 days or any previous exposure to trandolapril
History of chronic alcohol or drug abuse within 2 years
Terminal illness
Blood donation within 1 month

Subjects were instructed to take their study drug at 0900±1 hour, but the relationship to meals was not specified.

Safety data included medical history, physical exam, 12-lead ECG, and routine hematology, clinical chemistry, and urinalysis. There were no explicit criteria for dropping a subject because of lack of efficacy.

Blood samples were obtained for ACE activity, aldosterone, and renin activity levels at baseline and at the final double-blind visit. Trandolapril and trandolaprilat levels were obtained at three double-blind treatment period visits.

The primary analysis performed was an intent to treat analysis of supine diastolic blood pressure at trough (trough initially defined as immediately prior to the morning study dose—definition later modified). Secondary end points included peak supine diastolic pressure (peak defined as 4-5 hours after the morning dose), and peak and trough sitting and standing diastolic blood pressures, and supine, sitting, and standing systolic blood pressures. The study had 39-65% power to detect a 5 mmHg change from baseline with a two-sided $\alpha=0.05$.

As an amendment to the protocol, a 4.0 mg dosing group was added as one of the randomization arms.

Several procedures were added after study commencement. First, the definition of "trough" was extended from immediately prior to administration of the morning study dose to at least 20 hours post-dose. Secondly, the sponsor performed an analysis of "responders" (defined as patients with a trough supine diastolic pressure <90 mmHg and/or a decrease from baseline of at least 10 mmHg as measured at the final double-blind visit). Lastly, an interim review was done when approximately half the patients were enrolled. The results were presented with treatment groups blinded. The sponsor states that no changes in patient enrollment were involved, and that the final p-values were not affected.

A32.5. Results

One hundred thirty-six subjects enrolled in the trial, of whom 125 completed the final washout period. Individual study centers enrolled 17 to 30 subjects. Demographic and

baseline characteristics of subjects are shown in Table 264 below. The treatment

Table 264. Demographic and baseline characteristics of subjects in Study TR-21-HTN.

	Placebo n=23	Trandolapril				
		0.25 mg n=23	0.5 mg n=22	1 mg n=23	2 mg n=22	4 mg n=23
Male	11	12	9	7	10	8
Female	12	11	13	16	12	15
Age±SD	54±10	49±13	49±12	53±11	53±10	53±10
Range	38 to 72	26 to 71	26 to 71	28 to 66	38 to 77	32 to 72
Caucasian	0	0	0	0	0	0
Black	23	23	23	23	23	23
Other	0	0	0	0	0	0
Weight±SD	88±13	91±13	85±14	80±15	83±11	82±12
Range	65 to 115	68 to 122	61 to 109	54 to 113	60 to 100	63 to 103

groups were reasonably matched demographically as well as for concurrent diagnoses, duration and severity of hypertension, and previous and concurrent medications.

The disposition of subjects is shown in Table 265 below.

Table 265. Disposition of subjects in Study TR-21-HTN.

	Randomized	Double-blind				Wash-out	
		Week 1	Week 2	Week 4	Week 6	Week 1	Week 3
Remaining in study	136	132	128	126	126	125	125
Dropouts		4	4	2	0	1	0
Adverse reaction		0	0	0	0	0	0
Lack of efficacy		0	0	0	0	1	0
Not meet entry criteria		0	1	0	0	0	0
Compliance		2	1	1	0	0	0
Other ^a		1	0	1	0	0	0

a. Both listed as "therapy refusal".

No subjects were excluded from efficacy or safety analyses because of protocol violations or lack of compliance. One hundred thirty-five subjects were evaluated for efficacy. One subject, randomized to placebo, had no post-baseline efficacy measurements and thus was excluded from efficacy analysis.

An intent-to-treat approach was used to analyze the efficacy variables. All subjects with data for at least one post-baseline visit were included and the last measurements for dropouts were carried forward in the analyses.

The sponsor's analysis of change in supine diastolic pressure at trough (change from baseline and placebo) as a function of time in trial is shown in Figure 119 below.

According to the sponsor, the supine diastolic pressure at trough was analyzed using analysis of covariance (ANCOVA) with treatment and center as factors and baseline supine diastolic pressure as a covariate. A significant treatment difference was found at end point based on this ANCOVA model ($p=0.015$). The study report also gave estimated mean supine diastolic pressure for each treatment group, as shown in Table 266 below. No treatment-by-center or treatment-by-baseline interactions were found. Paired comparisons of adjusted mean supine diastolic blood pressures between each

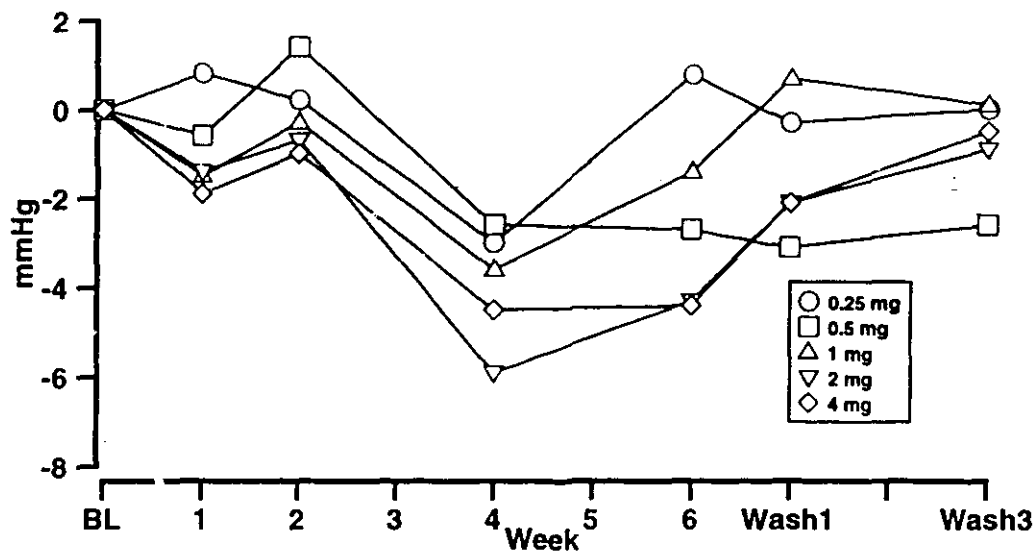


Figure 119. Change in supine diastolic pressure at trough by time in Study TR-21-HTN.

Table 266. Diastolic pressure data in Study TR-21-HTN^a.

	Placebo n=22	Trandolapril				
		0.25 mg n=23	0.5 mg n=22	1 mg n=23	2 mg n=22	4 mg n=23
Mean supine diastolic pressure at trough and end point						
Baseline	99.7	101.7	101.6	99.7	99.1	101.5
End point	98.5	98.8	96.3	98.0	94.5	93.7
Change from baseline and placebo	—	0.3	-2.2	-0.5	-4.0	-4.8
p-value		NS	NS	NS	0.026	0.009
Mean supine diastolic pressure at peak and end point						
Baseline	97.2	99.7	99.5	97.4	97.1	99.2
End point	96.6	93.5	93.6	91.9	87.9	89.3
Change from baseline and placebo	—	-3.1	-3.0	-4.7	-8.7	-7.3
p-value		NS	NS	0.039	<0.001	0.002

a. Statistical reviewer's analysis, with adjustment for baseline and investigator.

trandolapril group and the placebo group showed a significant decrease in supine diastolic pressure from baseline for the 2 and 4 mg groups ($p=0.026$ for 2 mg and $p=0.009$ for 4 mg, Table 266). Significant decreases in diastolic pressure from baseline at trough were observed for all treatment groups except the placebo and 0.25 mg groups.

A similar ANCOVA model was used to analyze supine diastolic pressure at peak at end point. The results are presented in Table 266. Again significant decreases from baseline in diastolic pressure were found. In this case, a significant treatment difference was found using ANCOVA ($p=0.003$). No treatment-by-center or treatment-by-baseline interactions were found. The decreases from baseline in diastolic pressure at peak ranged from 3.1 to 7.3 mmHg among the dosing groups. Analyses of sitting and standing diastolic pressure were done using ANCOVA models. A significant treatment difference was found for sitting diastolic pressure at trough at end point ($p=0.035$). However, significant treatment-by-center and treatment-by-baseline

interactions were observed at $\alpha=0.1$ level for standing diastolic pressure at trough at end point.

Based on the estimates of mean diastolic pressure at trough and peak, the trough-to-peak ratios were obtained, as shown in Table 267 below.

Table 267. Supine diastolic pressure peak/trough ratio in Study TR-21-HTN^a.

Trandolapril				
0.25 mg n=23	0.5 mg n=22	1 mg n=23	2 mg n=22	4 mg n=23
-0.14	0.54	0.13	0.48	0.57

a. Statistical reviewer's analysis.

Approximately 15% of the subjects were age >65. In these subjects, the effects of trandolapril 0.5, 1.0, and 2.0 mg on trough supine diastolic pressure were twice as great as the effects seen in subjects <65. The effects of trandolapril 0.25 and 4.0 mg doses were similar in both age groups.

Changes (group mean double differences from baseline and placebo) in sitting and standing diastolic pressure are shown in Table 268 below. Both sets of data also

Table 268. Changes in trough sitting and standing diastolic pressure in Study TR-21-HTN^a.

	Sitting					Standing				
	0.25 mg n=23	0.5 mg n=22	1 mg n=23	2 mg n=22	4 mg n=23	0.25 mg n=23	0.5 mg n=22	1 mg n=23	2 mg n=22	4 mg n=23
Baseline	102.6	102.1	101.7	100.2	102.9	104.1	104.5	103.2	102.8	104.0
Week 1	-1.2	-0.8	-2.3	0.0	-1.1	0.7	0.7	-1.7	0.4	-1.1
Week 2	0.8	0.2	-2.1	0.1	-3.3	1.8	2.5	-1.2	-3.5	-2.4
Week 4	-0.6	-1.2	-2.9	-4.8	-4.0	-0.5	-0.5	-2.5	-2.0	-3.1
Week 6	2.0	-0.3	0.7	-0.8	-3.2	3.4	-0.5	-2.0	-2.0	-3.1
End point	2.4	0.1	1.4	-0.7	-3.3	3.4	0.1	-1.7	-1.9	-3.4
Washout 1	-1.0	-2.7	0.8	-1.5	-2.7	1.7	-0.6	2.5	-0.6	-0.2
Washout 3	2.0	-0.3	0.5	1.5	0.2	3.0	-0.4	1.0	1.2	1.9

a. Sponsor has calculated endpoint values using analysis of covariance adjustments with baseline as a covariate and investigator as a factor.

appeared only weakly dose-dependent. Only the 2.0 and 4.0 mg doses consistently achieved a statistically significant difference from baseline (sponsor's analysis, without subtraction from placebo).

Responder (subjects with a trough supine diastolic pressure <90 mmHg and/or a decrease from baseline of at least 10 mmHg as measured at the final double-blind visit) rates were as follows: placebo = 14%; 0.25 mg = 9%; 0.5 mg = 18%; 1.0 mg = 13%; 2.0 mg = 41%; and 4.0 mg = 43%. The analysis of responders at end point using χ^2 test showed a significant difference in proportion of responders among the treatment groups ($p=0.011$). The proportion of responders in the 4 mg group was significantly higher than that in the placebo group (43% vs. 14%, $p=0.047$).

Regressions on dose were done by the statistical reviewer. In the final model, the dependent variable was change in supine diastolic pressure at trough at end point and

the regression terms included dose and baseline. The estimated response curve was

$$\Delta SupDP = -2.226 - 1.23 \times Dose$$

where the estimated coefficient for the linear dose terms was significantly different from zero ($p=0.0013$) indicating a linear dose-response relationship.

The duration of treatment effect was determined by comparing trough blood pressure measurements obtained during the placebo washout weeks 1 and 3 to those at baseline and at the last double-blind visit (week 6). Significant decreases from baseline in supine diastolic pressure still could be seen for the 0.5, 2, and 4 mg groups at washout week 1 and for the 0.5 mg group at washout week 3, as shown in Table 269 below.

Table 269. Duration of treatment effect in Study TR-21-HTN^a.

	Placebo n=22	Trandolapril				
		0.25 mg n=23	0.5 mg n=22	1 mg n=23	2 mg n=22	4 mg n=23
Mean change from baseline						
Washout week 1	-2.2	-2.5	-5.3†	-1.5	-4.3†	-4.3†
Washout week 3	-1.3	-1.3	-3.9†	-1.2	-2.2	-1.8
Mean change from end of double-blind treatment period						
Washout week 1	-0.1	-1.2	-0.4	2.0	2.1	2.2
Washout week 3	0.8	0.1	1.0	2.3	4.2†	4.7†

a. Statistical reviewer's analysis. *Significantly different from placebo, $p<0.05$. †Significantly different from placebo, $p<0.01$.

Increases in supine diastolic pressure from the corresponding measurements at week 6 were seen for all treatment groups but reached statistical significance only for the 2 and 4 mg groups at washout week 3. However no significant differences in such changes were found among the treatment groups (ANOVA).

Pulse rate changes from baseline to end point were minimal and generally not statistically significant for any treatment group.

Trough (immediately prior to drug administration) plasma trandolapril and trandolaprilat levels were obtained during weeks 2, 4, and 6, and at peak (4-5 hours after dosing) during weeks 2 and 6. Concentrations of trandolaprilat greatly exceeded those of trandolapril.

Renin, aldosterone, and ACE activity were measured at baseline and at the last double-blind visit (week 6). These data are shown as changes from baseline in Table 270

below. Little dose-response is evident. ACE inhibition ranged from 77 to 88% in the

Table 270. Changes from baseline in renin, aldosterone, and ACE activity in Study TR-21-HTN.

	Placebo n=22	0.25 mg n=23	0.5 mg n=22	1 mg n=23	2 mg n=22	4 mg n=23
Renin (ng/ml/h)	-0.2	0.2	1.0	0.4	1.2	0.9
Aldosterone (ng/dl)	-0.1	-0.3	2.6	-1.3	-3.5	-1.4
ACE activity (U/L)	-4.2	-35	-46	-36	-37	-32

actively treated groups.

A32.6. Safety.

Thirty-five of 136 subjects reported at least one adverse reaction. These adverse events are summarized in Table 271 below. There were no statistically significant differences

Table 271. Adverse events (%) in Study TR-21-HTN.

	Placebo n=23	Tran n=113	Events reported by ≥2 Trandolapril subjects	Tran	Placebo
Any	8 (35)	27 (24)	URI	7 (6)	0
Cardiovascular	2 (9)	7 (6)	Headache	4 (4)	2 (9)
CNS	3 (13)	7 (6)	1st deg AV block	2 (2)	0
EENT	1 (4)	9 (8)	Peripheral edema	2 (2)	1 (4)
Gastrointestinal	2 (9)	3 (3)	Anemia	2 (2)	0
General	2 (9)	4 (4)	Nausea	2 (2)	0
Hematopoietic	0	2 (2)	Sinus bradycardia	12 (11)	2 (9)
Musculoskeletal	2 (9)	1 (1)	Non-specific ST changes	7 (6)	1 (5)
			Prem ventricular contractions	2 (2)	0
			Sinus tachycardia	2 (2)	0

between the trandolapril and placebo groups in the proportion of subjects experiencing an adverse event during the double-blind phase. There did not appear to be a relationship between adverse events and dose. The sponsor theorizes that some investigators may have classified "cough" as an upper respiratory infection. No conclusions regarding adverse events in those >65 versus <65 were possible.

The sponsor inconsistently reported some of the adverse events. For example, although one part of the report states that peripheral edema occurred as an adverse event in 2 trandolapril treated subjects and one placebo subject, the "New physical findings" section lists that 4 trandolapril subjects developed peripheral edema, and 1 subject while on placebo. Similarly, although the sponsor's adverse events sections states 2 trandolapril subjects and no placebo subject experienced first degree AV block, the "New ECG findings" section states that one subject in each treatment group developed first degree AV block.

No deaths were reported for any randomized subject. One subject died subsequent to a myocardial infarction which occurred during the placebo-withdrawal period. Subjects who discontinued because of adverse reactions are listed in Table 272 below.

Laboratory data are summarized in Figure 120 to Figure 123 below.

Although there were a variety of new finding on ECG, physical exam, and laboratory testing, none appeared serious or to be obviously related to the study drug. Three

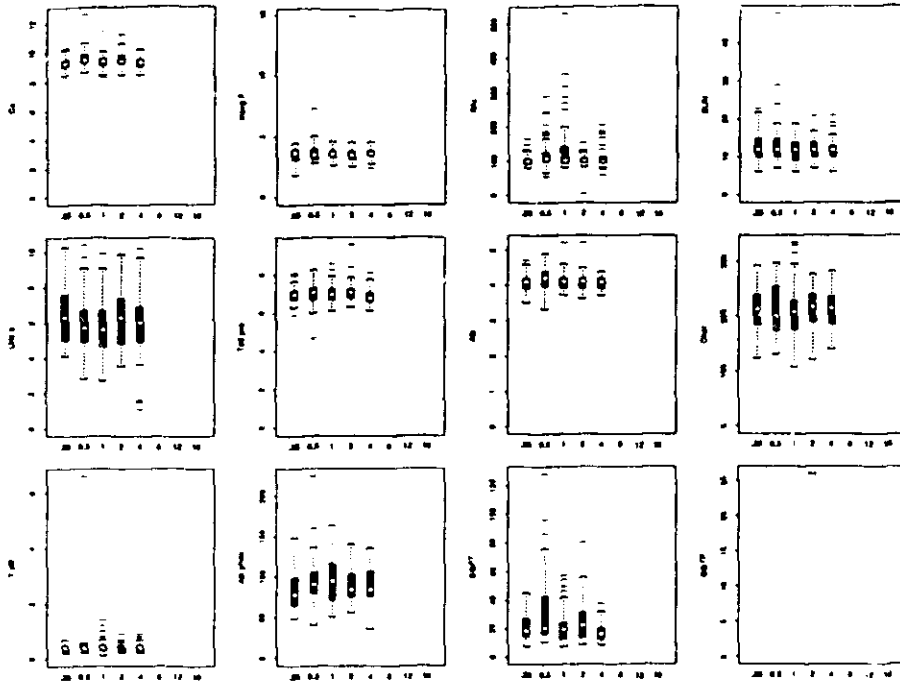


Figure 120. Clinical chemistry data (part A) in Study TR-21-HTN.

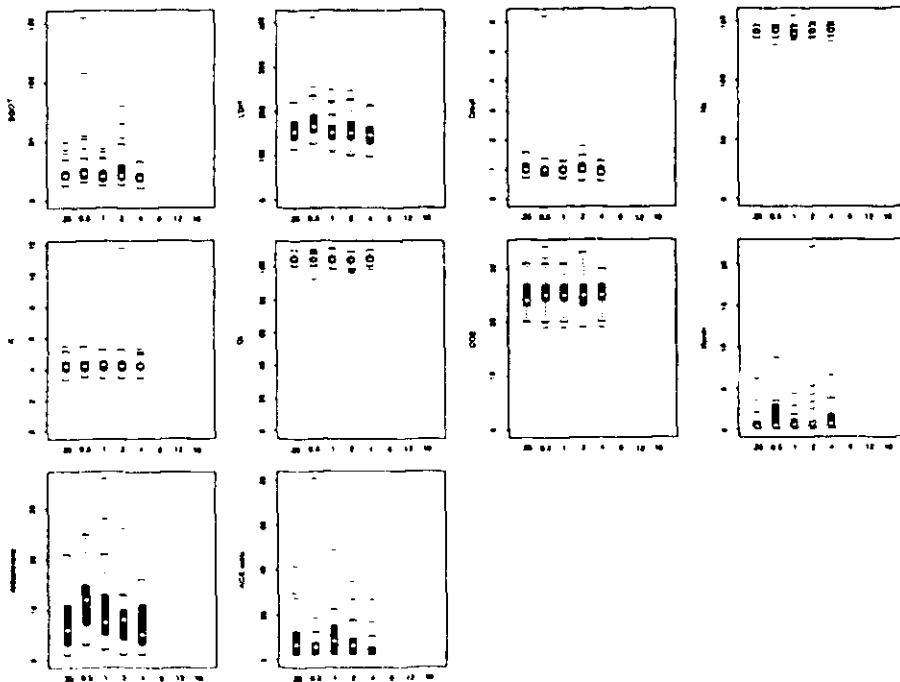


Figure 121. Clinical chemistry (part B) in Study TR-21-HTN.

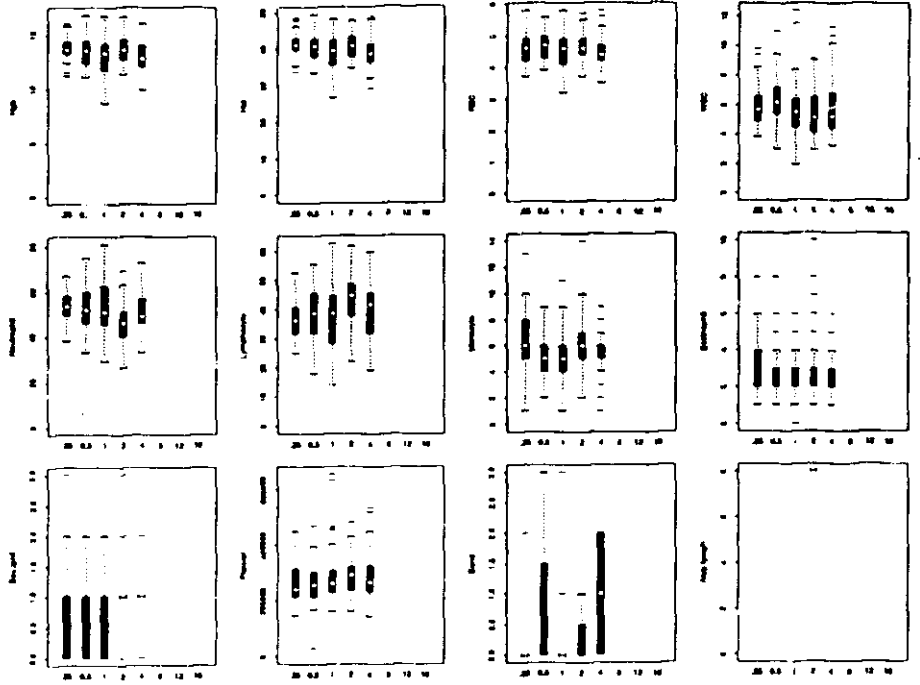


Figure 122. Hematology data in Study TR-21-HTN.

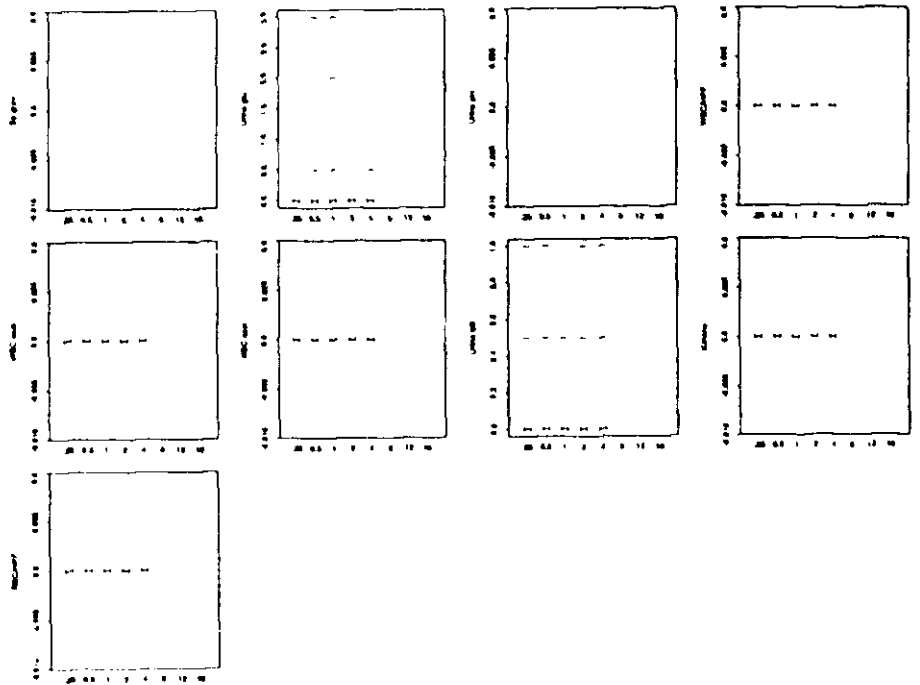


Figure 123. Urinalysis data in Study TR-21-HTN.

Table 272. Discontinuations for adverse events in Study TR-21-HTN.

Center	Subj	Group	Day	ADR
2105	16	0.25 mg	16	60 year old Black male with 9-year history of hypertension, experienced transient focal weakness, admitted to hospital, treated with heparin, diagnosed as a TIA.
2101	22	0.5 mg	21	54 year old Black female with 5-year history of hypertension, developed urticarial lesion beneath chin, angioedema (swelling of hands, eyelids, choking in throat).

subjects treated with trandolapril developed laboratory abnormalities that will be mentioned:

Subject 2103-16, a 70 year old Black female, insulin-dependent diabetic, had a glucose level of 54 mg/dl at week 6, and a level of 55 mg/dl during washout. Symptoms were not described.

Subject 2106-4, a 42 year old Black male, had an isolated platelet count of 31,000/mm³ at week 6.

Subject 2106-11, a 34 year old Black female, had a urinary RBC/HPF of 26-50 at week 6.

There were no significant differences between any of the trandolapril groups and the placebo group in the proportion of subjects with orthostatic hypotension (defined as a reduction in systolic blood pressure of >20 mmHg when subject changes from a supine to a standing position) during the double-blind phase (incidence ranged from 9% for trandolapril 0.25mg and 4.0 mg to a high of 23% for placebo).

A32.7. Summary.

Black subjects with mild-to-moderate hypertension were randomized to placebo or fixed doses of trandolapril 0.25, 0.5, 1.0, 2.0, and 4.0 mg for 6 weeks, followed by a 3-week single-blind washout period. Statistically significant differences from baseline for the primary end point, supine diastolic blood pressure, without subtraction from placebo, were seen after one week and maintained throughout treatment for all the trandolapril treatment groups except for the 0.25 mg dose, whose results were inconsistent. The dose-response relationship was weak. The greatest effect on supine diastolic pressure at trough occurred with the 4.0 mg dose which produced a reduction of 4.4 mmHg (double difference from baseline and placebo) at week 6. The largest peak supine diastolic pressure effect occurred with the 2.0 mg dose: a reduction of 8.7 mmHg measured at endpoint. Only the 2.0 and 4.0 mg doses produced consistent statistically significant effects on trough standing and sitting diastolic blood pressure. Only the 4.0 mg dose produced consistent statistically significant effects on systolic blood pressure. There were no deaths in the randomized portion of the trial. Angioedema and transient ischemic attacks occurred as serious adverse events. Adverse events with possible association with treatment included upper respiratory infection, headache, first degree AV block, peripheral edema, anemia, and nausea.

A33. Study TR-21A-HTN (Study report: vols 1.181-1.198; case report tabulations: vols 1.316-1.326; case report forms for deaths and discontinuations for adverse events: vols 1.381-1.382) "Double-blind, randomized, placebo-controlled, five-way parallel dose-ranging study to evaluate the safety and efficacy of oral trandolapril therapy (4, 8, 12, and 16 mg/day) in Black patients with mild to moderate essential hypertension".

A33.1. Investigators The investigators are listed in Table 273 below.

Table 273. Investigators in Study TR-21A-HTN.

Investigator	Center	City	Investigator	Center	City
W Applegate, MD	2121	Memphis, TN	V Papademetriou, MD	2126	Washington, DC
J Gray, MD	2122	Houston, TX	CVS Ram, MD	2127	Dallas, TX
AK Jain, MD	2123	New Orleans, LA	RW Simpson, MD	2128	Denver, CO
P Jenkins, MD ^a	2124	Baltimore, MD	M Weir, MD	2129	Baltimore, MD
JH Levine, MD	2125	Nashville, TN	S Younkin, MD	2130	Spartanburg, SC

a. DSI was unable to verify any of the data from this center. The analyses in this review include the contribution of this center (4 subjects per group). The primary efficacy analysis was repeated without this center and the effect on the analysis was insignificant.

A33.2. Study dates February 1991 to February 1993.

A33.3. Drug formulation

Table 274. Drug substance used in Study TR-21A-HTN.

Lot	Form	Type	Trandolapril	Source lot	Batch size
TRA-580	Cap	Plcbo	—	L-32	1,200,000
TRA-0210	Cap	Plcbo	—	L-21	1,200,000
99-0190	Cap	Tran	4.0 mg	99-0190	31,595
99-0290	Cap	Tran	4.0 mg	99-0290	86,218
99-0391	Cap	Tran	4.0 mg	99-0391	85,963

Table 275. Capsule composition in Study TR-21A-HTN.

	4 mg
Trandolapril	4.0
Corn starch	
Lactose, hydrous	
Povidone	
Sodium stearyl fumarate	
Total	

A33.4. Study design

There was a 4-week single-blind, placebo lead-in phase, followed by a 6-week double blind treatment period, and a 3-week single-blind, placebo washout period. Forty subjects per treatment group were planned, 20 per center. Subjects were to be age ≥ 21 years, $< 30\%$ above ideal body weight, with supine diastolic pressure between 95 and 114 mmHg at the end of the placebo run-in phase. Women were permitted if neither childbearing nor lactating, and if practicing recognized forms of contraception. Exclusion criteria are listed in Table 276 below. Other excluded concomitant drugs were antiarrhythmics, digitalis, MAO inhibitors, psychotropic drugs, chronic NSAIDs, phenylpropanolamine, and pseudophedrine.

Table 276. Exclusion criteria in Study TR-21A-HTN.

Hypertension other than essential and mild-to-moderate
History of evidence of malignant, accelerated, or severe hypertension
Cerebrovascular accident, convulsions, or hypertensive encephalopathy within 1 year
Clinically significant congestive heart failure or EF<40%
Use of antihypertensives within 4 weeks of double-blind period or spironolactone within 6 weeks
Heart rate <50 bpm, significant arrhythmia, sinus node dysfunction, or conduction abnormalities
Myocardial infarction within 3 months
Hypersensitivity or serious adverse reaction to any ACE inhibitor
Clinically significant renal failure, e.g., creatinine >2 mg/dl
Clinically significant hepatic failure, e.g., SGOT or SGPT >2 times ULN
Significant electrolyte imbalance
Significant hematologic abnormalities
Medical or surgical conditions affecting absorption, metabolism, or excretion
Previous clinical trial experience within 30 days or any previous exposure to trandolapril
History of chronic alcohol or drug abuse within 2 years
Terminal illness
Blood donation within 1 month

Subjects who qualified at the end of the placebo run-in period were randomized to placebo or trandolapril 4, 8, 12, or 16 mg q.d. and returned after 1, 2, 4, and 6 weeks. All subjects took the same number of capsules. Subjects were instructed to take their study drug at 0900±1 hour, but the relationship to meals was not specified.

Safety data included medical history, physical exam, 12-lead ECG, and routine hematology, clinical chemistry, and urinalysis. There were no explicit criteria for dropping a subject because of lack of efficacy.

Blood samples were obtained for ACE activity, aldosterone, and renin activity levels at baseline and at the final double-blind visit. Trandolapril and trandolaprilat levels were obtained at two double-blind treatment period visits.

The primary analysis was an intent-to-treat analysis of supine diastolic pressure at the final double-blind visit. Secondary end points were sitting and standing diastolic pressure and percentage of responders (no criteria pre-specified). The study had 80% power to detect a 5 mmHg change from baseline with two-sided $\alpha=0.05$.

There was one unimplemented amendment to the protocol, calling for an interim analysis.

A33.5. Results

One hundred eighty-six subjects enrolled in the trial, of whom 153 completed the final washout period. Individual study centers enrolled 8 to 40 subjects. Demographic and baseline characteristics of subjects are shown in Table 277 below. The treatment groups were reasonably well matched for these factors, for general health, duration and severity of hypertension, and baseline drug use.

The disposition of subjects is shown in Table 278 below.

No subjects were excluded from efficacy or safety analyses because of protocol violations.

Table 277. Demographic and baseline characteristics of subjects in Study TR-21A-HTN.

	Placebo n=37	Trandolapril			
		1mg n=37	2mg n=38	4mg n=38	6mg n=36
Male	16	20	19	19	15
Female	21	17	19	19	21
Age±SD	53±10	54±11	55±12	53±14	54±12
Range	34 to 75	28 to 72	31 to 79	30 to 78	30 to 76
Caucasian	0	0	0	0	0
Black	37	37	38	38	36
Other	0	0	0	0	0
Weight±SD	189±33	185±33	182±28	175±25	179±29
Range	130 to 278	130 to 250	125 to 240	102 to 228	111 to 250

Table 278. Disposition of subjects in Study TR-21A-HTN.

	Randomized	Double-blind				Wash-out	
		Week 1	Week 2	Week 4	Week 6	Week 1	Week 3
Remaining in study	186	183	174	167	158	156	153
Dropouts		3	9	7	9	2	3
Adverse reaction		1	3	3	1	0	0
Lack of efficacy		0	3	4	8	2	0
Not meet entry criteria		1	0	0	0	0	0
Compliance		1	3	0	0	0	2
Other		0	0	0	0	0	1

An intent-to-treat approach was used to analyze the efficacy variables. All subjects with data for at least one post-baseline visit were included and the last measurements for dropouts were carried forward in the analyses.

The sponsor's analysis of change in supine diastolic pressure at trough (change from baseline and placebo) as a function of time in trial is shown in Figure 124 below.

The sponsor's analysis of blood pressure considered 'trough' as any measurement at least 10 hours after dosing; 'peak' was considered 4 to 5 hours after dosing. Few trough measurements were actually outside the interval 24 ± 2 hours, but this was the interval used in the analyses performed for this review.

According to the sponsor, the supine diastolic pressure was analyzed using analysis of covariance (ANCOVA) with treatment and center as factors and baseline supine diastolic pressure as a covariate. A significant treatment difference was found based on this ANCOVA model ($p=0.001$). This analysis also gave the estimated mean supine diastolic pressure for each treatment group, as shown in Table 279 below. No treatment-by-center and treatment-by-baseline interactions were found. Paired comparisons of adjusted mean supine diastolic blood pressures between each trandolapril group and the placebo group showed that the trandolapril therapy significantly lowered supine diastolic pressure at trough from baseline at end point for the dosing groups. Significant decreases from baseline in diastolic pressure at trough were observed for all treatment groups.

A similar ANCOVA model was used to analyze peak supine diastolic pressure at end point. The results are presented in Table 279. Again significant decreases from baseline in diastolic pressure were found. In this case, a significant treatment

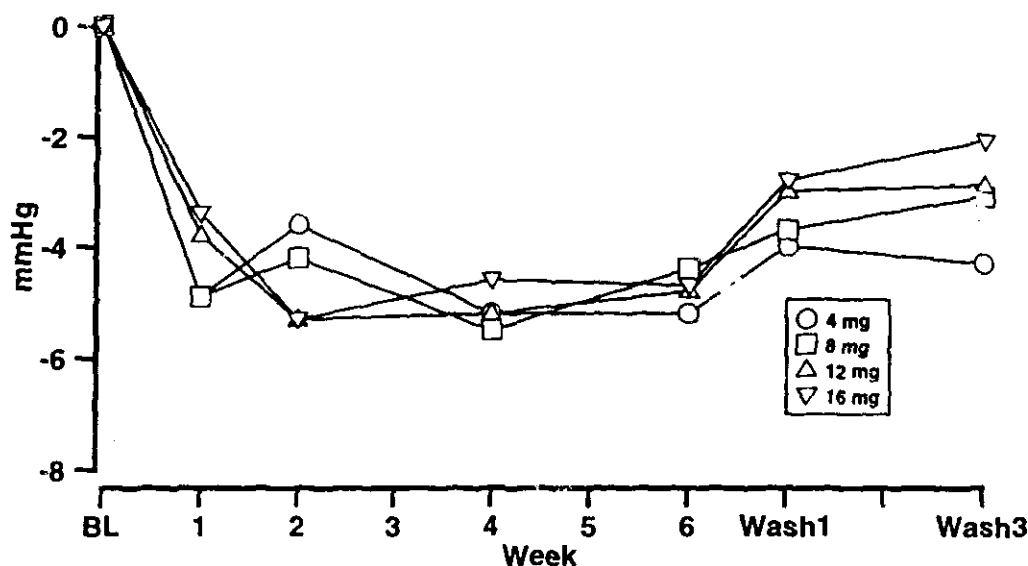


Figure 124. Change in supine diastolic pressure at trough by time in Study TR-21A-HTN.

Table 279. Diastolic pressure data in Study TR-21A-HTN^a.

	Placebo n=37	Trandolapril			
		4 mg n=37	8 mg n=38	12 mg n=37	16 mg n=36
Mean supine diastolic pressure at trough and end point					
Baseline	101.2	101.8	101.4	100.9	100.5
End point	101.5	94.9	96.6	95.4	95.5
Change from baseline and placebo	—	-6.6	-4.9	-6.1	-6.0
p-value		<0.001	0.006	<0.001	0.001
Mean supine diastolic pressure at peak and end point					
Baseline	97.7	97.6	96.8	98.0	97.1
End point	96.4	89.4	90.0	90.6	91.4
Change from baseline and placebo	—	-7.0	-6.4	-5.8	-5.0
p-value		0.001	0.002	0.006	0.017

a. Statistical reviewer's analysis, with adjustment for baseline and investigator.

difference was found using ANCOVA ($p=0.008$). No treatment-by-center or treatment-by-baseline interactions were found.

Analyses of sitting and standing diastolic pressure at trough at end point were done using ANCOVA models. A significant treatment difference was found for sitting and standing diastolic pressure at trough ($p=0.024$ for sitting and $p=0.003$ for standing diastolic pressure). Neither treatment-by-center nor treatment-by-baseline interactions were found.

Based on the estimates of mean diastolic pressure at trough and peak, the trough-to-peak ratios were obtained, as shown in Table 280 below.

The sponsor reasonably, but retrospectively, defined responders as subjects with supine diastolic pressure <90 mmHg or a ≥ 10 mmHg reduction at end point. The analysis of responders at end point using a χ^2 test showed significant difference in

Table 280. Supine diastolic pressure peak/trough ratio in Study TR-21A-HTN^a.

Trandolapril			
4 mg n=37	8 mg n=38	12 mg n=37	16 mg n=36
0.93	0.90	1.10	1.31

a. Statistical reviewer's analysis.

proportion of responders among the treatment groups ($p < 0.01$). The proportions of responders for all dosing groups were significantly higher than that for the placebo group (38% for 4 mg, 24% for 8 mg, 38% for 12 mg and 33% for 16 mg, vs. 5% for the placebo).

Quadratic regression on dose was done by the statistical reviewer. In the model, the dependent variable was change in supine diastolic pressure at trough at end point and the regression terms included dose, dose², and baseline. The estimated response curve was

$$\Delta SupDP = -0.799 - 1.053 \times Dose + 0.047 \times Dose^2$$

where the estimated coefficients for dose and dose² were significantly different from zero ($p = 0.005$ for dose and $p = 0.035$ for dose²), indicating a strong quadratic dose-response relationship.

The duration of treatment effect was determined by comparing trough blood pressure measurements obtained during the placebo washout weeks 1 and 3 to those at baseline and at the last double-blind visit (week 6). Significant decreases from baseline in supine diastolic pressure were still seen for all dosing groups at washout week 1 but only for the 4 mg group at washout week 3, as shown in Table 281 below. Significant

Table 281. Duration of treatment effect in Study TR-21A-HTN^a.

	Placebo n=37	Trandolapril			
		4 mg n=37	8 mg n=38	12 mg n=37	16 mg n=36
Mean change from baseline					
Washout week 1	-0.3	-4.3†	-5.0†	-3.3†	-3.1†
Washout week 3	1.1	-3.2*	-2.0	-1.8	-1.0
Mean change from end of double-blind treatment period					
Washout week 1	0.7	2.5	2.7*	3.7†	3.2*
Washout week 3	2.0	3.7†	4.8†	5.2†	5.5†

a. Statistical reviewer's analysis. *Significantly different from placebo, $p < 0.05$. †Significantly different from placebo, $p < 0.01$.

increases in supine diastolic pressure from the corresponding measurements at week 6 were found for all dosing groups at washout weeks 1 and 3, except for the 4 mg group at washout week 1. However no significant difference was found among treatment groups (ANOVA).

Changes in sitting and standing diastolic pressure are shown in Table 282 below. Changes in sitting and standing diastolic pressure were slightly smaller than changes

Table 282. Changes in trough sitting and standing diastolic pressure in Study TR-21A-HTN^a.

	Sitting				Standing			
	4 mg n=37	8 mg n=38	12 mg n=38	16 mg n=36	4 mg n=37	8 mg n=38	12 mg n=38	16 mg n=36
Baseline	101.2	101.0	100.3	100.7	103.4	103.1	103.5	104.1
Week 1	-2.6	-3.4	-3.1	-4.2	-2.7	-3.1	-3.9	-3.2
Week 2	-1.9	-3.1	-4.3	-2.9	-3.1	-3.2	-5.9	-5.0
Week 4	-5.1	-4.2	-5.4	-4.8	-4.7	-3.8	-4.6	-4.4
Week 6	-4.4	-3.8	-4.1	-3.7	-4.7	-3.5	-5.9	-5.1
End point	-4.3	-3.3	-5.0	-4.5	-4.1	-3.5	-6.7	-5.8
Washout 1	-1.9	-3.0	-3.0	-2.3	-3.9	-4.0	-4.6	-4.2
Washout 3	-3.5	-2.9	-1.9	-1.6	-3.8	-3.1	-3.7	-3.1

a. The sponsor's analysis of group means, with adjustment for baseline and investigator.

in supine diastolic pressure. Changes in systolic pressure (group mean double-differences from baseline and placebo) at end point are summarized in Table 283 below.

Table 283. Changes in trough systolic pressure in Study TR-21A-HTN^a.

	4 mg n=37	8 mg n=38	12 mg n=38	16 mg n=36
Supine	-6.3	-4.4	-5.7	-7.1
Sitting	-3.5	-2.9	-3.5	-4.4
Standing	-5.9	-5.7	-5.4	-8.0

a. The sponsor's analysis, with adjustment for baseline and investigator.

Pulse rate changes from baseline to end point were not statistically significant for any treatment group.

Pharmacokinetic data were obtained only once at trough and 4 to 5 hours after dosing. Trandolapril and trandolaprilat are both present at low levels at trough. Post-dosing, trandolaprilat levels are some 9- to 10-fold higher than trandolapril levels.

Renin, aldosterone, and ACE activity are shown in Table 284 below. There is not much of a dose-response relationship evident. Renin levels increased and aldosterone levels decreased slightly in the active treatment groups. ACE inhibition was about 85 to 90% in all actively treated groups.

A33.6. Safety.

Fifty-eight of 186 subjects reported at least one adverse reaction. These adverse events are summarized in Table 285 below. Counts of events were too low to discern any relationship to dose. Subjects who discontinued because of adverse reactions are listed in Table 286 below.

Twenty-nine subjects were deemed to have had orthostatic hypotension, defined as a >20 mmHg reduction in systolic pressure in moving from a supine to standing position; the incidence of orthostatic hypotension was 19% on placebo, 22% on 4 mg, 29% on 8 mg, 19% on 12 mg, and 17% on 16 mg. The incidence was 11% during the washout phase.

Table 284. Renin, aldosterone, and ACE activity in Study TR-21A-HTN.

	Plcbo n=28	4 mg n=31	8 mg n=32	16 mg n=33
Renin (ng/ml/h)				
Baseline	1.0	0.9	1.0	0.5
End point	0.6	4.1	2.9	2.5
Change	-0.5	3.2	1.9	2.0
Aldosterone (ng/dl)				
Baseline	7.8	7.3	10.3	10.2
End point	9.3	7.1	7.4	7.9
Change	1.5	0.1	-2.9	-2.3
ACE activity (U/L)				
Baseline	27.1	26.2	33.2	37.2
End point	25.8	3.4	3.1	4.5
Change	-1.4	-22.4	-30.2	-26.1

Table 285. Adverse events (%) in Study TR-21A-HTN.

	Plcbo n=37	Tran n=149	Events reported by ≥2 Trandolapril subjects	
Any	15 (41)	52 (35)	Edema	5
Cardiovascular	3 (8)	5 (3)	Nausea	2
CNS	4 (11)	10 (7)	Dizziness	4
Dermatologic	0	5 (3)	Vomiting	4
EENT	5 (14)	17 (11)	Headache	6
Gastrointestinal	2 (5)	11 (7)	Chest pain	2
General	3 (8)	8 (5)	Fatigue	3
Genitourinary	0	3 (2)	Rash	4
Metab/endoc	0	2	Influenza	2
Musculoskeletal	2 (5)	12 (8)	URI symptoms	6
Pulmonary	0	10 (7)	Elev SGPT	2
			Abdominal pain	3
			Arthritis	2
			Constipation	2
			Back pain ^a	5
			Diarrhea	4
			Cough ^b	9
			Dyspepsia	4

a. Vs. 1 in placebo group.

b. Vs. 0 in placebo group.

Table 286. Discontinuations for adverse events in Study TR-21A-HTN.

Center	Subj	Group	Day	ADR
2121	5	Plcbo	34	Epistaxis, severe headache
2121	13	Plcbo	15	Palpitations
2130	5	Plcbo	5	Headache, dizziness
2125	17	4 mg	43	42 year old Black female with 12 year history of hypertension. Baseline supine BP was 175/100 mmHg. Terminated for lightheadedness and fundoscopic changes, both described as mild. Final supine BP was 178/114 mmHg.
2123	37	8 mg	6	58 year old Black female with 16 year history of hypertension. Subject was discontinued for epigastric & RUQ pain, thought to be cholecystitis, but with negative ultrasound. Event resolved on Ceclor.

Table 286. Discontinuations for adverse events in Study TR-21A-HTN. (Continued)

Center	Subj	Group	Day	ADR
2125	11	8 mg	37	63 year old Black female with 30 year history of hypertension. Baseline supine BP was 168/86 mmHg. Subject had multiple complaints during study, including joint pain, headaches, and bilateral pedal edema. She terminated because of dizziness, described as severe but not serious. At the last study visit, supine BP was 190/114 mmHg.
2130	2	8 mg	22	46 year old Black female with diabetes mellitus and 22 year history of hypertension. Baseline supine BP was 190/110 mmHg. Subject was discontinued because of right pedal edema and S3 gallop, neither considered serious.
2126	3	12 mg	12	60 year old Black male with 6 year history of hypertension. No other cardiovascular problems by history or screening. Supine BP at baseline was 178/104 mmHg. Subject discontinued with atypical chest pain, described as moderate and serious. He underwent cardiac catheterization and angioplasty.
2123	27	16 mg	7	41 year old Black male with 17 year history of hypertension. Mild lymphocytosis (50%) and ↑SGOT, ↑SGPT (<2 x ULN) at baseline. Enzymes essentially unchanged at end of run-in, but ~3 x ULN at 7 days. Enzymes near baseline at follow-up 2 weeks after termination. Subject "[a]dmitted to having been drinking heavily".
2125	5	16 mg	21	40 year old Black male with 15 year history of hypertension. Discontinued with 'mild' prickling, itching sensation, and treated with benadryl. No subsequent follow-up.

Laboratory data are summarized in Figure 125 to Figure 128 below.

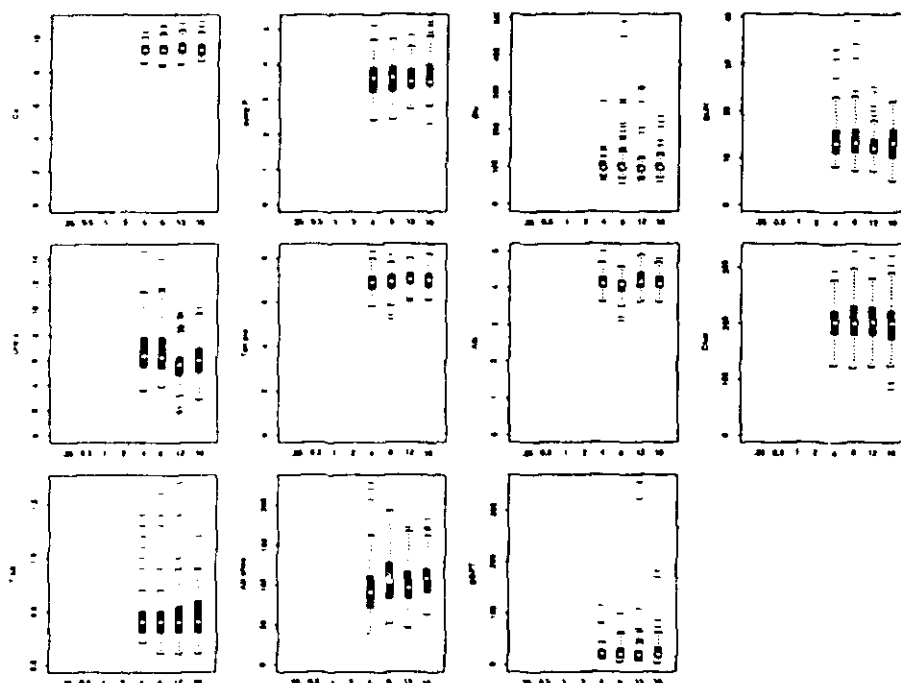


Figure 125. Clinical chemistry data (part A) in Study TR-21A-HTN.

There were a variety of new findings on the final physical exam and ECG; none were serious or had apparent relationship to the study drug.

A33.7. Summary.

Black subjects with mild-to-moderate hypertension were randomized to placebo or fixed doses of trandolapril 4, 8, 12, or 16 mg q.d. for 6 weeks, followed by a 3-week single-blind washout period. Statistically significant differences between placebo and the active treatment groups in the primary end point—supine diastolic pressure—appeared at 1 week and were maintained to the final double-blind visit. No differences were apparent among the active treatment groups. The magnitude of effect is about 6 mmHg at 'trough' and 8 mmHg at 'peak'; however, the sponsor's statistical analysis is flawed by considering all measurements ≥10 hours after dosing as 'trough'. Consistent and statistically significant differences between placebo and active treatment groups were also found for secondary end points of sitting and standing

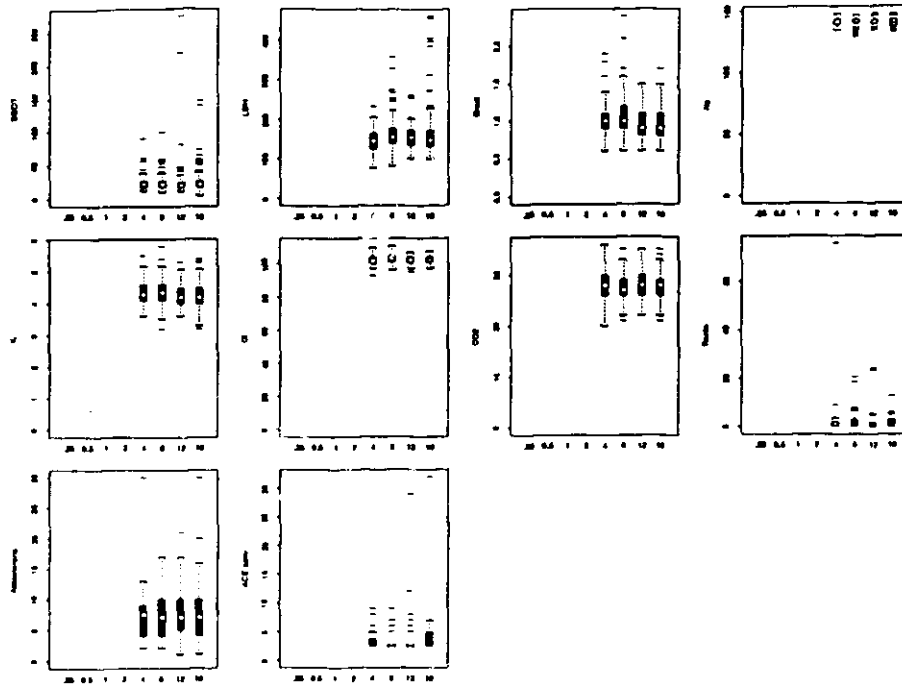


Figure 126. Clinical chemistry (part B) in Study TR-21A-HTN.

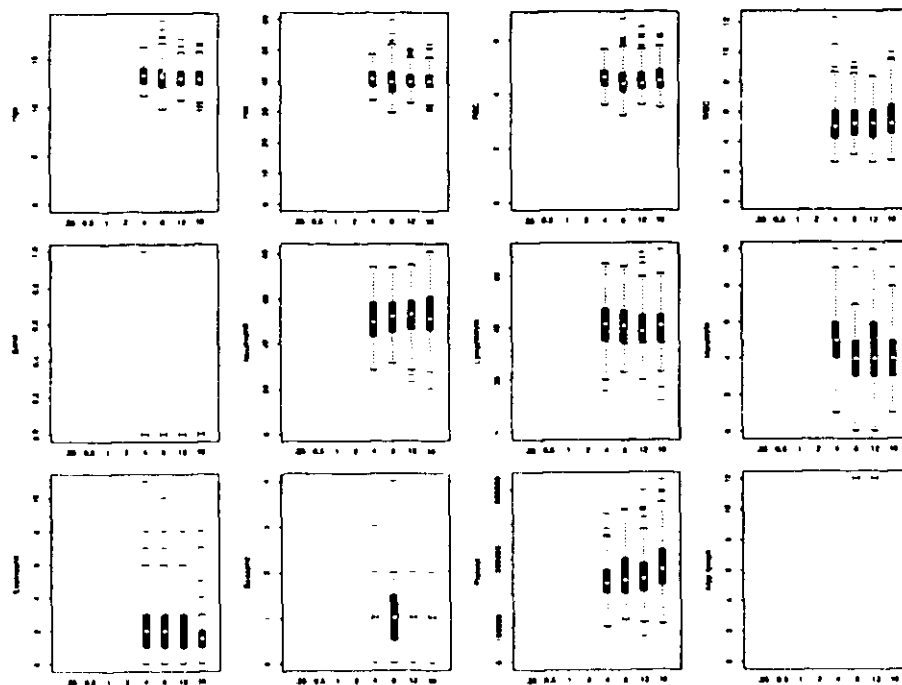


Figure 127. Hematology data in Study TR-21A-HTN.

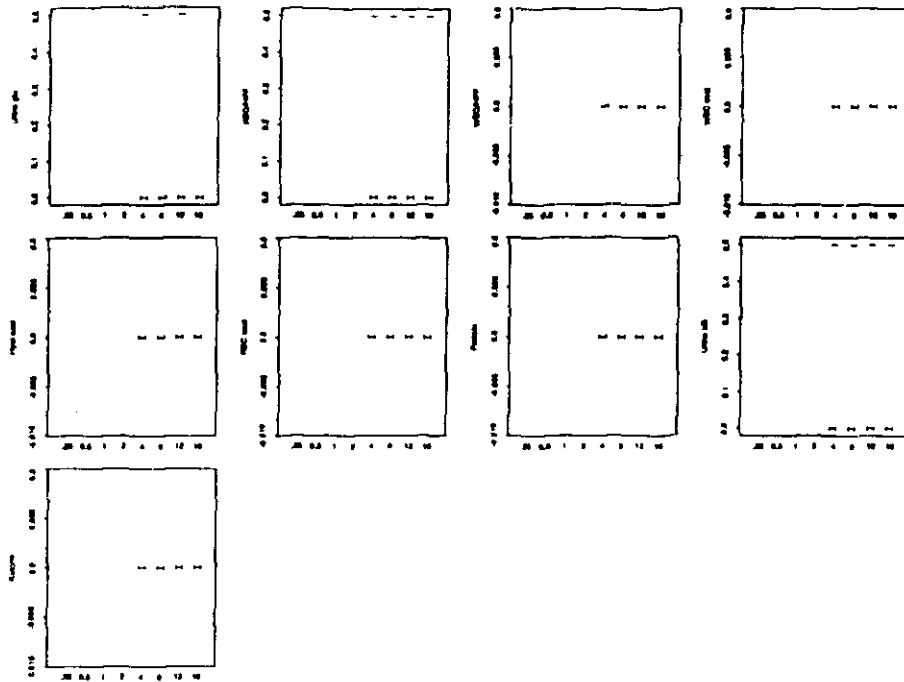


Figure 128. Urinalysis data in Study TR-21A-HTN.

diastolic pressure, systolic pressure, and response rate. There were no deaths or serious adverse events. Adverse events with an apparent association with treatment included cough, back pain, gastrointestinal symptoms (nausea, vomiting, diarrhea), rash, and pruritus.

..t.

A34. Study TR-22-HTN (Study report: vol 6.2; case report tabulations: vols 6.2-6.10, 6-12-6.20; case report forms for deaths and discontinuations for adverse events: vol 6.11) "Double-blind, randomized, placebo-controlled, parallel study to evaluate the safety and efficacy of oral trandolapril once-a-day vs. twice-a-day therapy in patients with mild to moderate essential hypertension".

A34.1. Investigators The investigators are listed in Table 287 below.

Table 287. Investigators in Study TR-22-HTN.

Investigator	Center	City	Investigator	Center	City
L Horwitz	2203	Denver, CO	S Rosenblatt	2207	Irvine, CA
P Kaihlanen	2204	San Antonio, TX	H Schnaper	2201	Birmingham, AL
J Levine	2205	Nashville, TN	H Serfer	2202	Hollywood, CA
A Lewin	2210	Los Angeles, CA	W Stein	2208	Rochester, NY
S Lewis	2206	Tampa, FL	M Strauss	2209	Little Rock, AK

A34.2. Study dates April 1992 to January 1993.

A34.3. Drug formulation

Table 288. Drug substance used in Study TR-22-HTN.

Lot	Form	Type	Trandolapril	Source lot	Batch size
TRA-0252	Cap	Plcbo	--	L-37	6,000,000
TRA-0082	Cap	Plcbo	--	L-37	6,000,000
TRA-0120A	Cap	Tran	2.0 mg	L-11	600,000
99-0391	Cap	Tran	4.0 mg	99-0391	85,963

Table 289. Capsule composition in Study TR-22-HTN.

	2 mg	4 mg
Trandolapril	2.0	4.0
Corn starch		
Lactose, hydrous		
Povidone		
Sodium stearyl fumarate		
Total		

A34.4. Study design

This placebo-controlled, double-blind, parallel-group study randomized 199 adults with mild-to-moderate essential hypertension to 6 weeks of oral treatment with placebo twice-daily, 2 mg trandolapril twice-daily, or 4 mg trandolapril once-daily (given as an active morning dose, and a placebo evening dose). A 4-week single-blind placebo run-in preceded randomized therapy. The objective was to assess comparative pharmacokinetics, efficacy, and safety.

Enrolled subjects had reproducible evidence of supine diastolic blood pressure ≥ 95 and ≤ 114 mmHg during the placebo run-in.

The exclusion criteria are shown in Table 290 below.

The primary efficacy end point was the change from baseline (end of run-in) supine diastolic blood pressure at trough (defined as 24 vs. 12 hours post-dose for the once-

Table 290. Exclusion criteria in Study TR-22-HTN.

Pregnant or lactating women, or women of childbearing potential not using contraception
Malignant or secondary hypertension
Cerebrovascular accident, seizure or hypertensive encephalopathy within 1 year
Myocardial infarction during the previous 3 months
Clinically significant congestive heart failure
Major cardiac rhythm disorders or conduction disturbances
Presence of hypersensitivity, diseases of the gastrointestinal, or hematologic systems, or clinically significant renal or hepatic insufficiency
History of angioneurotic edema
Blood donation during the previous month
Serum potassium <3.0 or >5.5 mEq/L
Serum sodium <135 mEq/L
History of non-medicinal drug use (including chronic ethanol use)
Use within one month of antihypertensives, antiarrhythmics, digitalis, psychotropics, chronic NSAIDs or other analgesics, decongestants, or bronchodilators
Use within 2 weeks of diuretics, ACE inhibitors, vasodilators, or anxiolytics

vs. twice-daily groups, respectively) during the last double-blind visit. Blood pressure and heart rate measures were obtained at weeks 1, 2, 4, and 6 of the double-blind period.

Other efficacy variables were supine diastolic pressure at peak, supine and standing diastolic pressure at peak and trough, supine sitting, and standing systolic pressure at peak and trough, and responder rate (supine diastolic pressure at trough <90 mmHg or reduction by ≥ 10 mmHg from baseline).

Blood samples for the determination of parent drug and metabolite levels were collected at baseline, and during double-blind week 6 (at trough, and at 4 to 5 hours post-dose). Laboratory evaluations were obtained at baseline, and at double-blind week 6. Physical examinations were obtained just prior to the run-in, and at double-blind week 6.

The primary efficacy analysis was based on the intent-to-treat principle. Pre-specified was a parametric model which included baseline, study center, and treatment. The study was reportedly powered to detect a 4.3 mmHg treatment difference in diastolic blood pressure at a β level of 0.85 and an α level of 0.05, assuming a mean square error of 50. Dropouts were included in the analyses of those time points for which the data were not missing. No interim analyses were performed.

Pharmacokinetic assays were assessed as shown in Table 291 below. The assays have

Table 291. Pharmacokinetic assays in Study TR-22-HTN.

Linearity	Accuracy	Precision	Sensitivity	Specificity
Plasma samples—trandolapril and trandolaprilat—				
Urine samples—trandolaprilat—				
Plasma samples—ACE inhibition—				

been validated over the range of plasma and urine concentrations observed in the study.

A34.5. Results

Demographic and baseline characteristics of subjects are shown in Table 292 below.

Table 292. Demographic and baseline characteristics of subjects in Study TR-22-HTN.

	Placebo n=68	Trandolapril	
		4 mg q.d n=66	2 mg bid n=65
Male	40	32	39
Female	28	34	26
Age mean	53.2	50.3	50.7
Caucasian	58	52	58
Black	9	13	5
Oriental	1	0	1
Hispanic	0	1	1
Weight mean	83.8	83.2	84.5
Baseline supine diastolic pressure	100.4	101.7	99.5

There was an excess of Black subjects in the 4 mg once-daily group (19.7%) compared to the 2 mg twice-daily active group (7.7%), and the placebo group (13.2%). Baseline mean supine diastolic pressure was slightly, but statistically distinguishably ($p=0.033$), higher in the once-daily trandolapril group (101.7 mmHg) than in the placebo (100.4 mmHg) or twice-daily trandolapril group (99.5 mmHg). Nine subjects had baseline supine diastolic pressures lower than the protocol's criterion for enrollment; how these were distributed among treatment groups was not specified.

The disposition of subjects is shown as a function of dose in Table 293 below, and by time in Table 294 below. There was a much higher withdrawal rate in the once-daily treatment group than in the twice-daily group. Some of this was a late-appearing excess of dropouts for inefficacy in the once-daily group (6.1%) relative to the twice-daily group (1.5%). This affects the interpretation of the time course of antihypertensive effects shown below.

Mean supine blood pressure effects are shown in Figure 129 below and Table 295 below. According to the sponsor, most trough blood pressure measurements were made at about the interdosing interval for the regimen. The sponsor analyzed trough

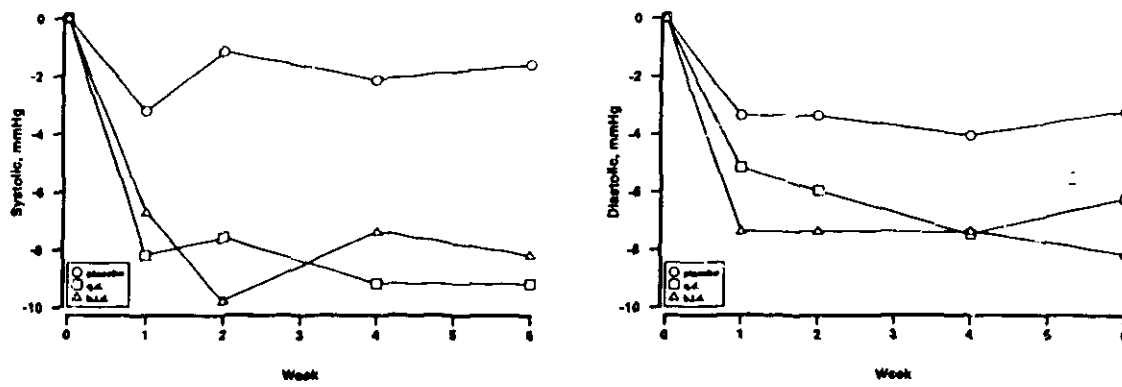


Figure 129. Blood pressure effects in Study TR-22-HTN.

Table 293. Disposition of subjects by dose in Study TR-22-HTN.

	Placebo n=68	Trandolapril	
		4 mg q.d. n=66	2 mg bid n=65
Randomized	68	66	65
Withdrawn	8	11	1
Adverse events	2	2	0
Lack of efficacy	4	4	1
Failure to follow schedule	2	4	0
Unrelated medical event	0	1 ^a	0
Completed	60	55	64

a. Underwent hysterectomy

Table 294. Disposition of subjects by time in Study TR-22-HTN.

	Randomized	Double blind period week			
		1	2	4	6
Remaining in study	199	198	194	183	179
Withdrawn		1	4	11	4
Adverse events		0	1	3	0
Lack of efficacy		0	2	7	0
Failure to follow schedule		1	1	0	4
Unrelated medical event		0	0	1	0

Table 295. Supine diastolic blood pressure changes in Study TR-22-HTN.

	Placebo n=68	Trandolapril	
		4 mg q.d. n=66	2 mg bid n=65
Baseline	100.4	101.7	99.5
Change from placebo ^a			
Peak		-4.7*	-5.0*
Trough		-3.5*	-6.0*

a. Sponsor's analysis confirmed by statistical reviewer, with adjustment for baseline and center effects; *p<0.01.

supine diastolic pressure using analysis of covariance (ANCOVA) with treatment and center as factors and baseline supine diastolic pressure at trough as a covariate. A significant treatment-by-center interaction was found at the $\alpha=0.10$ level ($p=0.075$). As part of the statistical review, the mean change from baseline was compared by centers and it was found that center #2208 contributed most to the interaction, where the estimated mean reduction from baseline in supine diastolic pressure at trough was smallest for once-daily treatment (1.7 mmHg) compared with placebo (5.5 mmHg) or twice-daily (14.9 mmHg). The interaction was significantly reduced after elimination of this center ($p>0.25$). Since the interaction does not seem essential, the sponsor's analysis, based on a model including only terms for treatment, center, and baseline diastolic pressure, is reasonable. This analysis showed a statistically significant treatment effect ($p<0.001$). Paired comparisons showed statistically significant differences in mean change from baseline in trough supine diastolic pressure, adjusted for baseline and center effects, between the placebo and either active treatment group. The difference between once- and twice daily treatment was not statistically significant ($p=0.062$). However, with the observed mean difference and population standard deviation, the power to distinguish these groups was only 0.6, considerably lower than the sponsor's planned power of 0.85. The 95% and 90% confidence intervals for the difference between treatment groups (once-daily minus twice-daily) were (-5.24, 0.16) and (-4.76, -0.32), respectively. It is, therefore, more likely that twice-daily dosing is somewhat superior to once-daily dosing than that they are equivalent.

ANCOVA was used to analyze supine diastolic pressure at peak (4 to 5 hours after dosing) at end point. There was a statistically significant treatment-by-center interaction ($p=0.016$), but the results of ANCOVA without this interaction term were similar, so the ANCOVA model with only baseline, treatment, and center as terms was used to estimate the mean supine diastolic pressure at peak and end point. The p-value for testing for differences among treatment groups was <0.001 . Paired comparisons showed statistically significant differences in mean change from baseline in peak supine diastolic pressure between the placebo and either active treatment group ($p<0.001$). The difference between once- and twice daily treatment was not statistically significant ($p=0.817$).

Mean sitting blood pressure effects are shown in Table 296 below. Analyses of sitting

Table 296. Sitting diastolic blood pressure changes at trough in Study TR-22-HTN.

	Placebo n=68	Trandolapril	
		4 mg q.d. n=66	2 mg bid n=63
Baseline	100.0	101.0	98.9
Change from placebo ^a		-3.9*	-6.0*

a. Sponsor's analysis confirmed by statistical reviewer, with adjustment for baseline and center effects; * $p<0.01$.

and standing diastolic pressure at trough and end point were done using ANCOVA. A significant treatment difference was found for trough standing diastolic pressure ($p=0.001$), and there were no significant treatment-by-center or treatment-by-baseline interactions. For trough sitting diastolic pressure at end point, there was a significant treatment-by-center interaction ($p=0.038$). The mean changes from baseline in either sitting or standing diastolic pressure were not statistically significantly different between the active treatment groups.

Estimated trough-to-peak ratios were 0.68 for once-daily dosing and 1.23 for twice-daily dosing.

Analysis of responders at end point was by χ^2 test, and it showed a significant difference among treatment groups ($p=0.015$). The proportion of responders was higher in the active treatment groups (42% on once-daily, 46% on twice-daily) than in the placebo group (24%), but the difference between active treatment groups was not statistically significant ($p=0.67$).

The sponsor's analysis showed the mean diastolic changes from baseline for each active group to be statistically distinguishable from placebo at end point (at trough). At end point, the twice-daily regimen tended to have a greater effect at the interdosing interval than did once-daily trandolapril (-6.0 vs. -3.5 mmHg, respectively; $p=0.06$). Mean peak reductions in supine diastolic pressure were approximately 5 mmHg for each active group at end point. Mean trough systolic pressure reductions at end point were modest (approximately 5 to 9 mmHg, placebo-corrected).

A retrospective analysis of antihypertensive response as a function of sex was performed by the sponsor, as depicted in Table 297 below. Based upon the sponsor's

Table 297. Supine diastolic blood pressure changes by sex in Study TR-22-HTN.

	4 mg q.d.		2 mg bid	
	Male n=32	Female n=34	Male n=39	Female n=26
Baseline	101.7	101.7	99.0	100.3
Change from placebo ^a	-4.9	-1.8	-6.7	-5.6

a. At trough, by sponsor's analysis, with adjustment for baseline and center effects.

analysis, all but the once-daily female group manifested statistically significant ($p < 0.05$) blood pressure reductions at end point. In each sex there was a tendency for twice-daily trandolapril to have greater efficacy at the interdosing interval than did once-daily treatment, and this approached statistical significance in females ($p=0.06$, sponsor's analysis). The role of other demographic covariates was not assessed by the sponsor since, for example, only small numbers of elderly (≥ 65 years old) subjects ($n=13$), and Black subjects ($n=27$) received any double-blind trandolapril.

Heart rate changes from baseline were not statistically significant.

A34.6. Safety.

There were reportedly no deaths. Among trandolapril-exposed subjects, there were 2 discontinuations for adverse events. Case report forms for these discontinuations were evaluated and the pertinent findings are summarized in Table 298 below.

Table 298. Discontinuations for adverse events in Study TR-22-HTN.

Center	Subj	Group	Day	ADR
2206	11	q.d.	20	33 year old Caucasian female reportedly experienced "moderate" hypotension after 20 days treatment. She discontinued herself from the study. Dropout-related blood pressures were not provided in the CRF.
2207	13	q.d.	3	53 year old Caucasian male initiated the dropout after experiencing reduced libido on treatment day 3, and subsequent headache.

One subject receiving trandolapril 2 mg b.i.d. (2205-9) was reported to have systolic hypotension (her supine pressure had fallen to an unspecified level below 100 mmHg), although this did not result in withdrawal from the study.

Orthostatic hypotension, although reported at a non-zero rate, was not appreciably more frequent in the active groups (14 to 19%) than it was in the placebo group (18%).

One 68 year old placebo-treated male (2204-19) with a history of diabetes experienced an acute cerebral vascular accident on day 20. He developed right hemiparesis, and 6 weeks later had a fatal gastrointestinal bleed.

Adverse events with $n > 1$ among actively treated subjects are shown in Table 299 below. For several adverse events (joint pain, chest pain, asthma, edema, and upper

Table 299. Adverse events ($n > 1$) in Study TR-22-HTN.

	Placebo n=58	Trandolapril			Placebo n=60	Trandolapril	
		4 mg q.d. n=60	2 mg bid n=65			4 mg q.d. n=60	2 mg bid n=65
Headache	6 (8.8%)	8 (12.1%)	4 (6.1%)	Urinary tract infection	0	2 (3.0)	1 (1.5)
Throat inflammation	2 (2.9)	3 (4.5)	2 (3.0)	Upper respiratory tract congestion	1 (1.5)	0	3 (4.5)
Cough	2 (2.9)	1 (1.5)	3 (4.5)	Rash	2 (2.9)	0	2 (3.0)
Dyspepsia	0	1 (1.5)	2 (3.0)	Joint pain	1 (1.5)	1 (1.5)	1 (1.5)
Bradycardia	0	1 (1.5)	2 (3.0)	Chest pain	2 (2.9)	1 (1.5)	1 (1.5)

respiratory infection), the reported rates among actively treated subjects did not exceed those found in the placebo group.

Not all laboratory findings outside of the normal range were presented in line listings, but rather only those falling outside of arbitrarily defined thresholds of abnormality (generally defined, depending on the laboratory parameter, as a 5-25% difference from the normal limit¹). For example, subject 2206-18 had a slight, treatment-emergent reduction in white count to 3.6 K/ μ L, but was not identifiable on the line listings of suprathreshold abnormalities. Noteworthy among the treatment-emergent laboratory abnormalities which were greater than these thresholds were:

Two of 61 subjects on once-daily trandolapril (subject 2201-3, a 63 year old Black female, and subject 2204-9, a 54 year old Caucasian male) developed high proportions of atypical lymphocytes (9-15%).

One trandolapril subject (2208-12) had apparent evidence of hypoglycemia (37 mg/dL) which the investigator attributed to laboratory error.

Three trandolapril subjects reportedly had 4 incidents of SGPT elevation. Nonetheless, the overall rate of this abnormality was higher in the placebo group (4.9 vs. 3.4%, respectively). In one actively treated subject (2202-14) this was reportedly associated with concurrent cholelithiasis and cholecystitis. A 56 year old female (2202-3) had an SGPT of 85 U/L noted on day 40 of exposure to the 4 mg q.d. dose. Another trandolapril subject (2208-17) was noted to have a mild SGPT elevation (to 70 U/L) on day 42 of treatment with 4 mg once-daily.

An LDH elevation (to 313 U/L) was observed in a 41 year old male (subject 2203-18) at the end of 46 days exposure to trandolapril 4 mg q.d. This reportedly resolved when drug was discontinued at the end of the trial.

Subject 2208-6 had a transient reduction of platelet count to 99 K/ mm^3 after 5 weeks of active treatment with once-daily dosing.

A.34.7. Summary.

This placebo-controlled, double-blind, parallel-group study randomized 199 adults with mild-to-moderate essential hypertension to 6 weeks of oral treatment with

¹ See page 117 of volume 6 5

placebo, or 4 mg trandolapril (given either once-daily, or as equally divided twice-daily doses) subsequent to a 4 week placebo run-in. The sponsor's analyses showed the mean 'trough' diastolic pressure changes from baseline for each active group (-6.0 vs. -3.9 mmHg for twice-daily vs. once-daily trandolapril, respectively) to be statistically distinguishable from placebo at end point. The apparently greater effect of the twice-daily regimen approached statistical significance ($p=0.06$). There were 2 withdrawals for adverse events (one for hypotension, and the other for loss of libido and headache), and several adverse events reported at rates exceeding those in the placebo group. The sponsor only presented laboratory abnormalities which exceeded arbitrarily defined thresholds. There were several such treatment-emergent abnormalities (including 3 cases of atypical lymphocytosis, 1 case of elevated LDH, and 1 case of transiently low platelet count).

d.

A35. Study TR-30-HTN (Study report: vols 1.251-1.259; case report tabulations: vols 1.345-1.362A; case report forms for deaths and discontinuations for adverse events: vols 1.387-1.390) "Open-label study of the long-term safety of oral trandolapril therapy in patients with mild to moderate essential hypertension".

A35.1. Investigators The investigators are listed in Table 300 below.

Table 300. Investigators in Study TR-30-HTN.

Investigator	Center	City	Investigator	Center	City
J Allen, MD	3005	Los Angeles, CA	R Paster, MD	3001	Oregon, WI
P Barden, MD ^a	3004	Albuquerque, NM	W Pleskow, MD	3007	Encinitas, CA
B Beller, MD	3013	San Antonio, TX	CVS Ram, MD	3019	Dallas, TX
JR Benz, MD	3016	Cedar Rapids, IA	R Reeves, MD	3010	Olympia, WA
R Goldstein, MD	3006	Tampa, FL	S Savran, MD	3011	Las Vegas, NV
J Gray, MD	3002	Houston, TX	H Schnaper, MD	3009	Birmingham, AL
PK Kaihlanen, MD	3003	San Antonio, TX	D Shaw, MD	3008	San Diego, CA
D Kelley, MD	3012	Olympia, WA	WJ Stein, MD	3014	Rochester, NY
JH Levine, MD	3017	Nashville, TN	M Weir, MD	3015	Baltimore, MD

a. Succeeded by F Snyder, MD

A35.2. Study dates February 1990 to March 1992.

A35.3. Drug formulation

Table 301. Drug substance used in Study TR-30-HTN.

Lot	Form	Type	Trandolapril	Source lot	Batch size
TRA-580	Cap	Plcbo	—	L-32	200,000
MB22633-172	Cap	Tran	0.25 mg	MB22633-172	20,000
MB22633-145	Cap	Tran	0.5 mg	MB22633-145	20,000
L-12	Cap	Tran	1.0 mg	L-12	546,100
TRA-0140A	Cap	Tran	1.0 mg	L-12	546,100
L-10	Cap	Tran	2.0 mg	L-10	583,100
TRA-0120A	Cap	Tran	2.0 mg	L-11	600,000
MB22327-163	Cap	Tran	2.0 mg	MB22327-163	600,000

Table 302. Capsule composition in Study TR-30-HTN.

	0.25 mg	0.5 mg	1 mg	2 mg
Trandolapril	0.25	0.5	1.0	2.0
Corn starch				
Lactose, hydrous				
Povidone				
Sodium stearyl fumarate				
Total				

A35.4. Study design Eighteen centers participated in this one-year, open-label study. Each was to enroll between 20 and 40 subjects. These subjects could have completed a previous short-term study or start receiving the study drug under this protocol. Subjects were to be age ≥21 years. Women were permitted if neither childbearing nor lactating, and if

practicing recognized forms of contraception. Exclusion criteria are listed in Table 303 below. Other excluded concomitant drugs were antiarrhythmics, digitalis, MAO

Table 303. Exclusion criteria in Study TR-30-HTN.

Hypertension other than essential and mild-to-moderate
History of evidence of malignant, accelerated, or severe hypertension
Cerebrovascular accident, convulsions, or hypertensive encephalopathy within 1 year
Clinically significant congestive heart failure or EF<40%
Use of antihypertensives within 4 weeks of double-blind period or spironolactone within 6 weeks
Heart rate <50 bpm, significant arrhythmia, sinus node dysfunction, or conduction abnormalities
Myocardial infarction within 3 months
Hypersensitivity or serious adverse reaction to any ACE inhibitor
Clinically significant renal failure, e.g., creatinine >2 mg/dl
Clinically significant hepatic failure, e.g., SGOT or SGPT >2 times ULN
Significant electrolyte imbalance
Significant hematologic abnormalities
Medical or surgical conditions affecting absorption, metabolism, or excretion
Previous clinical trial experience within 30 days, except short-term exposure to trandolapril
History of chronic alcohol or drug abuse within 2 years
Terminal illness
Blood donation within 1 month

inhibitors, psychotropic drugs, chronic NSAIDs, phenylpropanolamine, and pseudephedrine.

Subjects were to have their dose titrated as needed from 1 to 2 to 4 mg q.d. The titration interval was to 1 month or longer. Subjects who did not have adequate blood pressure control on trandolapril 4 mg q.d. were to have added Isoptin SR (verapamil) 180 mg q.d. If blood pressure were not adequately controlled for 1 month on the combination, then the subject was to be discontinued.

Follow-up visits were scheduled for 1, 2, 3, 4, 6, 9, and 12 months. The 4-month visit was only for subjects placed on combination therapy at 3 months.

Safety data included medical history, physical exam, 12-lead ECG, and routine hematology, clinical chemistry, and urinalysis. There were no explicit criteria for dropping a subject because of lack of efficacy.

Amendment I called for numbering subjects to track in which trial, if any, the subject had been. Amendment II specified a 4-week placebo run-in for subjects newly exposed under this protocol, and set the qualifying supine diastolic pressure to be 95 to 114 mmHg. These subjects had additional blood pressure assessments at the end of 1, 2, and 3 weeks of treatment. Amendment III¹ described a double-blind, placebo-withdrawal study to follow the one-year study in subjects treated only with trandolapril. Amendment X was for Doppler echocardiography to monitor left ventricular mass and function at baseline, 3 months, and 6 months, in subjects on trandolapril alone or in combination with verapamil.

A35.5. Results

Four hundred forty-four subjects enrolled in the trial, of whom 280 had previously participated in short-term trials. Individual study centers enrolled 3 to 42 subjects.

¹ The review of this study is found in section A36. on page 277

Demographic and baseline characteristics of subjects are shown in Table 304 below.

Table 304. Demographic and baseline characteristics of subjects in Study TR-30-HTN.

	Trandolapril only			Verapamil 180 mg+		All n=444
	1 mg n=68	2 mg n=77	4 mg n=121	2 mg n=16	4 mg n=89	
Male	43	59	81	35	58	276
Female	25	38	63	11	31	168
Age $\pm$ SD	54 $\pm$ 12	54 $\pm$ 12	52 $\pm$ 11	54 $\pm$ 10	53 $\pm$ 11	53 $\pm$ 11
Range	25 to 73	27 to 75	25 to 82	32 to 74	24 to 78	24 to 82
Caucasian	59	84	102	33	74	352
Black	7	10	38	13	14	82
Other	2	3	4	0	1	10
Weight $\pm$ SD	195 $\pm$ 43	182 $\pm$ 37	183 $\pm$ 33	194 $\pm$ 24	196 $\pm$ 38	188 $\pm$ 37
Range	96 to 324	105 to 280	116 to 286	116 to 256	110 to 325	96 to 325

Subjects had a variety of other diagnoses, including arthritis (8.3%) and diabetes (7.0%). Other cardiac diagnoses were uncommon: valvular heart disease (1.4%), angina (1.1%), coronary artery disease (0.9%).

The disposition of subjects is shown in Table 305 below.

Table 305. Disposition of subjects in Study TR-30-HTN.

Interval	Began n	Drop-outs	
		n	rate ^a
$\leq$ 2 weeks	444	2	2.25
2 to 6 weeks	442	8	4.52
6 weeks to 3 months	434	19	6.25
3 to 6 months	415	71	13.16
6 to 9 months	344	31	6.93
9 to 12 months	313	19	4.67
>12 months	195	7	—

a. Per week per 1000 subjects

No subjects were excluded from safety analyses because of protocol violations.

An analysis of the dose at last visit is shown in Table 306 below. Overall exposure is characterized in Figure 130 below.

The sponsor presented several analyses of efficacy. Because of the open-label nature of the trial, these are difficult to interpret. Responders were considered subjects whose trough supine diastolic pressure was <90 mmHg or decreased by 10 mmHg. The response rate at end point is shown in Table 307 below as a function of dose.

A35.6. Safety.

Two hundred ninety-two of 444 subjects reported at least one adverse reaction. Adverse events with a >1% incidence are summarized in Table 308 below. Subjects who discontinued because of adverse reactions are listed in Table 309 below. Six subjects (4 on trandolapril only) had dosage reductions because of adverse events, none of which were considered serious. Ten subjects had treatment interruptions because of adverse events.

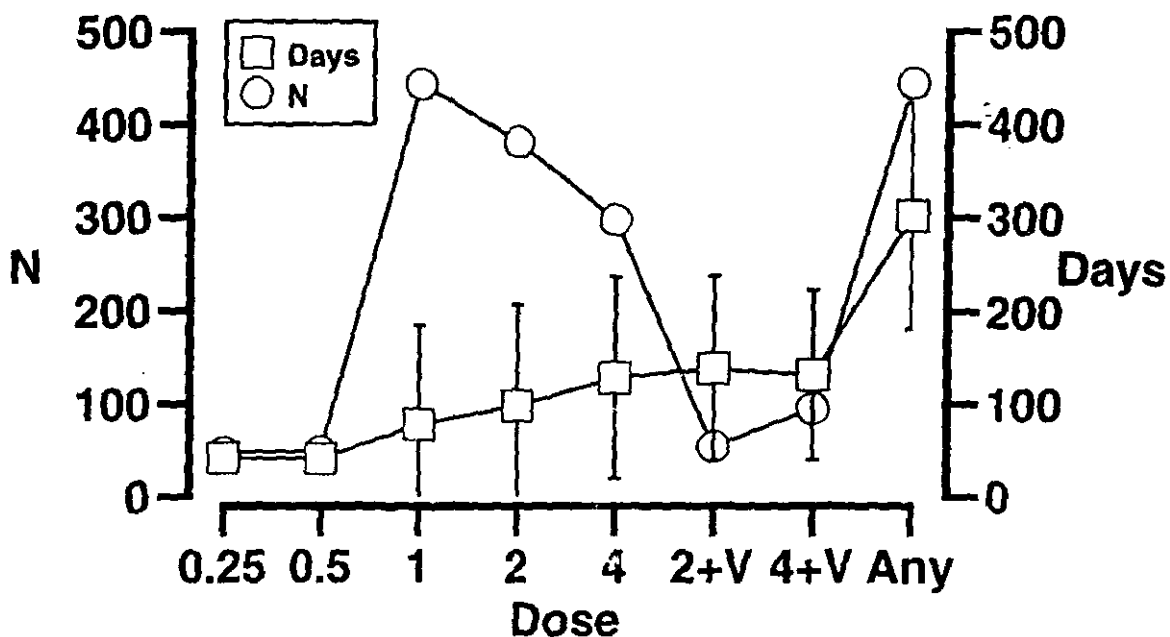


Figure 130. Exposure (subjects and duration) by dose in Study TR-30-HTN.

Table 306. Dose at last visit and principal causes for withdrawal in Study TR-30-HTN.

Dose	Trandolapril only			Verapamil 180 mg +		All n=444
	1 mg. n=68	2 mg n=97	4 mg* n=144	2 mg n=46	4 mg n=89	
Drop-outs (%)	23 (34)	26 (27)	51 (35)	19 (41)	38 (43)	157 (35)
ADR	10	8	6	5	4	33
Lack of efficacy	3	4	28	13	31	79
Intercurrent illness	2	4	2	0	1	9
Need for concomitant meds	1	2	1	0	0	4
Not follow schedule	4	3	4	0	1	12
Voluntary	0	1	7	0	0	8
Administrative problems	1	0	0	0	0	1
Death	0	0	1	0	0	1
Other ^a	2	4	2	1	1	10

a. Includes 5 withdrawals of consent, 4 moved out of area, 1 withdrawn by sponsor.

Fifteen percent of subjects had new physical exam findings during the study, the most common of which were trauma, cardiac murmurs, S4, and upper respiratory congestion, all with incidence <2%.

The sponsor did several analyses to look for hypotension. No subjects had supine systolic pressure <100 mmHg. Almost 20% of subjects of subjects receiving

Table 307. Response rate at end point (%) in Study TR-30-HTN.

Dose	Trandolapril-only			Verapamil 180 mg +	
	1 mg n=70	2 mg n=89	4 mg n=141	2 mg n=55	4 mg n=88
<90 mmHg	45 (64)	55 (62)	57 (40)	7 (16)	28 (32)
10 mmHg decrease	36 (51)	40 (45)	67 (48)	10 (22)	32 (36)
Either	47 (67)	57 (64)	78 (55)	13 (29)	38 (43)

Table 308. Adverse events (%) in Study TR-30-HTN.

	Mono n=444	Comb n=139		Mono n=444	Comb n=139
Cardiovascular			General		
Angina	5 (1.1)	1 (0.7)	Chest pain	7 (1.6)	0 (0.0)
Bradycardia	5 (1.1)	2 (1.4)	Fatigue	21 (4.7)	2 (1.4)
Edema	9 (2.0)	0 (0.0)	Influenza	6 (1.4)	1 (0.7)
Palpitations	6 (1.4)	0 (0.0)	Trauma	3 (1.8)	0 (0.0)
Nonspecific ST changes	0 (0.0)	2 (1.4)	Genitourinary		
CNS			Urinary tract infection	9 (2.0)	2 (1.4)
Dizziness	14 (3.2)	7 (5.0)	Metabolic/endocrine	—	—
Headache	61 (13.7)	9 (6.5)	Musculoskeletal		
Paresthesia	5 (1.1)	0 (0.0)	Arthritis	8 (1.8)	2 (1.4)
Dermatologic			Muscle cramps	5 (1.1)	0 (0.0)
Rash	9 (2.0)	1 (0.7)	Back pain	16 (3.6)	2 (1.4)
Emotional			Extremities pain	8 (1.8)	0 (0.0)
Anxiety	13 (2.9)	1 (0.7)	Joint pain	21 (4.7)	2 (1.4)
Impotence	5 (1.1)	3 (2.2)	Muscle pain	5 (1.1)	0 (0.0)
EENT			Neck pain	7 (1.6)	1 (0.7)
Epistaxis	5 (1.1)	0 (0.0)	Pulmonary		
Throat infection	11 (2.5)	0 (0.0)	Bronchitis	8 (1.8)	1 (0.7)
Upper resp congestion	19 (4.3)	1 (0.7)	Cough	42 (9.5)	7 (5.0)
Upper resp infection	60 (13.5)	8 (5.8)			
Upper resp inflammation	31 (7.0)	2 (1.4)			
Gastrointestinal					
Abdominal pain	6 (1.4)	1 (0.7)			
Constipation	5 (1.1)	5 (3.6)			
Diarrhea	14 (3.2)	0 (0.0)			
Dyspepsia	15 (3.4)	2 (1.4)			
Nausea	10 (2.3)	5 (3.6)			
Vomiting	2 (0.5)	2 (1.4)			

Table 309. Discontinuations for adverse events in Study TR-30-HTN.

Cntr	Subj	Dose	Day	ADR
3001	2029	2 mg	7 mo	70 year old Caucasian male; participated in TR-20-HTN. By adverse event report form, subject discontinued with chest pain (angina) of 3-months duration. ECG report shows subject was hospitalized with acute MI, confirmed with enzymes.
3003	3027	1 mg	2 mo	61 year old Caucasian male with 10-year history of hypertension; no prior exposure to trandolapril. Subject discontinued subsequent to trauma (assault).
3004	2004	1 mg	5	68 year old Caucasian female with 5-year history of hypertension; previously a participant in TR-20-HTN. She discontinued after 5 days on 1 mg trandolapril, with angioedema.
3004	2013	2 mg +V	9 mo	50 year old Caucasian male. He had multiple complaints including lumbar pain, lightheadedness, headache, muscle spasms, impotence, and cough. The latter, the sole cause listed for discontinuation, was described as mild.
3004	3001	2 mg	3 mo	50 year old Caucasian male with a 3-year history of hypertension. Missing prior exposure CRF. Subject discontinued with dry cough, described as moderately severe. There was no follow-up.

Table 309. Discontinuations for adverse events in Study TR-30-HTN. (Continued)

Cntr	Subj	Dose	Day	ADR
3004	3006	2 mg +V	6 mo	34 year old Caucasian male with 7-year history of hypertension; no previous exposure to trandolapril. Subject discontinued because of lethargy and irritability. BP was adequately controlled at time of withdrawal.
3005	3010	2 mg +V	4 mo	60 year old Black male with 7 year history of hypertension, no previous exposure to study drug. He discontinued after 4 months for neutropenia. Baseline WBC was 4.3 with 37% neutrophils. Subsequent monthly values were WBC 3.8 to 4.7 with neutrophils 22 to 43%. No clear trend was evident. Three days after discontinuing treatment, WBC was 4.7 with 53% neutrophils.
3006	3002	2 mg +V	12 mo	55 year old Caucasian male with 11-year history of hypertension, diabetes mellitus; no prior exposure to trandolapril. Discontinued with new onset of chest tightening. Subsequent to cardiac catheterization, he was recommended for CABG.
3007	2013	4 mg	6 mo	52 year old Caucasian male; participated in TR-20-HTN. Last recorded supine BP was 125/83. Subject died in hospital with acute myocardial infarction.
3007	2018	4 mg	3 mo	60 year old Caucasian male; participated in TR-20-HTN. Discontinued with cough which began during first week of treatment.
3007	2022	1 mg	1 mo	44 year old Caucasian male; participated in TR-20-HTN. Discontinued with tachycardia which responded to labetolol.
3007	2025	1 mg	3 mo	Missing demographic data form; participated in TR-20-HTN. Discontinued with cough of moderate severity. Also complaints of sore throat and lightheadedness. No follow-up.
3007	3005	2 mg +V	3 mo	57 year old Caucasian female with 5-year history of hypertension; no prior exposure to trandolapril. Subject discontinued for headaches, constipation, nose bleeds, insomnia, and mood swings, all of which were said to begin with the addition of verapamil.
3007	3006	1 mg	2	57 year old Caucasian male with 5-year history of hypertension; no prior exposure to trandolapril. Subject discontinued because of weakness and shakiness, both of which resolved 3 days later.
3007	3008	2 mg	1 mo	52 year old Caucasian male with 3-month history of hypertension; no prior exposure to trandolapril. Subject discontinued because of cough, associated with URI symptoms.
3007	3010	2 mg	2 mo	57 year old Caucasian male with 15-year history of hypertension; no prior exposure to trandolapril. Subject discontinued because of impotence.
3008	2001	2 mg +V	7 mo	55 year old Caucasian male with 8-year history of hypertension; previously in study TR-20-HTN. Subject discontinued for lightheadedness, beginning the day verapamil was started and resolving when both drugs were discontinued.
3008	2008	2 mg +V	4 mo	66 year old Caucasian female; participated in TR-20-HTN. Subject discontinued because of cough and inadequate blood pressure control.
3008	2009	1 mg	3 mo	63 year old Caucasian female; participated in TR-20-HTN. Subject discontinued because of cough. Also complaints of dizziness and allergic rhinitis.
3008	2010	4 mg +V	6 mo	50 year old Caucasian male; participated in TR-20-HTN. Subject withdrew because of cough.
3011	2003	1 mg	6 mo	52 year old Caucasian female; participated in TR-20-HTN. Subject discontinued for cough and chest soreness.
3011	2005	4 mg	3 mo	67 year old Caucasian female; participated in TR-20-HTN. Subject discontinued for cough. Also complained of nervousness and heart pounding. Last ECG showed new non-specific ST-T changes. There was no further follow-up.
3011	3005	2 mg	1 mo	66 year old Caucasian male with 12-year history of hypertension, diabetes mellitus, and hearing loss; no prior exposure to trandolapril. Subject discontinued with unilateral leg pain, but also complained of headaches, epigastric discomfort, hand pain, fatigue, and decreased appetite. Two weeks after withdrawal, following subacute mental status changes, subject underwent subtotal resection of glioblastoma multiforme and had a less than 1-year expected life span. There was no follow-up after this hospitalization.
3011	3006	4 mg	3 mo	42 year old Caucasian female with 12-year history of hypertension; no prior exposure to trandolapril. Subject discontinued because of persistent cough. Also complained of headaches, anxiety, and insomnia.
3011	3007	2 mg +V	3 mo	72 year old Caucasian male with a 1-year history of hypertension; no prior exposure to trandolapril. Subject discontinued with nausea, vertigo, and pre-syncope, all of which began one day after addition of verapamil. Other complaints of headaches, dizziness upon arising quickly, and dry cough predated addition of verapamil, but were not said to be contributory to withdrawal.
3011	3011	4 mg +V	7 mo	48 year old Caucasian female with 18-year history of hypertension; no prior exposure to trandolapril. Subject discontinued because of elevated enzymes (SGPT 299, SGOT 102, LDH 275), all of which were near normal or WNL at 2-week follow-up. There was no rechallenge.
3013	3003	2 mg	20	68 year old Mexican-American male with a 5-year history of hypertension; no previous exposure to trandolapril. History included possible old myocardial infarction and carotid endarterectomy. Baseline supine BP was 164/103; ECG showed non-specific ST-T changes. BP lower at only interim visit. Subject admitted to hospital for angina said to begin 3 days after starting trandolapril. Underwent CABGx3; no further follow-up.
3014	2010	1 mg	1 mo	Missing demographic data form; participated in TR-20-HTN. Subject discontinued because of angina (onset prior to open-label study), and subsequently underwent angioplasty.
3015	2112	2 mg	2 mo	56 year old Black male; participated in TR-21-HTN. Subject discontinued because of cough, of 3-weeks duration.
3015	2113	1 mg	7 mo	65 year old Black female; participated in TR-21-HTN. Subject hospitalized subsequent to right occipital infarct. Study drug was not discontinued until several weeks after discharge.
3016	2013	4 mg +V	9 mo	Missing demographic data form; participated in TR-20-HTN. She discontinued because of symptomatic cholelithiasis predating entry. Subject had multiple other complaints—cough, back pain, hiatal hernia, vomiting, flu symptoms, RUQ pain—not considered contributory to withdrawal. No further follow-up.
3016	2015	2 mg	9 mo	Missing demographic data form; participated in TR-20-HTN. Discontinued with atypical chest pain and for cholecystectomy. Also complaint of reflux esophagitis. No further follow-up.
3016	2020	2 mg	2 mo	Missing demographic data form; participated in TR-20-HTN. Subject discontinued with mild epigastric pain ("definitely not" related). "Pt will undergo gallbladder surgery." Also complaint of back pain secondary to snow shoveling.

Table 309. Discontinuations for adverse events in Study TR-30-HTN. (Continued)

Cntr.	Subj	Dose	Day	ADR
3017	3002	2 mg +V	4 mo	45 year old Black female with 1-year history of hypertension; no previous exposure to trandolapril. Discontinued because of nausea of 1-month duration. Also complaints of constipation and paresthesias. There is no follow-up.
3019	3001	2 mg	2 mo	35 year old Caucasian male with a 15-year history of hypertension; no prior exposure to trandolapril. Subject was dropped because of mildly elevated SGPT and AlkPhos, thought to be related to alcohol abuse. No follow-up.
3019	3003	1 mg	1 mo	44 year old Caucasian female with a 2-year history of hypertension; no prior exposure to trandolapril. Subject withdrew because of persistent cough.
3019	3006	4 mg +V	7 mo	39 year old Black male with a 1-year history of hypertension; no previous exposure to trandolapril. Subject discontinued with 2-week history of nausea and 2-month history of cough.
3019	3008	1 mg	3	58 year old Black female with a 50 ⁺ -year history of hypertension; no prior exposure to trandolapril. Subject discontinued because of double vision, which resolved the next day.
3019	3009	4 mg	3 mo	46 year old Black male with 5-year history of hypertension; previously enrolled in TR-20-HTN. Subject developed asymptomatic RBBB and was discontinued. Last supine BP was 170/114. There was no follow-up.
3019	3010	2 mg	5 wk	55 year old Caucasian female with 20-year history of hypertension; no previous exposure to trandolapril. Subject discontinued with swelling of ankles and abdomen, skin rash, and heartburn. Except later, symptoms resolved in 4 days.

a. Enrollment CRF clearly says 602 months, but this same investigator had, for another subject, equated 440 months and 20 years.

trandolapril alone had >20 mmHg reduction in systolic pressure upon moving from a supine to a standing position. Three of these subjects had orthostatic hypotension reported as an adverse event. Two subjects had standing blood pressures <90 mmHg systolic and >10 mmHg reduction upon standing; both were asymptomatic.

Thirty-one percent of subjects had new ECG findings, the most common of which were sinus bradycardia (13%) and non-specific ST-T changes (6%). Twenty-two percent of subjects showed elevated cholesterol as a new laboratory finding. Six percent of subjects showed newly elevated SGPT. Seventeen percent of subjects had new urinalysis findings of or elevated hyaline casts.

Laboratory data are summarized in Figure 131 to Figure 134 below.

In addition to subject 3005-3010 discussed in Table 309, several subjects had significant reductions in neutrophils, but were not discontinued from treatment.

Subject 3009-2108 was a 56 year old Black male with neutrophil counts ranging from 858 to 1645/ μ L during open-label treatment. He received doses of 1 to 4 mg trandolapril. At the lowest neutrophil count, he was receiving 2 mg trandolapril plus verapamil. The neutrophil count was unchanged a month later.

Subject 3014-2009 was a 71 year old Caucasian male whose low neutrophil count (%) was related to a lymphocytosis which predated study entry.

Subject 3015-3018 was a 61 year old Black female who recorded an absolute neutrophil count of 912 at visit 7, having crept down progressively over the preceding several visits. She also showed a progressive moderate increase in hepatic enzymes (SGPT and SGOT both about 3x ULN, which the investigator attributed to alcohol abuse.

A35.7. Summary.

This was a one-year, open-label study, in which dose was titrated for efficacy. Approximately 1/3 of subjects were last on doses of 1 or 2 mg trandolapril alone, 1/3 on 4 mg trandolapril alone, and 1/3 on 2 or 4 mg trandolapril plus verapamil. The dropout rate peaked during the 3-to-6 month interval. Overall, 1/3 of subjects withdrew. Half of the withdrawals were for lack of efficacy. There was one death (acute myocardial infarction).

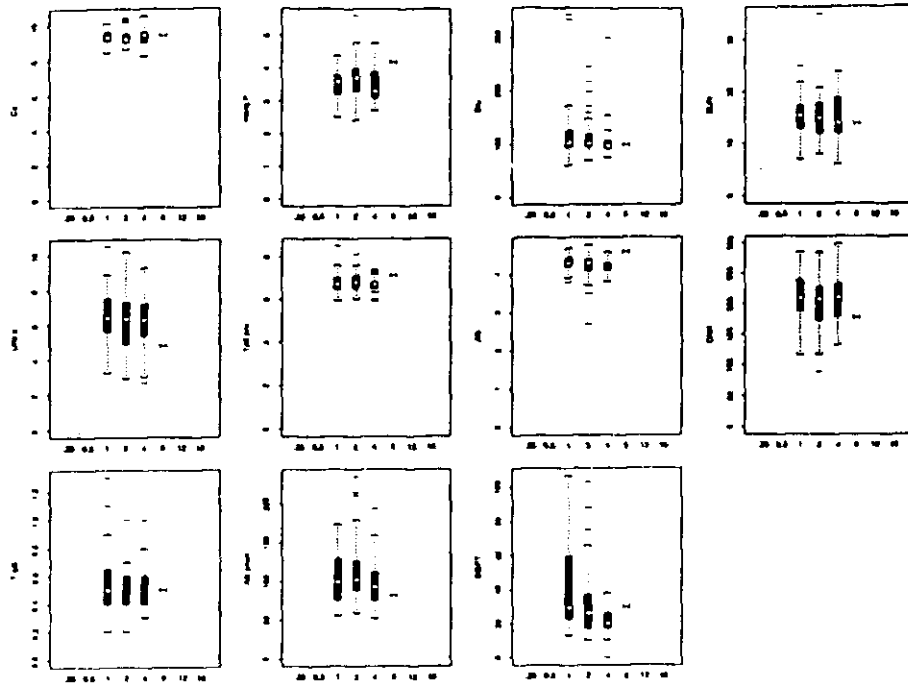


Figure 131. Clinical chemistry data (part A) in Study TR-30-HTN.

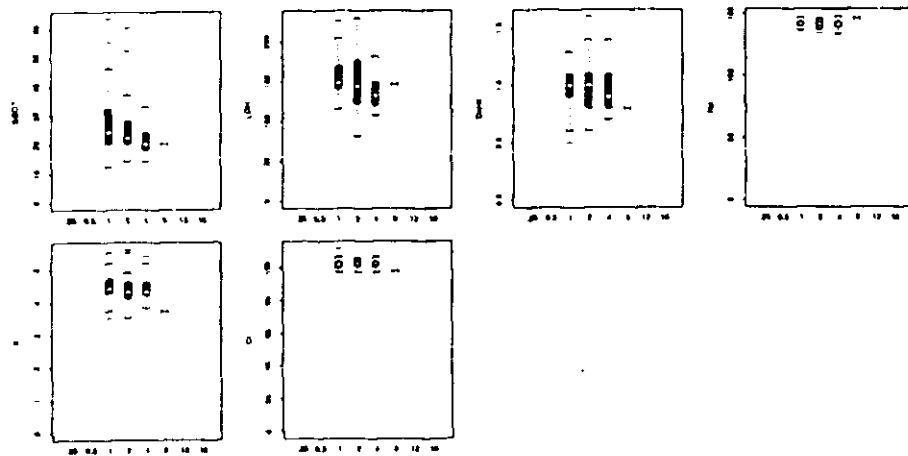


Figure 132. Clinical chemistry (part B) in Study TR-30-HTN.

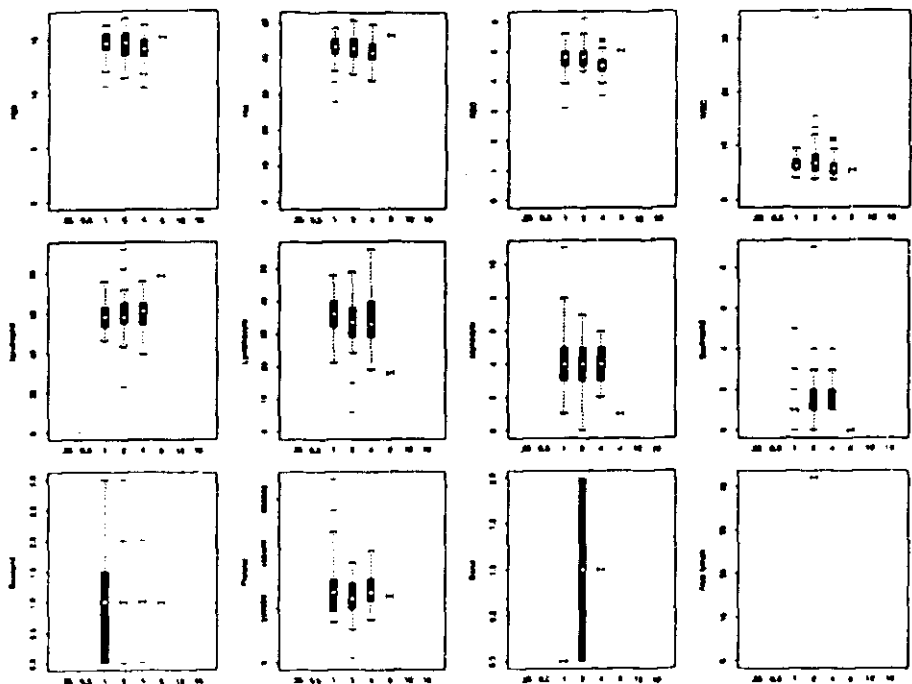


Figure 133. Hematology data in Study TR-30-HTN.

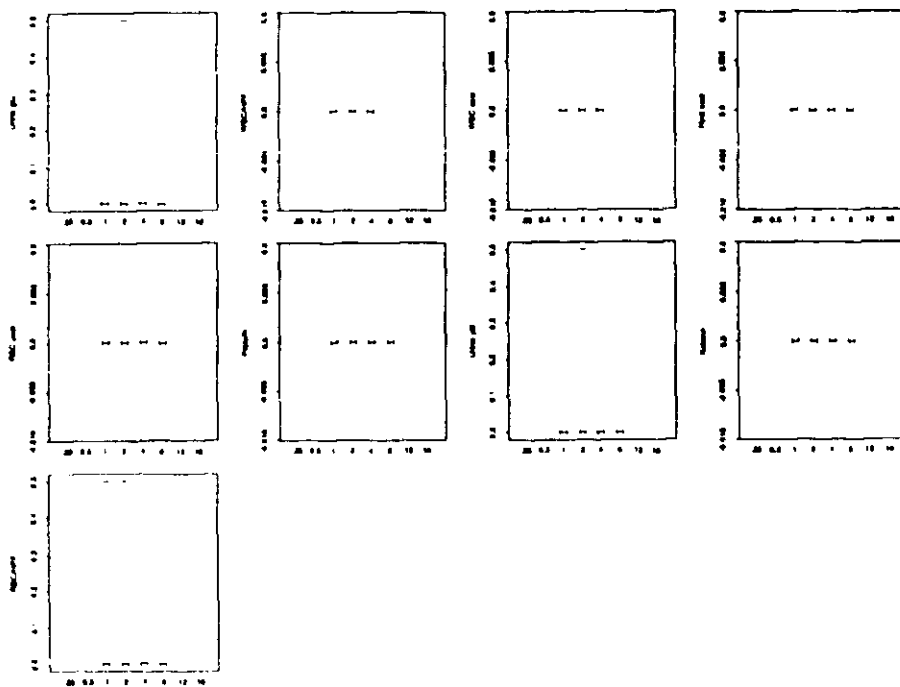


Figure 134. Urinalysis data in Study TR-30-HTN.

A36. Study TR-30-HTN-Amendment III (Study report: vols 1.208-1.211; case report tabulations: vol 1.330-1.344; case report forms for deaths and discontinuations for adverse events: vols 1.387-1.390) "Randomized, placebo-controlled withdrawal from long-term trandolapril therapy in patients with mild to moderate essential hypertension".

A36.1. Investigators The investigators are listed in Table 310 below.

Table 310. Investigators in Study TR-30-HTN-III.

Investigator	Center	City	Investigator	Center	City
P Barden, MD	3004	Albuquerque, NM	W Pleskow, MD	3007	Encinitas, CA
JR Benz, MD	3016	Cedar Rapids, IA	R Reeves, MD	3010	Olympia, WA
R Goldstein, MD	3006	Tampa, FL	H Schnaper, MD	3009	Birmingham, AL
PM Kaihlanen, MD	3003	San Antonio, TX	D Shaw, MD	3008	San Diego, CA
D Kelly, MD	3012	Olympia, WA	W Stein, MD	3014	Rochester, NY
J Levine, MD	3017	Nashville, TN	M Weir, MD	3015	Baltimore, MD
R Paster, MD	3001	Oregon, WI			

A36.2. Study dates May 1991 to January 1992.

A36.3. Drug formulation

Table 311. Drug substance used in Study TR-30-HTN-III.

Lot	Form	Type	Trandolapril	Source lot	Batch size
TRA-0140A	Cap	Tran	1.0 mg	L-12	546,100
TRA-0120A	Cap	Tran	2.0 mg	L-11	600,000
99-0290	Cap	Tran	4.0 mg	99-0290	86,218

Table 312. Capsule composition in Study TR-30-HTN-III.

	1 mg	2 mg	4 mg
Trandolapril	1.0	2.0	4.0
Corn starch			
Lactose, hydrous			
Povidone			
Sodium stearyl fumarate			
Total			

A36.4. Study design

Subjects were those completing a one-year open-label study (TR-30-HTN, see appendix A35, on page 268 for a description of the population studied). Subjects qualified for inclusion in this withdrawal study on the basis of average supine diastolic pressure <90 mmHg 2 weeks prior to completion of TR-30-HTN and at the final study visit for TR-30-HTN ("responders"). The qualifying study permitted use of verapamil; subjects on verapamil were excluded from this withdrawal trial. Subjects in Study TR-30-HTN were on doses of 1, 2, or 4 mg. Each baseline dosing group was randomized to the same dose or placebo. Subjects were followed at 2 and 4 weeks. The primary efficacy parameter was percentage of responders at the end of 4 weeks. With 60 subjects per group, the trial had an estimated 80% power to detect such differences with a one-sided $\alpha=0.05$.

Safety data included history, physical exam, 12-lead ECG, and routine laboratory, all performed at baseline and at end point.

A36.5. Results

Eighty-five subjects enrolled in the trial, of whom 84 completed¹. Twelve subjects are listed with protocol violations; 11 subjects had baseline supine diastolic pressure ≥ 90 mmHg (of whom 8 were randomized to placebo) and 2 subjects received the wrong drug. One subject who was randomized in violation did not complete the study. Individual study centers enrolled 1 to 17 subjects. Demographic and baseline characteristics of subjects are shown in Table 313 below. The treatment groups were

Table 313. Demographic and baseline characteristics of subjects in Study TR-30-HTN-III.

	Plcbo n=38	Tran n=47
Male	23	31
Female	15	16
Age $\pm$ SD	54 $\pm$ 13	53 $\pm$ 11
Range	25 to 75	27 to 72
Caucasian	34	45
Black	4	2
Other	0	0
Weight $\pm$ SD	184 $\pm$ 43	193 $\pm$ 35
Range	113 to 286	105 to 324

reasonably well matched for these factors, for general health, duration and severity of hypertension, and baseline drug use.

No subjects were excluded from efficacy or safety analyses because of protocol violations.

The sponsor's analysis of blood pressure considered 'trough' as any measurement at least 10 hours after dosing.

Responder rate data are shown in Table 314 below. An intent-to-treat analysis was

Table 314. Responder rate data (%) in Study TR-30-HTN-III^a.

	Trandolapril						Pooled	
	Plcbo n=9	1 mg n=15	Plcbo n=14	2 mg n=19	Plcbo n=15	4 mg n=18	Plcbo n=37	Any n=47
Week 2	5 (56)	11 (73)	8 (57)	13 (68)	8 (57)	8 (62)	21 (57)	32 (68)
Week 4	6 (67)	8 (53)	6 (43)	11 (58)	4 (27)	4 (33)	16 (42)	23 (49)

a. Sponsor's analysis.

performed by the sponsor; the overall difference (42% for placebo vs. 49% for trandolapril) was not statistically significant. This was consistent with the statistical reviewer's analysis with χ^2 ($p=0.53$) and Fisher's exact test ($p=0.66$), ignoring differences in dosing among trandolapril groups, and the Mantel-Haenszel test ($p=0.74$), controlling by dose level.

Changes in blood pressure are shown in Figure 135 below (supine only) and Table 315 below (again with the sponsor's definition of 'trough'). The sponsor's analysis of

¹ One subject in the 4 mg group was withdrawn because of compliance problems.

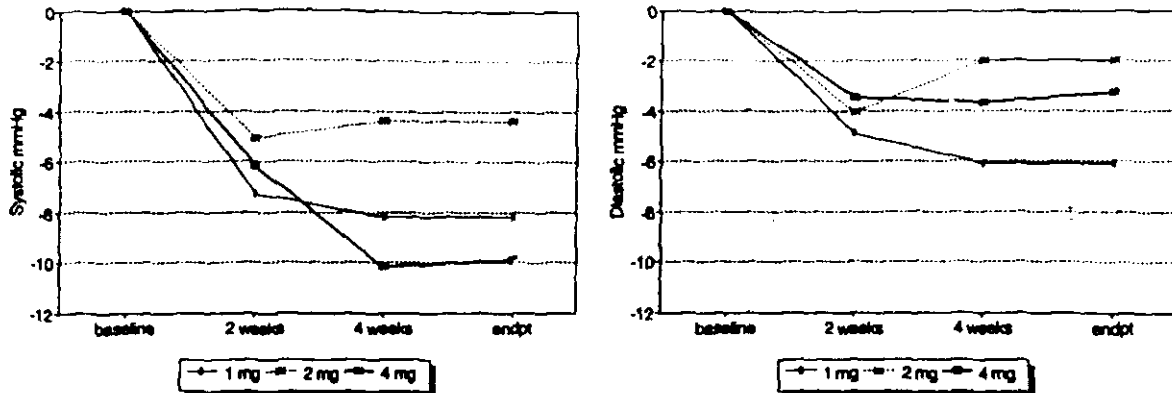


Figure 135. Changes in supine systolic and diastolic pressure in Study TR-30-HTN-Amendment III.

Table 315. Changes from baseline and placebo in trough blood pressure in Study TR-30-HTN-III^a.

	Diastolic			Systolic		
	1 mg n=15 ^a	2 mg n=19 ^a	4 mg n=13 ^a	1 mg n=15 ^a	2 mg n=19 ^a	4 mg n=13 ^a
Supine						
2 weeks	-4.9	-4.1	-3.5	-7.3	-5.1	-6.2
4 weeks	-6.1	-2.0	-3.7	-8.2	-4.4	-10.2
End point	-6.1	-2.0	-3.3	-8.2	-4.4	-9.9
Sitting						
2 weeks	-2.6	-3.9	-4.4	-2.1	0.2	-3.1
4 weeks	-2.4	-4.5	-5.6	-1.1	-4.1	-7.0
End point	-2.8	-2.1	-6.7	-1.1	-3.3	-6.5
Standing						
2 weeks	-2.9	-2.0	-6.2	-6.5	-3.3	-4.4
4 weeks	-2.4	-4.5	-5.2	-6.8	-6.1	-4.4
End point	-2.9	-1.7	-6.1	-6.8	-4.9	-4.3

a. Sponsor's analysis.

supine diastolic pressure used analysis of covariance (ANCOVA), with treatment, center, and stratum (defined as the 3 treatments prior to the double-blind period) as factors and baseline supine diastolic pressure as a covariate. This model ignores differences in dosing level during treatment. The statistical reviewer's analysis (Table 316 below) found a significant treatment effect in overall mean change of supine

Table 316. Change from baseline and placebo in trough blood pressure in Study TR-30-HTN-III^a.

	1 mg		2 mg		4 mg	
	Plcbo	Tran	Plcbo	Tran	Plcbo	Tran
n	9	15	14	19	15	13
Baseline	81.8	85.8	87.5	85.8	86.0	87.8
Δ from baseline	7.0	1.5	5.2	3.2	6.2	2.9
Δ from placebo		-6.1		-2.0		-3.3
p		0.019		0.158		0.160

Table 316. Change from baseline and placebo in trough blood pressure in Study TR-30-HTN-III^a. (Continued)

	1 mg		2 mg		4 mg	
	Plcbo	Tran	Plcbo	Tran	Plcbo	Tran
	Pooled placebo			Pooled trandolapril		
n	38			47		
Baseline	84.9			86.3		
Δ from baseline	6.2			2.6		
Δ from placebo				-3.6		
p				0.007		

a. Statistical reviewer's analysis.

diastolic pressure from baseline ($p < 0.007$). No center or stratum effects were found. Omitting placebo subjects, mean changes in supine diastolic pressure at trough and end point were compared among active treatment groups using ANOVA; no significant differences were found. An ANOVA analysis with stratum and treatment nested within stratum was also used to analyze differences in mean change in supine diastolic pressure at trough and end point between trandolapril-treated subjects and placebo subjects in at least one stratum. This result was significant ($p = 0.042$). Paired comparisons showed that the mean change in supine diastolic pressure at trough and end point for placebo subjects was significantly higher than in subjects receiving trandolapril 1 mg. The increase in diastolic pressure for subjects remaining on trandolapril is possibly a "regression to the mean" effect, since all subjects were selected with baseline diastolic pressure < 90 mmHg.

Changes in pulse rate, supine, sitting, or standing, were at no time statistically significantly different from placebo.

A36.6. Safety.

Nine of 47 subjects on trandolapril and 2 of 40 subjects receiving placebo reported at least one adverse reaction². These adverse events are summarized in Table 317 below.

Table 317. Adverse events in Study TR-30-HTN-III.

	Group		Group		Group
Headache	Plcbo	URI symptoms	2 mg	URI symptoms	4 mg
URI symptoms	Plcbo	URI symptoms	2 mg	URI symptoms	4 mg
Tinnitus	Plcbo	URI symptoms	2 mg	Thyroid nodule	4 mg
Influenza	1 mg	Dysuria	2 mg	Dyspnea	4 mg

Counts of events were too low to discern any relationship to dose. No adverse events led to withdrawal, dose reduction, or interruption of study drug.

The data were not examined for orthostatic hypotension.

There were a variety of new findings on the final physical exam and clinical laboratory; none were serious or had apparent relationship to the study drug.

A36.7. Summary.

Eighty-five subjects participated in this 4-week randomized withdrawal phase of a one-year open-label assessment. The primary end point, responder rate, did not differ significantly between subjects on placebo vs. those who remained on their open-label dose. Most subjects had some increase in blood pressure during the withdrawal phase;

² Numbers take into account several subjects who, for some time, received a treatment other than the one to which they were randomized.

the magnitude of the increase was consistently and statistically significantly greater in the placebo group. There was no consistent trend for dose-dependence to the change in blood pressure. Adverse events were few and not serious.

A37. Study TR-42-CP (Study report: vols 1.199-1.208; case report tabulations: vols 1.327-1.329; case report forms for deaths and discontinuations for adverse events: none) "Ambulatory monitoring for the circadian variation in blood pressure during oral trandolapril therapy".

A37.1. Investigators The investigators are listed in Table 318 below.

Table 318. Investigators in Study TR-42-CP.

Investigator	Center	City
MA Weber, MD	4201	Long Beach, CA
JA Schoenberger, MD H Black, MD		Chicago, IL

A37.2. Study dates February 1991 to February 1993.

A37.3. Drug formulation

Table 319. Drug substance used in Study TR-42-CP.

Lot	Form	Type	Trandolapril	Source lot	Batch size
TRA-0321	Cap	Plcbo	—	L-37	6,000,000
TRA-0120A	Cap	Tran	2.0 mg	L-11	600,000
99-0290	Cap	Tran	4.0 mg	99-0290	86,218
99-0391	Cap	Tran	4.0 mg	99-0391	85,963

Table 320. Capsule composition in Study TR-42-CP.

	2 mg	4 mg
Trandolapril	2.0	4.0
Corn starch		
Lactose, hydrous		
Povidone		
Sodium stearyl fumarate		
Total		

A37.4. Study design There was a 4-week single-blind, placebo lead-in phase, followed by an 8-week double blind treatment period, and a 1-week single-blind, placebo washout period. Forty subjects were planned, 20 per center. Subjects were to be age ≥ 21 years, $< 30\%$ above ideal body weight, with supine diastolic pressure between 95 and 114 mmHg at the end of the placebo run-in phase. Women were permitted if neither childbearing nor lactating, and if practicing recognized forms of contraception. Exclusion criteria are listed in Table 321 below. Other excluded concomitant drugs were digitalis, MAO

Table 321. Exclusion criteria in Study TR-42-CP.

Hypertension other than essential and mild-to-moderate
History of evidence of malignant, accelerated, or severe hypertension
Cerebrovascular accident, convulsions, or hypertensive encephalopathy within 1 year
Clinically significant congestive heart failure or EF $< 40\%$
Use of antihypertensives within 4 weeks of double-blind period or spironolactone within 6 weeks
Heart rate < 50 bpm, significant arrhythmia, sinus node dysfunction, or conduction abnormalities
Myocardial infarction within 3 months

Table 321. Exclusion criteria in Study TR-42-CP. (Continued)

Hypersensitivity or serious adverse reaction to any ACE inhibitor
Clinically significant renal failure, e.g., creatinine >2 mg/dl
Clinically significant hepatic failure, e.g., SGOT or SGPT >2 times ULN
Significant electrolyte imbalance
Significant hematologic abnormalities
Medical or surgical conditions affecting absorption, metabolism, or excretion
Previous exposure to trandolapril
History of chronic alcohol or drug abuse within 2 years
Terminal illness
Blood donation within 1 month

inhibitors, psychotropic drugs, chronic NSAIDs, phenylpropanolamine, and pseudoephedrine.

Subjects who qualified at the end of the placebo run-in period were randomized to placebo or trandolapril 2 mg q.d. and returned after 1 week. Subjects whose supine diastolic pressure was not <90 mmHg or reduced by ≥ 10 mmHg had their doses increased to 4 mg q.d.¹ Subjects were instructed to take their study drug at the same time in the morning, but neither that time nor the relationship to meals was specified.

Subjects underwent 2 48-hour ABPM sessions, the first at the end of the placebo run-in period, and second beginning with the last dose in the double-blind treatment period. The ABPM equipment was SpaceLabs 90207 System and was programmed to take readings at 20-minute intervals from 0600 to 2200 hours, and at 30-minute intervals at night.

Safety data included medical history, physical exam, 12-lead ECG, and routine hematology, clinical chemistry, and urinalysis.

Blood samples were obtained for trandolapril and trandolaprilat levels at one of the double-blind visits.

The study had an estimated 65% power ($\alpha=0.05$) to detect a 5 mmHg change in diastolic pressure from baseline. Treatment groups were to be compared in 2-hour blocks. An interim analysis was planned after 20 subjects had completed study².

A37.5. Results

Forty-one subjects enrolled in the trial, of whom 39 completed the final washout period. Two placebo group subjects completed <2 or <4 weeks of double-blind treatment, 1 being withdrawn for failure to meet entry criteria and the other withdrawn because of lack of efficacy. Demographic and baseline characteristics of subjects are shown in Table 322 below. The treatment groups were reasonably well matched for these factors, for general health, duration and severity of hypertension, and baseline drug use. There were 21 subjects with reported protocol violations, including failure to meet entry criteria (2), use of proscribed concomitant drugs (4), administration of the wrong study drug (2), and performance of the second ABPM while on double-blind treatment (9).

Compliance was at least 70% for all subjects at all visits.

All but 2 of the active treatment group subjects received the 4 mg dose.

¹ Amendment I (January 1991) changed to these doses; the original protocol specified 1 and 2 mg.

² Amendment II (signed by investigators in March and May of 1993) dropped the interim analysis, and the final study report denies there was an interim analysis.

Table 322. Demographic and baseline characteristics of subjects in Study TR-42-CP.

	Plcbo n=21	Tran n=20
Male	17	14
Female	4	6
Age $\pm$ SD	52 $\pm$ 12	51 $\pm$ 13
Range	27 to 70	27 to 68
Caucasian	15	17
Black	3	3
Other	3	0
Weight $\pm$ SD	188 $\pm$ 37	185 $\pm$ 27
Range	118 to 250	118 to 222
Supine systolic $\pm$ SD	155 $\pm$ 17	154 $\pm$ 15
Supine diastolic $\pm$ SD	100 $\pm$ 5	101 $\pm$ 5

Cuff diastolic blood pressure, shown in Table 323 below, was subjected to analysis of

Table 323. Cuff blood pressure data in Study TR-42-CP.

	Plcbo n=21	Tran n=20	Diff	Plcbo n=21	Tran n=20	Diff
	Supine			Sitting		
Baseline	99.7	100.6	—	100.8	97.7	—
Week 2	96.5	93.2	-2.1	97.4	94.8	+0.5
Week 4	98.7	93.1	-5.9	99.4	91.7	-4.0
Week 6	98.2	91.8	-7.2	99.2	91.6	-3.9
Week 8	98.7	93.3	-6.2	99.5	93.4	-2.4
End point	99.5	93.2	-6.3	98.9	93.4	-2.4
Washout	97.5	98.0	-0.2	99.3	97.8	+2.1

covariance with treatment and center as factors and baseline as a covariate. A statistically significant difference in mean supine diastolic pressure at end point was found between treatment groups (trandolapril 93.2 vs. placebo 99.5 mmHg; $p=0.008$). No treatment-by-center or treatment-by-baseline interactions were found. Data for standing diastolic blood pressure are similar. The analysis of covariance for trough sitting diastolic pressure did not show a significant treatment effect, although the adjusted mean diastolic pressure at trough at end point was smaller in the trandolapril group than in the placebo group. Data for systolic pressure are qualitatively similar. There were no significant changes in heart rate.

Statistical analyses for treatment effect on ambulatory blood pressure were based upon the subjects' hourly mean data. The sponsor's analysis showed a statistically significantly lower mean diastolic pressure for both 24-hour post-treatment time blocks for the active treatment group compared with placebo. The reductions were 6.9 ($p<0.001$) and 5.7 mmHg ($p=0.03$) for the first and second post-treatment days, respectively. In this analysis, subjects who mistakenly received trandolapril during the second 24-hour post-treatment period were excluded. Mean ambulatory diastolic pressures by 2-hour averages are shown as changes from baseline in Figure 136 below. Differences between the treated group and the placebo group are plotted in this figure with $\square$ at time points statistically significantly different from zero. The statistical reviewer used analysis of covariance to obtain baseline- and center-adjusted estimates of the mean difference at 6 and 44 hours, the times of the largest group differences. The

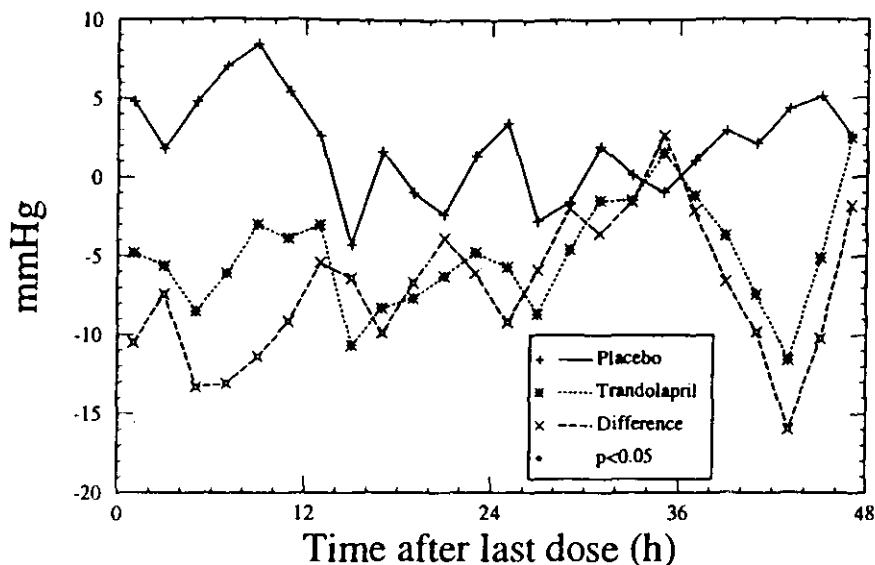


Figure 136. Changes from baseline in diastolic ABPM in Study TR-42-CP.

differences were -15.2 mmHg ($p=0.0001$) at 6 hours and -14.9 mmHg ($p=0.0001$) at 44 hours.

A37.6. Safety.

Headache was the only adverse event reported by at least 2 subjects in the active treatment group and at a greater frequency than in the placebo group ($n=5$). Eleven subjects in each group reported adverse events during the double-blind treatment period. No subject discontinued, had treatment interrupted, or had a dose reduction because of an adverse event. Two subjects in each treatment group had edema noted as either a new physical finding or adverse event. No subject had a supine systolic pressure <100 mmHg; 6 trandolapril and 4 placebo subjects had orthostatic hypotension³, none of which was reported as an adverse reaction. Two trandolapril subjects had ECG changes (sinus bradycardia and non-specific ST-T wave changes) noted at the final evaluation.

Laboratory data are summarized in Figure 137 to Figure 140 below.

One subject on trandolapril experienced a moderate elevation in SGPT (to about 4 x ULN at washout); there was no follow-up.

A37.7. Summary.

This was an 8-week double-blind, placebo-controlled study with ambulatory blood pressure monitoring and a 1-week washout. The trandolapril treatment group was titrated as needed from 2 to 4 mg q.d.; virtually all trandolapril subjects received the higher dose. The sponsor's statistical analysis found significant reductions in cuff blood pressure in the trandolapril group at most time points. Differences in cuff blood pressure were largely gone at the end of the washout period. ABPM data appear to show a reduction in effect to baseline by about 36 hours after the last dose; however, at 37 to 45 hours, a difference in diastolic pressure between treatment groups reappears with larger magnitude than that seen in the first 24 hours. There were no safety-related withdrawals. One subject had moderately elevated hepatic enzymes at the final study visit and was not followed-up.

³ Defined by sponsor as >20 mmHg difference in supine and standing systolic pressure.

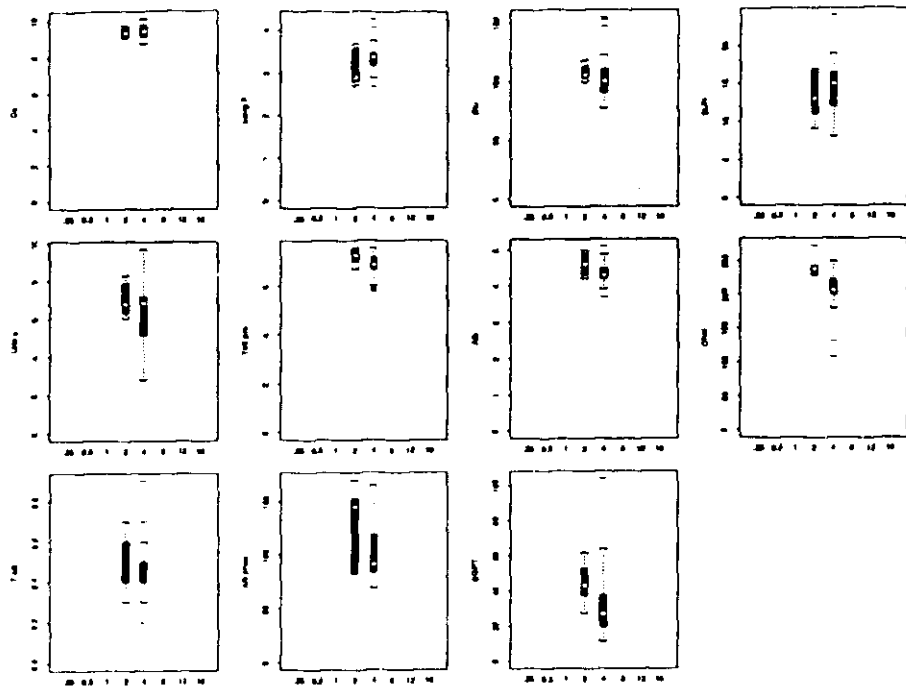


Figure 137. Clinical chemistry data (part A) in Study TR-42-CP.

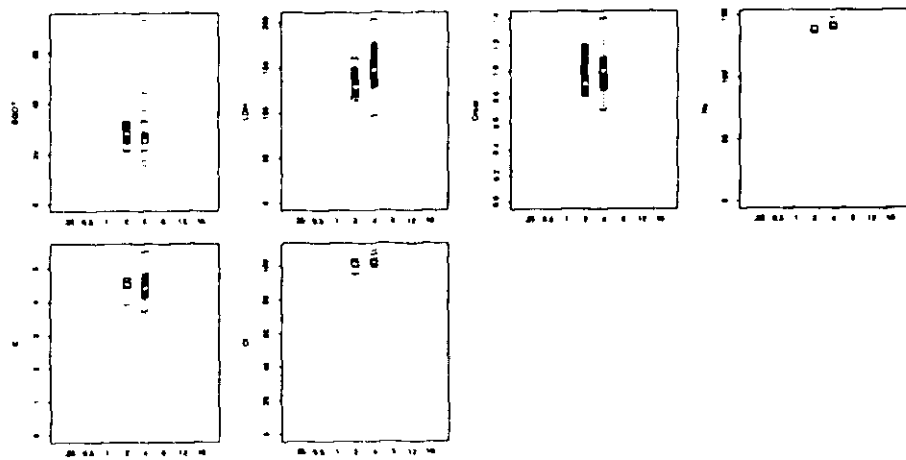


Figure 138. Clinical chemistry (part B) in Study TR-42-CP.

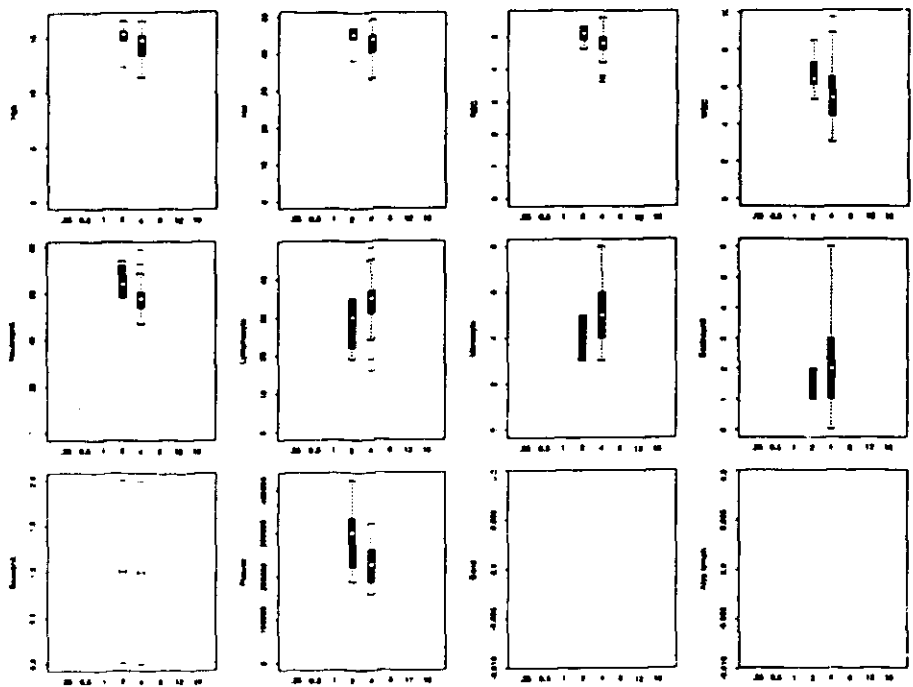


Figure 139. Hematology data in Study TR-42-CP.

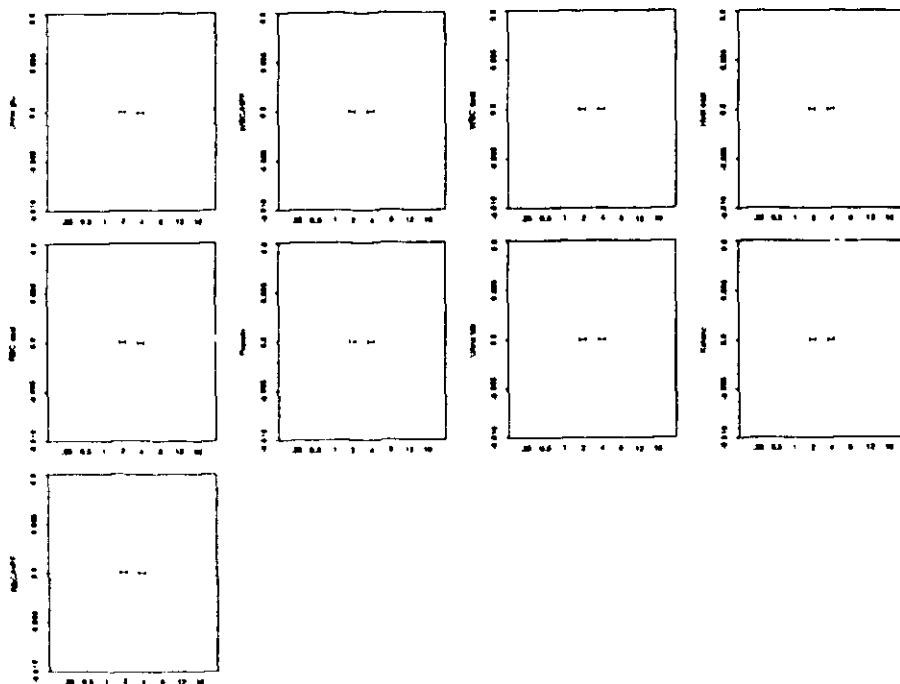


Figure 140. Urinalysis data in Study TR-42-CP.

A38. Study TV-40-HTN (Study report: vols 1.260-1.270; case report tabulations: vols 1.363-1.374; case report forms for deaths and discontinuations for adverse events: vol 1.391) "Evaluation of the safety and efficacy of oral trandolapril in combination with verapamil in patients with severe essential hypertension".

A38.1. Investigators The investigators are listed in Table 324 below. A twelfth investigator is said to have

Table 324. Investigators in Study TV-40-HTN.

Investigator	Center	City	Investigator	Center	City
JE Angelo, DO	4012	New Orleans, LA	LK Smith, MD	4004	Phoenix, AZ
R Esham, MD	4009	Mobile, AL	J Soler, MD	4002	Seffner, FL
R Goldstein, MD	4001	Tampa, FL	CT Spalding, MD	4005	Albuquerque, NM
PK Kaihlanen, MD	4013	San Antonio, TX	MA Weber, MD	4007	Long Beach, CA
HB Karunaratne, MD	4003	Winter Park, FL	M Weir, MD	4006	Baltimore, MD
HA Punzi, MD	4008	Carrollton, TX			

enrolled no subjects.

A38.2. Study dates March 1991 to October 1992.

A38.3. Drug formulation

Table 325. Drug substance used in Study TV-40-HTN.

Lot	Form	Type	Trandolapril	Source lot	Batch size
TRA-0120A	Cap	Tran	2.0 mg	L-11	600,000
99-0190	Cap	Tran	4.0 mg	99-0190	31,595
99-0290	Cap	Tran	4.0 mg	99-0290	86,218
99-0391	Cap	Tran	4.0 mg	99-0391	85,963

Table 326. Capsule composition in Study TV-40-HTN.

	2 mg	4 mg
Trandolapril	2.0	4.0
Corn starch		
Lactose, hydrous		
Povidone		
Sodium stearyl fumarate		
Total		

A38.4. Study design

Eleven centers participated in this open-label study. Each was to enroll approximately 10 subjects. Subjects were to be age 21 to 70 years, with untreated baseline supine diastolic pressure 115 to 135 mmHg. Women were permitted if neither childbearing nor lactating, and if practicing recognized forms of contraception. Exclusion criteria are listed in Table 327 below. Other excluded concomitant drugs were antiarrhythmics, digitalis, MAO inhibitors, psychotropic drugs, chronic NSAIDs, phenylpropanolamine, and pseudephedrine.

Subjects were to have their dose titrated as needed from 1 to 2 to 4 mg q.d. The titration interval was to 1 month or longer. Subjects who did not have adequate blood pressure control on trandolapril 4 mg q.d. were to have added Isoptin SR (verapamil) 180 mg

Table 327. Exclusion criteria in Study TV-40-HTN.

Hypertension other than severe essential
History of evidence of malignant or accelerated hypertension
Cerebrovascular accident, convulsions, or hypertensive encephalopathy within 1 year
Clinically significant congestive heart failure or EF<40%
Use of antihypertensives within 3 days of dose-titration period
Heart rate <50 bpm, significant arrhythmia, sinus node dysfunction, or conduction abnormalities
Myocardial infarction within 3 months
Hypersensitivity or serious adverse reaction to any ACE inhibitor
Clinically significant respiratory or connective tissue disorder
Clinically significant renal failure, e.g., creatinine >2 mg/dl
Clinically significant hepatic failure, e.g., SGOT or SGPT >2 times ULN
Significant electrolyte imbalance
Significant hematologic abnormalities
Unstable diabetes mellitus
History of angioneurotic edema
Proteinuria >300 mg/day
Medical or surgical conditions affecting absorption, metabolism, or excretion
Previous clinical trial experience within 30 days, or any exposure to trandolapril
History of chronic alcohol or drug abuse within 2 years
Terminal illness

q.d. If blood pressure were not adequately controlled for 1 month on the combination, then the subject was to be discontinued.

Subjects underwent a 3 to 14 day placebo-withdrawal. Subjects qualified on the basis of supine diastolic pressure 0, 1, 2, and 3 hours after placebo administration. Subjects then entered a 2-week dose-titration phase. The starting dose for Caucasians was 2 mg q.d. and for Blacks it was 4 mg q.d. The dose was doubled if necessary at 1 week, sustained release verapamil 180 mg was added at 3 weeks, and HCTZ 12.5 to 25 mg was added at 5 weeks. Subjects whose diastolic pressure was >110 mmHg could be titrated more quickly.

The maintenance period was 19 weeks with visits scheduled at 1, 3, 7, 9, 11, 15, and 19 weeks.

The primary efficacy criterion was supine diastolic pressure at trough, with satisfactory therapeutic response defined as diastolic pressure <90 or a reduction by ≥ 10 mmHg at end point.

Safety data included medical history, physical exam, 12-lead ECG, and routine hematology, clinical chemistry, and urinalysis. There were no explicit criteria for dropping a subject because of lack of efficacy.

Amendment I called for the addition of a baseline 12-lead ECG.

A38.5. Results

Ninety subjects enrolled in the trial. Individual study centers enrolled 1 to 20 subjects. Demographic and baseline characteristics of subjects are shown in Table 328 below. Only 4 subjects had secondary cardiovascular diagnoses (2 myocardial infarctions, 1 palpitations, 1 PVCs). Seven subjects had diabetes mellitus.

Table 328. Demographic and baseline characteristics of subjects in Study TV-40-HTN.

	Trandolapril only			Verapamil (80mg)+						All n=90
				HCTZ (12.5mg)+		HCTZ (25mg)+				
	1mg n=3	2mg n=7	4mg n=1	1mg n=3	2mg n=4	1mg n=10	2mg n=5	1mg n=34	2mg n=22	
Male	1	7	0	3	2	9	3	24	14	63
Female	2	0	1	2	1	1	2	10	8	27
Age±SD	39±10	55±15	40	43±14	43±13	55±10	58±12	50±10	53±10	51±11
Range	27 to 45	38 to 71		24 to 56	28 to 51	41 to 69	45 to 71	31 to 69	31 to 70	24 to 71
Caucasian	3	4	0	4	1	10	0	34	2	58
Black	0	3	1	1	2	0	5	0	20	32
Other	0	0	0	0	0	0	0	0	0	0
Weight±SD	186±53	218±45	193	190±54	195±54	233±63	210±25	219±49	196±25	211±45
Range	135 to 241	159 to 272		126 to 265	133 to 232	145 to 348	188 to 243	138 to 343	152 to 242	126 to 348

The disposition of subjects is shown in Table 329 below. A total of 8 subjects

Table 329. Disposition of subjects in Study TV-40-HTN.

Visit	n	Drop-outs				Visit	n	Drop-outs			
		ADR	LOE	Other	Total			ADR	LOE	Other	Total
Entry	90					Week 6	69	1	3	1	5
Day 1	89	0	0	1	1	Week 8	62	0	7	0	7
Week 1	84	2	1	2	5	Week 12	53	2	4	3	9
Week 2	80	1	2	1	4	Week 14	52	0	0	1	1
Week 3	79	1	0	0	1	Week 16	51	0	1	0	1
Week 4	78	1	0	0	1	Week 20	49	0	0	2	2
Week 5	74	0	4	0	4	Week 24	49	0	0	0	0

withdrew for adverse events¹, 22 for lack of efficacy, 11 for other reasons (7 for failure to follow appointment schedule, 2 for withdrawal of consent, and 2 moved from area).

No subjects were excluded from safety analyses because of protocol violations. One subject withdrew during the baseline period and was excluded from the efficacy assessment.

The sponsor presented several analyses of efficacy. Because of the open-label nature of the trial, these are difficult to interpret. Responders were considered subjects whose trough supine diastolic pressure was <90 mmHg or decreased by 10 mmHg. The sponsor considered "trough" any measurement at least 10 hours after dosing. For 87 subjects who received trandolapril monotherapy, the mean change in supine diastolic pressure at the last such dose was -9.0 mmHg. For 75 subjects who received trandolapril plus verapamil, the mean change at the last such dose was -13.9 mmHg. For 72 subjects who received trandolapril, verapamil, and HCTZ, the mean change was -19.0 mmHg at the last such dose. The responder rates at end point were 44% (5/9) for subjects who remained on monotherapy, 88% (7/8) for subjects who received no HCTZ, and 79% (55/70) for subjects who received all three agents.

¹ The sponsor tabulated and provided narrative summaries for 8 withdrawals for adverse events, but provided CRFs for 9 such subjects, as described below

A38.6. Safety.

The number of subjects reporting adverse events was 36 of 90 on trandolapril alone, 27 of 78 on trandolapril plus verapamil, and 35 of 73 subjects on all three agents. Headache, dizziness and cough were the most common adverse events. Dizziness and cough occurred somewhat more often with combination treatment, but this result must be interpreted in the light of a much briefer average exposure time to trandolapril alone.

Subjects who discontinued because of adverse reactions are listed in Table 330 below.

Table 330. Discontinuations for adverse events in Study TV-40-HTN.

Cntr	Subj	Dose	Day	ADR
4001	10	8+V	2 mo	61 year old Black female discontinued because of nonproductive cough of 1-month duration.
4003	6	4+V+ HCTZ	3 mo	51 year old Caucasian male with 32-year history of hypertension, discontinued 14 days after onset of cardiac chest pain and left arm pain, subsequent to positive exercise tolerance test. The investigator considered the event of moderate severity and probably not related to treatment. He was referred for cardiac catheterization. Last BP was 135/81 mmHg.
4005	3	4	10	45 year old Caucasian male with 23-year history of hypertension, discontinued upon hospitalization to rule out myocardial infarction following severe chest pressure. Last BP was 197/128 mmHg.
4005	11	4	17	58 year old Caucasian male with 18-year history of hypertension, discontinued with worsening headaches and nausea, of which the former began during placebo-withdrawal, and the latter resolved within 2 days of withdrawal.
4005	15	4+V	14	56 year old Caucasian male with diabetes mellitus and 6-year history of hypertension, discontinued because of paresthesias of the face and scalp, associated with sinus infection, and which resolved 6 days later.
4007	3	4+V+ HCTZ	7 wk	63 year old Caucasian male with 15-year history of hypertension, discontinued because of impotence, attributed by the investigator to HCTZ. Last BP was 164/99 mmHg.
4008	3	8	1 mo	40 year old Black female with 8-year history of hypertension, discontinued because of tiredness and excessive perspiration. Last BP was 190/120 mmHg.
4008	10	2	12	27 year old Caucasian female with 6-year history of hypertension, discontinued because of vomiting, headache, dizziness, and shortness of breath.
4013	4	4	9	70 year old Caucasian male with a 6-year history of hypertension, discontinued because of hospitalization for severe "neuro-orthopedic" symptoms, which were "definitely not" attributable to study drug.

In addition, one subject (4005-1), a 55-year old Black male, was receiving 8 mg trandolapril plus verapamil (for 66 days) when he experienced hypotension (BP 79/59, pulse 89). His dose was reduced to 4 mg trandolapril with no change in verapamil, and he completed the study. No subjects had treatment interruptions because of adverse events.

Few other subjects had orthostatic symptoms reported; no other subjects were treated for orthostatic symptoms. Eighteen percent (16/87) subjects had a 20 mmHg difference between standing and supine systolic pressures at end point trough. Less than 10% of subjects met this criterion 1 to 3 hours after the last dose.

Few subjects had new physical exam findings during the study, the most common of which were trauma and hypertensive retinopathy.

The most common new ECG findings were sinus bradycardia (n=12) and non-specific ST-T changes (n=24). Laboratory abnormalities considered marked occurred with 11 subjects. Most were of doubtful relationship to treatment. Three subjects had at least transient increases in WBC counts on urinalysis. One subject had a transiently low serum potassium (2.8 mEq/L) after 12 weeks on 4 mg trandolapril plus verapamil plus HCTZ.

A38.7. Summary.

This was an open-label study, in which subjects had severe hypertension and the dose was titrated for efficacy. Verapamil and HCTZ were sequentially added as necessary. Most subjects were on the highest allowed doses at the time of completion or withdrawal. One-third of subjects withdrew either for lack of efficacy or adverse events. Seventy-nine percent of subjects receiving all three drugs met criteria for efficacy at end point.

A39. Study TV-41-CP (Study report: vol 1.119; case report tabulations: vol 1.120; case report forms for deaths and discontinuations for adverse events: 1.392) "Systemic and regional hemodynamic effects of trandolapril and trandolapril + verapamil in patients with mild to moderate essential hypertension" and follow-on Study TV-31-HTN (case report tabulations: vols 1.375-1.376) "Open label study of the long-term safety of oral trandolapril in combination with verapamil (Isoptin SR)".

A39.1. Investigators The investigator was FH Messerli (center 4101¹ and 3141²), New Orleans, LA.

A39.2. Study dates September 1991 to July 1992 (as center 4101) and July 1992 to December 1992 (as center 3141).

A39.3. Drug formulation

Table 331. Drug substance used in Study TV-41-CP.

Lot	Form	Type	Trandolapril	Source lot	Batch size
TRA-0580	Cap	Plcbo	—	L-32	1,200,000
TRA-0120A	Cap	Tran	2.0 mg	L-11	600,000
99-0391	Cap	Tran	4.0 mg	99-0391	85,963

Table 332. Capsule composition in Study TV-41-CP.

	2 mg	4 mg
Trandolapril	2.0	4.0
Corn starch		
Lactose, hydrous		
Povidone		
Sodium stearyl fumarate		
Total		

A39.4. Study design This was a single center study with a 4-week single-blind placebo run-in phase, and a 10-week open-label titration study. Twenty subjects were to have stable essential hypertension with supine diastolic pressure 95 to 114 mmHg at the end of placebo-withdrawal, age 21 or older, not more than 30% above ideal body weight, and of low childbearing potential. Exclusion criteria are listed in Table 333 below. Other excluded

Table 333. Exclusion criteria in Study TV-41-CP.

Hypertension other than severe essential
History of evidence of malignant or accelerated hypertension
Cerebrovascular accident, convulsions, or hypertensive encephalopathy within 1 year
Clinically significant congestive heart failure or EF<40%
Use of antihypertensives within 4 weeks or spironolactone within 6 weeks of dose-titration period
Heart rate <50 bpm, significant arrhythmia, sinus node dysfunction, or conduction abnormalities
Myocardial infarction within 3 months
Hypersensitivity or serious adverse reaction to any ACE inhibitor
Clinically significant renal failure, e.g., creatinine >2 mg/dl
Clinically significant hepatic failure, e.g., SGOT or SGPT >2 times ULN
Significant electrolyte imbalance

¹ Subjects in center 4101 were enrolled in Study TV-41-CP and then follow-on Study

² Subjects in center 3141 were enrolled in Study and then follow-on Study

Table 333. Exclusion criteria in Study TV-41-CP. (Continued)

Significant hematologic abnormalities	
Medical or surgical conditions affecting absorption, metabolism, or excretion	
Previous clinical trial experience within 30 days, or any exposure to trandolapril	
History of chronic alcohol or drug abuse within 2 years	
Terminal illness	

concomitant drugs were antiarrhythmics, digitalis, MAO inhibitors, psychotropic drugs, chronic NSAIDs, phenylpropanolamine, and pseudoephedrine.

All subjects received trandolapril 2 mg/day during the first 2 weeks. Those not meeting efficacy criteria (supine diastolic pressure <85 mmHg) had their dose increased to 4 mg/day for another 2 weeks. Those not meeting efficacy criteria at 4 weeks had verapamil (Isoptin SR) 120 mg/day added, and then increased to 180 mg/day at 6 weeks, if needed. There were no specific criteria for withdrawal for lack of efficacy.

Hemodynamic measurements included plasma volume (^{125}I -albumin), cardiac output (indocyanine green dilution), renal blood flow (clearance of ^{131}I -PAH), and splanchnic blood flow (clearance of indocyanine green). Plasma drug levels, renin, aldosterone, and catecholamines were measured.

Safety data included medical history, physical exam, 12-lead ECG, and routine hematology, clinical chemistry, and urinalysis.

A39.5. Results

Eleven subjects enrolled in Study TV-41-CP. Demographic and baseline characteristics of subjects are shown in Table 334 below.

Table 334. Demographic and baseline characteristics of subjects in Study TV-41-CP.

	n=11
Male	8
Female	3
Age $\pm$ SD	52 $\pm$ 15
Range	30 to 74
Caucasian	5
Black	6
Other	0
Weight $\pm$ SD	196 $\pm$ 51
Range	135 to 288

One subject withdrew for adverse events, and none withdrew for any other reason. All subjects had protocol violations noted. Three subjects were missing more than one hemodynamic measurement and were excluded from efficacy assessments. No subjects were excluded from safety analyses because of protocol violations.

Of 10 subjects completing study, 4 completed on 2 mg trandolapril, 2 on 4 mg, 4 on 4 mg trandolapril plus 120 mg verapamil, and none were on 4 mg trandolapril plus 180 mg verapamil. One subject was said to be <70% compliant.

For 6 subjects on monotherapy at study end, mean supine diastolic pressure fell from 99 to 90 mmHg. For 4 subjects on combination therapy, supine diastolic pressure fell from 110 to 97 mmHg.

All but two subjects had invasive hemodynamic measurements at baseline, 6 at the end of trandolapril monotherapy, and 2 subjects at the final study visit. Only the data from these measurements were analyzed by the sponsor.

There were other serious departures from protocol with regard to missing or timing of other measurements. Because of these defects and the small numbers of subjects overall, these data were not reviewed.

Nine subjects continued in the 12-month follow-on study. At the time the study was halted by Knoll, 2 subjects had discontinued for failure to follow appointment schedules and 7 subjects had been enrolled for 2 weeks to 9 months. At the time of discontinuation, 6 subjects were on trandolapril 2 mg plus verapamil 180 mg (the highest allowed doses of both agents), and 3 subjects were on trandolapril 2 mg plus verapamil 120 mg. Mean trough supine diastolic pressure was 103 mmHg at baseline and 86 mmHg at end point, where "trough" was defined as at least 10 hours after dosing.

A39.6. Safety.

Subject 4101-8 was a 50 year old Black female with diabetes mellitus who became anxious, nauseated, and vomited during baseline hemodynamic testing. She was hospitalized but later received one dose of 2 mg trandolapril. She discontinued the next day.

Subject 4101-9 was a 44 year old female who developed a serious depression after 31 days on 2 mg trandolapril. No treatment was deemed necessary and the event was not considered drug-related.

No subject met criteria for hypotension or orthostatic hypotension.

Significant laboratory abnormalities included one subject with >75 RBC/HPF, and one subject with low neutrophil count (960/mm³) and high atypical lymphocyte count (260/mm³).

There were no serious adverse events or deaths in the follow-on study. No subject experienced hypotension or orthostatic hypotension. There were no new and clinically significant findings on physical exam or laboratory assessments.

A39.7. Summary.

This was a short-term open-label study with a long-term open-label follow-on, in which subjects had mild to moderate hypertension and the doses of trandolapril and verapamil was titrated for efficacy. Technical problems in the design and conduct of the trial prevent conclusions regarding efficacy. In the follow-on study, most subjects were on the highest allowed doses of both drugs at the time of completion or withdrawal.

A40. Study ZA/88/570/28 (Study report: vol 1.121; case report tabulations: 1.121-1.122; case report forms for deaths and discontinuations for adverse events: none) "Interaction between warfarin and trandolapril"

A40.1. Investigators The investigator was B Meyer, Bloemfontein. South Africa.

A40.2. Study dates May 1989 to June 1989.

A40.3. Drug formulation

Table 335. Drug substance used in Study ZA/88/570/28.

Lot	Form	Type	Trandolapril	Source lot	Batch size
21662-144	Cap	Plcbo	—	—	30,000
MB 22327-149	Cap	Tran	2.0 mg	18	25,000

Table 336. Capsule composition in Study ZA/88/570/28.

	2 mg
Trandolapril	2.0
Corn starch	
Lactose, hydrous	
Povidone	
Sodium stearyl fumarate	
Total	

A40.4. Study design

This placebo-controlled, double-blind, sequence-randomized crossover study assessed the effect of multi-dose trandolapril therapy (2 mg/day) on single-dose warfarin (25 mg) pharmacodynamics in 19 healthy males. Trandolapril or placebo was randomly administered during each of two 13-day treatment periods (study days 8 to 20, and 22 to 34). Single warfarin doses were administered (under fasting conditions) one week prior to randomized therapy (study day 1), and on the eighth day of each of the randomized treatment periods (study days 15 and 29).

Enrolled subjects were healthy, adult, male Caucasians. They were to abstain from non-trial medication (beginning 2 weeks prior to the trial), as well as from the use of tobacco, ethanol, and xanthine-containing beverages (during the days before and after drug administration).

The exclusion criteria are shown in Table 337 below.

Table 337. Exclusion criteria in Study ZA/88/570/28.

Weight 10 to 15% more or less than ideal
Presence of hypersensitivity, any disease of the renal, hepatic, or gastrointestinal systems, abnormal blood pressure or EKG, or abnormal chest radiograph or pulmonary function test
Use in the previous 3 months of a drug with the potential for major organ toxicity
Regular use of medication or ethanol
Blood donation >500 ml during the previous 3 months
Participation in another clinical trial in the previous 3 months

The International Normalized Ratio (INR), and the prothrombin time (PT) were measured at baseline (prior to each warfarin treatment), at hours 12 and 24 after each

warfarin dose, and at the end of post-dose days 2, 3, 4, 5, and 6. Clotting factors II, VII, IX, and X were also evaluated, but technical problems precluded a reliable analysis of these results.

Hematology and clinical chemistry measures were obtained at baseline and 24 hours after the final study medication. Blood pressure, and heart rate were determined at baseline (pre-treatment), and after completion of the trial.

Descriptive statistics (including 90% confidence intervals) were generated for INR data.

A40.5. Results

The demographic characteristics of the subjects are presented in Table 338 below.

Table 338. Demographic and baseline characteristics of subjects in Study ZA/88/570/28.

	n=20
Male	20
Female	0
Caucasian	20
Other	0
Age—mean	21
Weight—mean	81

There was reportedly one dropout. Although minimally described, this withdrawal was attributed by the sponsor to a leg fracture. No protocol violations were reported.

INR data are depicted in Figure 141 below, and, as changes from placebo, in Table 339

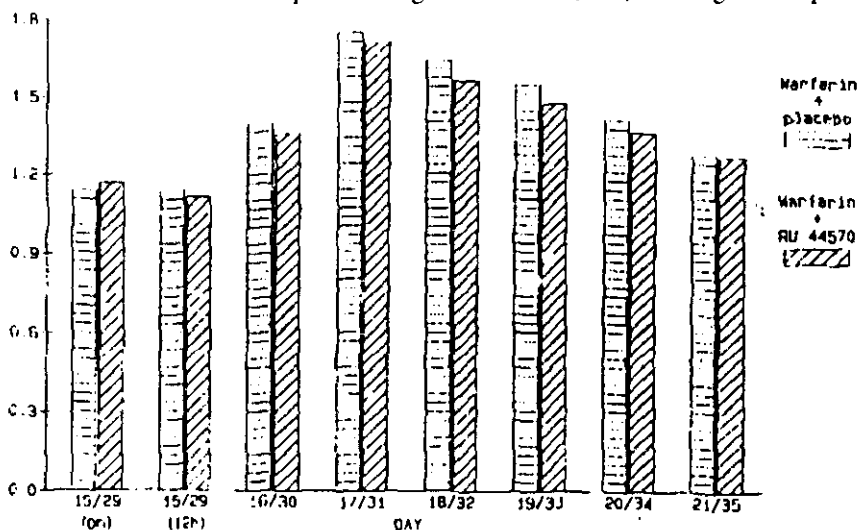


Figure 141. Mean INR in Study ZA/88/570/28.

below. The maximal mean INR levels attained after single 25 mg warfarin doses were

Table 339. Change from placebo in INR in Study ZA/88/570/28.

Time	Mean	90% CI	Time	Mean	90% CI
Baseline	0.03	-0.05, 0.11	3 days	-0.09	-0.28, 0.09
12 hours	-0.02	-0.08, 0.03	4 days	-0.07	-0.21, 0.06
1 day	-0.05	-0.16, 0.06	5 days	-0.04	-0.12, 0.04
2 days	-0.04	-0.16, 0.07	6 days	-0.01	-0.08, 0.06

in all periods sub-therapeutic (1.75 to 1.85)¹. During trandolapril-treated periods the mean baseline (pre-warfarin) INR tended to be higher than during placebo periods. There was a tendency towards a small (no higher than 0.09) reduction in mean INR in the presence of trandolapril, but the confidence intervals for the difference between trandolapril and placebo-associated INRs included zero at all observed time points after exposure to warfarin.

For completeness, prothrombin time data are presented in Figure 142 below (although

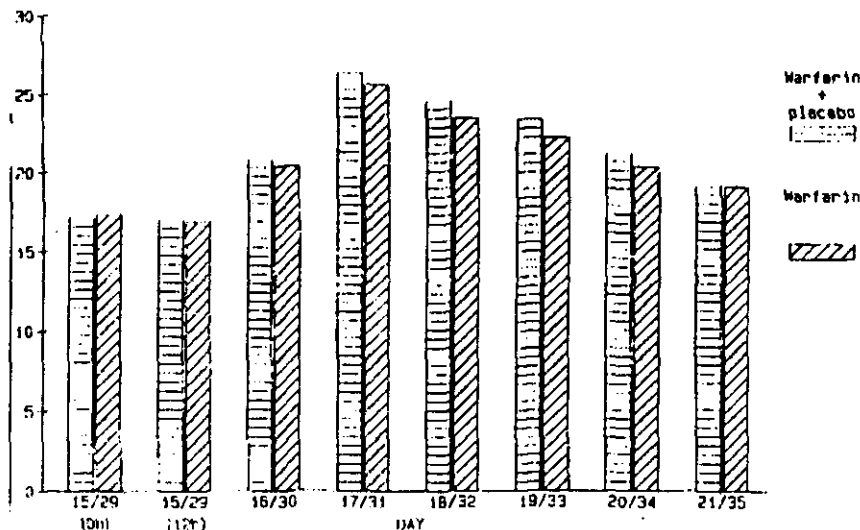


Figure 142. Mean prothrombin time in Study ZA/88/570/28.

these are assay-dependent and therefore less useful than INR data).

The reported differences (baseline to post-trial) in blood pressure, and heart rate were clinically nonsignificant.

A40.6. Safety.

Case report forms were not submitted for evaluation. There were no adverse events reported.

The following study-emergent laboratory abnormalities were observed 24 hours after the final study drug administration: subject 10 had moderate elevations of SGPT (42 U/L) and total bilirubin (28 μ M); and subject 18 had a minimal leukocytosis (3.9 K/ μ L). The report did not contain a description of the reversibility, clinical antecedents, or sequelae of these findings.

A40.7. Summary.

This placebo-controlled, sequence-randomized crossover study assessed the effect of multi-dose trandolapril therapy (2 mg/day) on single-dose warfarin (25 mg) pharmacodynamics in 19 healthy males. During trandolapril-treated periods the mean baseline INR tended to be higher than during placebo periods. There was a tendency towards a small reduction in mean warfarin-associated INR in the presence of trandolapril, but the confidence intervals for the difference between trandolapril and placebo effects on this included zero during all post-warfarin observations. The following three study-emergent laboratory abnormalities (of indeterminate causality given the data submitted) were observed at the end of the trial: moderate elevations of SGPT and total bilirubin, and minimal leukocytosis. There was one minimally described dropout which was attributed to a leg fracture.

¹ with respect to the goals for treatment of, for example, non-valvular atrial fibrillation or venous thromboembolism.

A41. Study ZA/89/570/43 (Study report: vol 1.94; case report tabulations: 1.94-1.101; case report forms for deaths and discontinuations for adverse events: none) "Pharmacokinetic interaction study between trandolapril and furosemide".

A41.1. Investigators The investigator was B Meyer, Bloemfontein, South Africa.

A41.2. Study dates June 1989 to August 1989.

A41.3. Drug formulation

Table 340. Drug substance used in Study ZA/89/570/43.

Lot	Form	Type	Trandolapril	Source lot	Batch size
EF 20183-48	Cap	Plcbo	—	—	16,870
MB 22327-149	Cap	Tran	2.0 mg	18	25,000

Table 341. Capsule composition in Study ZA/89/570/43.

	2 mg
Trandolapril	2.0
Corn starch	
Lactose, hydrous	
Povidone	
Sodium stearyl fumarate	
Total	

A41.4. Study design

This single-blind, placebo-controlled¹, 3-way crossover study assessed bidirectional kinetic and dynamic drug-drug interactions in 12 healthy adult males randomized (on 3 discrete treatment days separated by 3 week intervals) to a single dose of trandolapril (2 mg/day), a single dose of furosemide (40 mg), or to their combination. Subjects were to abstain from non-trial medication (beginning 2 weeks prior to the trial), as well as from the use of tobacco, ethanol, and xanthine-containing beverages (during the days before and after drug administration). The timing and content of meals were standardized during drug administration days.

The exclusion criteria are shown in Table 342 below.

Table 342. Exclusion criteria in Study ZA/89/570/43.

Weight 10 to 15% more or less than ideal
Presence of hypersensitivity, any disease of the renal, hepatic, or gastrointestinal systems, abnormal blood pressure or EKG, or abnormal chest radiograph or pulmonary function test
Use in the previous 3 months of a drug with the potential for major organ toxicity
Regular use of medication or ethanol
Blood donation >500 ml during the previous 3 months
Participation in another clinical trial in the previous 3 months

In each treatment period blood samples for the determination of ACE activity were collected at baseline, at post-dose hours 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24, and at the end of post-dose days 2 and 3. Baseline and 24 hour post-dose urine samples were obtained for the determination of creatinine and electrolytes. Hematology and clinical

¹ Placebo was used in a dummy-pill technique, but there was no placebo monotherapy control.

chemistry measures were obtained at baseline and 24 hours after the final study medication.

Blood pressure, and heart rate measures were obtained at baseline, at post-dose hours 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24, and at the end of post-dose days 2 and 3.

Blood samples for the determination of trandolapril, trandolaprilat, and furosemide levels were collected at baseline, at post-dose hours 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, and 24, and at the end of post-dose days 2 and 3. Urine samples for assay of trandolaprilat and furosemide were collected pre-dose (-24 to 0) and at post-dose intervals 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, and 12 to 24 hours. Plasma and urine samples were stored at -20°C until analyzed.

Pharmacokinetic assays were assessed as shown in Table 343 below. The assays have

Table 343. Pharmacokinetic assays in Study ZA/89/570/43.

Linearity	Accuracy		Precision			Sensitivity	Specificity
Plasma samples—trandolapril and trandolaprilat—							
Satisfactory.	Satisfactory.		Satisfactory.			LOQ—0.1 ng/ml.	Satisfactory.
Standard curves from 0.02 to 10 ng/ml for trandolapril and trandolaprilat	ng/ml	T'april	T'aprilat	ng/ml	T'april	T'aprilat	
	0.155	105.0%	99.4%	0.155	7.9%	9.2%	
	0.309	105.0%	99.4%	0.309	7.8%	8.7%	
	0.824	105.0%	102.0%	0.824	7.7%	6.1%	
	2.060	102.0%	94.3%	2.060	7.7%	7.5%	
Urine samples—trandolaprilat—							
Satisfactory. Standard curves from 7.8 to 125 ng/ml.	Satisfactory. 98.0% at 10 ng/ml, 102.0% at 20 ng/ml, 106.8% at 40 ng/ml, and 101.1% at 80 ng/ml		Satisfactory. %CV— 11.4% at 10 ng/ml, 7.6% at 20 ng/ml, 6.1% at 40 ng/ml, 6.9% at 80 ng/ml			LOQ—10 ng/ml	Satisfactory
Plasma samples—ACE inhibition—							
"	Satisfactory. 50.4 U/L for expected values of 44 to 58 U/L and 58.3 U/L for expected values of 50 to 67 U/L.		Satisfactory. C.V. 3.3% at 50.4 U/L and 3.3% at 58.3 U/L.				
Plasma samples—furosemide—							
Satisfactory. Standard curves from 10 to 1000 ng/ml.	Satisfactory. 97.4% at 150 ng/ml, 95.9% at 300 ng/ml, 97.2% at 600 ng/ml.		Satisfactory. C.V. 2.7% at 150 ng/ml, 2.9% at 300 ng/ml, 2.4% at 600 ng/ml.			LOQ—5 ng/ml	Satisfactory. Chromatograms submitted.
Urine samples—furosemide—							
Satisfactory. Standard curves from 0.5 to 10 µg/ml.	Satisfactory. 99.3% at 1.0 µg/ml, 97.8% at 2.5 µg/ml, and 96.5% at 10.1 µg/ml.		Satisfactory. C.V. 6.4% at 1.0 µg/ml, 5.1% at 2.5 µg/ml, and 1.0% at 10.1 µg/ml.			LOQ—0.05 µg/ml	Satisfactory. Chromatograms submitted.

been validated over the range of plasma and urine concentrations observed in the study.

AUC_∞, C_{max}, t_{1/2}, CL_r, and percentage of dose secreted in urine were calculated.

There was no sequence randomization and, as a result, the study was sequentially unbalanced. ANOVA was used to compare end points in the presence and absence of trandolapril.

A41.5. Results

The demographic characteristics of the subjects are presented in Table 344 below.

Table 344. Demographic and baseline characteristics of subjects in Study ZA/89/570/43.

	n=12
Male	12
Female	0
Age—mean	21.5
Weight—mean	77.3

There were reportedly no protocol violations and no dropouts.

Blood pressure effects are shown in Figure 143 below and Table 345 below. Although

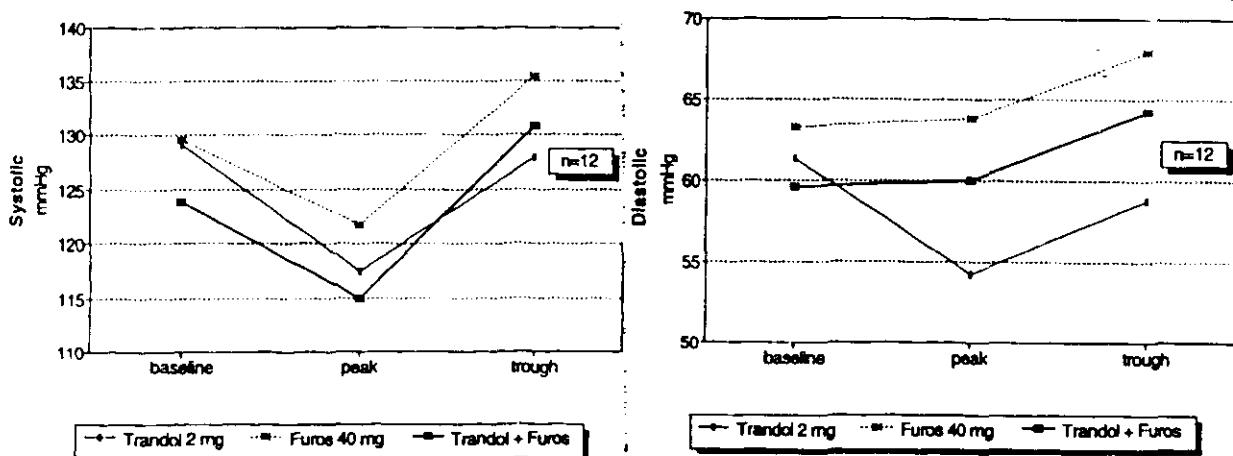


Figure 143. Blood pressure data in Study ZA/89/570/43.

Table 345. Blood pressure in Study ZA/89/570/43.

	Trand alone	Furos alone	Trand + furos		Trand alone	Furos alone	Trand + furos
Diastolic (mean)				Systolic (mean)			
Baseline	61.3	63.3	59.6	Baseline	129.1	129.6	123.8
Peak	54.2	63.8	60.0	Peak	117.5	121.7	115.0
Trough	58.8	67.9	64.2	Trough	127.9	135.4	130.8

not placebo monotherapy-controlled, the mean blood pressure-lowering effect associated with trandolapril appeared to be slightly-to-moderately larger than the effect associated with the combination of trandolapril and furosemide.

Heart rate changes from baseline were clinically nonsignificant during each treatment phase.

The trandolapril effects on furosemide-induced diuresis, natriuresis, and kaliuresis are shown in Figure 144 to Figure 146 below. The cumulative (0-12 or 0-24 hour) furosemide-induced excretion of urine and electrolytes was not appreciably altered by

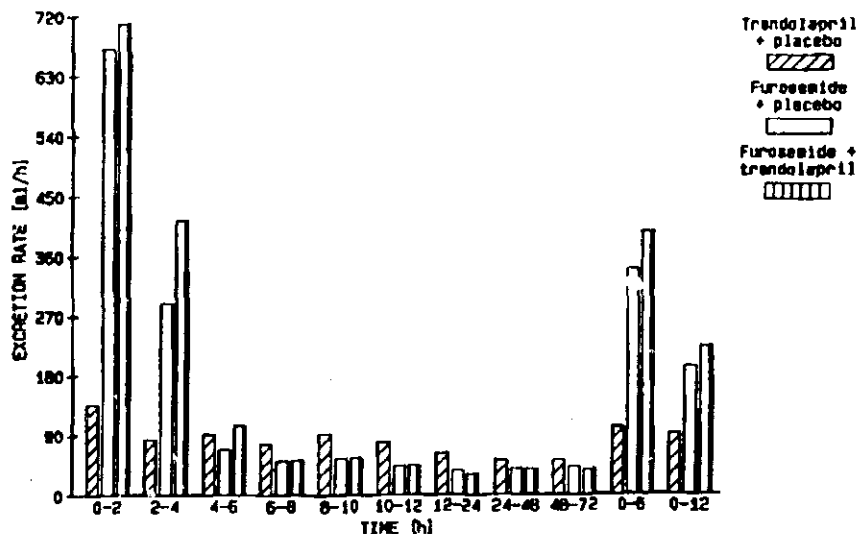


Figure 144. Mean urinary excretion rate in Study ZA/89/570/43.

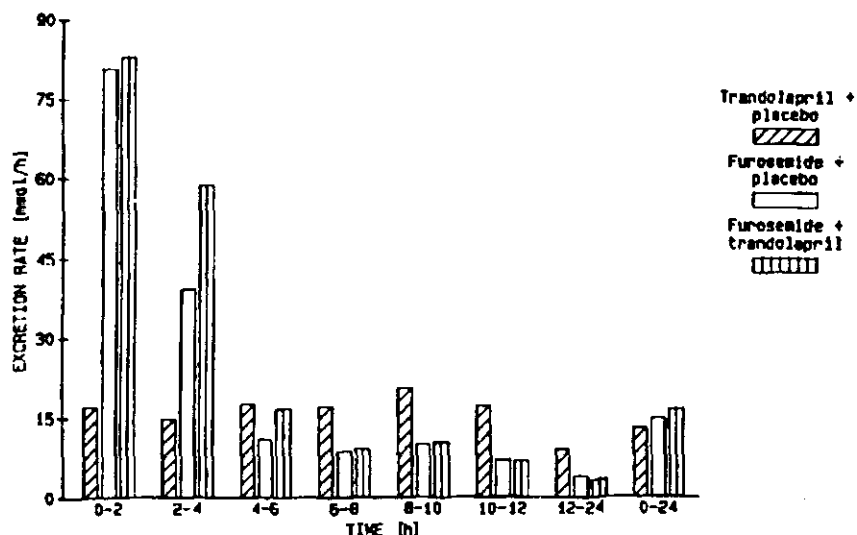


Figure 145. Mean urinary sodium excretion in Study ZA/89/570/43.

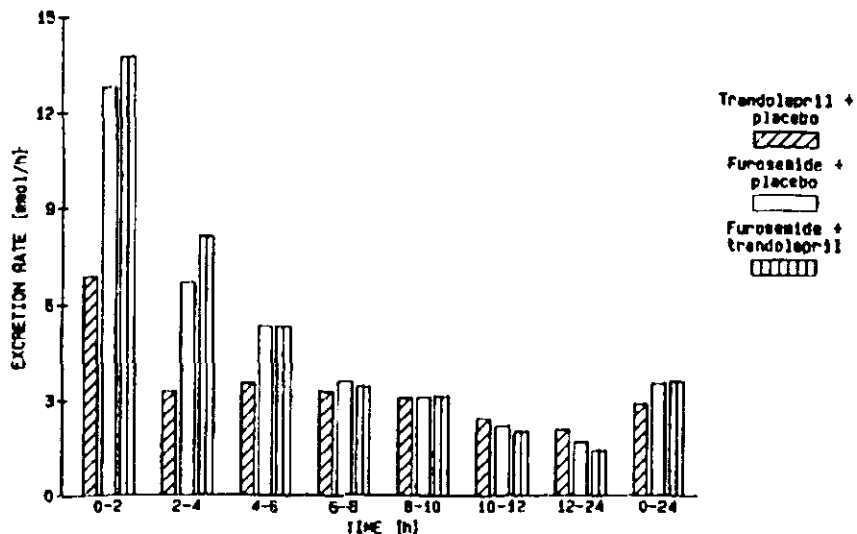


Figure 146. Mean urinary potassium excretion in Study ZA/89/570/43.

trandolapril, although trandolapril appeared to potentiate the peak rates (2 to 4 hours post-dose) of excretion of urine, sodium, and potassium.

Pharmacokinetic data are summarized in Table 346 below. Plasma levels of

Table 346. Pharmacokinetic parameters in Study ZA/89/570/43.

	Trandolapril + Placebo	Placebo + Furosemide	Trandolapril + Furosemide		Trandolapril + Placebo	Placebo + Furosemide	Trandolapril + Furosemide
Trandolapril				Furosemide			
AUC ₀₋₄ (h·ng/ml)	4.43±1.63	—	4.98±1.80	AUC ₀₋₁ (h·ng/ml)	—	2209±728	2257±479
C _{max} (ng/ml)	3.37±1.57	—	3.61±1.52	AUC _{0-∞} (h·ng/ml)	—	2245±724	2300±473
T _{max} (h)	0.63±0.27	—	1.02±0.39	C _{max} (ng/ml)	—	1097±658	954±311
				T _{max} (h)	—	1.60±0.92	1.96±0.72
				t _{1/2 α}	—	0.89±0.18	0.75±0.07
				t _{1/2 β}	—	3.47±1.37	3.53±1.43
				Ae ₀₋₇₂ (%dose)	—	44.4±6.76	48.5±7.60
				CL _r (ml/min)	—	138±27.7	144±23.8
Trandolaprilat				ACE inhibition			
AUC ₀₋₂₄ (h·ng/ml)	46.5±12.5	—	44.0±11.9	I _{max} (%)	85.6±11.6	—	84.0±9.44
AUC ₀₋₇₂ (h·ng/ml)	101±18.7	—	95.8±20.8	AUCI ₀₋₂₄ (%·h)	1574±403	—	1649±335
C _{max} (ng/ml)	3.41±2.08	—	3.06±1.23	AUCI ₀₋₇₂ (%·h)	4424±927	—	4637±1037
T _{max} (h)	5.25±2.05	—	5.08±2.31	I ₂₄ (%)	62.8±16.8	—	62.8±22.6
Ae ₀₋₂₄ (%dose)	9.57±4.56	—	11.9±8.42	TI _{max} (h)	7.25±12.9	—	8.88±12.7
Ae ₀₋₇₂ (%dose)	14.1±5.52	—	15.7±8.63	TI _{>80%} (h)	10.0±14.1	—	12.2±18.2
CL _r (ml/min)	62.8±24.1	—	78.3±40.4				

trandolapril and trandolaprilat are shown in Figure 147 below. Furosemide levels are

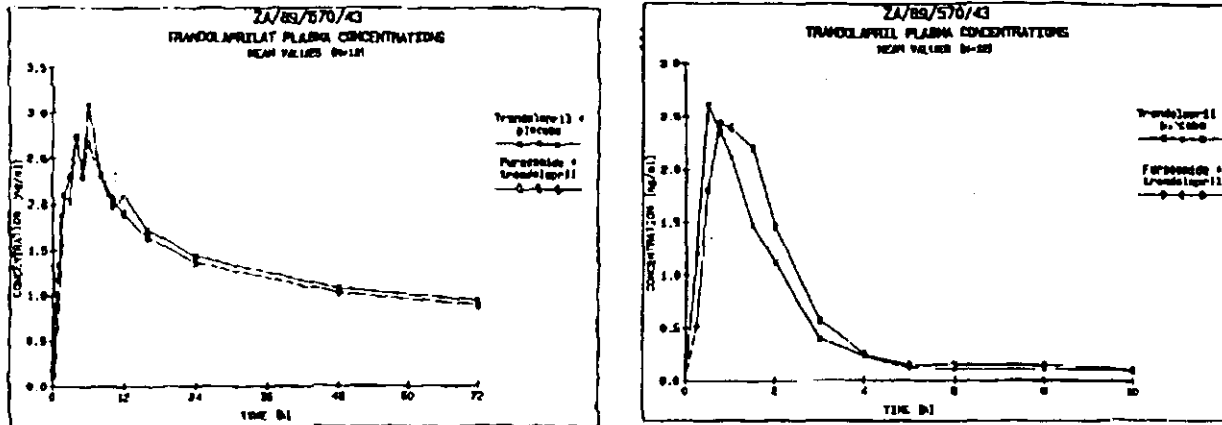


Figure 147. Plasma trandolapril and trandolaprilat levels in Study ZA/89/570/43.

shown in Figure 148 below. Cumulative urinary excretion of trandolaprilat and furosemide are shown in Figure 149 below. Plasma ACE inhibition as functions of time and plasma trandolaprilat is shown in Figure 150 below.

A41.6. Safety.

Case report forms were not submitted for evaluation. Only brief descriptions of adverse effects were provided. Two subjects complained of headache during combined therapy with trandolapril and furosemide, yet a greater number (n=3) had this complaint during exposure to furosemide plus placebo.

Potentially clinically significant, treatment-emergent laboratory abnormalities were as follows:

Subject 4, a 21 year old and 79 kg male, manifested an isolated,

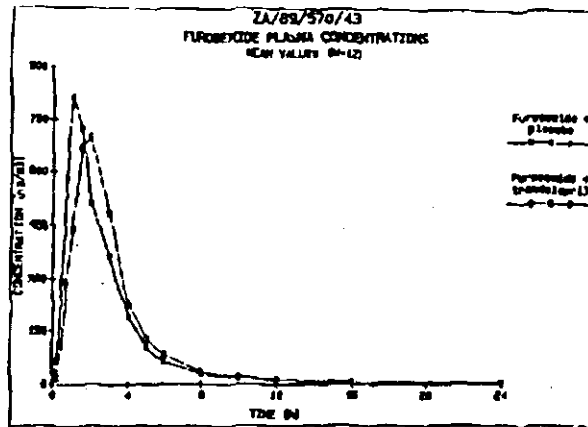


Figure 148. Plasma furosemide levels in Study ZA/89/570/43.

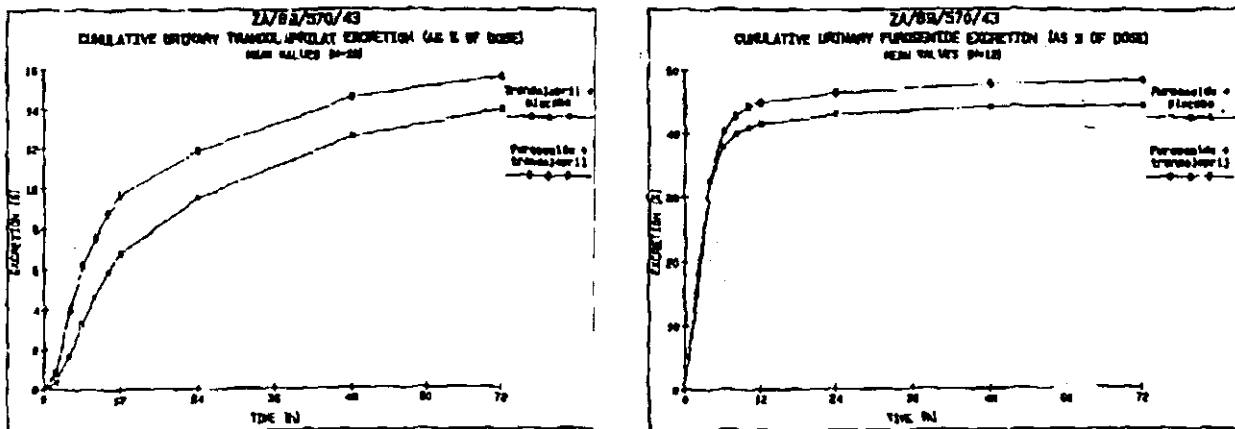


Figure 149. Urinary excretion of trandolaprilat and furosemide in Study ZA/89/570/43.

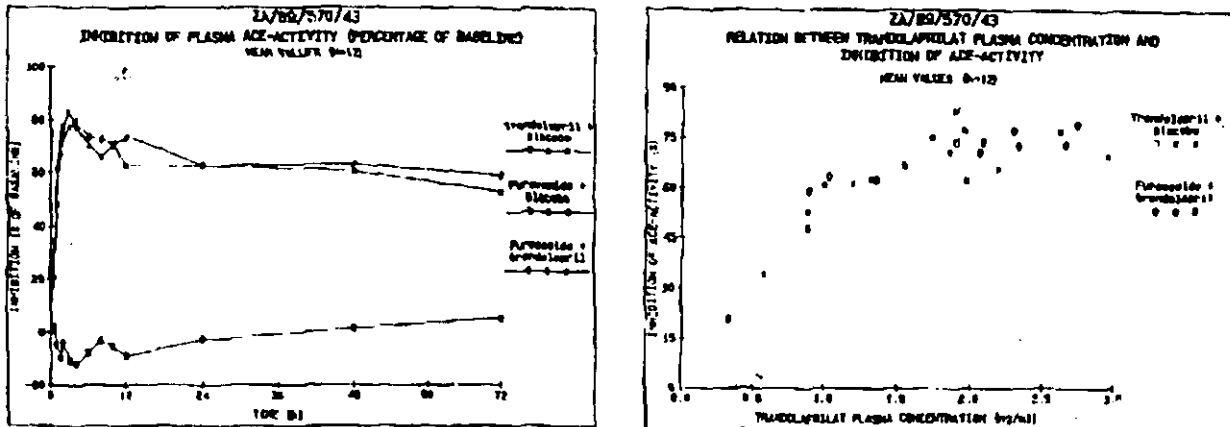


Figure 150. Plasma ACE inhibition in Study ZA/89/570/43.

further elevation of a baseline-elevated SGPT (initially 41 U/L). The enzyme level fluctuated at higher than baseline levels throughout all treatment periods, to a maximum level of 77 and a last reported value of 55 U/L. Clinical sequelae (if any) were not described.

Subject 9 had a considerable baseline elevation of SGPT (100 U/L) which was lower (at some times normal) throughout the treatment periods. At the end of the trial, SGPT was down to 51 U/L, but at this point gamma-GT was mildly elevated (to 73 U/L). Clinical antecedents and sequelae (if any) were not described.

Subject 10, a 20 year old and 74 kg male, had a treatment-emergent reduction of leukocyte count (3.92 K μ L) initially observed after furosemide treatment. This was further reduced to 3.13 K μ L — during exposure to trandolapril in the subsequent period, and was higher, although still reduced (3.68 K μ L) at the end of the trial. Clinical antecedents and sequelae (if any) were not described.

Subject 11, a 19 year old and 70 kg male, had elevations of SGOT and SGPT in association with each trandolapril-exposure. SGOT and SGPT levels were 100 and 121 U/L, respectively at maxima, and 78 and 116 U/L, respectively at the end of the study. These abnormalities were temporally associated with rigorous exercise, reportedly resolved 2 weeks later, and re-appeared with exercise re-challenge.

A41.7. Summary.

This single-blind, crossover study assessed the non-steady-state kinetic and dynamic interactions between single doses of trandolapril (2 mg/day) and furosemide (40 mg) in 12 healthy males. These were not placebo monotherapy-controlled data. The 12 or 24 hour cumulative furosemide-induced excretion of urine and electrolytes was not appreciably altered by trandolapril, although trandolapril appeared to potentiate the peak rates of diuresis and electrolyte excretion. Furosemide had no clinically significant effect on trandolapril-induced inhibition of ACE. Co-administration of furosemide and trandolapril did not affect pharmacokinetics of furosemide, but resulted in a 25% increase in renal clearance of trandolaprilat, possibly accounting for the somewhat smaller mean blood pressure-lowering effect than that associated with trandolapril as sole active agent. There were no safety-related withdrawals, but there were cases of elevated hepatocellular enzymes and leukopenia for which a contributory role of trandolapril cannot be excluded.

B. Narrative summaries of dropouts for adverse reactions

B1. Withdrawals from U.S. hypertension trials

B1.1. Short-term, placebo-controlled trials

Arrhythmia (atrial)	<i>Subject 2008-4 in Study TR-20-HTN, a 67 year old Caucasian male with 12-year history of hypertension, experienced atrial flutter during the double-blind phase after receiving 2 mg/day trandolapril for 1 day. The subject was then given 0.5 mg digoxin and 240 mg verapamil and subsequently discontinued from the study because of atrial flutter.</i>
Angioneurotic edema	<i>Subject 2101-22 in Study TR-21-HTN, a 54 year old Black female with a 5-year history of hypertension, experienced angioneurotic edema, urticaria, and pruritus after receiving 0.5 mg/day trandolapril during the double-blind phase for 21 days. This subject was discontinued by the investigator 3 days after onset and the event resolved in 3 days.</i>
Angioneurotic edema	<i>Subject 2079-11 in Study TR-20-HTN was a 55 year old Caucasian male with a 15-year history of hypertension who experienced hives and facial and lip edema after receiving 0.25 mg/day trandolapril for 38 days. This subject was discontinued from the study and was placed on diphenhydramine, prednisone and enalapril. At a 2-week follow-up, the subject stated that "he has no further problems".</i>
Blurry vision	<i>Subject 2012-8 in Study TR-20-HTN, a 69 year old Caucasian female with a 5-year history of hypertension and non-insulin dependent diabetes mellitus, experienced blurred vision during the double blind phase after receiving trandolapril 2 mg/day for 11 days. The adverse event resolved on the same day as the onset. The subject was discontinued from the study one day after the onset of the adverse event.</i>
Cough	<i>Subject 2022-7 in Study TR-20A-HTN, a 54 year old Oriental male with a history of diabetes mellitus and a 4-year history of hypertension, experienced a dry, nonproductive cough during the double-blind phase after receiving 12 mg/day trandolapril for 2 days. The subject was discontinued from the study because of cough.</i>
Death— cardiorespiratory failure, possibly secondary to myocardial infarction	<i>Subject 2013-8 in Study TR-20-HTN, a 59 year old Caucasian female, complained of neck, arm, and chest pain at day 17 of the double-blind phase while receiving trandolapril 4 mg/day. Her symptoms were thought to be related to a known back and neck problem; ECG was normal and unchanged from baseline and CPK enzymes were normal. Subject was found dead at home 2 days later. The subject's private physician stated that the cause of death was cardiorespiratory failure probably the result of myocardial infarction.</i>
Death—pulmonary embolus	<i>Subject 2126-6 in Study TR-21-HTN, a 66 year old Black male had been randomized to 12 mg/day of trandolapril. Twenty-nine days after completion of the study, and 49 days after the last dose of trandolapril, the subject presented to the emergency room with acute shortness of breath. An echocardiogram showed a large clot in the right ventricle. The subject died the next day from a pulmonary embolus. In the investigator's opinion, the pulmonary embolus and death were not related to the HCTZ. The investigator did not comment regarding the relationship of these events to trandolapril.</i>

Dizziness	<i>Subject 2125-11 in Study TR-21A-HTN, a 63 year old Black female with 30-year history of hypertension, experienced dizziness during the double blind phase after receiving trandolapril 8 mg/day for 36 days. The subject was discontinued from the study on the same day as the onset of the adverse event which was still ongoing at the time of termination. This adverse event was considered by the investigator to be "severe".</i>
Dizziness, headache	<i>Subject 2031-14 in Study TR-20A-HTN, a 66 year old Caucasian male with a 32-year history of hypertension, experienced dizziness and headaches during the double-blind phase after receiving 8 mg/day trandolapril for 11 days. The subject was discontinued from the study because of dizziness. Both events resolved 4 days after discontinuation.</i>
Epigastric, right upper quadrant pain	<i>Subject 2123-37 in Study TR-21A-HTN, a 58 year old Black female with a 16-year history of hypertension, experienced epigastric pain and right upper quadrant pain after receiving 8 mg/day trandolapril during the double-blind phase for 6 days. These complaints were assumed to be the result of cholecystitis. However, ultrasound showed no gall bladder disease. The subject was discontinued from the study by the investigator 7 days after the onset of the adverse reaction. The event resolved two weeks after discontinuation of the drug and treatment with Ceclor.</i>
Gallop (S-3), pedal edema	<i>Subject 2130-2 in Study TR-21-HTN, a 46 year old Black female with diabetes mellitus and a 22- year history of hypertension, experienced an S-3 gallop and edema of the right foot after receiving 8 mg/day trandolapril during the double-blind phase for 22 days. This subject was discontinued from the study by the investigator 5 days after the onset of the adverse reaction. The S-3 gallop was of mild severity and the edema of the right foot was of moderate severity.</i>
Headache	<i>Subject 2005-15 in Study TR-20-HTN, a 58 year old Caucasian male, experienced intermittent (migraine?) headaches during the run-in phase after receiving placebo for 15 days. The subject then completed the double-blind phase receiving trandolapril 1 mg/day for 42 day and was then discontinued from the study on Day 7 of the washout phase which was 64 days after the onset of the adverse event. The headaches were treated with Tylenol. This adverse event was ongoing at the time of termination.</i>
Hypotension	<i>Subject 2206-11 in Study TR-22-HTN, a 33-year-old Caucasian female with a 10-year history of hypertension for 10 years and an allergy to β-blockers, experienced hypotension during the double-blind phase after receiving 4-mg once-a-day trandolapril for 20 days. The subject was discontinued from the study because of hypotension. This adverse reaction was considered by the investigator to be "moderate" and possibly related to the study drug. The event resolved one day later.</i>
Ischemic heart disease, angioplasty	<i>Subject 2126-3 in Study TR-21A-HTN, a 60 year old Black male experienced moderate to severe atypical chest pain after receiving 12 mg/day trandolapril during the double-blind phase for 12 days. The subject was discontinued from the study on the date of onset of the adverse reaction. He subsequently underwent cardiac catheterization and angioplasty.</i>
Itching	<i>Subject 2125-5 in Study TR-21A-HTN, a 40 year old Black male with 15-year history of hypertension, experienced a prickling itching sensation after receiving 16 mg/day trandolapril during the</i>

	<i>double-blind phase for 21 days. This subject was discontinued from the study by the investigator 6 days after the onset of the adverse reaction. The subject was treated with Benadryl 25 mg/day.</i>
Lightheadedness, grade 1 retinopathy	<i>Subject 2125-17 in Study TR-21A-HTN, a 42 year old Black female with 12-year history of hypertension, experienced dizziness in the run-in phase beginning on day 20 and resolving on day 27. On day 27 of the run-in phase, the subject also experienced orthostatic hypotension with average blood pressure readings of 175/103 mmHg supine and 149/105 mmHg standing. The subject then completed the double-blind phase receiving trandolapril 4 mg/day for 42 days and then experienced lightheadedness and had bilateral grade 1 funduscopic changes (hypertensive retinopathy) noted on day 2 of the washout phase. The subject was discontinued on day 2 of the washout phase and both adverse events were ongoing at the time of termination.</i>
Liver enzyme elevation, mild lymphocytosis	<i>Subject 2123-27 in Study TR-21A-HTN, a 41 year old Black male with a previous history of hepatic dysfunction (enzymes at baseline: SGOT 98 [normal 0-55] and SGPT 99 [normal 0-55]), experienced increased SGOT and SGPT (3 times upper normal limit) and mild lymphocytosis after receiving 16 mg/day trandolapril during the double-blind phase for 7 days. The subject also admitted to drinking heavily. Enzymes were back to near baseline at 10-day follow-up.</i>
Myocardial infarction	<i>Subject 2008-14 in Study TR-20-HTN, a 51 year old Caucasian male had a myocardial infarction during the washout phase (day 51) after receiving 1 mg/day trandolapril for 42 days. The subject was hospitalized and discontinued from the study. This adverse reaction was considered by the investigator to be "severe" and "definitely not" related to study drug.</i>
Sex drive loss	<i>Subject 2207-13 in Study TR-22-HTN, a 53-year-old Caucasian male with a 15-year history of hypertension, experienced loss of sex drive during the double-blind phase after receiving trandolapril 4 mg daily for 3 days, and experienced headache during the double-blind phase after receiving trandolapril 4 mg daily for 21 days. Both adverse events were considered by the investigator to be "moderate" and "possibly" related to study drug. Both events had resolved at a 19-day follow-up visit.</i>
Thrombocytopenia	<i>Subject 2014-4 in Study TR-20-HTN, an 84 year old Caucasian male with a 17-year history of hypertension and COPD, experienced thrombocytopenia during the double-blind phase after receiving trandolapril 2 mg/day for 28 days. The subject's pretreatment platelet count was 110,000/μl (normal is 140,000 to 350,000/μl). At the start of the double-blind phase, the subject's platelet count had decreased to 69,000/μl at which time the investigator noted that the low count was the result of congestive splenomegaly and hypersplenism. One day before being discontinued, the platelet count was 57,000/μl. The subject was discontinued from the study 2 days after the onset of the adverse event. This adverse event was ongoing at the time of termination and resolved 30 days after the onset (28 days after the time of termination).</i>
Transient ischemic attack	<i>Subject 2105-16 in Study TR-21-HTN, a 60 year old Black male with a 9-year history of hypertension and myocardial infarction, experienced a transient ischemic attack during the double-blind phase after receiving trandolapril 0.25 mg/day for 16 days. The subject was admitted to the hospital and started on intravenous heparin.</i>

B1.2. Long-term, open-label studies with trandolapril alone

Angioedema	<i>Subject 3004-2004 in Study TR-30-HTN, a 68 year old Caucasian female with a 5-year history of hypertension, developed angioedema after taking trandolapril 1 mg/day for 5 days during the long-term open-label phase. This subject had swelling and drooping of the right lower face and lips. Upon examination, no breathing or neurological problems were noted and CAT scan and ECG were normal. The subject was terminated from the study by the investigator on the day of onset of the adverse reaction. The event completely resolved within 2 days.</i>
Assault	<i>Subject 3003-3027 in Study TR-30-HTN, a 61 year old Caucasian male with a 10-year history of hypertension, had been receiving 1 mg/day of trandolapril for 7 days when he was assaulted and suffered multiple facial fractures. The subject was hospitalized and required surgery. Trandolapril treatment was discontinued.</i>
Bundle branch block	<i>Subject 3019-3009 in Study TR-30-HTN, a 46 year old Black male, developed a symptomatic right bundle branch block after receiving trandolapril that was titrated upward as follows: 1 mg/day for 28 days, 2 mg/day for 36 days and then 4 mg/day for 40 days during the long-term open-label phase. This subject was discontinued from the study, by the investigator, on the day of onset of the adverse reaction. The right bundle branch block was moderate in severity and was considered, by the investigator, to be 'probably not' study drug related.</i>
Cancer	<i>Subject 3011-3005 in Study TR-30-HTN was a 66 year old Caucasian male with a history of diabetes mellitus and a 12-year history of hypertension. He experienced a decrease in appetite and unilateral leg pain after receiving trandolapril 1 mg/day for 25 days and then 2 mg/day for 7 days during the long-term open-label phase. Two weeks after withdrawal, following subacute mental status changes, the subject underwent subtotal resection of a glioblastoma multiforme and had a less than 1-year expected life span.</i>
^{4t} Cerebrovascular accident	<i>Subject 3015-2113 in Study TR-30-HTN, a 65 year old obese Black female, experienced a cerebrovascular accident with decreased peripheral vision in the left eye after receiving trandolapril 1 mg/day for 157 days during the long-term open-label phase. The subject denied weakness, numbness, and paresthesia, or bulbar symptoms. She did not experience pain, other than a headache, on the night of admission. The loss of vision remained constant since onset. A head CT scan with contrast confirmed the presence of an infarction in both the right occipital lobe and right cerebellar hemisphere. The subject was terminated from the study by the investigator 15 days after the onset of the event. The subject was discharged from the hospital 5 days after admission. The adverse reactions were severe and were considered by the investigator to be "probably not" study drug related.</i>
Cerebrovascular accidents, severe lumbar degenerative disease	<i>Subject 4013-4 in Study TV-40-HTN, a 70 year old Caucasian male, was receiving trandolapril 2 mg/day for 1 day and then 4 mg/day for 5 days during the open-label phase when he was hospitalized because of severe lumbar degenerative joint disease and bilateral cerebral infarctions on the day of onset. This subject was terminated from the study by the investigator on the day of onset of the adverse reaction. This adverse reaction was considered by the investigator to be "severe" and "definitely not" study drug related.</i>

Cholelithiasis, cholecystectomy, hysterectomy	<i>Subject 3016-2015 in Study TR-30-HTN, a 69 year old Caucasian female, experienced atypical chest pain and symptoms of reflux esophagitis diagnosed as cholelithiasis, after receiving trandolapril 1 mg/day for 55 days and 2 mg/day for 207 days. The subject continued on 2 mg/day of trandolapril and was then discontinued from the study 26 days after the onset of the adverse event. The adverse event resolved on the same day as the onset. The subject was hospitalized for laparoscopic cholecystectomy and ovarian cystectomy.</i>
Cough	<i>Subject 3004-3001 in Study TR-30-HTN, a 50 year old Caucasian male with a 3-year history of hypertension, experienced a dry cough after receiving 1 mg/day of trandolapril for 22 days during the long-term open-label phase. This subject discontinued the study on his own, 53 days after the onset of the adverse reaction. The cough was ongoing at the time of termination.</i>
Cough	<i>Subject 3007-2018 in Study TR-30-HTN, a 60 year old Caucasian male, experienced cough after receiving 1 mg/day of trandolapril for 9 days during the long term open label study. The cough was moderate in severity and no treatment was required. The subject was terminated from the study by the investigator approximately 5 and 1/2 months after the onset of the adverse reaction.</i>
Cough	<i>Subject 3007-2025 in Study TR-30-HTN, a 54 year old Caucasian male, experienced a cough after receiving 1 mg/day of trandolapril for 63 days during the long-term open-label phase. This subject discontinued the study on his own, 30 days after the onset of the adverse reaction. Earlier in the study, this subject had an upper respiratory infection with cough, sore throat, and lightheadedness that resolved with no sequelae.</i>
Cough	<i>Subject 3007-3008 in Study TR-30-HTN, a 52 year old Caucasian male with a 3-month history of hypertension, experienced cough associated with URI symptoms after receiving 1 mg/day of trandolapril for 24 days during the long-term open-label phase. The cough was treated with doxycycline for 7 days starting 20 days after the onset of the adverse reaction. The subject was discontinued from the study by the investigator 35 days after the onset of the adverse reaction. The cough resolved 15 days after the subject was dropped from the study.</i>
Cough	<i>Subject 3008-2009 in Study TR-30-HTN, a 63 year old Caucasian female, experienced cough after receiving 1 mg/day of trandolapril for 7 days during the long-term open-label phase. The subject also complained of dizziness and allergic rhinitis. The subject was discontinued from the study by the investigator 94 days after the onset of the adverse reaction, which was still ongoing at the time of termination.</i>
Cough	<i>Subject 3008-2010 in Study TR-30-HTN, a 50 year old Caucasian male, experienced cough after receiving trandolapril that was titrated upward as follows: 1 mg/day for 28 days, 2 mg/day for 27 days then 4 mg/day for 63 days, during the long-term open-label phase. The subject was discontinued from the study by the investigator 60 days after the onset of the adverse reaction, which was still ongoing at the time of termination.</i>
Cough	<i>Subject 3011-2003 in Study TR-30-HTN, a 52 year old Caucasian female, experienced cough and chest soreness after receiving 1 mg/day of trandolapril for 154 days during the open-label long-term phase. Robitussin was started 14 days after the onset of the</i>

	<i>cough. However, the cough was still ongoing when, 35 days after the onset of the adverse event, the subject was terminated from the study by the investigator.</i>
Cough	<i>Subject 3011-2005 in Study TR-30-HTN, a 67 year old Caucasian female, experienced a persistent cough after receiving trandolapril that was titrated upward as follows: 1 mg/day for 27 days and then 2 mg/day for 27 days and then 4 mg/day for 10 days during the long-term open-label phase. Contac and Dimetapp were given for the relief of the cough. The subject also complained of nervousness and heart pounding. The last ECG also showed non-specific ST-T segment changes. The subject discontinued the study on her own, 19 days after the onset of the adverse reaction, which was still ongoing at the time of termination.</i>
Cough	<i>Subject 3011-3006 in Study TR-30-HTN, a 42 year old Caucasian female with a 12-year history of hypertension, experienced a cough after receiving trandolapril that was titrated upward as follows: 1 mg/day for 27 days, 2 mg/day for 27 days and then 4 mg/day for 2 days during the long-term open-label phase. Robitussin was given for the relief of the cough. The subject discontinued the study, on her own, 33 days after the onset of the adverse reaction, which was still ongoing at the time of termination. Subject had also complained of headaches, anxiety, and insomnia. The cough resolved 4 days after discontinuation.</i>
Cough	<i>Subject 3015-2112 in Study TR-30-HTN, a 56 year old Black male, experienced cough after taking trandolapril that was titrated upward as follows: 1 mg/day for 26 days and then 2 mg/day for 8 days. No treatment was given for the cough. The subject was terminated from the study by the investigator 21 days after the onset of the cough, which had resolved one day earlier.</i>
Cough	<i>Subject 3019-3003 in Study TR-30-HTN, a 43 year old Caucasian female with a 2-year history of hypertension, experienced a persistent cough after receiving 1 mg/day of trandolapril for 35 days during the long-term open-label phase. This subject discontinued the study on her own, 14 days after the onset of the adverse reaction, which was still ongoing at the time of termination.</i>
Death—myocardial infarction	<i>Subject 3007-2013 in Study TR-30-HTN, a 52 year old Caucasian male, experienced jaw pain, chest pain and diaphoresis after receiving trandolapril 1 mg/day for 26 days, 2 mg/day for 30 days and then 4 mg/day for 206 days, during the long-term open-label phase. He was admitted to an emergency room, where an EKG revealed an acute anterolateral myocardial infarction. He could not be resuscitated. An autopsy revealed occlusion of the left main coronary artery. This adverse reaction was considered by the investigator to be "definitely not" study drug related.</i>
Diplopia	<i>Subject 3019-3008 in Study TR-30-HTN, a 58-year-old Black female with a 50-year history of hypertension, experienced double vision after receiving trandolapril 1 mg/day for 4 days during the long-term open-label phase. The subject had no prior exposure to trandolapril. The subject was dropped from the study on the day of onset of the adverse reaction. The double vision, which was moderate in severity and was considered by the investigator to be 'possibly' related to study drug, resolved the day after trandolapril was discontinued.</i>
Epigastric pain, cholecystectomy?	<i>Subject 3016-2020 in Study TR-30-HTN, a 43 year old Caucasian female, experienced intermittent epigastric pain after receiving</i>

	<i>trandolapril 1 mg/day for 27 days and 2 mg/day for 16 days. The subject continued on 2 mg/day of trandolapril and was discontinued from the study 219 days after the onset of the adverse drug reaction. The subject was treated with the following drugs that were ongoing at termination: Alka-Seltzer, Bufferin, Motrin, Regular and Extra Strength Tylenol, Tempo, Tums, and Zantac. This adverse event was ongoing at the time of termination and was considered by the investigator to be 'mild' in severity and 'definitely not' related to study drug. Follow-up notes that the subject is to undergo gall bladder surgery. Subject also complained of back pain secondary to snow shoveling.</i>
Headache, liver enzyme elevation	<i>Subject 3019-3001 in Study TR-30-HTN, a 35 year old Caucasian male experienced headaches after receiving trandolapril 1 mg/day for 36 days, 2 mg/day for 35 days, and 4 mg/day for 2 days during the long-term open-label phase. The subject was discontinued from the study by the investigator 12 days after the onset of the adverse reaction and was also dropped because of mildly elevated SGPT and alkaline phosphatase (although both of these elevation were present at baseline and were considered to be related to alcohol abuse).</i>
Impotence	<i>Subject 3007-3010 in Study TR-30-HTN, a 57-year-old Caucasian male with a 15-year history of hypertension, experienced impotence after receiving 2 mg/day of trandolapril for 36 days during the long-term open-label phase. The subject discontinued the study, on his own, 35 days after the day of onset of the adverse reaction.</i>
Ischemic heart disease	<i>Subject 4005-3 in Study TV-40-HTN, a 45 year old Caucasian male experienced chest pressure (angina) during the open-label phase after receiving trandolapril 2 mg/day for 2 days and 4 mg/day for 6 days. The subject was admitted to the hospital for a suspected myocardial infarction. The adverse event resolved on the same day as the onset, and the subject was discharged the next day after treadmill testing. The investigator concluded the subject experienced "chest pain secondary to supply/demand mismatch secondary to high afterload". The subject was discontinued from the study 4 days after the onset of the adverse event. This adverse event was considered by the investigator to be severe and "probably not" related to study drug.</i>
Ischemic heart disease, angioplasty	<i>Subject 3014-2010 in Study TR-30-HTN, a 62 year old Caucasian male experienced angina in between the short-term (double-blind) and the long-term (open-label) study period (while not receiving drug). The subject had received trandolapril 1 mg/day for 41 days followed by a 20 day washout period on placebo. The subject entered the long-term open-label study and received 1 mg/day of trandolapril for 29 days, at which time trandolapril was stopped because of angina. The investigator noted that the subject was scheduled to have angioplasty.</i>
Ischemic heart disease, coronary artery bypass	<i>Subject 3013-3003 in Study TR-30-HTN, a 68 year old Mexican-American male experienced angina pectoris after receiving trandolapril 1 mg/day for 21 days and then 2 mg/day for 4 days during the long-term open-label phase. His history included a possible old myocardial infarction and carotid endarterectomy. The subject was discontinued from the study 17 days after the onset of the adverse reaction. ECG showed non-specific ST-T changes. Subject was admitted to a hospital and underwent subsequent 3-vessel CABG.</i>

Myocardial infarction	<i>Subject 3001-2029 in Study TR-30-HTN, a 70 year old Caucasian male, experienced anginal pain after receiving 1 mg/day of trandolapril for 111 days during the long-term open-label phase. The subject was treated with nitroglycerin p.r.n. starting 73 days after the onset of the adverse event. This subject was terminated from the study by the investigator 104 days after the onset of the adverse reaction, when subject was hospitalized with acute myocardial infarction, confirmed with enzymes.</i>
Nausea, headache	<i>Subject 4005-11 in Study TV-40-HTN, a 58 year old Caucasian male with an 18-year history of hypertension, experienced headaches with increasing intensity and nausea after receiving trandolapril that was titrated upward as follows: 2 mg/day for 2 days and then 4 mg/day for 2 days during the open-label phase. This subject also had moderate headaches ongoing from the placebo run-in phase. Eleven days after the onset of the adverse reactions, the subject discontinued study medication. Two days later this subject discontinued from the study, on his own, at which time the nausea had resolved. These adverse reactions were considered by the investigator to be 'moderate' in severity and 'possibly' study drug related.</i>
Nausea, vomiting, anxiety	<i>Subject 4101-8 in Study TV-41-CP, a 50-year-old Black female with diabetes mellitus, became anxious, nauseated, and vomited upon 45o upright tilting for the hemodynamic study during the placebo run-in phase of the study. Blood pressure before the maneuver was 170/110. The subject was not sure if she had taken her insulin dose in the morning (Humulin N, 35 units), and she did not eat breakfast. Blood pressure increased to 180/120. Because of repeated vomiting, the fact that she had been on insulin, and because of her past cardiac history, she was hospitalized for control of blood pressure and follow up of carbohydrate status. The subject received the first dose of 2 mg trandolapril later that day (while hospitalized) and was discontinued from the study on the following day, Study Day 2. After two days hospitalization, the subject's symptoms had resolved and she was discharged with instructions to remain on nifedipine. The nausea and vomiting were considered severe and serious; the anxiousness was considered moderate and not serious. The adverse events were considered by the investigator to be 'probably not' related to study drug.</i>
Rash, ankle edema, abdominal swelling, heartburn	<i>Subject 3019-3010 in Study TR-30-HTN, a 55 year old Caucasian female with a 20-year history of hypertension, experienced heartburn, a skin rash, ankle edema, and abdominal swelling after receiving 1 mg/day trandolapril for 36-38 days during the long-term open-label phase. The skin rash, edema and abdominal swelling resolved, without treatment, 4 days after onset. The subject's heartburn was treated with Maalox; however, the heartburn was still ongoing at the time of termination from the study. This subject stopped taking trandolapril after the onset of the adverse reactions. However, she did not notify the investigator until the next study visit, which was 20-22 days after the onset of the events. She was then terminated from the study.</i>
Tachycardia	<i>Subject 3007-2022 in Study TR-30-HTN, a 44 year old Caucasian male, developed tachycardia (heart rate 120 bpm, baseline = 49 bpm) after receiving 1 mg/day of trandolapril for 31 days during the long-term open-label phase. The subject was treated with labetalol and the episode resolved within 1 hour. This subject was discontinued from the study 2 days after the onset of the adverse</i>

	<i>reaction. The investigator considered the tachycardia to be moderate in severity but 'probably not' study drug related.</i>
Tiredness, excessive perspiration	<i>Subject 4008-3 in Study TV-40-HTN, a 40 year old Black female with an 8-year history of hypertension, experienced extreme tiredness and excessive perspiration (DBP 2 days before the events was 110 mmHg and 3 days after the events was 120 mmHg) after receiving trandolapril that was titrated upward as follows: - 4 mg/day for 11 days and then 8 mg/day for 10 days during the open label phase. This subject discontinued from the study, on her own, 3 days after the onset of the adverse reactions which had resolved after 1 day.</i>
Vomiting, headache, dizziness, shortness of breath	<i>Subject 4008-10 in Study TV-40-HTN, a 27 year old Caucasian female with a 6-year history of hypertension, experienced vomiting, headache, dizziness and shortness of breath after receiving trandolapril 2 mg/day for 4 days during the open-label phase. It is noted that this subject had similar reactions when on an unknown combination therapy in the past. This subject was discontinued from the study by the investigator on the day of onset of the adverse reactions which were still ongoing at termination of the study.</i>
Weakness, shakiness	<i>Subject 3007-3006 in Study TR-30-HTN, a 57-year-old Caucasian male with a 5-year history of hypertension, experienced weakness and shakiness after receiving 1 mg/day of trandolapril for 2 days during the long-term open label phase. The subject admitted to feeling weak and flu-like 7 days before the onset of the adverse reactions (prior to the start of the study). This subject was discontinued from the study by the investigator on the day of onset of the adverse reactions and both symptoms resolved 3 days later.</i>

B1.3. Long-term, open-label studies with trandolapril plus verapamil

AV block (first degree), paresthesia of the face and scalp	<i>Subject 4005-15 in Study TV-40-HTN, a 56 year old Caucasian male with diabetes mellitus and a 6-year history of hypertension, developed a new ECG finding of first degree AV block, and experienced paresthesia of the face and scalp (associated with sinus infection) after receiving trandolapril (T) and verapamil (V) that were titrated upward as follows: 2 mg/day T for 7 days then 4 mg/day T for 1 day and then 4 mg/day T + 180 mg/day V for 2 days during the open-label phase. This subject was terminated from the study by the investigator 1 day after the onset of the adverse reaction which was still ongoing at the time of termination from the study. Both events resolved 6 days after onset.</i>
Cholelithiasis, cholecystectomy	<i>Subject 3016-2013 in Study TR-30-HTN, a 61 year old Caucasian female, experienced worsening cholelithiasis (pre-dated entry into trial) after receiving trandolapril 1 mg/day for 31 days, 2 mg/day for 55 days, 4 mg/day for 27 days, and then 4 mg/day of trandolapril with 180 mg/day of verapamil for 130 days. The subject was then discontinued from the study (17 days after the onset of the adverse event) and hospitalized for a laparoscopic cholecystectomy. This adverse event was considered by the investigator to be "moderate" in severity and "probably not" related to study drug. Subject had multiple other complaints such as cough, back pain, hiatal hernia, vomiting, flu symptoms, right upper quadrant pain which were not considered contributory to withdrawal.</i>
Cough	<i>Subject 3004-2013 in Study TR-30-HTN, a 50 year old Caucasian male, experienced cough after receiving trandolapril for a total of 3.1 days. The subject initially received trandolapril 0.25 mg/day for</i>

	42 days during the double-blind phase of a short-term study. In the open-label, long-term study, he received trandolapril 1 mg/day for 29 days, 2 mg/day for 84 days, 4 mg/day for 43 days, and 4 mg/day of trandolapril with 180 mg/day of verapamil for 113 days. The subject discontinued the study on his own on the day of onset of the adverse reaction.
Cough	Subject 3008-2008 in Study TR-30-HTN, a 66 year old Caucasian female, experienced cough after receiving trandolapril that was titrated upward as follows: 1 mg/day for 27 days, 2 mg/day and verapamil 180 mg/day for 27 days, during the long-term open-label phase. The subject was discontinued from the study by the investigator 60 days after the onset of the adverse reaction, which was still ongoing at the time of termination.
Cough, nausea	Subject 3019-3006 in Study TR-30-HTN, a 39 year old Black male with a 1-year history of hypertension, experienced a cough after receiving trandolapril that was titrated upward as follows: 1 mg/day for 28 days, 2 mg/day for 42 days and then 4 mg/day for 70 days during the long-term open-label phase. The subject continued in the study. After the subject received 4 mg/day of trandolapril for 119 days, 180 mg/day of verapamil was added. Twenty-seven days later, the subject experienced nausea. The subject was discontinued from the study, by the investigator, because of both adverse reactions, which were still ongoing at the time of termination.
Headache, nose bleed, constipation, insomnia, mood swings	Subject 3007-3005 in Study TR-30-HTN, a 57-year-old Caucasian female with a 5-year history of hypertension, experienced headaches, nose bleeds, constipation, insomnia and mood swings after receiving trandolapril that was titrated upward as follows: 1 mg/day for 28 days, 2 mg/day for 28 days, 4 mg/day for 28 days, 2 mg/day of trandolapril with 180 mg/day of verapamil for 1 day and then 2 mg of trandolapril for 2 days during the long-term open-label phase. This subject discontinued the study, on her own, 21 days after the onset of the adverse reactions. The subject indicated that all of the symptoms started when the verapamil was added. The headache resolved in 2 weeks, whereas 3 weeks were necessary for the other symptoms.
Ischemic heart disease	Subject 3006-3002 in Study TR-30-HTN, a 55 year old Caucasian male with an 11-year history of hypertension and diabetes mellitus, experienced new onset of chest tightness (angina) after receiving trandolapril that was titrated upward as follows: 1 mg/day for 28 days, 2 mg/day for 36 days, 4 mg/day for 135 days and then 2 mg/day of trandolapril with 180 mg/day of verapamil for 143 days during the long-term open-label phase. This subject was discontinued from the study on the day of onset of the adverse reaction. Subsequent to cardiac catheterization, he was recommended for CABG.
Lethargy	Subject 3004-3006 in Study TR-30-HTN, a 40 year old Caucasian male with a 7-year history of hypertension, experienced lethargy and irritability after receiving trandolapril that was titrated upward as follows: 1 mg/day for 64 days then 2 mg/day for 32 days, then 4 mg/day and verapamil 180 mg/day for 18 days, during the long-term open-label phase. The subject discontinued the study on his own 89 days after the onset of the adverse reaction which was ongoing at the time of termination. The subject had no previous exposure to trandolapril.

Light-headedness	<i>Subject 3008-2001 in Study TR-30-HTN, a 54 year old Caucasian male with an 8-year history of hypertension, experienced lightheadedness during the open-label phase after receiving trandolapril that was titrated upward as follows: 1 mg/day for 69 days, 2 mg/day for 34 days, 4 mg/day for 97 days and then 4 mg/day of trandolapril with 180 mg/day of verapamil for 8 days. Verapamil was discontinued on the same day of the onset of the adverse drug event and the subject continued treatment with 4 mg/day of trandolapril alone for 18 more days and then was discontinued from the study (21 days after the adverse event onset.) The lightheadedness resolved one day after discontinuation of verapamil. The investigator commented that 'subject unable to tolerate Isoptin-SR (adverse event) and trandolapril alone did not control blood pressure.</i>
Liver enzyme elevation	<i>Subject 3011-3011 in Study TR-30-HTN, a 48 year old Caucasian female, developed elevated SGOT 102 U/L, (baseline was 25 U/L; normal range is 7 to 39 U/L), SGPT of 299 U/L, (baseline 31 U/L; normal range 2 to 54 U/L) and LDH 275 U/L, (baseline 180 U/L; normal range 88 to 230 U/L) after receiving trandolapril 1 mg/day for 26 days, 2 mg/day for 31 days, 4 mg/day for 107 days and then 2 mg/day trandolapril plus 180 mg/day verapamil for 28 days during the long-term open label phase. The subject was discontinued from the study by the investigator 7 days after the onset of the adverse reactions. The SGOT, SGPT and LDH elevations returned to normal ranges at 2-week posttreatment follow-up.</i>
Nausea	<i>Subject 3017-3002 in Study TR-30-HTN, a 45 year old Black female with a 1-year history of hypertension, experienced nausea during the open label phase after receiving trandolapril that was titrated upward as follows: 1 mg/day for 35 days, 2 mg/day for 33 days, 4 mg/day for 35 days, and then 4 mg/day of trandolapril with 180 mg/day of verapamil for 2 days. Three days after the onset of adverse event, verapamil was discontinued and the study continued with 4 mg/day of trandolapril alone for 22 days. 180 mg/day of verapamil was then added back again and the subject was discontinued from the study after three days which was 26 days after the adverse event onset. The investigator did not indicate the relationship between the subtraction and addition of verapamil from the study regimen and the incidence of nausea. Subject also complained of constipation and paresthesia.</i>
Nausea, pre-syncope, and vertigo	<i>Subject 3011-3007 in Study TR-30-HTN, a 72 year old Caucasian male with a 1-year history of hypertension, experienced nausea, pre-syncope and vertigo after receiving trandolapril that was titrated upward as follows: 1 mg/day for 27 days, 2 mg/day for 28 days, 4 mg/day for 26 days and then 2 mg/day of trandolapril with 180 mg/day of verapamil for 2 days during the long-term open-label phase. The subject was discontinued from the study, by the investigator, 6 days after the onset of the adverse reactions. Other complaints of headaches, dizziness upon arising quickly, and dry cough predated addition of verapamil, but were not said to be contributory to withdrawal.</i>
Neutropenia	<i>Subject 3005-3010 in Study TR-30-HTN, a 60 year old Black male, had a severely low neutrophil count of 22% with a WBC count of 4.4 Kμl. At baseline, the subject's neutrophil count was 37% with a WBC count of 4.3 Kμl. The subject had received trandolapril 1 mg/day for 35 days, 2 mg/day for 35 days, and 4 mg/day for 35 days. At the time of the decreased neutrophil count, the subject's</i>

trandolapril dose was changed to 2 mg/day with 180 mg/day of verapamil. After 40 days of combination trandolapril/verapamil treatment, the neutrophil count was 27% with a WBC count was 4.1 K μ l. The subject was discontinued from the study at that time. Three days after the last dose of study medication, the neutrophil count was 53% with a WBC count of 4.7 K μ l, and the absolute neutrophil count was 2491.

B1.4. Long-term, open-label studies with trandolapril, verapamil, and HCTZ

Cough

Subject 4001-10 in Study TV-40-HTN, a 61 year old Black female, experienced a non-productive cough after receiving trandolapril and verapamil that were titrated upward as follows: trandolapril 4 mg/day for 3 days then trandolapril 8 mg/day for 3 days and then trandolapril 8 mg/day plus verapamil 180 mg/day for 5 days during the open-label phase. HCTZ 12.5 mg/day was added 12 days after the onset of the adverse reaction. The dose of HCTZ was increased to 25 mg/day 7 days later. This subject discontinued from the study, on her own, 35 days after the onset of the adverse reaction.

Impotence

Subject 4007-3 in Study TV-40-HTN, a 63 year old Caucasian male with a 15-year history of hypertension, experienced impotence after receiving trandolapril, verapamil and HCTZ that were titrated upward as follows: trandolapril 2 mg/day for 8 days then trandolapril 4 mg/day for 14 days then trandolapril 4 mg/day plus verapamil 180 mg/day for 14 days and then trandolapril 4 mg/day plus verapamil 180 mg/day plus HCTZ 12.5 mg/day for 2 days during the open-label phase. This subject discontinued from the study, on his own, 5 days after the onset of the adverse reaction which was still ongoing at the time of discontinuation. The investigator commented that impotence was a result of HCTZ usage.

Ischemic heart disease

Subject 4003-6 in Study TV-40-HTN, a 51 year old Caucasian male experienced chest pain and left arm pain subsequent to positive exercise tolerance test after receiving trandolapril, verapamil, and HCTZ as follows: trandolapril 2 mg/day for 8 days then trandolapril 4 mg/day for 5 days then trandolapril 4 mg/day plus verapamil 180 mg/day for 7 days then trandolapril 4 mg/day plus verapamil 180 mg/day plus HCTZ 12.5 mg/day for 9 days and then trandolapril 4 mg/day plus verapamil 180 mg/day plus HCTZ 25 mg/day for 56 days during the open-label phase. This subject was terminated from the study 14 days after the onset of the adverse reactions, both of which resolved one day later. Subject was subsequently referred for cardiac catheterization.

B1.5. Placebo subjects in controlled studies

Broken heel

Subject 2011-17 in Study TR-20-HTN, a 37 year old Caucasian male with a 3-month history of hypertension, experienced a broken heel from a boating accident during the double-blind phase (day 26) after receiving placebo for 25 days. The subject was hospitalized and was discontinued from the study 11 days after the accident occurred. The subject was still hospitalized at the time of discontinuation.

Cough, ankle edema

Subject 2202-4 in Study TR-22-HTN, a 54-year-old Black postmenopausal female with a 4-year history of hypertension, as

	well as upper respiratory infection, dermatitis, and allergy to aspirin, experienced ankle swelling +? during the run-in phase (day -4) after receiving placebo for three days, and experienced a dry non productive cough during the double-blind phase (day 29) after receiving placebo for 28 days. The subject was discontinued from the study because of ankle swelling and cough. Both adverse reactions were considered by the investigator to be 'moderate' and 'probably' related to the study drug. The cough resolved in 9 days; the ankle edema in 13 days.
Death— cerebrovascular accident, gastrointestinal bleeding	Subject 2204-19 in Study TR-22-HTN, a 68 year old Caucasian male, experienced an acute cerebral vascular accident with right hemiparesis during the double-blind phase after receiving placebo for 20 days. The subject was hospitalized and discontinued from the study because of the cerebrovascular accident. This adverse reaction was considered by the investigator to be "severe" and "definitely not" related to study drug. The subject died from gastrointestinal bleeding 6 weeks later. The investigator considered the cause of death to be related to the anti-coagulant therapy which was being administered to the subject.
Death—myocardial infarction	Subject 2101- a 64 year old Black female, died during the placebo run-in phase of study TR-21-HTN. A few days into the run-in period, the subject experienced persistent chest discomfort, and was admitted with a diagnosis of acute myocardial infarction. The cause of death was listed as cerebral hypoperfusion and ventricular pump failure resulting from myocardial infarction. Although the study screening ECG suggested a possible old anteroseptal scar, there were no symptoms of ischemic heart disease.
Epistaxis, headache	Subject 2121-5 in Study TR-21A-HTN experienced nose bleeding and severe headache after receiving placebo during the double-blind phase (Study Day 34) for 34 days. The subject was seen in an emergency room, but any medications given are not available. The subject was discontinued from the study by the investigator the day of onset of the adverse reactions. Both events resolved the next day.
Headache	Subject 2002-7 in Study TR-20-HTN, a 59 year old Caucasian female with a 19-year history of hypertension, experienced headache during the double blind phase after receiving placebo for 6 days. The subject was discontinued from the study 9 days after the onset of the adverse event. The investigator noted 'subject complaining of headache and flushed feeling-believes that it is a result of poor blood pressure control'. The investigator indicated that there was 'no response' to the study medication at study termination. The subject's average supine blood pressure at the start of the double blind phase was 168/110 mmHg and 195/104 mmHg at discontinuation. This adverse event was ongoing at the time of termination.
Headache	Subject 2013-11 in Study TR-20-HTN, a 76 year old Caucasian male with a 25-year history of hypertension, experienced headache during the double blind phase (Study Day 3) after receiving placebo for 2 days. The subject was discontinued from the study 5 days after the onset of the adverse event which was still ongoing at the time of termination.
Headache, dizziness	Subject 2130-5 in Study TR-21A-HTN, a 57 year old Black female with a new diagnosis of hypertension, experienced headache and dizziness after receiving placebo during the double-blind phase (Study Day 5) for 5 days. This subject was discontinued from the

	<i>study by the investigator 1 day after the onset of the adverse reaction. Both events resolved within a day of discontinuation.</i>
Ischemic heart disease	<i>Subject 2004-7 in Study TR-20-HTN, a 62 year old Caucasian male experienced ECG changes indicative of myocardial ischemia during the double-blind phase after receiving placebo for 42 days. The subject was discontinued from the study because of myocardial ischemia. Subsequent cardiac catheterization revealed no significant coronary artery lesions.</i>
Liver enzyme elevation	<i>Subject 2015-1 in Study TR-20-HTN, a 66 year old Caucasian female, had increased SGOT and SGPT during the double-blind phase after receiving placebo for 29 days. The subject was discontinued from the study because of elevated SGOT (188 U/L) and SGPT (173 U/L). One week earlier, the subject had received an injection of triamcinolone and had been started on Clinoril 200 mg twice daily. Three days after drug discontinuation, the enzymes remained elevated: SGOT 174 U/L, and SGPT 171 U/L. This adverse reaction was considered by the investigator to be "probably not" related to study drug.</i>
Myocardial infarction, coronary artery bypass	<i>Subject 2029-3 in Study TR-20A-HTN, a 39 year old Caucasian male experienced an acute myocardial infarction during the double-blind phase after receiving placebo for 5 days. The subject was admitted into an acute coronary care unit and discontinued from the study because of myocardial infarction. The subject subsequently underwent a CABG.</i>
Palpitations	<i>Subject 2121-13 in Study TR-21A-HTN, a 35 year old Black male with a new diagnosis of hypertension, experienced palpitations after receiving placebo during the double-blind phase for 15 days. This subject discontinued his treatment the day of onset of the adverse reaction and began therapy with Maxzide before notifying the investigator. The palpitations were of mild severity and were considered by the investigator as "probably not" related to the study drug. The palpitations resolved the day of discontinuation.</i>
Paresthesia, numbness in right arm	<i>Subject 2023-23 in Study TR-20A-HTN, a 55 year old Caucasian male with a 5-month history of hypertension, experienced tingling of the right hand and arm and numbness of the right hand and arm during the double-blind phase after receiving placebo for 16 days. The subject was discontinued from the study because of tingling of the right hand and arm and numbness of the right hand and arm. The events were resolved at 3-week follow-up.</i>
Periorbital edema, lethargy	<i>Subject 2024-16 in Study TR-20-HTN, a 54 year old Caucasian female with 4-year history of hypertension, hyperlipidemia, and COPD, experienced periorbital edema and lethargy during the double-blind phase after receiving placebo for 17 days. The subject was discontinued from the study because of periorbital edema and lethargy. The subject was put on a low salt diet for the edema. Both events were resolved at follow-up 4 weeks later.</i>

B2. Withdrawals from foreign hypertension trials

Events were reported for the following foreign clinical trials: FF/86/570/05¹, FF/86/570/06², FF/86/570/14, FF/87/570/21³, U/89/570/62⁴, UK/89/570/05⁵,

¹ Study FF/86/570/05, reviewed on page 130.

² Study FF/86/570/06, reviewed on page 136.

³ Study FF/87/570/21, reviewed on page 149.

FF/87/570/02⁶, FF/87/570/03⁷, FF/87/570/04⁸, FF/87/570/05, FF/87/570/06⁹,
 FF/87/570/07¹⁰, UK/89/570/01¹¹, FF/87/570/08¹², FF/87/570/22¹³, FF/88/570/29¹⁴,
 FF/88/570/33¹⁵.

B2.1. Controlled and uncontrolled studies with trandolapril alone

Adenocarcinoma (caecum)	<i>Subject 105/357 in Study FF/87/570/22 was a 70 year old female who after one month of trandolapril 2 mg/day, adenocarcinoma of the caecum was diagnosed. Trandolapril was discontinued, and the subject was admitted for surgery and chemotherapy.</i>
Angioedema	<i>Subject 278/109 in Study FF/87/570/07 was a 64 year old male smoker reported to be suffering from asthma before entry into the trial and taking salbutamol (inhaler) and beclomethasone (inhaler). After 5 weeks of trandolapril 2 mg/day, the subject developed an angioneurotic reaction, with swollen lips, tongue and face (intensity: severe). He was examined by his physician who prescribed chlorpheniramine maleate. Treatment with trandolapril was stopped as soon as the symptoms appeared and the symptoms resolved within 2 days.</i>
Angioedema (presumed—facial edema)	<i>Subject 99 in Study FF/87/570/21, a 34 year old Caucasian male with a 1-year history of hypertension, discontinued on day 7 of treatment with trandolapril 2 mg/day with facial edema of several days' duration.</i>
Atrial fibrillation	<i>Subject 4 in Study FF/88/570/39, a 70 year old female, with chronic renal insufficiency, and of cholecystectomy, and hypertension since 1977. On January 17, 1990 trandolapril 2 mg daily was initiated. After 8 days on trandolapril 2 mg daily, the subject presented with diarrhea, fever, nausea and vomiting. The next day, heart rate was 156 bpm and irregular. The subject had lost 1.2 kg and she was slightly dehydrated. ECG showed atrial fibrillation. The subject was given digoxin 25 mg i.v. Trandolapril was not given and the study was discontinued.</i>
Bradycardia (alleged)	<i>Subject 75/531 in Study FF/87/570/07 was a 42 year old female had no particular history of note. On entry, her ECG was normal, with supine heart rate of 66 bpm. After 2 months on trandolapril 2 mg/day, the subject reported episodes of bradycardia (alleged heart rate: 40 bpm). These episodes were not documented. At the last visit while on study drug, the electrocardiogram was normal and heart rate was 60 to 70 bpm. The subject decided to drop from the study. No additional information is available.</i>
Bronchitis, lack of efficacy	<i>Subject 1305/40 in Study FF/87/570/22 was a 63 year old female with a history of cholecystectomy and asthma. After 11 months of trandolapril 4 mg/day, she presented with acute bronchitis</i>

⁴ No study report submitted with NDA.

⁵ No study report submitted with NDA.

⁶ No study report submitted with NDA.

⁷ No study report submitted with NDA.

⁸ Study FF/87/570/04, reviewed on page 145.

⁹ No study report submitted with NDA.

¹⁰ No study report submitted with NDA.

¹¹ No study report submitted with NDA.

¹² No study report submitted with NDA.

¹³ No study report submitted with NDA.

¹⁴ No study report submitted with NDA.

¹⁵ No study report submitted with NDA.

	<i>(intensity: moderate), requiring treatment with amoxicillin. Blood pressure was at that time 195/125 mmHg (supine), whereas the values on the previous visit had been respectively 128/75 mmHg and 130/78 mmHg. The investigator judged this increase in blood pressure to be a result of the intercurrent bronchitis, and decided to withdraw the subject from the trial.</i>
Bronchospasm, dyspnea, precordial pain	<i>Subject 249 in Study FF/87/570/07 was a 34 year old female with a history of spasmophilia. Three weeks after treatment with trandolapril 2 mg/day was started, the subject developed severe bronchospasm, requiring hospitalization and treatment with intravenous aminophylline and salbutamol. Nine days later, after recovery, the subject was discharged from hospital still taking aminophylline and trandolapril. One week later, aminophylline was stopped, and 2 days thereafter, the subject presented with severe dyspnea, precordial pain radiating to the left arm and mild bronchospasm. Trandolapril was stopped the next day, and the subject recovered uneventfully.</i>
Carcinoma, lung	<i>Subject 280/651 in Study FF/87/570/05 was a 55 year old female with no history of smoking. At the beginning of the placebo period, a chest X-ray was taken and showed a lung opacity of the left lower zone. Tomography confirmed the presence of a tumor, and biopsy performed two-and-a-half months after initiating trandolapril revealed a squamous cells carcinoma in the lower left lobe of the lung. At the subsequent visit scheduled in the protocol, the investigator decided to withdraw trandolapril.</i>
Cerebrovascular accident	<i>Subject 72/225 in Study FF/87/570/22 was a 64 year old male with a history of coronary artery disease and a gastric ulcer. From the third day of treatment with trandolapril 2 mg/day, he complained of fatigue (intensity: moderate) lasting 4 days. After 2 months, he presented with a rash on his back (intensity: mild) which lasted for 2 weeks; the attending dermatologist concluded that this cutaneous event was unrelated to the test drug. Ten days after recovery, the subject developed shingles on his neck (intensity: severe), and was treated with acyclovir. Two months thereafter, he experienced a sudden severe rise in blood pressure which resulted in a left sided stroke (intensity: mild). He was admitted to hospital and withdrawn from the trandolapril trial. Complete recovery occurred within a few hours.</i>
Chest pain (presumed infection)	<i>Subject 229 in Study FF/86/570/06, a 48 year old Caucasian male with newly diagnosed hypertension, had onset of severe left-sided chest pain on day 7 while taking trandolapril 4 mg/day. ECG and enzymes were normal and he was treated with amoxicillin for a presumed chest infection with resolution of symptoms.</i>
Chest pain	<i>Subject 256/592 in Study FF/87/570/05, a 62 year old male with no pertinent medical history, experienced fatigue (intensity: mild) during the first week of the placebo phase. Then, one week before starting active treatment, he developed a rash (intensity: moderate) from scabies, which required treatment with terfenadine, benzylbenzoate and chlorpheniramine maleate. After one day of trandolapril treatment, he complained of a chest pain (intensity: severe) radiating to his back ("like an indigestion") although his ECG remained normal. In spite of stopping trandolapril 3 days later, the chest pain persisted. Esophagitis was suspected and simethicone was prescribed. Recovery occurred within one week.</i>

Chest and epigastric pain	<i>Subject 266/350 in Study FF/87/570/05 was a 56 year old female smoker with no pertinent medical history. Towards the end of the placebo period (on day -3), she began suffering from leg cramps (intensity: mild) for one day. An ECG carried out at that time showed occasional ventricular ectopic beats. After one month of trandolapril, she experienced chest and epigastric pains (intensity: severe). On admission to hospital, cardiac enzymes and an ECG showed no evidence of myocardial infarction. A diagnosis of esophageal spasm was proposed. The subject improved after trandolapril was withdrawn.</i>
Cough	<i>Subject 185 in Study FF/87/570/04, a 64 year old Caucasian male with 6-year history of hypertension, discontinued trandolapril 2 mg/day on day 33 because of cough that began on day 31.</i>
Cough	<i>Subject 154/31 in Study FF/88/570/29 was a 54 year old female who was previously enrolled in Study FF/87/570/21 (placebo group). One month after entry into the long-term study and on trandolapril 2 mg/day, she began suffering from cough; after trandolapril was stopped, cough improved. She had previously suffered from this problem with other angiotensin converting enzyme inhibitors (captopril, lisinopril and enalapril).</i>
Cough	<i>Subject 162/50 162/50 in Study FF/88/570/29 was a 51 year old female who was previously entered into Study FF/87/570/21 (placebo group) and experienced headache. At the end of the second month on trandolapril 2 mg/day, she complained of throat pain (intensity: mild). Two months later, she experienced cough (intensity: severe), and after two weeks, she decided to stop taking trandolapril. Recovery occurred within 2 weeks.</i>
Cough	<i>Subject 53/277 in Study FF/87/570/22 was a 56 year old female with no medical history of note. After one month of taking trandolapril 2 mg/day, the dose was doubled and over the following days, HCTZ 2.5 mg (sic) was added, and the subject experienced faintness at home (intensity: mild). At the next visit, supine blood pressure was 168/92 mmHg. At the beginning of the fifth month, she had flu and experienced cough. In spite of taking several self-prescribed medications the subject went on suffering from cough (intensity: moderate) and after 2 months decided to stop trandolapril.</i>
Cough	<i>Subject 703/84 in Study FF/87/570/22 was a 49 year old female had a history of hysterectomy. After 1-1/2 months of trandolapril 2 mg/day, she experienced unproductive cough (intensity: severe), with progressive worsening. The investigator decided to withdraw her from the study at the next visit, i.e. 3 weeks after the beginning of the symptoms. Complete recovery occurred within one week.</i>
Cough	<i>Subject 119/377 in Study FF/87/570/22 was a 72 year old female who after one month of trandolapril 2 mg/day experienced cough (intensity: severe). The cough worsened progressively over a week and she decided to stop her treatment. No further details are available.</i>
Cough	<i>Subject 69/493 in Study FF/87/570/07 was a 60 year old male who suffered from intermittent claudication. After 3-1/2 months of treatment with trandolapril 2 mg/day, he experienced persistent nocturnal cough (intensity: moderate). The study drug was stopped two weeks later, and recovery occurred within 2-1/2 months.</i>
Cough	<i>Subject 1304/7 in Study FF/87/570/07 was a 54 year old female with no previous history of allergy or of lung disease. After 6 months of</i>

	<i>treatment with trandolapril 4 mg/day, she experienced nocturnal cough (intensity: mild at that time). Symptomatic treatment was prescribed (astemizole), but the symptoms gradually worsened, occurring also in the daytime and becoming severe. After 5 weeks, the investigator decided to stop the drug treatment, and complete recovery occurred in 2 weeks.</i>
Cough	<i>Subject 267/779 in Study FF/87/570/08 was a 55 year old female who had entered into Study FF/87/570/05 (atenolol group), where she experienced very cold feet. After starting on trandolapril 2 mg/day, the subject presented with a bronchospasm (intensity: moderate), cough and purulent sputum diagnosed as a chest infection. The chest infection resolved within 15 days. One month later, however, some degree of dry cough persisted, especially at night. After 12 weeks, the subject decided to stop her treatment because of the persistence of the nocturnal cough. One week later, the cough resolved.</i>
Cough, angina	<i>Subject 68/245 in Study FF/87/570/22 was a 55 year old male with a history of smoking and arteritis of the lower limbs with a surgical history of aortoiliac bypass; he also suffered from incomplete right heart block. After 3 weeks of treatment with trandolapril 2 mg/day, the subject suffered for 3 weeks from rhinitis and pharyngitis with cough (intensity: moderate). One week after recovery, the subject developed bronchitis, presenting with dyspnea and cough (intensity: moderate). The subject recovered. One month later, he experienced, for the first time, an attack of angina (intensity mild) while walking. Two months later, he again had a cough (intensity: mild). After an initial recovery and more than 8 months of treatment with trandolapril, the cough recurred and its intensity was moderate. One week earlier, the subject had also experienced further angina attacks (intensity: moderate). After trandolapril was stopped, the subject recovered at first, within 2 weeks, but his cough recurred intermittently. Follow-up information suggested that the subject had spastic bronchitis.</i>
Cough, dry mouth	<i>Subject 52/497 in Study FF/87/570/07 was a 62 year old female with stenosis of the subclavian artery. After one year of treatment with trandolapril 4 mg/day, she complained of cough and dry mouth (intensity: moderate for both symptoms). The reduction in the dose of trandolapril to 2 mg/day resulted one week later in an improvement in cough. However, because of the persistence of this symptom, the investigator decided to stop the treatment one month later. Recovery occurred within 3 weeks.</i>
Crohn's disease, perineal fistula	<i>Subject 701/301 in Study FF/87/570/08 was a 61 year old male who was previously enrolled in Study FF/87/570/06 (trandolapril 2 mg plus nifedipine). Four-and-a-half months after starting trandolapril 2 mg/day, he was diagnosed with Crohn's disease (intensity: severe). Three months later, he had a perineal fistula for which he underwent surgery. The test drug was thus stopped.</i>
Diabetes	<i>Subject 288/621 in Study FF/87/570/05 was a 56 year old female whose entry blood glucose was 5 mM. After 3 weeks of active treatment, hyperglycemia (17.3 mM) and glucosuria were reported. One week later, after blood glucose was 12.5 mM and diabetes was diagnosed, the investigator decided to stop trandolapril.</i>
Diarrhea	<i>Subject 68/467 in Study FF/87/570/07 was a 60 year old male who had non-insulin-dependent diabetes with mild retinopathy. He was being treated with glibenclamide, metformin and calcium</i>

	<i>dobesilate. After one month of treatment with trandolapril 2 mg/day, he experienced diarrhea (intensity: moderate). Two months later, the persistence of this symptom led the subject to report it to the investigator. At that point, his treatment was stopped. Recovery occurred within one week.</i>
Dizziness	<i>Subject 198 in Study FF/86/570/14, a 51 year old Caucasian male with a 10-year history of hypertension, discontinued for severe dizziness on day 13 of treatment with trandolapril 4 mg/day. His hypertension had been untreated at time of enrollment, and baseline supine blood pressure was 155/100 mmHg. Symptoms resolved after termination.</i>
Dizziness, light-headedness	<i>Subject 211/78 in Study FF/87/570/08 was a 48 year old female with a history of chronic constipation who before entry into the long-term trial was in Study FF/87/570/06 (trandolapril 2 mg plus nifedipine). On entry into Study FF/87/570/08, her blood pressure was 150/90 mmHg (supine). One week later, she complained of exacerbated constipation (intensity: severe). After 2 months, the investigator reported hypotension (intensity: mild, with a supine and standing blood pressure of 120/80 mmHg). The subject complained of dizziness, with light-headedness. She decided to stop trandolapril and after 4 weeks without any known treatment, her blood pressure was 130/80 mmHg (supine).</i>
Dizziness, tiredness	<i>Subject 256/796 in Study FF/87/570/08 was a 52 year old female who had experienced occasional vertigo for 10 years. Prior to the long-term study, she was in Study FF/87/570/05 (atenolol) where she experienced vertigo and headache, both assessed by the investigator as possibly related to the test drug. Two days after starting trandolapril 2 mg/day, the subject complained of dizziness and tiredness (intensity: moderate for both symptoms). These symptoms persisted for 3 weeks and the test treatment was thus discontinued. Within 4 days, the subject recovered.</i>
Dizziness and a feeling of upper abdominal distention	<i>Subject 256/762 in Study FF/87/570/08 was a 62 year old female who, prior to the long-term study, was in Study FF/87/570/05 (atenolol). Her blood pressure at the inclusion visit of this study was 180/110 mmHg (supine). The day she was entered into Study FF/87/570/08, her blood pressure was 174/98 mmHg (supine). After three days on trandolapril 2 mg/day, she complained of dizziness and a feeling of upper abdominal distention (intensity: moderate for both symptoms). These symptoms persisted for 4 weeks, until the next visit scheduled by the protocol. On this visit, her blood pressure was 200/116 mmHg (supine). The investigator decided to stop trandolapril. Within three days, the subject recovered.</i>
Duodenal ulcer, cholecystectomy	<i>Subject 256/612 in Study FF/87/570/08 was a 49 year old female who had been in Study FF/87/570/05 (trandolapril 2 mg/day), where she twice developed an allergic rash on her face and a dental abscess. After one month on trandolapril 2 mg/day, she presented with a dental abscess (intensity: moderate), for which she was treated with pericillin and paracetamol. Trandolapril was reduced for 3 days. Subsequently, the dose was increased to 4 mg/day according to the protocol. One month later, the subject experienced epigastric pain and vomiting (intensity: moderate), ascribed by the investigator to gastritis. The test drug was stopped. Endoscopy performed in the following days disclosed duodenal ulcer, for which she was subsequently treated with ranitidine. Two months later, she underwent also a cholecystectomy.</i>

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Duodenal ulcer, hematemesis	<i>Subject 281, 524 in Study FF/87/570/08 was a 38 year old male who first entered into Study FF/87/570/05 (trandolapril 2 mg). Prior to the trial, he suffered from migraine and nausea. On trandolapril, he complained of an exacerbation of his headache. After 1.5 months on 2 mg/day in the long-term trial, he complained of nausea (intensity: moderate). One week later, he had hematemesis, requiring his hospitalization and blood transfusion. The test drug was stopped, and cimetidine was started. A barium X-ray showed the presence of a duodenal ulcer.</i>
Dyspnea	<i>Subject 205/004 in Study FF/87/570/06 was a 41-year-old overweight male who had been recently diagnosed as hypertensive. EEG was normal and no chest X-ray was done. Two days after starting trandolapril, the subject went to the ER complaining of severe dyspnea. Clinical examination was normal and this was diagnosed as a hysterical reaction. One day later, the dyspnea reappeared and the investigator decided to stop trandolapril the following day. The subject recovered rapidly.</i>
Dyspnea	<i>Subject 68/246 in Study FF/87/570/22 was a 67 year old female with a history of arteritis of the right lower limb. She also had complete right bundle branch block. The subject was taking pentoxifylline and naftidrofuryl. After 6 months of treatment with trandolapril 2 mg/day, she presented with ventricular ectopic beats (intensity: moderate) for which she was prescribed nadolol. With nadolol, the ectopic beats ceased, but after 3 months, she developed dyspnea (intensity: moderate). The investigator stopped nadolol treatment, prescribed furosemide, and discontinued trandolapril.</i>
Eyelid edema, dry skin	<i>Subject 337 in Study UK/89/570/01 was a 62 year old female with no medical history of note. After 2 weeks of treatment with trandolapril, she developed edema of the upper eyelids (intensity: mild). One week later, she complained of dry skin (intensity: moderate). After 2 weeks, the test drug was stopped. Within two weeks, her skin had returned to normal. The edema resolved two weeks later.</i>
Gastrectomy	<i>Subject 206/009 in Study FF/87/570/06 was a 45 year old female, with a history of duodenal ulcer in 1980, two abdominal operations in January and March 1987, and hypertension since 1960. Three days after trandolapril was started, the subject complained of dizziness and numbness in the extremities. These symptoms lasted 5 days. Six days after trandolapril was started, the subject reported a viral infection with cough. The subject had recovered 7 days later. After 1 month in the study, gastroscopy was performed and 3 days later, the subject underwent gastrectomy. Trandolapril was discontinued at that time.</i>
Gastric upset	<i>Subject 213 in Study UK/89/570/01 was a 48 year old male who occasionally experienced atrial ectopic beats and occasionally took naproxen. On the first day of treatment with trandolapril, he experienced gastric upset (intensity: moderate). The test drug was immediately withdrawn, and the subject recovered.</i>
Gastrointestinal pain	<i>Subject 287/429 in Study FF/87/570/05 was a 50 year old female with a history of hysterectomy. After 4 weeks of trandolapril, she experienced gastrointestinal pain (intensity: mild), for which sodium alginate plus sodium bicarbonate and calcium carbonate were prescribed. Apparently, these gastrointestinal symptoms represented an exacerbation of a pre-existing condition. Four weeks thereafter, because of persisting high blood pressure</i>

	<i>(160/100 mmHg), the dose of trandolapril was doubled and the subject reported worsening of the same symptoms (intensity: moderate) and stopped treatment with trandolapril. Recovery occurred within 2 weeks. One month later, the subject started a new treatment, with lisinopril, and after one week, she complained again of epigastric pain, leading once more to the cessation of treatment. X-ray examination showed no abnormality.</i>
Headache	<i>Subject 408/244 in Study FF/88/570/33 was a 49 year old female who was previous enrolled in Study FF/87/570/02 (enalapril). During the placebo period, she complained of headaches, which persisted into the beginning of her treatment with enalapril. During this initial study, increased blood pressure was reported; it was ascribed by the investigator to the subject's poor compliance. After 6 months on trandolapril 2 mg/day, she complained of headache (intensity: moderate). This symptom was related, according to investigator, to "the increase in blood pressure" on trandolapril, although her blood pressure was 150/95 mmHg (supine and standing) at that time. Finally, the subject refused to continue in the study.</i>
Headache	<i>Subject 201/165 in Study FF/88/570/33 was a 27 year old male who had been in Study FF/87/570/04 (trandolapril 2 mg plus HCTZ). Ten months after being on trandolapril 2 mg/day, he began experiencing headaches. The practitioner judged that his blood pressure was too high. He prescribed captopril and the subject was withdrawn. On the last visit while he was still taking the test drug, the subject's blood pressure was 145/95 mmHg (supine).</i>
Headache	<i>Subject 272/372 in Study FF/87/570/05 was a 52 year old female who, after six weeks of treatment with trandolapril, complained of headache (intensity: severe). At the previous visit 3 weeks earlier, her systolic blood pressure was normalized, although the diastolic pressure remained between 96 and 102 mmHg. In spite of stopping trandolapril treatment, the symptoms persisted one week later.</i>
Headache	<i>Subject 75/527 in Study FF/87/570/07 was a 34 year old female who suffered from headaches before she was entered into the trial. On day 3 of trandolapril 2 mg/day, she complained of headaches (intensity: moderate), and after 4 weeks in the trial, it was decided to stop treatment with trandolapril.</i>
Headache	<i>Subject 211/015 in Study FF/87/570/06 was a 55-year-old female, who was recently diagnosed as hypertensive. Twenty-five days before inclusion in the study, during the placebo phase, the subject began to complain of dizziness and mild headaches. On month after trandolapril was started, the subject suffered from severe headaches. Her blood pressure was 160/100 mmHg. The headaches persisted for 1 month after which trandolapril was discontinued. No other data are available.</i>
Headache	<i>Subject 201/160 in Study FF/88/570/33 was a 32 year old male smoker who had been enrolled in Study FF/87/570/04 (trandolapril 2 mg plus HCTZ). One month after being on trandolapril 2 mg/day, he developed bronchitis (intensity: moderate), for which cephalixin was prescribed. Seven months later, he complained of headache (intensity: moderate). The investigator decided to stop the test drug. Further details of the adverse event are unknown.</i>
Headache, epigastric pain	<i>Subject 1304/5 in Study FF/87/570/22 was a 62 year old female with a history of uterine fibroids. After 1-1/2 months of treatment with trandolapril 4 mg/day, she complained of headache and epigastric</i>

	<i>pain (intensity: moderate for both symptoms). One week later, she decided to withdraw from the trial. Her blood pressure at that time was 158/96 mmHg (supine), whereas the value at the end of the placebo period was 176/102 mmHg.</i>
Headache, dizziness	<i>Subject 199 in Study FF/86/570/14, a 60 year old Caucasian male with a 3-year history of hypertension, discontinued because of headache and dizziness after 25 days of double-blind treatment with trandolapril 2 mg/day.</i>
Headache, tremors, scotomas, malaise, tachycardia, lack of efficacy	<i>Subject 3 in Study FF/87/570/21, a 48 year old Caucasian female with 10-year history of hypertension, discontinued on day 17 of treatment with trandolapril 0.5 mg/day because of headache, tremors, scotomas, malaise, and tachycardia beginning on day 7. Lack of efficacy was also a reason for termination. The headache resolved within 10 days, although it is not clear if the other symptoms had resolved as well.</i>
Headache, vertigo	<i>Subject 703/85 in Study FF/87/570/22 was a 63 year old female with a history of cholecystectomy. After 2 days of trandolapril 2 mg/day, she complained of headache and vertigo (intensity: mild for both symptoms). She immediately discontinued treatment with trandolapril, and recovered within one week.</i>
Hip replacement surgery	<i>Subject 703/84 in Study FF/87/570/22 was a 63 year old male who was reported to have arthritis of the hip. During the sixth month of treatment with trandolapril 2 mg/day, he was withdrawn from the study to undergo hip replacement surgery and rehabilitation.</i>
Hyperglycemia, vomiting	<i>Subject 68/257 in Study FF/87/570/22 was a 66 year old female had a past history of diabetes, arteritis, and hemiplegia. She was being treated with glibenclamide, cefiedil and naftidrofuryl. While taking trandolapril 2 mg/day for 1 month, she experienced vomiting for 2 days (intensity: moderate), and was hyperglycemic (2.84 g/L). According to the investigator, her blood sugar during the run-in phase ranged between 1.5 to 2 g/L. The subject was withdrawn from the study.</i>
Hypertensive crisis	<i>Subject 130/151 in Study FF/87/570/06 was a 51-year-old female, with a history of leg venous insufficiency and hypertension since 1960. Baseline supine blood pressure was 180/110 mmHg. Two months after trandolapril was started, her supine pressure was 170/100 mmHg. After 3 months in the study, a hypertensive crisis with epistaxis occurred, at which time, her supine blood pressure was 205/115 mmHg. Two days later, the investigator stopped trandolapril.</i>
Hypertensive crisis, acute glaucoma	<i>Subject 1851/076 in Study FF/87/570/06 was a 58-year-old female with a 5-year history of hypertension. Trandolapril was started at a dose of 2 mg daily. Her baseline blood pressure was 180/104 mmHg and heart rate was 64 bpm. Laboratory data were normal and ECG revealed left axis deviation. Forty-four days after the start of trandolapril, a hypertensive crisis occurred (200/110 mmHg) and, at the same time, acute glaucoma of left eye was diagnosed (left intra ocular pressure was 40 mmHg). After 49 days in the study, trandolapril was stopped. Three months after trandolapril cessation, the subject was well.</i>
Hypotension	<i>Subject 275/707 in Study FF/87/570/08 was a 49 year old female previously in Study FF/87/570/05 (atenolol). On entry in the long-term study, her blood pressure was 115/84 mmHg (supine). After 6 months on trandolapril 2 mg/day, the subject was withdrawn by</i>

	<i>the investigator because of asymptomatic hypotension; her blood pressure at that time was 120/78 mmHg (supine) and 118/74 mmHg (standing).</i>
Hypotension, vertigo	<i>Subject 58 in Study FF/86/570/14, a 54 year old Caucasian male with newly diagnosed hypertension, developed severe morning vertigo after 15 days of treatment with trandolapril 2 mg/day. Baseline supine blood pressure was 120/50 mmHg and it is unclear why he was randomized. His blood pressure at discontinuation was 100/30 mmHg. Symptoms resolved 1 week after termination.</i>
Hypotension, headache, dizziness	<i>Subject 207/2 in Study FF/88/570/29 was a 32 year old female who was previously entered into Study FF/87/570/21 (placebo). After one month of treatment with trandolapril 2 mg/day, she complained of headache (intensity: mild), which resolved within 3 weeks without special treatment. One month thereafter, she reported dizziness (intensity: mild) lasting three weeks. Two months later, while in the long-term study for 5 months, she again experienced headache and dizziness (intensity: severe), and was reported to have severe "postural hypotension". At this time, her blood pressure was 100/60 mmHg (supine) and 110/70 mmHg (standing). After a month, she refused to continue with the treatment. Four days later, the subject had recovered, although her blood pressure was unchanged.</i>
Influenza	<i>Subject 53 in Study FF/87/570/04, a 55 year old Caucasian male with history of hypertension of unknown duration, discontinued on day 46 of trandolapril 2 mg/day because of a 2-week bout of the flu.</i>
Influenza	<i>Subject 154 in Study FF/87/570/02 was a 53-year-old male with no relevant history. Seventeen days before trandolapril was started, the subject complained of "congestion in the head", headache and dizziness. Baseline blood pressure was 175/100 mmHg. After starting trandolapril 4 mg/day, he presented with a flu which resolved quickly. Nine days later, the subject decided to withdraw, at which time his blood pressure was 193/118 mmHg.</i>
Knee swelling, arthritis	<i>Subject 107/11 in Study FF/88/570/33 was a 60 year old female with no pertinent medical history who was previously enrolled in Study FF/87/570/02 (trandolapril 4 mg). Two months after being on trandolapril 2 mg/day, she developed swelling of both knees (intensity: severe), attributed by the investigator to arthritis. She decided to stop trandolapril.</i>
Leukopenia	<i>Subject 207/6 in Study FF/88/570/29 was a 24 year old male who was entered into FF/87/570/21 (trandolapril 1 mg group). On entry to the long-term study on trandolapril 2 mg/day, his WBC count was 3.1 K/μl. After one-and-a-half months of trandolapril treatment, the subject was reported to have a low WBC count (2.3 K/μl). Persistently low values over one year led the investigator to withdraw the subject from the trial, when his WBC count was 3.6 K/μl.</i>
Liver enzyme elevation	<i>Subject 27 in Study F/86/570/10, a 25-year-old healthy male volunteer, was enrolled to in the 16 mg trandolapril group. Eight days before trandolapril was started, laboratory data were normal. Serologic tests for hepatitis B were negative. The volunteer complained of abdominal pain on day 2 and loose stools on day 3 of trandolapril and reported muscular pain on days 1, 4, and 5. On day 6, mild isolated increase of SGPT (to 2x normal) was noted and the subject was withdrawn from the study. Three days later, SGPT reverted to normal.</i>

Liver enzyme elevation	<i>Subject 58 in Study FF/86/570/06, a 56 year old male not previously treated for hypertension, experienced elevation of liver enzymes. Enzymes were normal at baseline, but at the end of run-in, SGOT was 73, SGPT was 80, and AlkPhos was 108. At the end of double-blind treatment (day 8), SGOT was 479, SGPT was 828, and AlkPhos was 140. Enzymes climbed further during wash out, then declined, but were still not normal range at 3 weeks. Subject tested positive for HBS antibody at 3 weeks.</i>
Liver enzyme elevation	<i>Subject 267/719 in Study FF/87/570/05 was a 61 year old male who complained of tiredness and lethargy (intensity: moderate) after one day of trandolapril. Laboratory results were within normal ranges. Tiredness persisted to a lesser extent throughout the study. After 12 weeks of active treatment, liver enzymes were raised ASAT 230 (normal is <41), ALAT 152 (normal is <41) and AlkPhos 264 (normal is <115). Despite the absence of symptoms of liver disease, the investigator decided to stop trandolapril and one week after drug withdrawal, enzymes were within the normal range.</i>
Lower limb edema	<i>Subject 130/154 in Study FF/87/570/06 was a 47-year-old female who had been hypertensive since 1965. Five days after trandolapril was started, lower limb edema appeared. After 18 days in the study, trandolapril was discontinued because of the edema. The edema disappeared 2 weeks after cessation of trandolapril.</i>
Lumbar osteophytes	<i>Subject 352/278 in Study FF/87/570/03 was a 59-year-old female with a history of cervicarthrosis and lumbar arthrosis (unspecified) and recently diagnosed hypertension. Twenty-two days after the start of trandolapril, the subject complained of left sciatica and crural pain which required non-steroidal anti-inflammatory drug treatment. Trandolapril was stopped at that time. Six days later, an X-ray revealed osteophytes between the L4 and L5 lumbar vertebra. The subject's condition improved but did not resolve.</i>
Myocardial infarction	<i>Subject 279/465 in Study FF/87/570/05 was a 52 year old male who had been a smoker. On entry into the study, his ECG was normal. At the end of the second month of trandolapril, persistent high blood pressure led the investigator to double the dose of trandolapril as per the protocol. One-and-a-half months later, when the subject's blood pressure was still high (supine: 170/110 mmHg; standing: 160/110 mmHg), he had an antero-lateral myocardial infarction (intensity: severe). The investigator stopped the test drug.</i>
Myocardia. infarction, anterior	<i>Subject 258/672 in Study FF/87/570/08 was a 63 year old female who was in the FF/87/570/05 study (atenolol group). One day after starting trandolapril 2 mg/day, she awoke in the night with chest pain. The next night, she had the same symptoms and on the next day, she decided to stop trandolapril. Five days later, ECG displayed changes related to a recent myocardial infarction, with frequent unifocal ventricular ectopic beats. She refused hospitalization as well as blood sampling. Subsequent ECG displayed the normal evolution of an anterior myocardial infarction, and the subject made an uneventful recovery.</i>
Nausea, asthenia	<i>Subject 83 in Study FF/87/570/0, a 27 year old Caucasian female with 5-year history of hypertension, discontinued on day 3 of trandolapril 2 mg/day because of nausea and asthenia that began with the first dose. Both events resolved within 2 days.</i>
Neutropenia	<i>Subject 141 in Study UK/89/570/01 was a 63 year old female smoker who had a history of hypertension for an unspecified period. One month prior to starting the study drug, laboratory data showed a</i>

	slight increase in WBC count (11.3 K/μl) with 75% neutrophils. A blood sample taken at that time was lost so that no baseline values are available. One week later, the investigator reported neutropenia (1.95 K/μl; normal is >2.2 K/μl). After 44 days in the study, the neutrophil count remained low (1.6 K/μl). Trandolapril treatment was discontinued at that time. A follow-up blood sample was taken 4 months later and showed an increased neutrophil count (3.65 K/μl).
Nightmares	Subject 280/499 in Study FF/87/570/05 was a 58 year old male with no pertinent medical history. After 4 weeks of trandolapril, the subject had a sty which was treated during one week with amoxicillin and chloramphenicol eye drops. One week later, he experienced nightmares (intensity: moderate), but reported these persisting symptoms only after 10 weeks. The investigator stopped trandolapril. Shortly thereafter, the subject returned to normal.
Paresthesia	Subject 703/82 in Study FF/87/570/22 was a 64 year old female had a history of uterine cancer. At the end of the placebo period, her blood pressure was 175/105 mmHg (supine). After 1 week of trandolapril 2 mg/day, her blood pressure dropped to 140/80 mmHg. The next week, the investigator reported a fall in blood pressure to 112/68 mmHg. At this time, the subject complained of paraesthesia in her right arm, legs, and face (intensity: severe), and of dizziness and faintness. She stopped her treatment and was withdrawn from the study.
Pneumopathy	Subject 40/113 in Study FF/88/570/29 was a 60 year old female who had participated in Study FF/87/570/21 (trandolapril 1 mg) without experiencing adverse events. Two months after inclusion in the long-term study on trandolapril 2 mg/day, this subject presented with a pneumopathy (intensity: moderate), and the investigator decided to withdraw her from the study.
Polyneuropathy	Subject 702/103 in Study FF/87/570/22 was a 55 year old female with a history of cerebrovascular disease (unspecified) at the age of 44. After one month of trandolapril 2 mg/day, she presented with polyneuropathy (intensity: mild) and the investigator decided to stop the test drug. The symptoms persisted after drug withdrawal.
Premature ventricular beats	Subject 271/522 in Study FF/87/570/05 was a 64 year old male smoker, who had reported noticing irregular heart beats in the past (not documented). An ECG was judged normal at inclusion. After two months of treatment with trandolapril, he had a cold with cough (intensity mild) lasting one week. Just at the end of this episode, when the dose of trandolapril was doubled, the investigator reported premature ventricular beats (intensity: moderate). This abnormality was still present on ECG after 2 weeks, and treatment with trandolapril was withdrawn. Four months thereafter, the subject felt well, but premature ventricular beats were still present on ECG.
Psychotic reaction	Subject 113/570 in Study FF/87/570/07 was a 54 year old male was reported to be suffering from insomnia, depression, and peptic ulcer. He was taking sulpiride, dihydroergotoxine, flunitrazepam, quinidine, urgenin and an unspecified H₂-antagonist. After about one month of treatment with trandolapril 2 mg/day, the subject developed a psychotic reaction, and was referred to a psychiatrist. According to the investigator, the subject's compliance with the drug treatment was questionable, and he decided to stop further treatment.
Rash	Subject 532 in Study FF/87/570/05 was a 63 year old female with no

	<i>pertinent medical history who developed a rash on both forearms. Trandolapril was discontinued, but 8 days later the rash persisted. No further follow-up information was available.</i>
Rash	<i>Subject 220 in Study UK/89/570/01 was a 41 year old female. After 3 days of treatment with trandolapril, she had a grazing injury to her right knee and the top of her foot, probably related to a fall. After 10 days of trandolapril, she developed a rash on the trunk and the limbs (intensity: moderate), and the test drug was discontinued. One day later, the rash improved. Three days later the subject had completely recovered.</i>
Rash	<i>Subject 267/756 in Study FF/87/570/08 was a 60 year old male who was previously in Study FF/87/570/05 (atenolol). Eleven days after starting trandolapril 2 mg/day, he developed a maculo-papular pruriginous rash (intensity: severe), initially generalized. Despite trandolapril interruption and treatment with triprolidine hydrochloride, the rash took five weeks to resolve.</i>
Rash	<i>Subject 52/249 in Study FF/87/570/22 was a 62 year old female who developed an erythematous pruritic rash over the whole body (intensity: severe) after 3 days of trandolapril 2 mg/day treatment. Trandolapril was stopped and she recovered the next day.</i>
Renal impairment	<i>Subject 68/490 in Study FF/87/570/07 was a 65 year old female was reported to be suffering from abnormal renal function. Two weeks after treatment with trandolapril 2 mg/day, her serum potassium was 4.3 mM and serum creatinine 180 µM. After one month of treatment, persistent high blood pressure led the investigator to double the dose and to add spironolactone 75 mg/day. Two weeks later, serum potassium was 4.8 mM and serum creatinine was 148 µM. At the sponsor's request, the subject was withdrawn from the study (subject should not have been entered into the study)</i>
Serum creatinine increase	<i>Subject 78/292 in Study FF/87/570/22 was a 68 year old male whose non-insulin-dependent diabetes was treated with metformin. After 2 months of trandolapril 2 mg/day, he experienced cough (intensity: mild) for one month, making a spontaneous recovery. One week later, he was injured in a car crash. While in the hospital, he received heparin and tiaprofenic acid. After two days, he developed a pneumonia for which he was prescribed erythromycin and oxacillin. Ten days after his hospital admission, laboratory tests showed raised levels of serum creatinine (189 µM). Thereafter, creatinine levels varied from 120 to 164 µM as the antibiotics, tiaprofenic acid and trandolapril were successively discontinued. Rechallenge with tiaprofenic acid was followed by another increase in serum creatinine.</i>
Sexual dysfunction	<i>Subject 112/18 in Study FF/88/570/33 was a 51 year old male had a history of smoking and probable alcohol abuse. He had been enrolled in Study FF/87/570/06 (trandolapril 2 mg plus nifedipine). Once in the long-term study on trandolapril 2 mg/day, the subject began to think that the test drug decreased his sexual potency (intensity: severe). At the end of the fourth month in the long-term study, he decided to stop trandolapril treatment, but the subject's sexual difficulties persisted after stopping the drug.</i>
Sinusitis	<i>Subject 207/1 in Study FF/88/570/29 was a 49 year old female who was previously in Study FF/87/570/21 (placebo). On entry into the long-term study (trandolapril 4 mg/day), she was reported to have a low WBC count (3.0 K/µl) which persisted to some extent throughout the study. This pre-existing low WBC was judged by the</i>

Sinus node arrest, syncope	investigator probably related to the test drug. After almost one year in the study, the subject suffered from sinusitis which was resistant to treatment, at which time her WBC count was 4.5 K/ μ l. The investigator decided to stop her treatment. The outcome is unknown.
Sinus tachycardia	Subject 279/438 in Study FF/87/570/05 was a 58 year old male. After one month of treatment with trandolapril and while traveling in Spain, he began to have 3 or 4 blackouts per day (intensity: severe), often when sitting. He was admitted to a clinic and where his ECG was normal. When he returned to the U.K., he was immediately admitted to hospital and trandolapril was stopped. An ECG tracing showed sinus node arrest. Within 48 hours, the subject's syncopal problems ceased. ECG examinations performed 4.5 months later confirmed that the subject had completely recovered.
Syncope, tachycardia, headache	Subject 1304/25 in Study FF/87/570/07 was a 44 year old male with no medical history of note. On entry into the study, ECG was judged normal (heart rate: 96 to 98 bpm). After one month of treatment with trandolapril, the daily dose of was doubled (4 mg/day). After 3 months of treatment with trandolapril, ECG revealed sinus-tachycardia (heart rate: 106 to 108 bpm). Four weeks later, at which time the ECG findings persisted, the subject complained of tachycardia and palpitations (intensity: moderate). At the visit scheduled for that time, the subject's heart rate was 92 bpm (supine) and 100 bpm (standing). Apparently, the palpitations resolved over 3.5 months, but recurred 2.5 months later (i.e., after 10 months of treatment with trandolapril). As their intensity had become severe according to the subject, he decided to stop his treatment claiming to have experienced a prompt recovery one day after stopping. Four days later and with no treatment, his ECG still showed sinus tachycardia: heart rate 106 bpm (supine) and 114 bpm (standing).
Tinnitus, fatigue	Subject 73/272 in Study FF/87/570/22 was a 69 year old female with no medical history of note. After 3 months of treatment with trandolapril 2 mg/day, she reported a "malaise" (intensity: mild) which was poorly described. One week later, she reported fainting spells (intensity: severe) lasting 3 days. She also suffered from severe headaches and tachycardia. The investigator decided to stop the treatment. The blood pressure at this time was 150/80 mmHg (supine) and 160/90 mmHg (standing) and an electrocardiogram could not be performed. Three weeks thereafter, the investigator reported that his subject had improved after withdrawal of trandolapril.
Transient ischemic attack	Subject 117/553 in Study FF/87/570/07 was a 41 year old male alcoholic. On day 4 of trandolapril 2 mg/day, he experienced headache and dizziness which lasted for 3 days. The subject also complained of tinnitus and fatigue on day 9 (intensity: moderate for all these symptoms). He decided to stop his treatment, and claimed to have completely recovered the next day.
	Subject 185/152 in Study FF/87/570/03 was a 56 year old man with a history of hypertension. On inclusion, blood pressure was 160/110 and EEG was normal. Trandolapril 4 mg/day was started. Sixty-seven days after trandolapril start, the subject had an acute onset of right hemiparesis and speaking problem. On clinical examination, the subject had aphasia, facial paralysis and right hemiparesis. Blood pressure was 230/130 mmHg. The subject was admitted to the hospital and trandolapril was stopped. Four days after trandolapril was stopped, the neurological symptoms had

	<i>disappeared. Ultrasound imaging of carotid bifurcation was performed and revealed a 1 mm reduction in left carotid lumen diameter with no atheromatous lesions.</i>
Transient ischemic attack	<i>Subject 154/28 in Study FF/88/570/29 was a 56 year old female with no medical history of note. She had been enrolled in FF/87/570/21 (trandolapril 2 mg group) with a blood pressure on entry of 170/100 mmHg (supine). After having been enrolled in the long-term study on trandolapril 4 mg/day, she experienced a transient ischemic attack. EEG showed signs of vascular insufficiency. One month after trandolapril cessation, she still complained of slight paresis of right arm.</i>
Urinary tract infection	<i>Subject 78 in Study FF/86/570/14, a 22-year-old male, with a history of hypertension since 1984. After 12 days on trandolapril, the subject complained of left sided thoracic pain associated with vertigo, hot flushes, which occurred repeatedly, but spontaneously disappeared. Nineteen days after the start of trandolapril, the subject had a urinary tract infection and stopped the study drug himself. Urine culture yielded staphylococcus epidermidis; leucocyturia and proteinuria were present. Trandolapril was discontinued.</i>
Urinary tract infection, orthostatic hypotension	<i>Subject 207/15 in Study FF/87/570/21 was an overweight 43-year-old male smoker with history of hypertension for 8 years. Trandolapril 0.5 mg daily was started. Nine days later the subject was hospitalized for a severe urinary tract infection and severe postural hypotension (unspecified blood pressure) for 24 hours. Trandolapril was stopped and cefazolin and scopolamine were prescribed. One day after the discontinuation of trandolapril supine blood pressure was 110/90 mmHg and standing blood pressure was 100/70 mmHg. Six days after discontinuation, the subject had completely recovered.</i>
Urticaria	<i>Subject 7 in Study F/89/570/54 a 20-year old healthy volunteer with no pertinent medical history took a single-dose of trandolapril 2 mg. Three weeks later, a second dose of trandolapril 4 mg was taken and was well tolerated. During the night following the third dose of trandolapril 0.5 mg, 20 days after the second dose, urticaria occurred and disappeared 10 days later. The subject was withdrawn from the study.</i>
Vasovagal episode	<i>Subject 114/563 in Study FF/87/570/07 was a 55 year old female had no particular history of note. On day 3 of trandolapril 2 mg/day, she experienced a vaso-vagal attack (intensity: moderate) and decided at once to stop treatment with trandolapril. There is no record of her blood pressure during the vasovagal episodes.</i>
Vertigo	<i>Subject 41 in Study FF/86/570/06, a 60 year old Caucasian female, discontinued with vertigo on the first day of double-blind with treatment trandolapril 4 mg/day, with prompt resolution of symptoms.</i>
Vertigo	<i>Subject 106/5 in Study FF/88/570/33 was a 65 year old female with no pertinent medical history who was previously enrolled in Study FF/87/570/02 (enalapril) where she developed acute bronchitis. After one month on trandolapril 2 mg/day, she developed another episode of bronchitis, requiring treatment with antibiotics. During the fifth month of the long-term study, the subject experienced vertigo (intensity: mild), which lead the investigator to discontinue the test drug 1.5 months later. Her blood pressure was 180/90 mmHg (supine and standing) at that time. Further details</i>

are not known.

B2.2. Trandolapril plus amiloride, HCTZ, bendroflumethiazide, or furosemide

Chest pain	<i>Subject 414/179 in Study FF/87/570/02 was a 49-year-old male, with a history of chest pain in 1987 and hypertension since 1986. After two months of trandolapril, hydrochlorothiazide was added because blood pressure was not controlled (160/95 mmHg). After 3 months in the study, the subject began to complain of moderate chest pain. Neither medical examination nor ECG were conclusive. Four days later, the subject stopped trandolapril. One week later, chest pain persisted. The follow-up is not known.</i>
Cough	<i>Subject 278/147 in Study FF/87/570/07 was a 68 year old female with a history of chronic obstructive airway disease. After two months of treatment with trandolapril 4 mg/day and hydrochlorothiazide, she experienced cough (intensity: moderate). One-and-a-half months later, the study drug was stopped, and recovery occurred within 2 weeks.</i>
Depression	<i>Subject 71 in Study FF/87/570/04, a 55 year old Caucasian female with recently diagnosed hypertension, discontinued on day 4 of combination treatment with HCTZ 25 mg/day plus trandolapril 2 mg/day because of depression which predated study entry.</i>
Digestive troubles, pins and needles in arms, hot flushes, nausea, sweating	<i>Subject 168 in Study FF/87/570/04, a 60 year old Caucasian male with a 4-year history of hypertension, discontinued because of digestive troubles, pins and needles feeling in arms, hot flushes, nausea, and sweating on day 9 of combination treatment of HCTZ 25 mg/day plus trandolapril 2 mg/day. Symptoms actually initially began during placebo withdrawal. The digestive troubles, nausea, hot flushes, and sweating resolved after a few days, but the pins and needles feeling was still ongoing.</i>
Headache	<i>Subject 152/120 in Study FF/87/570/02 was a 58-year-old male, with a history of hypertension since 1975. One month and a half after the start of trandolapril, HCTZ was added. Seven days after the start of HCTZ, the subject experienced severe headache. The subject stopped taking HCTZ 8 days later and stopped trandolapril a further 6 days after that. Two weeks after discontinuing trandolapril, the subject completely recovered.</i>
Hypokalemia	<i>Subject 51/501 in Study FF/87/570/07 was a 50 year old female with no pertinent medical history. On entry into the study, her serum potassium was 3.3 mM. After 4 weeks in the study, it was 3.2 mM. After 2 months of treatment with trandolapril 4 mg/day, her persistent high diastolic blood pressure (142/100 mmHg supine) led the investigator to add HCTZ (12.5 mg/day) to her treatment regimen. The subject's serum potassium was 3.5 mM at that time. Ten days later, it was 3.1 mM, and it remained unchanged the following week when she was withdrawn from the study. The study protocol prohibited addition of potassium to the subjects' regimen.</i>
Insomnia, slight changes in WBC differential	<i>Subject 82/517 in Study FF/87/570/07 was a 54 year old female with no pertinent medical history. After one month of treatment with trandolapril 4 mg/day and furosemide, the investigator reported a slight drop in neutrophils from 56% (2.2 K/μl) to 46% (1.9 K/μl) and an increase in lymphocytes from 32% (1.3 K/μl) to 41% (1.7 K/μl). This modification persisted throughout the study and, in addition, during the fifth month, the subject complained of insomnia. The investigator decided to stop the study drug.</i>

Lack of efficacy	<i>Subject 417/145 in Study FF/87/570/02 was a 55-year-old female, with a history of hypertension since 1983. After two months on trandolapril, HCTZ was added. A half hour after this first dose, vertigo occurred and persisted until the next day when HCTZ was stopped. Trandolapril was continued. After 3 months in the study, trandolapril was stopped because of lack of efficacy (blood pressure was 170/105 mmHg).</i>
Libido decreased, impotence	<i>Subject 260/776 in Study FF/87/570/08 was a 56 year old male who was reported to have renal stones as a concomitant disease. He had been in Study FF/87/570/05 (trandolapril 2 mg) where he had complained of lethargy. Four days after starting trandolapril 2 mg/day in the long-term trial, the subject experienced palpitations on exertion (intensity: mild). One month later, dizziness on standing up was reported (supine blood pressure was 142/100 mmHg). One week later, he complained of decreased libido and erectile failure (intensity: moderate). Two weeks later, bendroflumethiazide 2.5 mg/day was added to his drug regimen. The treatment was stopped two months later. At that time, the subject had recovered from his dizziness although the sexual problems were reported to have persisted for 3 weeks.</i>
Nausea, vertigo	<i>Subject 417/149 in Study FF/87/570/02 was a 63 year old female with a history of hypertension since 1986. After 2 months of trandolapril, HCTZ was added. Eight days after the start of HCTZ, the subject complained of nausea and vertigo. Two days later, trandolapril and HCTZ were stopped by the subject. The day after, the subject had completely recovered.</i>
Pneumonia	<i>Subject 75/533 in Study FF/87/570/07 was a 65 year old male subject with chronic bronchitis and mild repolarization ECG abnormalities. On entry, his blood glucose was 12.7 mM, and he had no glucosuria. After one month of treatment, blood glucose was 15.0 mM, but the investigator interpreted this result as a consequence of a non-fasting sample. One month later while taking trandolapril 2 mg/day and furosemide, the subject developed an infectious bronchopneumonia, leading to the administration of amoxicillin. His blood glucose was then 14.0 mM (without glucosuria), and he started taking metformin. At the sponsor's request, the subject was withdrawn from the study. No additional information is available.</i>
Sinus tachycardia, palpitations, and precordial pain	<i>Subject 1304/11 in Study FF/87/570/07 was a 56 year old male with a history of kidney stones and suffering from cerebral insufficiency. He was taking acetylsalicylic acid and dipyridamole. On entry into the study, ECG was normal and his heart rate was 75 to 94 bpm. After one month of active treatment, the dose of trandolapril was doubled (to 4 mg/day) as prescribed by the protocol, because of persistent high blood pressure. His heart rate was 98 bpm at this time. One month later, his diastolic blood pressure was still above 95 mmHg. The dose of trandolapril was reduced to 2 mg/day and HCTZ 25 mg/day was started in accordance with the protocol. At the same visit (i.e., after 2 months of treatment with trandolapril), ECG revealed sinus tachycardia with a heart rate of 100 bpm (supine) and 108 bpm (erect). His blood pressure was 157/93 mmHg (supine) and 152/96 mmHg (standing). One month thereafter, with the ECG abnormality persisting, the subject began complaining of palpitations and, two days later, of precordial pain (intensity: severe). As the intensity of the symptoms was "intolerable", the subject decided the next week to stop taking</i>

	<i>trandolapril and HCTZ. Clinical and ECG recovery occurred within 3 days. Two days after his treatment was stopped, his heart rate was 91 bpm (supine) and 96 bpm (standing).</i>
Tiredness, shortness of breath	<i>Subject 161/54 in Study FF/88/570/29 was a 50 year old male who was enrolled in Study FF/87/570/21 (placebo). After six weeks of trandolapril 2 mg/day, amiloride 5 mg/day, and HCTZ 50 mg/day, he developed psoriasiform skin lesions on his back (intensity: moderate), which resolved within 6 weeks, after a transient worsening. A relationship to the test drug was assessed probable by the investigator. Just after recovery, while still taking trandolapril, the subject reported tiredness and shortness of breath on exercise (intensity: mild), ascribed these effects to his diuretics, and refused to go on taking his treatment.</i>
Vomiting, sweating, influenza	<i>Subject 152/136 in Study FF/87/570/02 was a 58-year-old female smoker, with a recent diagnosis of hypertension. After 10 days of trandolapril treatment, she complained of excessive perspiration. One month later, the subject complained of vomiting, but trandolapril was continued. One week later, HCTZ was added. Vomiting and sweating persisted. Twelve days after beginning HCTZ, the subject reported influenza for one week and then recovered. She stopped trandolapril one week later (2.5 months after initiation). Vomiting and sweating disappeared in a few days.</i>

B2.3. Placebo subjects in controlled studies

Nausea and vomiting	<i>Subject 47 in Study FF/86/570/14, a 51 year old Caucasian female with a 1-year history of hypertension, discontinued after 5 days of placebo treatment in the double-blind phase because of nausea and vomiting, described as medium in severity.</i>
Neutropenia	<i>Subject 280/456 in Study FF/87/570/05 was 45 year old male who, while taking placebo, presented with influenza from day -15 to day 7, for which Bactrim was prescribed for one week. Since his neutrophil count (1.7 Kμl) on entry was lower than permitted in the protocol, the subject was withdrawn at the sponsor's request, after one week of active treatment. One week later, his neutrophil count was 2.5 Kμl.</i>

B2.4. Trandolapril plus nifedipine plus furosemide or HCTZ

Cough	<i>Subject 424/148 in Study FF/87/570/22 was a 75 year old female who suffered from type II diabetes, hypercholesterolemia, and heart failure. In addition, her ECG showed signs of ischemia. She was treated with glibenclamide and digoxin. After 6 weeks of treatment with trandolapril 4 mg/day, and 2 weeks after nifedipine (slow-release 40 mg) and HCTZ 2.5 mg were added to her drug regimen, she experienced cough (intensity: mild). This symptom persisted during trandolapril treatment for 4 months. After the investigator withdrew the test drug, the subject recovered within 2 weeks.</i>
Myocardial infarction	<i>Subject 201/167 in Study FF/88/570/33 was a 47 year old obese male. Two months after entry into the long-term study, persistent high blood pressure led the investigator to add nifedipine to his treatment regimen. Two months later, hydrochlorothiazide was added because of high diastolic blood pressure. The next month, he had a myocardial infarction (intensity: severe) and was hospitalized. The test drug was stopped and cardiac surgery was recommended. Throughout the study ECGs had been judged normal.</i>

Renal artery lesion

Subject 42/284 in Study FF/87/570/22 was a 47 year old male. After 2 months on trandolapril 4 mg/day, persistent high blood pressure (180/110 mmHg) resulted in the addition of nifedipine to treatment; three months later, furosemide was also added. At the beginning of the eighth month, while his blood pressure was still high (170/96 mmHg supine), an abdominal murmur was heard on auscultation which led to the discovery of an atheromatous lesion of the right renal artery. Renal function remained normal. Trandolapril was discontinued, and the subject was prescribed labetalol while awaiting for surgical treatment.

B2.5. Trandolapril plus nifedipine or nitrendipine**Cough**

Subject 1304/2 in Study was a 59 year old female, with no pertinent medical history. She presented with a dry cough (intensity: moderate) after 3 months of trandolapril 4 mg/day and nitrendipine 20 mg/day. One month later, since the subject's blood pressure remained elevated (188/114 mmHg supine), the investigator decided to stop the test drug. Within 3 days, the subject recovered from her cough.

Cough, liver enzyme elevation

Subject 42/286 in Study FF/87/570/22 was a 51 year old male. After 3 months of treatment with trandolapril 2 mg/day, persistent high diastolic blood pressure (94 mmHg supine) led to the addition of nifedipine to the subject's treatment. Two weeks earlier, he had begun to experience a daytime cough (intensity: severe). Symptomatic treatment with triprolidine, pseudoephedrine, and paracetamol was ineffective, and the subject was withdrawn after one month. He recovered within 2 weeks. Laboratory tests on withdrawal showed raised LDH (643 IU/L; normal is <480) and GGT (280 IU/L; normal is <43); these values normalized one month after he stopped treatment.

Hot flushes

Subject 201/161 in Study FF/88/570/33 was a 60 year old female who had been in Study FF/87/570/04 (trandolapril 2 mg plus HCTZ). After initiating trandolapril 2 mg/day, she complained of intermittent, productive cough (intensity: moderate). This cough persisted throughout the trial, and its relationship to trandolapril was judged remote by the investigator. On week 12 of the long-term study, persistent high blood pressure (170/110 mmHg supine) led the investigator to add nifedipine. One month later, the subject experienced hot flushes (intensity: moderate). The investigator decided to withdraw the subject from the trial.

Palpitations

Subject 112/184 in Study FF/87/570/06 was a 52-year-old male who had been hypertensive since May 1986. Trandolapril and nifedipine were started. Supine blood pressure was 150/100 mmHg and ECG was normal. After 3 months of treatment, the subject complained of palpitations. Two days later, he was withdrawn from the study; however, the palpitations did not disappear. No other information is available.

Peptic ulcer

Subject 201/182 in Study FF/88/570/33 was a 49 year old male who had been in Study FF/87/570/04 (trandolapril 2 mg plus HCTZ). After 3 weeks on trandolapril 2 mg/day, he complained of headaches (intensity: moderate) for one day, and a causal relationship to the drug was judged remote. Three weeks later, he suffered for 5 days from mild bronchitis, the causal relationship of which was judged unevaluable. During the fourth month, nifedipine was added to the subject's drug regimen. His blood pressure at that time was 150/100 mmHg (supine) and 150/95 mmHg (standing). One-and-a-

half months later, the subject experienced backache for one week (intensity: moderate), and a causal relationship to the drug was judged unevaluable. He was prescribed naproxen. Five days later, gastroscopy identified a peptic ulcer. Naproxen and nifedipine were stopped.

Urinary tract infection with renal impairment

Subject 701/341 in Study FF/87/570/06 was a 55 year old male, with a history of a congenital abnormality of the left kidney (prominent Bertin's column). Two weeks before inclusion serum creatinine was within normal range. Trandolapril 2 mg and nifedipine SR 40 mg daily were started. Eighteen days after treatment was started, the subject experienced fever (40°C) and dysuria and was admitted to hospital. Clinical examination disclosed no flank pain but albumin and red blood cells were present in urine. Serum creatinine level was high (1.5 mg/dl). Mezlocillin and oxacillin were started. After 25 days of treatment, trandolapril and nifedipine were interrupted, since his blood pressure was 95/60 mmHg. Twelve days after discontinuation of study treatment, his blood pressure was 175/95 mmHg and trandolapril plus nifedipine were restarted. Five days after this restart, an isotope nephrogram was performed which showed decreased renal function on both sides and an obstruction in the pelvi-ureteric region. Eleven days after the treatment was restarted, an intravenous pyelogram was performed, which showed no abnormalities. Trandolapril and nifedipine were stopped on the advise of the sponsor. Twenty days after trandolapril and nifedipine were discontinued, the creatinine level decreased to normal range.

Uterine fibroid surgery

Subject 120/361 in Study FF/87/570/22 was a 46 year old female with a history of nephrectomy. After taking trandolapril 4 mg/day and nifedipine for 9 months, she was withdrawn from the study to have uterine fibroid surgery.

B2.6. Other active drugs (atenolol or HCTZ)

Headache

Subject 51 in Study FF/87/570/04, a 52 year old Caucasian male with peptic ulcer disease and a 10-year history of hypertension, discontinued with headache after 77 days of treatment with HCTZ 25 mg/day. Event described as "moderate" severity, but resulted in hospitalization.

Headache, nervousness

Subject 264 in Study FF/87/570/04, a 22 year old Caucasian male with a 2-year history of hypertension, discontinued with new onset of headache and nervousness after 58 days of treatment with HCTZ 25 mg/day. The subject was lost to follow-up.

Hot flushes

Subject 70 in Study FF/87/570/04, a 54 year old Caucasian female with a 35-year history of hypertension, discontinued on day 21 of treatment with HCTZ 25 mg/day because of hot flushes that initially began during placebo withdrawal. The event attributed to menopause. The subject withdrew for medical treatment and the events resolved in 7 weeks.

Liver enzyme elevation

Subject 266/695 in Study FF/87/570/08 was a 63 year old female previously enrolled in Study FF/87/570/05 (atenolol). The laboratory tests performed on the day before her first intake of trandolapril showed elevated liver enzymes (ASAT 2.2x ULN; ALAT 1.4 x ULN), and she was withdrawn by the investigator.

B2.7. Deaths in European hypertension studies¹⁶

Cardio-respiratory arrest

Subject 35 in Study FF/89/570/44 was an 83 year old female with a history of hypertension and dementia, both diagnosed in 1979. The

subject presented with symptoms of intestinal obstruction (not specified), which resolved with nasogastric aspiration and enemas. Trandolapril was interrupted for 3 days. Eight weeks later, the subject again experienced symptoms of intestinal obstruction, and the subject was again treated with nasogastric aspiration and enema. Surgical procedure was not undertaken. Trandolapril was discontinued on this day. Two weeks later, the subject died from respiratory and cardiac arrest. In the investigator's opinion, the subject's death was not related to trandolapril.

**Cardio-respiratory
arrest**

Subject 0122 in Study FF/89/570/51 was a 50-year old female with hypertension diagnosed in 1985 and left ventricular hypertrophy of unknown duration. Three months after starting trandolapril 4 mg/day, the investigator reported that the subject, while at home, suffered a cardio-respiratory arrest. She was transferred to an Intensive Care Unit. Her cardiac status improved but she remained comatose. Trandolapril was discontinued. Four days later, the subject died despite intensive support (mechanical ventilation and hemodynamic support). The investigator believes that the cardio-respiratory arrest and coma were probably related to a stroke resulting from hypertension and not likely related to trandolapril.

**Cardio-respiratory
arrest**

Subject 125 in Study FFR/92/570/167 was a 68 year old overweight male with a history of hypertension, non-insulin-dependent diabetes mellitus, and hypertriglyceridemia, tobacco abuse and probable alcohol abuse (but denied by the subject himself). Nineteen days after randomization to unknown treatment, he had to be hospitalized for a lipothymic malaise with bradycardia (30 bpm) and normal blood pressure (140/90 mmHg). No etiology could be found at the origin of this malaise. There was no recurrence during the hospitalization. The subject also presented with epigastralgia. Gastroscopy was performed and disclosed esophageal varicosis, atrophic and purpuric gastritis, and corrosive duodenitis. The subject was given sucralfate and chlorpropamide, which led to improvement. The subject had also presented with obtundation and headache. CT scan showed no particular finding. During hospitalization, the subject presented with an episode of confusion with agitation, probably related to delirium tremens which recovered with rehydration, vitamin therapy and sedation. The subject was transiently treated with insulin because of worsening of his diabetes. He also presented with a biological cytolysis and cholestasis associated with an hyperamylasemia. No cause was found out and outcome was spontaneous recovery. The subject had a moderate hypoxia, pulmonary rales at both bases with radiological opacities at the right bases, and an inflammatory bronchial mucosa observed at the fibroscopy. He presented with a biological inflammatory syndrome. One month after randomization, at the scheduled end of the study, the study drug was discontinued. The clinical course at the hospital was marked by a general clinical improvement and the persistence of hypoxia and inflammatory syndrome. One month later, the subject experienced a sudden cardio-respiratory arrest. A resuscitation attempt was unsuccessful and final outcome was death. No autopsy was performed.

**Carcinoma,
bronchial**

Subject 72/416 in study FF/87/570/07 was a 56 year old male subject with angina pectoris, osteoarthritis, a lipid disorder and a

¹⁶. Full study reports not included in NDA: Studies FF/87/570/03, FF/87/570/05, FF/87/570/07, FF/87/570/08, FF/87/570/29, FF/89/570/44, FF/89/570/49, FF/89/570/51, FF/89/570/59, FFR/91/570/165, FFR/91/570/166, FFR/92/570/167, and UK/89/570/05.

	history of heavy smoking. His concomitant treatment was: pentaerythrityl tetranitrate, fenofibrate, paracetamol and vitamin C. One month before inclusion, the subject presented with persistent cough and hemoptysis; X-ray revealed an opacity of the upper left lobe. As a result of this radiological finding, bronchoscopy was undertaken 2 months later (the subject had been on trandolapril 2 mg/day for about 3 weeks) and showed a tumor with bronchial obstruction. Spinocellular carcinoma was confirmed by biopsy. The subject died unexpectedly at home a few days later.
Carcinoma, bronchial	Subject 269/469 in study FF/87/570/05 was a 54 year old male who smoked. A chest X-ray performed at inclusion was normal, but his WBC count was high (13.8). One week before starting active treatment, while still taking placebo, the subject had a cold with occasional headaches (intensity: mild), for which paracetamol was prescribed. Recovery occurred within 10 days. At the end of the first month of active treatment, an increased leucocytosis was reported (WBC: 28.3). One week later, the subject experienced nausea and anorexia (intensity: moderate), and a weight loss (-6.5 kg) was noted. He was admitted to hospital for investigation and carcinoma of the bronchus was diagnosed. The investigator decided to discontinue the test drug. He died within 5 months and there was no postmortem examination.
Carcinoma, bronchial	Subject 15080 in Study FFR/91/570/166 was a 72 year old man with a history of hypertension (unspecified beginning date), and non-insulin-dependent diabetes mellitus treated with gliclazide. After one month on trandolapril 2 mg/day, he had to be hospitalized because of aggravation of his diabetes, bronchial infection, and mental status alteration. Bronchial carcinoma was diagnosed and led to death within 3 weeks. In the investigator's opinion, these events were unrelated to study drug.
Carcinoma, lung	Subject 2361 in Study FFR/91/570/166 was a 73 year old non-smoking female with no previous relevant history. The subject, experiencing asthenia, was started on trandolapril 2 mg/day. Fifteen days later, blood pressure was 160/80 mmHg, but asthenia persisted and was associated with anorexia, weight loss (5 to 6 kg within 15 days) and cough. The subject was admitted to hospital and carcinoma of lung was diagnosed. One month after beginning treatment, the subject died from her carcinoma. In the investigator's opinion, the carcinoma is unrelated to study drug.
Carcinoma, pancreatic	Subject 434 in Study UK/90/570/01 (Local) and Study FF/89/570/44 (International) was an 80 year old female with a medical history of hypertension. The subject started at 1 mg/day. One month later, the dose was increased to 2 mg. After 5 months of treatment, the subject experienced moderate vomiting, which resolved spontaneously over 4 days. Three weeks later, the subject began to complain of severe abdominal pain and trandolapril was withdrawn, after approximately 6 months of treatment. The subject was admitted to hospital and had a laparotomy at which time the subject was diagnosed as having inoperable carcinoma of the pancreas. The subject was discharged from hospital with dexamethasone 1 mg, cyclizine 50 mg and morphine sulphate 10 mg. The subject died from carcinoma of the pancreas, 7 months after starting trandolapril. In the investigator's opinion pancreatic carcinoma is unrelated to trandolapril.
Cerebrovascular accident	Subject 4937 in Study FFR/91/570/166 was a 63 year old man. Six weeks after starting on trandolapril 2 mg/day, the subject has a

	cardiac arrest. Reactive coma was diagnosed, with intrabronchial aspiration of gastric juice and status epilepticus. The subject was intubated and mechanically ventilated. The ECG showed no abnormality. The subject was admitted to Intensive Care. A brain scan was performed and showed no abnormality. Convulsions persisted despite intravenous antiepileptic therapy. Final diagnosis was deep cerebrovascular accident probably at truncus cerebri level and the subject died 2 months later.
Cerebrovascular accident	Subject 11296 in Study FFR/91/570/166 was a 78 year old man with a previous history of hypertension who was began trandolapril 2 mg daily and died, apparently of a cerebrovascular accident, sometime in the next 30 days. No autopsy was performed.
Cerebrovascular accident	Subject 7409 in Study FFR/91/570/166 was a 72 year old man with a history of recently-diagnosed hypertension and a past history of cerebral hemorrhage. He began on trandolapril 2 mg/day, and 30 days later, since hypertension was not well controlled (185/110 mmHg), furosemide 40 mg/day was added. Two weeks later, the subject was hospitalized because of cerebral ischemia secondary to embolism from cardiac origin (atrial fibrillation). This ischemia was in the same cerebral area as the previous one. Trandolapril was discontinued. After transient improvement, the subject died one month later. In the investigator opinion, cerebral hemorrhage was unrelated to study drug.
Coronary arterial thrombosis	Subject 257/766 in study FF/87/570/08 was a 44 year old male with sinus tachycardia and sebaceous cysts on the face. He was also a smoker. Prior to the long-term study, he was in Study FF/87/570/05 (trandolapril 2 mg), where he presented a red lumpy rash on the face, which was assessed as probably related to the test drug by the investigator. Two months after his entry into the long-term study, and while taking trandolapril 2 mg/day, he died from a coronary arterial thrombosis. He had been on trandolapril for 6 months.
Coronary artery bypass graft and mitral prosthesis, post-operative.	Subject 1702 in Study FFR/91/570/166 was an 81 year old female with a 10-year history of hypertension (unspecified duration), and transient ischemic attack in 1991. Two months after starting trandolapril 2 mg/day, she was hospitalized because of hypotension and tachycardia (unspecified values of blood pressure and heart rate). During hospitalization, ECG disclosed lateral myocardial necrosis without Q wave (it appeared that myocardial infarction was present on admission). Mitral insufficiency was diagnosed. The subject presented with signs of biventricular heart failure. Outcome was favorable with diuretics, vasodilators and anticoagulants. Trandolapril was apparently discontinued. The subject was discharged with a treatment including furosemide, amiodarone, nitroglycerin patch, lisinopril, enoxaparin, omeprazole, alginic acid, and sodium (given for esophageal ulcer diagnosed during hospitalization). Three days later, she had to be hospitalized again because of a malaise and aggravation of heart failure. One month later, she underwent cardiac surgery for CABG and mitral valve replacement. The investigator believes that the myocardial infarction was unlikely to be related to study drug.
Cor pulmonale (pulmonary embolism)	Subject 98 in Study FFR/89/570/59 was a 77 year old female with a history of hypertension since 1980, treated previously with methyl dopa. Medical history included myocardial infarction in 1983, angina pectoris treated with isosorbide dinitrate and diltiazem, non-insulin-dependent diabetes since 1984 treated with diet, glibenclamide and metformin, hyperlipidemia since 1987

treated with fenofibrate. The baseline ECG was normal. The baseline chest x-ray was normal with cardiothoracic ratio 0.48. Two months after starting trandolapril 1 mg daily, her supine blood pressure was 140/88 mmHg and standing blood pressure was 140/86 mmHg. Fasting plasma glucose concentration was 8.4 mM and post prandial plasma glucose concentration was 15.3 mM. Urinalysis showed 2+-glucosuria. ECG was normal. That evening, the subject suffered from severe epigastric pain and sudden exertional dyspnea. She was hospitalized. ECG showed subepicardial ischemia in anterior leads. The enzymes were not elevated. The next day, trandolapril was discontinued and replaced by ramipril. Dyspnea and epigastric pain disappeared. Four days later, she was discharged home. The next day, she again suffered from dyspnea and epigastric and right hypochondrium pain. She was readmitted to the hospital. Blood pressure was 120/70 mmHg and she was afebrile. Physical exam showed tachycardia, normal cardiopulmonary auscultation. ECG was unchanged. Chest x-ray showed cardiac enlargement. Arterial blood gases showed hypocapnia, hypoxemia, and lactic acidosis. Laboratory data showed hyperglycemia, renal failure and increased transaminases (with SGOT value up to 2000). Cardiac echo showed acute cor pulmonale, with dilatation of right cavities and increased pulmonary arterial pressure. Ascending contrast phlebography showed signs of thrombophlebitis. The diagnosis of acute cor pulmonale secondary to pulmonary embolism was made. When the umbrella was introduced, blood clots migrated and the subject died a few hours later. In the investigator's opinion, this was unlikely to be related to trandolapril.

Heart failure (right-sided), cardiorespiratory decompensation

Subject 9141 in Study FFR/91/570/166 was a female subject of unspecified age and race with a previous history of hypertension, chronic pulmonary heart disease secondary to pulmonary embolism in 1989 (treated with furosemide and trinitrine since 1990), and coronary disease treated with trimetaxidine since 1992. At an unspecified date, trandolapril 2 mg/day was started. On February 20, 1993, she was hospitalized for aggravation of right heart failure. Right catheterization showed a worsening of both preexisting pulmonary hypertension and right ventricular failure. Lung fibroscopy showed pulmonary edema. The dosage of furosemide was doubled and digoxin was added. Other drugs including trandolapril were continued. The subject transiently and partially improved for a few days but her cardiac status then worsened again. She was said to be in irreversible terminal cardiac failure. Two months later, a cardiorespiratory decompensation occurred which led to death the following day. No autopsy was performed. In the investigator's opinion, neither the attack of right-sided heart failure nor the cardiorespiratory decompensation was related to trandolapril.

Leiomyosarcoma

Subject 203/171 in study FF/87/570/07 was a 43 year old female with no pertinent medical history. On entry, she had an elevated erythrocyte sedimentation rate (26 mm/47 mm). After 2 months of treatment with trandolapril 4 mg/day, laboratory tests revealed a low hemoglobin (10 g/dl) and a persistent high sedimentation rate (41 mm/45 mm). One month later, the subject suffered severe headaches and abdominal pain. On gynecological examination, a large pelvic mass was discovered; laparotomy identified a tumor of the terminal ileum, which proved to be a leiomyosarcoma on histological examination. The subject did not recover after surgery.

	<i>and became comatose. A scan performed at this time showed several brain metastases. The subject died within 3 weeks.</i>
Motor vehicle accident	<i>Subject 7 in Study FF/87/570/29 was a 47 year old male with a history of hypercholesterolemia and a 2-year history of hypertension not previously treated. One month after starting trandolapril 2 mg, he continued in a long-term study. ECG was normal. Chest X-ray showed increased perihilar markings. Laboratory data showed high transaminases (1.8x ULN) and GGT (2.5x ULN). Transaminases reverted to normal range, but GGT persisted high throughout the study. Five months later, the subject died in a motor vehicle accident.</i>
Myocardial infarction	<i>Subject 8972 in Study FFR/91/570/166 was a 77 year old man. One month after starting trandolapril 2 mg/day, HCTZ was added because of continued high blood pressure (190/90 mmHg). One month later, he had to be hospitalized for an anteroseptal and lateral myocardial infarction (onset of 1 chest pain after climbing 1 floor). He had several slight constricting chest pains while walking during the previous weeks. Two weeks later, the subject was discharged with acebutolol, nitroglycerin patch, and aspirin. One month after discharge, he experienced recurrence of myocardial infarction with circumferential necrosis which led to death 3 days later. It is not known whether an autopsy was performed or not. In the investigator's opinion, myocardial infarctions were unlikely to be related to study drug.</i>
Myocardial infarction	<i>Subject 72 in study UK/89/570/05 was an overweight 69 year old man who upon entry into the placebo pre-selection period exhibited left ventricular hypertrophy with some unfolding of the aorta on chest X-Ray. ECG was normal. Trandolapril 1 mg daily was started but after 33 days of treatment, he suddenly collapsed and was dead on arrival at the hospital. Post mortem examination confirmed the cause of death as acute myocardial infarction.</i>
Myocardial infarction	<i>Subject 257/766 in study FF/87/570/09 was a 44 year old smoking male who suffered a fatal myocardial infarction after 174 days of treatment with trandolapril. No additional information is available.</i>
Myocarditis	<i>Subject 362 in Study UK/90/570/01 (FF/89/570/44) was an 80 year old female with a history of hypertension (unspecified beginning date), carcinoma of left ovary, and depression. Two months after starting trandolapril 1 mg/day, the subject was found dead. Postmortem examination was performed and revealed purulent material in the pericardial sac, epicardial congestion and hemorrhage as well as myocardial changes consistent with myocarditis which was assessed by the pathologist consultant as the cause of the death. In the investigator's opinion, death is unrelated to the study drug.</i>
Pancreatitis, necrotizing	<i>Subject 135/291 in study FF/87/570/03 was a 65-year-old overweight female, with a recent diagnosis of hypertension. She was not an alcoholic and had never suffered from biliary colic. Three months before entering the study, she complained of mild epigastric pain and upper gastrointestinal endoscopy revealed esophagitis. Twenty-two days after trandolapril 2 mg daily was started, after an especially fatty meal, the subject complained of severe abdominal pain during the night, especially in the right upper quadrant, with vomiting and nausea. The next morning, the subject was hospitalized and trandolapril was stopped. Cholangiography revealed a mechanical obstruction of the pancreatic duct resulting</i>

	from migration of the gallstones. Laboratory data showed an elevated serum amylase level and hyperleucocytosis. Two days after withdrawal of trandolapril, the clinical status worsened. Laparotomy showed a complete necrotizing pancreas with retroperitoneal and peritoneal necrotic zones. A cholecystectomy was performed and peritoneal drainage was started. Gallstones were found in the gallbladder. The subject died 4 days after cessation of trandolapril. No autopsy was performed. The investigator did not believe the event was related to trandolapril.
Pancreatitis (hemorrhagic, acute)	Subject 484 in Study FF/89/570/49 was a 51 year old non-alcoholic female with a history of hypertension since 1984 previously treated with methyldopa (stopped because of nausea). Four months after starting trandolapril 4 mg daily, HCTZ 25 mg daily was added because of uncontrolled blood pressure. Three months later, the subject complained of abdominal pain, nausea, vomiting and dizziness. The subject was hospitalized and trandolapril and HCTZ were stopped. Two days later, laboratory data showed metabolic acidosis, pre-renal azotemia, hyperglycemia, hyperamylasemia and hyperlipasemia: pH = 7.35, PCO₂ 84 mmHg, bicarbonate 16.8 mM, amylase 707 (normal is <1 UI), lipase 1462 (normal is <150), and LDH 5086 (normal is <240). Two days later, the subject was placed on assisted ventilation and she was treated with gentamicin and ampicillin for ascendant cholangitis. A biliary tree and pancreatic USG were done and showed a hemorrhagic acute pancreatitis. Neither pancreatic abscess nor biliary stone was found. One week after admission, the subject's metabolic condition deteriorated rapidly with a worsening of metabolic acidosis and she died. In the Investigator's opinion, acute pancreatitis was unlikely to be related to trandolapril, but he suspected HCTZ or group IV hyperlipidemia.
Parathyroid adenoma, coronary arterial disease	Subject 271/607 in Study FF/87/570/08 was a 65 year old male with no special medical history. One-and-one-half months after taking trandolapril 2 mg/day and bendroflumethiazide 2.5 mg/day in the long-term study, the subject complained of nausea and tiredness with poor concentration (intensity: severe for all these symptoms). Serum calcium was 2.89 mM. One month later, still symptomatic, he stopped trandolapril. He was referred to a hospital where his serum calcium was 5.38 mM. He was treated with prednisolone, suffered cardiac arrest but was successfully resuscitated. Three days later, he underwent surgery where a parathyroid adenoma was found and removed. Following the operation however, he developed renal failure and required dialysis. He died 2 days after surgery. Post mortem examination revealed a severe underlying coronary artery disease, without evidence of acute thrombosis.
Pulmonary embolism	Subject 18328 in Study FFR/91/570/166 was a 70 year old female with a history of hypertension and no past digestive history. Six weeks after starting trandolapril 2 mg daily, the subject suddenly complained of nausea, vomiting, slow bowel movements. She was diagnosed with ascites related to peritoneal carcinomatosis with hepatic and pleural metastases and colic linitis, secondary to a primitive gastric carcinoma. Trandolapril was discontinued. She was treated only with symptomatic treatments (no chemotherapy) during hospitalization and no surgery was performed. Four months later, the subject died apparently of pulmonary embolism
Pulmonary embolism	Subject 6223 in Study FFR/91/570/166, of unspecified sex and age with unknown past medical history apart from hypertension, was enrolled on an unspecified date and treated with trandolapril. At an

	<i>unspecified date during the course of the study, the subject died of a pulmonary embolism. It is not known whether an autopsy was performed. The investigator's opinion on causality is not yet known.</i>
Pulmonary embolism	<i>Subject 139 in Study FF/89/570/49 was a 71-year-old male with a history of hypertension since March 1989, osteosynthesis of left hip in 1985, and lower limb polyneuropathy. She previously completed Study FF/87/570/22. Seven months after starting open-label -trandolapril 2 mg daily, the subject presented with a hip fracture for which a hip prosthesis was inserted. Two weeks later, trandolapril was discontinued. One week later, the subject developed a pulmonary embolism and died. In the investigator's opinion, both the hip fracture and the pulmonary embolism were unlikely to be related to trandolapril.</i>
Pulmonary embolism (fracture of femoral neck)	<i>Subject 1544 in Study FFR/91/570/165 was a 65-year-old female. Two weeks after starting on unspecified dose of trandolapril, the subject was hospitalized because of a fracture of the femoral neck (the circumstances of the accident are not specified) leading to a surgical treatment. Trandolapril was discontinued on the same day. Following surgical treatment, a lower-limb thrombophlebitis developed (unspecified date) leading to pulmonary embolism and to death (unspecified date). The investigator believes that the fall leading to femoral neck fracture, and the thrombophlebitis and pulmonary embolism leading to death were unrelated to trandolapril.</i>
Septic shock, arterial cutaneous ulcer	<i>Subject 9 in Study FF/89/570/44 was a 75 year old female with a 16-year history of hypertension, dyslipidemia, and narrow lumbar canal diagnosed in 1984. Baseline blood glucose was 6.1 mM, other laboratory data were normal. ECG showed left posterior hemiblock. One month after starting trandolapril 1g/day, her blood pressure was 150/90 mmHg and blood glucose was 6.7 mM. Other laboratory data were normal. Two months later, her blood glucose was 5.7 mM. Another 2 months later, the subject presented with hyperglycemia (11 mM), hyponatremia (128 mEq/L), dehydration, fever, and severe pneumopathy. Acute ischemia occurred in the leg. Trandolapril was stopped. Pneumopathy was successfully treated with amoxicillin and clavulanic acid and resolved. Hyperglycemia required insulin. Leg ischemia was treated with naftidrofuryl. The subject subsequently experienced arterial cutaneous ulcers resulting in septicemia, and she died 2 months later. In the investigator's opinion the relationship between acute leg ischemia and the diabetes to the study drug is uninterpretable.</i>
Septic shock, pneumonia	<i>Subject 4 in Study FF/89/570/44 was a 74 year old male with a medical history of hypertension since 1967, treated previously with enalapril, and memory troubles since 1986. Ten days after starting trandolapril 1 mg/day, the subject became febrile, asthenic, anorectic. Cough with expectoration was present. Bronchi rales were heard over both lungs. Trandolapril was discontinued. Laboratory data showed leukocytosis and dehydration. Amoxicillin and clavulanic acid were started and associated with rehydration. Three days later, septic shock occurred leading rapidly to death. No postmortem examination was done. In the investigator's opinion, pneumonia was unrelated to trandolapril.</i>
Septic shock, pneumonia	<i>Subject 2³ in Study FF/89/570/44 was a 91 year old female, with a history of hypertension (beginning date unknown) and disorientation. Eleven months after starting trandolapril 2 mg/day, the subject presented with severe pneumopathy. Treatment with</i>

Septic shock, pneumonia, and cerebrovascular accident	<i>amoxicillin and clavulanic acid was started (1 g/day) and the study drug was discontinued. Septic shock occurred, and the subject died. In the investigator's opinion the relationship between pneumopathy and study drug is unlikely.</i>
Septic shock, pneumopathy, laryngeal carcinoma	<i>Subject 14 in Study FF/89/570/44 was a 84-year-old female with a medical history of hypertension since an unspecified date and mood disturbance since 1985. Two months after starting trandolapril 1 mg/day, blood pressure was not well controlled (170/90 mmHg), so trandolapril was reduced to 1 mg and furosemide 20 mg was added. Subsequently, blood pressure was well-controlled (140/80 mmHg). One year after starting, the subject presented with severe pneumonia. Amoxicillin and clavulanic acid 2 g per day was commenced. The following day, left hemiplegia and consciousness troubles occurred. Blood pressure was 145/80 mmHg. Trandolapril was discontinued, and nifedipine and digoxin were prescribed (unspecified reason). Despite antibiotic therapy, the pneumonia worsened and, 4 days later, the subject died secondary to septic shock. No post mortem examination was done. In the investigator's opinion, the pneumonia and the cerebrovascular accident were unrelated to trandolapril.</i>
Suicide	<i>Subject 15898 in Study FFR/91/570/166 was a 73 year old male with unspecified past medical history apart from hypertension. Trandolapril 2 mg/day was started. Further examination disclosed a laryngeal carcinoma with a quick extension toward vascular axes, making surgical removal impossible. Three weeks later, chemotherapy was started. The subject was also treated for left ventricular insufficiency. He next developed pneumopathy and consequent septic shock, leading to death two weeks later. No autopsy was performed. In the investigator's opinion, these events were unrelated to study drug.</i>
Superinfection, exacerbation of chronic bronchitis	<i>Subject 15531 in Study FFR/91/570/166 was a 66 year old male with a history of hypertension, alcoholism, and depression. One month after starting trandolapril 2 mg/day, he committed suicide with a firearm. Death was instantaneous and no autopsy was performed. In the investigator's opinion, death by suicide is unlikely to be related to study drug.</i>
Unknown	<i>Subject 11272 in Study FFR/91/570/166 was an 84 year old overweight male with a history of hypertension (unspecified beginning date) and chronic bronchitis. At an unspecified date, trandolapril 2 mg/day was started. The subject subsequently had an accidental fall leading to a fracture of femoral neck and was admitted to hospital and trandolapril was discontinued. Just after the operation, he presented with a voluminous decubitus ulcer in the sacral region with consequent superinfection. At the same time, he suffered from acute exacerbation of his chronic bronchitis. These events led to death. In the investigator's opinion, these events were unrelated to study drug.</i>
	<i>Subject 10398 in Study FFR/91/570/166 was a 77 year old female with a previous history of hypertension, diabetes mellitus (treated with metformin), lower limb arteritis, lower limb phlebitis, cardiac insufficiency and atrial fibrillation (treated with furosemide 40 mg/day and digoxin 0.25 mg/day), several cerebrovascular accidents (unspecified number and dates), from which he had completely recovered within 7 days except for the last episode which occurred prior to inclusion and led to permanent hemiplegia and aphasia. Trandolapril 2 mg/day was started. Three weeks later, the</i>

- subject suddenly died during evening trial. No autopsy was performed. In the investigator's opinion, the death was probably related to a pulmonary embolism.
- Unknown** Subject 96 in Study FF/89/570/49 was a 67 year old female with a medical history of hypertension diagnosed in 1984, venous insufficiency diagnosed in 1976, and a smoking habit (10 cigars daily during 30 years). The subject completed Study FF/87/570/07 on trandolapril 2 to 4 mg. Trandolapril 4 mg daily was continued for 13 months, and was well tolerated and blood pressure was normalized. At that time, the subject complained of asthenia and headache which improved with dextropropoxyphene. The next day, the subject was found dead in the afternoon on the toilet. In the investigator's opinion, the casual relationship between subject's death and trandolapril is not known.
- Unknown, angioedema** Subject 152 in Study FF/87/570/07, a 71 year old male with a 10-year history of hypertension, received trandolapril 2 mg/day. An electrocardiogram, performed at baseline, showed left ventricular hypertrophy and negative T waves. During trandolapril administration, 3 episodes of moderate facial swelling occurred over a 3-week period, each lasting about 24 hours. The subject complained of coryza from over the same period. Astemizole was given to the subject for urticaria. As scheduled in this study, the daily dose was increased to 4 mg and blood pressure was 160/80 mmHg. Laboratory's test performed on week 4, 8 and 12 of the study were normal and electrocardiograms remained unchanged on weeks 4 and 8. One month after the last visit, the subject died at after working in his garden. The general practitioner certified "Cause of death, myocardial infarction". No postmortem examination was carried out.
- Unknown** Subject 17913 in Study FFR/91/570/166 was a 74 year old male with a history of hypertension (unspecified beginning date) and lower limb arteritis (thigh amputation). Two months after starting trandolapril 2 mg/day, he had to be hospitalized because of malaise with stupor and confusion. Hypokalemia was then disclosed (unspecified value) and assessed as laxative abuse. It is not known if trandolapril was discontinued. The subject died 15 days later (unspecified reason and circumstances).
- Unknown** Subject 18152 in Study FFR/91/570/166 was an 82 year old female with a history of hypertension (long standing), several pulmonary emboli (anticoagulant therapy since 1980), and non-insulin-dependent diabetes mellitus treated with carbutamide since 1970. One week after starting trandolapril 1 mg/day, while attending a burial service, the subject suddenly died in front of her family. No resuscitation was attempted by the emergency service and no autopsy was performed. In the investigator's opinion, given her age, death is possibly related to study drug.
- Unknown** Subject 10/407 in Study FF/87/570/07 was a 51 year old male who had been taking trandolapril 4 mg/day for 13 months. ECG had revealed evidence of minor repolarization abnormalities for 5 years but without symptoms. On entry into the trial, cardiothoracic ratio was 0.41. After two months of active treatment, the daily dose of trandolapril was doubled because of persistent high blood pressure (165/95 mmHg). One morning, he died suddenly at home, having had no warning symptoms. No postmortem examination was performed.

Unknown	<i>Subject 68/441 in Study FF/87/570/07 was a 51 year old male with asymptomatic repolarization ECG abnormalities. "Vertigo" was reported as a concurrent condition. After more than 8 months of treatment with trandolapril 2 mg/day, during which time his blood pressure was normalized, the subject died while traveling abroad.</i>
Unknown	<i>Subject 278/135 in study FF/87/570/07 was a 63 year old male with a past history of amputation of the right leg (in 1945; cause - unspecified), smoking, and chronic obstructive airways disease. On inclusion, an ECG was said to be normal by the investigator and laboratory data were within the normal range. Four weeks after beginning trandolapril 2 mg/day, the subject experienced a pain (unspecified) while at home. He died before reaching the hospital with a suspected diagnosis of myocardial infarction. No postmortem examination was performed.</i>
Unknown	<i>Subject 428/381 in study FF/87/570/07 was a 64 year old female with no pertinent medical history. At inclusion, her ECG was judged normal and a chest X-ray revealed no abnormality; the cardiothoracic ratio was 0.48. After 5 weeks of trandolapril 2 mg/day, the daily dose was doubled. One-and-a-half months thereafter, the subject experienced severe epigastric pain during the night. Upon admission to a hospital, CPK was normal and the ECG did not suggest myocardial infarction; however palpation of the precordium induced moderate pain. After intake of nitrates by spray and by infusion, the pain resolved. However, the same day, the subject died. No postmortem examination was performed.</i>
Unknown	<i>Subject 156/279 in Study FF/87/570/08 was a 49 year old male subject with a history of myocardial infarction 10 years previously. He had first been in Study FF/87/570/04 (HCTZ) and was then entered into the long-term trial. Ten months after trandolapril 2 mg/day was started, he was found dead while watching television. Resuscitation was unsuccessful. No post mortem examination was performed.</i>
Ventricular fibrillation, myocardial infarction	<i>Subject 16020 in Study FFR/91/570/166 was a 65 year old man with a history of hypertension and tobacco abuse. Two weeks after starting an unspecified dose of trandolapril, he was hospitalized for acute myocardial infarction. The following night, the subject experienced ventricular fibrillation and died. It is not known whether an autopsy was performed. In the investigator's opinion, myocardial infarction and consequent death are unrelated to study drug.</i>

C. Review of clinical literature

A Medline search was conducted for clinical trials involving trandolapril. Where a copy of the reference was available, the reviewer's comments are noted by a hanging indent in italic font. In most other cases, the abstract is reproduced.

C1. Clinical trials in hypertension

Backhouse CI, Orofiamma B, and Pauly NC. Long-term therapy with trandolapril, a new nonsulphydryl ACE inhibitor, in hypertension: a multicenter international trial. Investigator Study Group. *J Cardiovasc Pharmacol* 1994;23 Suppl 4:S86-90.

This study may represent one of the foreign trials whose full report was not submitted to the NDA. Patients were treated (unblinded) for 1 year and initially began trandolapril 2 mg once daily. If blood pressure was not controlled, the dose was increased to 4 mg/day; then, after 1 month, diuretics and/or calcium antagonists were added if necessary. At the interim analysis, 481 subjects had been treated for over 12 months and 960 for 3 months. At end point, trandolapril significantly decreased blood pressure (-14 mmHg for mean supine diastolic blood pressure). Three point nine percent of subjects reported dry cough, and 3.8% withdrew from the study for treatment related reasons. Diastolic pressure changes in this study are difficult to compare to data from controlled studies, but appear similar to the higher end of changes seen in the controlled studies.

Cesarone MR, De Sanctis MT, Laurora G, Ambrosoli L, Marelli C, and Belcaro G. Effects of trandolapril on 24 hour ambulatory blood pressure in patients with mild-to-moderate essential hypertension. *J Cardiovasc Pharmacol* 1994;23 Suppl 4:S65-72.

The effects of trandolapril 1 mg (n=14) and 2 mg (n=13) once daily for 2 weeks on 24-h blood pressure in mild-to-moderate hypertensive subjects were investigated by clinic and ambulatory monitoring in a double-blind study. Both clinic and ambulatory measures demonstrated significant end-of-treatment decreases in diastolic and systolic pressure in the 1 and 2 mg groups. Although intergroup differences were not statistically significant, reductions in the 2 mg group tended to be consistently greater than those in the 1 mg group. There was no effect on the normal circadian rhythm. Two of three NDA randomized trials showed little difference between the 1 and 2 mg dose whereas the third study showed a proportionally greater blood pressure lowering effect of the 2 mg dose. The study did not allow for detection of a secondary peak 24-48 hours post-dose as noted in Study TR-42-CP.

De Bruijn JH, Orofiamma BA, and Pauly NC. Efficacy and tolerance of trandolapril (0.5-2 mg) administered for 4 weeks in patients with mild-to-moderate hypertension. Investigator Study Group. *J Cardiovasc Pharmacol* 1994;23 Suppl 4:S60-4.

This was NDA Study FF/88/570/39.

Mancia G, De Cesaris R, Fogari R, Lattuada S, Montemurro G, Palombo C, Porcellati C, Ranieri G, Tettamanti F, Verdecchia P, et al. Evaluation of the antihypertensive effect of once-a-day trandolapril by 24-hour ambulatory blood pressure monitoring. The Italian Trandolapril Study Group. *Am J Cardiol* 1992 Oct 29;70(12):60D-66D.

Trandolapril was compared to placebo in a double-blind fashion using 24-hour ambulatory blood pressure monitoring in subjects with mild-to-moderate essential hypertension. After washout, 42 subjects were randomized to trandolapril 2 mg once daily and 20 to placebo for 6 weeks. Then a second washout period of 4 weeks followed. Forty-eight subjects who met the criterion for the minimal

number of ambulatory blood pressure readings were assessed. In the trandolapril-treated group, pretreatment values were 160/102 (clinic, n=41) and 140/91 mmHg (24-hour mean blood pressure, n=31); values during the treatment period: 147/95 (clinic) and 131/84 mmHg (24-hour); post-treatment: 156/99 (clinic) and 140/91 mmHg (24-hour). The differences between the lower treatment values versus the higher pre- and post-treatment values were statistically significant ($p < 0.01$). The study did not allow for detection of a secondary peak 24-48 hours post-dose as noted in Study TR-42-CP.

Meyer BH and Pauly NC. Double-blind comparison of the efficacy and safety of trandolapril 2 mg and hydrochlorothiazide 25 mg in patients with mild-to-moderate essential hypertension. Investigator Study Group. *J Cardiovasc Pharmacol* 1994;23 Suppl 4:S77-80.

This study appears in the NDA as Study FF/88/570/39.

Patat A, Surjus A, Le Go A, and Granier J. Safety and tolerance of single oral doses of trandolapril, a new angiotensin converting enzyme inhibitor. *Eur J Clin Pharmacol* 1989;36(1):17-23. The safety and tolerance of single oral doses of a new angiotensin converting enzyme (ACE) inhibitor, trandolapril have been examined in 90 healthy male volunteers, in a randomized, double blind, placebo-controlled study. The subjects were divided into 10 groups, each of 9 subjects and treatments (6 subjects on trandolapril and 3 on placebo per group) were allocated by unbalanced randomization. Ten single, increasing oral doses were tested: 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 24 and 32 mg. The assessment criteria were clinical (monitoring of blood pressure, heart and respiratory rate, electrocardiogram, temperature and evaluation of behavior and side effects) and routine laboratory tests. Blood pressure did not fall except for a slight drop in diastolic pressure during the first 4 h following the 32-mg dose. However, although an effect of the compound cannot be excluded, the reduction in blood pressure may have reflected intersubject variability. No orthostatic hypotension was observed. There was no change in the other vital signs, and in particular no increase in heart rate was observed. No serious adverse effect was encountered. The pharmacological activity of the compound was studied by assaying plasma ACE activity. Inhibition of ACE was linearly dose-dependant from 0 (placebo) to 2 mg, and above that dose, the inhibition was nearly total. ACE activity was markedly reduced within 30 min after administration of trandolapril, and maximal inhibition was observed from 2-4 h onwards, lasting for up to 24 h after dosing. For doses above 2 mg, inhibition was still 40% of the basal activity on Day 8 after dosing.

Pauly NC and Safar ME. Comparison of the efficacy and safety of trandolapril and captopril for 16 weeks in mild-to-moderate essential hypertension. Investigator Study Group. *J Cardiovasc Pharmacol* 1994;23 Suppl 4:S73-6.

Subjects (n=180) were randomized to double-blind treatment for 16 weeks of either trandolapril 4 mg once daily or captopril 50 mg twice daily. If blood pressure remained high at 8 weeks, HCTZ 25 mg was added. At 8 weeks, there was a significant difference for supine diastolic pressure (mean±SEM) between the two groups: trandolapril -13.5±0.9 mmHg; captopril -10.1±1.0 mmHg (p=0.007). The proportion of subjects with normalized blood pressure was 61% for trandolapril and 44% for captopril (p=0.02). At 8 weeks, 26% of the trandolapril group received HCTZ compared with 38% taking captopril. The addition of HCTZ further increased the antihypertensive effect of both treatments. All treatments were well tolerated clinically. This study has the great potential to be used in trandolapril's marketing given the conclusions of the authors that "Trandolapril given once daily is an effective and well-tolerated antihypertensive agent that, in this study, exhibited better efficacy

compared with captopril." However, such use would be inappropriate, given that both ACE inhibitors were given at less than maximum doses.

Poirier L, Lefebvre J, Archambault F, Bourgeois J, Lacourciere Y. Antihypertensive effects of trandolapril and enalapril on casual and 24-hour ambulatory blood pressures (abstract). *Clin Invest Med* (1993a) 16(4 Suppl): B75.

In a randomized, double-blind, parallel group study, 65 subjects were randomized to trandolapril or enalapril, titrated to 0.5, 1, 2, 4 mg, and 2.5, 5, 10, and 20 mg, per day, respectively. Both drugs significantly and similarly reduced office systolic and diastolic, and mean 24-hour ambulatory blood pressures (both awake and asleep). There appeared to be no difference between these two agents.

Poirier L, Bourgeois J, and Lacourciere Y. Once-daily trandolapril compared with the twice-daily formulation in the treatment of mild to moderate essential hypertension: assessment by conventional and ambulatory blood pressures. *J Clin Pharmacol* 1993b Sep;33(9):832-6.

This was a double-blind, crossover study comparing trandolapril 1 mg once daily to 0.5 mg twice daily in 31 mild-to-moderate hypertensive subjects. After randomization, there was a single-blind placebo period of 4 weeks. After a 4 week double-blind treatment, subjects were crossed over for the last 4 weeks. Both cuff and 24-hour ambulatory systolic and diastolic pressures were significantly ($p < 0.001$) and similarly reduced in both groups. Also, the response rates (diastolic pressure ≤ 90 mmHg) were similar with both treatment regimens. However, trandolapril 0.5 mg twice daily had a significantly ($p = 0.03$) greater effect on systolic pressure during sleep. Safety issues were not thoroughly discussed, but both treatments appeared equally benign. The authors admit that higher doses should be studied. Indeed, TR-22-HTN investigated trandolapril 4 mg once daily versus 2 mg twice daily and found little difference in the systolic blood pressure effects of the two treatments. However, TR-22-HTN reported a greater number of dropouts from the 4 mg once daily group (because of lack of efficacy), and the 2 mg twice daily group exerted a nearly statistically significantly greater effect on trough supine diastolic blood pressure.

Ravogli A, Omboni S, De Cesaris R, Fogari R, Lattuada S, Montemurro G, Palombo C, Porcellati C, Ranieri G, and Tettamanti C. Twenty-four-hour ambulatory blood pressure monitoring and antihypertensive treatment: focus on ACE inhibitors. *J Cardiovasc Pharmacol* 1994;23 Suppl 1:S15-9.

Sixty-two mild to moderate hypertensive subjects were recruited into this double-blind parallel group study. After a 4-week washout, subjects received trandolapril 2 mg/day, or placebo for 6 weeks, followed by a second washout period. Clinic and 24 hour blood pressures were assessed at the end of each period. Trandolapril significantly reduced clinic and 24 hour blood pressures. The decrease was evident throughout the 24 hours and remained statistically significant in the last 4 hours of blood pressure monitoring. This study was not designed to tell if a second peak occurred in hours 24 to 48, as seen in Study TR-42-CP.

Steiner G and Pauly NC. Comparison of the efficacy and safety of trandolapril and nifedipine SR in mild-to-moderate hypertension. Investigator Study Group. *J Cardiovasc Pharmacol* 1994;23 Suppl 4:S81-5.

After a 4-week placebo run-in, 161 subjects were randomized to

trandolapril 2 mg/day (n=54), nifedipine slow-release (SR) 20 mg twice daily (n=55), or both drugs combined (n=52) for 16 weeks. At end point, the intent-to-treat analysis showed no significant difference in supine diastolic pressure (mean $\pm$ SEM) between trandolapril (-12.4 $\pm$ 1.5 mmHg) and nifedipine SR (-15.3 $\pm$ 1.4 mmHg; p=0.1). Combination therapy lowered blood pressure significantly as compared to trandolapril (p<0.01), but not compared to nifedipine SR. Blood pressures were normalized in 54% of trandolapril, 63% of nifedipine SR, and 77% of combination subjects. Adverse events occurred more frequently with nifedipine SR (34%) than with trandolapril (17%; p<0.05) or the combination (21%).

C2. Clinical studies other than hypertension

Banduceau B, Vaur L, Rezvani Y, Gautier D. Action of trandolapril on blood glucose control and microalbuminuria in hypertensive diabetics (French). *Ann Cardiol Angeiol* (1994) 43(2): 84-88.

Trandolapril was evaluated for its effects on blood glucose and microalbuminuria in mild to moderate hypertension in non-insulin-dependent diabetic patients. Sixty-seven subjects, aged between 33 and 79, were enrolled. Trandolapril monotherapy was given for 3 months between 1 and 4 mg/day according to antihypertensive response. Mean diastolic pressure fell from 101 $\pm$ 5 mmHg to 82 $\pm$ 7 mmHg (p<0.0001). Microalbuminuria normalized in 11 of the 13 subjects with baseline values above 21 μ g/min and increased to above normal in 2 of the 26 subjects with normal baseline values. Blood glycosylated hemoglobin, glucose, and creatinine, and creatinine clearance remained stable. Six adverse events were reported (4 coughs, 1 peripheral edema, 1 plantar mal perforans). One subject died from pulmonary embolism.

TRACE Study Group. The TRAndolapril Cardiac Evaluation (TRACE) study: rationale, design, and baseline characteristics of the screened population. The Trace Study Group. *Am J Cardiol* (1994) 73(10): 44C-50C.

The TRAndolapril Cardiac Evaluation (TRACE) study is evaluating the effect of trandolapril on mortality (overall and cardiovascular) and cardiovascular morbidity in subjects surviving a myocardial infarction with reduced left ventricular function. TRACE is a randomized, double-blind, placebo-controlled study conducted in 27 centers in Denmark. Eligible subjects had a myocardial infarction verified by enzymes as well as reduced left ventricular function, as shown by echo wall motion index $\leq$ 1.2. (A wall motion index $\leq$ 1.2 approximates left ventricular ejection fraction $\leq$ 35%). Trandolapril or placebo was added 3 to 7 days after myocardial infarction. Between May 1990 and June 1992, 6,674 patients were screened, with 1,749 included. Overall 1-year mortality of the subjects entered into the study was 24%. Treatment will be continued for 2 to 4 years ending in mid 1994.

C3. Studies in human pharmacology

Asmar RG, Benetos A, Darne BM, Pauly NC, and Safar ME. Converting enzyme inhibition: dissociation between antihypertensive and arterial effects. *J Hum Hypertens* 1992 Oct;6(5):381-5.

Arterial and antihypertensive dose-response curves of trandolapril were analyzed in a double-blind study carried out in 24 subjects with essential hypertension. The decrease in blood pressure

following trandolapril was used as a marker of the arteriolar effect while the effect on conduit arteries using carotid-femoral pulse wave velocity was used as an index of arterial distensibility. The antihypertensive effect was observed from 2 mg, where the plateau of blood pressure reduction was already achieved. There was no significant correlation between dose and blood pressure reduction whereas the dose was significantly related to the change in pulse wave velocity. There was no significant correlation between changes in blood pressure and change of pulse wave velocity.

de Champlain J, Yacine A, Le Blanc R, Bouvier M, Lebeau R and Nadeau R. Effects of trandolapril on the sympathetic tone and reactivity in systemic hypertension. *Am J Cardiol* 1994 Apr 7;73(10):18C-25C.

Hemodynamic and autonomic assessments were done after 4 weeks of trandolapril 2 mg/day in 18 hypertensive subjects (48 2 years). Trandolapril lowered the mean arterial pressure from 112 to 105 mmHg ($p < 0.05$) without significant changes in basal heart rate or norepinephrine plasma levels. The subjects were then split into 2 groups of 9 subjects: the "responders", those with an average decrease in mean arterial pressure of 12 mmHg, and the "non-responders" with a 2 mmHg average fall in mean arterial pressure. Before trandolapril, responders had a higher resting heart rate, a lower cardiac output (-16%), and a higher peripheral resistance (+22%) than the non-responders, although the differences were not statistically significant. Also, responders had a 33% higher plasma norepinephrine level ($p < 0.05$) while supine, a 52% higher norepinephrine response to standing ($p < 0.05$), and a 40% lower number of beta-receptors on lymphocytes, suggesting a higher sympathetic tone and reactivity in responders. After trandolapril, heart rate and plasma norepinephrine levels did not change significantly in either group, possibly indicating an attenuation of the baroreflex especially in the responders. While cardiac output remained the same in both groups, peripheral resistance decreased by 10% in the responders (not statistically significant). Norepinephrine responses to standing were blunted more in responders than in non-responders. The authors conclude that hypertensives who appear to have an increased sympathetic tone may better respond to trandolapril or other ACE inhibitors.

De Luca N, Rosiello G, Lamenza F, Ricciardelli B, Marchegiano R, Volpe M, Marelli C, Trimarco B. Reversal of cardiac and large artery structural abnormalities induced by long-term antihypertensive treatment with trandolapril. *Am J Cardiol* (1992) 70(12) 52D-59D.

Fifteen subjects with untreated mild to moderate hypertension and left ventricular hypertrophy, were assessed using blood pressure, echo left ventricular mass index, brachial artery compliance (pulsed doppler flowmetry), and calculated forearm vascular resistance (strain gauge plethysmography) before, during (6 and 12 months), and 1 month after (a washout period) 1 year of blood pressure normalization ($\leq 140/90$ mmHg) with trandolapril 2 mg once daily. While blood pressure was normalized during treatment, systolic and diastolic blood pressures, left ventricular mass index, and forearm vascular resistance were all reduced at both 6 and 12 months. Brachial artery compliance was significantly increased as well.

Dutrey-Dupagne C, Girard A, Ulmann A, and Elghozi JL. Effects of the converting enzyme inhibitor trandolapril on short-term variability of blood pressure in essential hypertension. *Clin Auton Res* 1991 Dec;1(4):303-7.

Blood pressure and heart rate short-term fluctuations were assessed in eight hypertensive males. Indirect finger blood pressure and heart rate was measured by a non-invasive device (Finapres) every second. Trandolapril 2 mg/day was administered for 7 days in a double-blind, randomized, placebo-controlled cross-over study. Trandolapril produced a significant reduction in systolic blood pressure (15 mmHg) although the reduction in diastolic blood pressure was not significant. Systolic (-20%) and diastolic (-22%) blood pressure standard deviations were significantly reduced. Trandolapril reduced the variability underlying the standard deviation changes of systolic and diastolic blood pressure. This could be mediated by reduced sympathetic outflow to vascular smooth muscle.

D'Yvoire MB, Caplain H, and Stepniewski J-P. Comparison of plasma ACE activity after discontinuation of repeated doses of trandolapril or enalapril (abstract). *Am J Hypertens* (1994) 7(4 part 2): 35A.

Two groups of young healthy male volunteers received either trandolapril 2 mg daily or enalapril 20 mg daily for 7 days in an open, randomized study. Both drugs were stopped and plasma ACE inhibition was measured daily in both groups for one week. Twenty-four hours after the last dose, trandolapril's ACE inhibition was significantly greater than enalapril's. This difference remained at one week. ACE inhibition remained above 50% 48 hours after the last dose of trandolapril. There appeared to be a rebound of ACE activity one week after enalapril that did not occur after trandolapril.

Guller B, Hall J, and Reeves RL. Cardiac effects of trandolapril in hypertension. *Am Heart J* 1993 May;125(5 Pt 2):1536-41.

Trandolapril was titrated from 1 to 4 mg for 3 months in 25 subjects with essential hypertension. Diastolic blood pressure decreased by 7.5% ($p < 0.0001$). As compared to baseline echocardiographic values, left ventricular hypertrophy regressed as evidenced by a 23.2% ($p < 0.0001$) decrease in left ventricular mass index at 3 months and a 12.4% ($p < 0.05$) reduction of relative wall thickness at 6 months. Afterload decreased by 12.4% ($p < 0.05$) at 3 months and left ventricular contractility improved. Also, left ventricular diastolic function (E/A ratio) improved in 15 of 25 subjects with low baseline values: from 0.96 ± 0.14 to 1.18 ± 0.25 (mean $\pm$ SD, $p < 0.0002$) at 3 months.

Leonetti G, Fogari R, Mazzola C, Pasotti C, Ponti GB, Marelli C, and Zanchetti A. Effects of blood pressure reduction with trandolapril and enalapril on left ventricular hypertrophy and exercise tolerance. *J Hypertens Suppl* 1993 Dec;11 Suppl 5:S356-7.

Li Kam Wa TC, Cooke ED, Beacham J, and Turner P. Absence of potentiation of the skin response to intradermal bradykinin by a long-acting angiotensin converting enzyme inhibitor, trandolapril, at conventional antihypertensive dosage in human volunteers: a double-blind, randomized, cross-over, placebo-controlled trial. *Postgrad Med J* 1992 Sep;68(803):742-5. A double-blind, randomized, cross-over, placebo-controlled study was carried out to determine the extent and duration of potentiation of the action of bradykinin introduced intradermally by a long-acting novel angiotensin converting enzyme (ACE) inhibitor, trandolapril. The investigations were performed in a temperature and humidity-controlled laboratory. Intradermal injections of 1 microgram, 2.5 micrograms and 5 micrograms of bradykinin and normal saline (as control) were made into the forearm skin of eight healthy normotensive male volunteers aged 21-33 years (mean 28 years) at baseline, 2, 4, 8, 24, 48, 72 and 96

hours after either 2 mg trandolapril or placebo given orally. Skin blood flow outside the induced weal was monitored by laser Doppler flowmetry (mean of recordings at four sites adjacent to the weal within the flare area). Flare area and weal volume were also measured. Trandolapril reduced the mean arterial pressure. However, there was no evidence that this activity was associated with a potentiation of the cutaneous action of bradykinin. In conclusion, it would appear that potentiation of the action of bradykinin may not be an important contributing factor to the fall in total peripheral vascular resistance associated with ACE inhibition in humans in the control of hypertension.

Mooser V, Nussberger J, Juillerat L, Burnier M, Waeber B, Bidiville J, Pauly N and Brunner HR. Reactive hyperreninemia is a major determinant of plasma angiotensin II during ACE inhibition. *J Cardiovasc Pharmacol* 1990 Feb;15(2):276-82.

Trandolapril was administered to 21 volunteers, age 21 to 30 at daily doses of 0.5, 2, and 8 mg for 10 days. Dose-dependent ACE inhibition was paralleled by a dose-dependent rise in renin and blood angiotensin I levels, most evident on day 10. However, plasma angiotensin II levels on day 10 were not responsive to dose.

Predel HG, Rohden C, Heine O, Rost RE. Influence of the nonsulphydryl angiotensin-converting enzyme inhibitor trandolapril on lipid and carbohydrate metabolism related to exercise capacity in healthy subjects. *Am Heart J* (1993) 125(5 Pt 2): 1532-5.

The effects of trandolapril 2 mg/day on the physical work capacity, the received perception of exertion, as well as LDL and HDL cholesterol, triglycerides, glucose, insulin, and C peptide were investigated in 10 well-trained, healthy males. Subjects underwent a bicycle ergometry until exhaustion to determine baseline readings. Ergometry was repeated after 14 days of trandolapril 2 mg/day. Treatment with trandolapril did not significantly alter maximum work capacity, perceived exertion, LDL and HDL cholesterol, triglycerides, glucose, insulin, and C peptide.

Predel HG, Rohden C, Heine O, Prinz U, and Rost RE. ACE inhibition and physical exercise: studies on physical work capacity, energy metabolism, and maximum oxygen uptake in well-trained, healthy subjects. *J Cardiovasc Pharmacol* 1994;23 Suppl 1:S25-8.

The effects of trandolapril 2 mg/day on the physical work capacity, the received perception of exertion, and the parameters determining physical performance (i.e., energy metabolism, lactate production, and oxygen uptake) were investigated in 20 well-trained, healthy males. Subjects underwent bicycle spiroergometry until exhaustion to determine baseline readings. Spiroergometry was repeated after 14 days of either trandolapril 2 mg/day or placebo. Treatment with trandolapril did not significantly alter maximum work capacity, perceived exertion, 4.0 mmol/L lactate threshold, heart rate, maximum oxygen uptake, plasma levels of total cholesterol, triglycerides, free fatty acids, glucose, insulin, cortisol, or human growth hormone. The authors conclude that ACE inhibitors should be considered among the first choices when treating active hypertensives.

Rett K, Jacob S, and Wicklmayr M. Possible synergistic effect of ACE inhibition and calcium-channel blockade on insulin sensitivity in insulin-resistant type II diabetic hypertensive patients. *J Cardiovasc Pharmacol* (1994) 23 Suppl 1:S29-33.

The authors discuss data suggesting a synergistic effect of ACE inhibitors and calcium antagonists to improve insulin sensitivity. They discuss plans for a double-blind study in 40 insulin-resistant subjects with non-insulin-dependent diabetes mellitus, comparing

trandolapril 1 mg/day, verapamil 180 mg/day, a combination of these two, or placebo. Ambulatory 24-hour blood pressure measurements and insulin resistance are to be monitored.

Weichenhain B, van Kooten HJ, Standl E. Hypertension and insulin resistance. Glycaemia and insulinaemia in overweight hypertensive patients. *Drugs* (1993) 46 Suppl 2: 183-8.

Preliminary results of an ongoing study investigating the effects of trandolapril or the diuretic combination of hydrochlorothiazide and triamterene are presented. Trandolapril, while reducing blood pressure in overweight hypertensives, did not affect post-absorptive blood glucose levels, and generally appeared to influence peripheral insulin resistance. The diuretic combination slightly raised post-absorptive blood glucose. Neither treatment modified serum insulin peaks.

C4. Studies of pharmacokinetics

Arner P, Wade A, Engfeldt P, Mouren M, Stepniewski JP, Sultan E, Bryce T, and Lenfant B. Pharmacokinetics and pharmacodynamics of trandolapril after repeated administration of 2 mg to young and elderly patients with mild-to-moderate hypertension. *J Cardiovasc Pharmacol* 1994;23 Suppl 4:S44-9.

This study appears in the NDA as two studies: FF/89/570/45 and S/87/570/23.

Bevan EG, McInnes GT, Aldigier JC, Conte JJ, Grunfeld JP, Harper SJ, Meyer BH, Pauly N, Wilkinson R. Effect of renal function on the pharmacokinetics and pharmacodynamics of trandolapril. *Br J Clin Pharmacol* (1993) 35(2): 128-35.

This study appears in the NDA as Study FF/87/570/25.

Danielson B, Querin S, LaRoche P, Sultan E, Mouren M, Bryce T, Stepniewski JP, and Lenfant B. Pharmacokinetics and pharmacodynamics of trandolapril after repeated administration of 2 mg to patients with chronic renal failure and healthy control subjects. *J Cardiovasc Pharmacol* 1994;23 Suppl 4:S50-9.

This study appears in the NDA as Study FF/88/570/39.

Lenfant B, Mouren M, Bryce T, De Lauture D, and Strauch G. Trandolapril: pharmacokinetics of single oral doses in healthy male volunteers. *J Cardiovasc Pharmacol* 1994;23 Suppl 4:S38-43.

This study appears in the NDA as Study FF/89/570/54.

C5. Pre-clinical studies

Amrani FC, Cheaw SL, Chevalier B, Paolaggi F, Jouquey S, Hamon G, Swynghedauw B. Regression of left ventricular hypertrophy by converting enzyme inhibition in 12-15-month-old spontaneously hypertensive rats: effects on coronary resistance and ventricular compliance in normoxia and anoxia. *J Cardiovasc Pharmacol* (1994) 23(1):155-65.

The effects of trandolapril on left ventricular diastolic stiffness and coronary vascular resistance, were studied with an isolated heart preparation in spontaneously hypertensive rats. Trandolapril completely normalized left ventricular weight and normalized the tissue stiffness of spontaneously hypertensive rats under anoxia. Trandolapril also improved coronary vascular resistance.

Bree F, Hamon G, Tillement JP. Evidence for two binding sites on membrane-bound angiotensin-converting enzymes (ACE) for exogenous inhibitors except in testis. *Life Sci* (1992) 51(10): 787-94.

Pre-clinical data were not fully reviewed; the authors concludes thattrandolapril binds to two different sites on the converting enzyme.

Chevalier B, Callens-el Amrani F, Heymes C, Swynghedauw B. Molecular basis of regression of cardiac hypertrophy. *Am J Cardiol* (1994) 73(10): 10C-17C.

Spontaneously hypertensive rats were treated for 3 months withtrandolapril. The treatment had a modest effect on blood pressure but markedly reduced cardiac hypertrophy and cardiac fibrosis, improved coronary reserve, and attenuated the effects of anoxia on left ventricular diastolic compliance and the incidence of ventricular arrhythmias.

Chevillard C, Jouquey S, Bree F, Mathieu MN, Stepniowski JP, Tillement JP, Hamon G, Corvol P. Compared properties oftrandolapril, enalapril, and their diacid metabolites. *J Cardiovasc Pharmacol* (1994) 23 Suppl 4: S11-5.

The effects on blood pressure and ACE activity (measured ex vivo in various organs) of 14-days oftrandolapril or enalapril in spontaneously hypertensive rats were studied. Whiletrandolapril was 30- and 400- to 1,000-fold more active than enalapril on blood pressure and ACE activity,trandolaprilat was only three- to five-fold more active than enalaprilat. Trandolaprilat was more lipophilic than enalaprilat.

Chobanian AV, Haudenschield CC, Nickerson C, Hope S, Nagano M, Higaki J, Nakamura F, Higashimori K, Nagano N, Mikami H, Ogihara T. Trandolapril inhibits atherosclerosis in the Watanabe heritable hyperlipidemic rabbit: Role of cardiac angiotensin II in isoproterenol-induced left ventricular hypertrophy; effects of angiotensins on cellular hypertrophy and c-fos expression in cultured arterial smooth muscle cells. *Eur J Biochem* (1992) 206(2): 367-72.

The effects oftrandolapril on aortic atherosclerosis were examined in the Watanabe heritable hyperlipidemic rabbit. Trandolapril caused a significant decrease in atherosclerotic involvement of the intimal surface of the aorta. To examine the role of angiotensin II in left ventricular hypertrophy, isoproterenol was infused into 9-week-old male Wistar rats for 7 days. Isoproterenol increased left ventricular weight and angiotensin II concentrations in plasma and in left ventricular tissue. Concomitant oral administration oftrandolapril and isoproterenol prevented increases in both left ventricular angiotensin II and left ventricular weight.

Corbier A, Lecaque D, Secchi J, Depouez B, Hamon G. Effects of 4 weeks of treatment withtrandolapril on renal hypertension and cardiac and vascular hypertrophy in the rat. *J Cardiovasc Pharmacol* (1994) 23 Suppl 4: S26-9.

The effects oftrandolapril versus a control group were studied using a Goldblatt rat model of renovascular hypertension after 4 weeks of oral treatment. Trandolapril produced a rapid, dose-dependent decrease in blood pressure. Dose-dependent inhibition of cardiac hypertrophy was also observed, heart:body weight ratio being decreased by 17 and 30% at 0.3 and 1 mg/kg, respectively, as compared with normotensive controls. Similarly,trandolapril produced a marked decrease in vascular wall hypertrophy.

Fornes P, Richer C, Pussard E, Heudes D, Domergue V, Giudicelli JF. Beneficial effects of trandolapril on experimentally induced congestive heart failure in rats. *Am J Cardiol* (1992) 70(12): 43D-51D.

Following induction of infarction in rats, systolic blood pressure decreased, left ventricular dP/dt decreased, and end-diastolic pressure increased. Cardiac remodeling (left ventricular dilation, myocardial hypertrophy, and fibrosis) occurred as early as 7 days after infarction and worsened throughout the study. Treatment with trandolapril over 1 year reduced pre- and afterload, increased cardiac index and reversed the infarction-induced cardiac morphological changes.

Freslon JL, Pourageaud F, Lecaque D, Secchi J. 1992. Effects of trandolapril on vascular morphology and function during the established phase of systemic hypertension in the spontaneously hypertensive rat. *Am J Cardiol* (1992) 70(12): 35D-42D.

Trandolapril decreased blood pressure of the spontaneously hypertensive rat by 15 to 18% compared with control animals. Trandolapril also reduced myocardial hypertrophy, the media thickness of the thoracic aorta and femoral arteries, and increased the compliance of the resistance arteries.

Himeno H, Crawford DC, Hosoi M, Chobanian AV, Brecher P. Angiotensin II alters aortic fibronectin independently of hypertension. *Hypertension* (1994) 23(6 Pt 2): 823-6.

This study assessed the changes in aortic fibronectin mRNA expression that occur in the rat in response to angiotensin II administration. Infusion with a pressor dose of angiotensin II increased fibronectin mRNA expression but not of several other extracellular matrix genes. This change was inhibited by losartan whereas trandolapril reduced fibronectin mRNA but did not reduce blood pressure.

Huber KC, Schwartz RS, Edwards WD, Camrud AR, Bailey KR, Jorgenson MA, Holmes DR, Jr. Effects of angiotensin converting enzyme inhibition on neointimal proliferation in a porcine coronary injury model. *Am Heart J* (1993) 125(3): 695-701.

Thirty-five animals received oral trandolapril, captopril, or no drug for 6 days before and 28 days after coronary artery injury. Twenty arterial lesions in the trandolapril group, 17 in the captopril group, and 18 in the control group were evaluated. There was no significant difference in neointimal thickness or luminal area stenosis between the groups. However, the fractional increase in mean neointimal thickness per unit of injury was significantly less with trandolapril ($p=0.019$) but not with captopril as compared with controls.

Illiano S, Mombouli JV, Nagao T, Vanhoutte PM. Potentiation by trandolapril of the endothelium-dependent hyperpolarization induced by bradykinin. *J Cardiovasc Pharmacol* (1994) 23 Suppl 4: S6-10.

Trandolaprilat potentiates endothelium-dependent relaxations evoked by bradykinin in canine coronary arteries. These experiments demonstrate that endothelium-derived hyperpolarizing factor contributes to the relaxation evoked by bradykinin in the canine coronary artery and that trandolaprilat potentiates the release of this factor.

Jouquey S, Stepniewski JP, Hamon G. Trandolapril dose-response in spontaneously hypertensive rats: effects on ACE activity, blood pressure, and cardiac hypertrophy. *J Cardiovasc Pharmacol* (1994) 23 Suppl 4: S16-8.

After 2-week oral treatment with trandolapril in adult spontaneously hypertensive rats, a dose-dependent decrease in mean blood pressure was seen. Dose-response curves were observed for trandolapril from 0.03 mg/kg upwards with respect to hypotensive effect and regression of cardiac hypertrophy, effects not seen with enalapril. These results also indicate that trandolapril is a more potent inhibitor of ACE than enalapril.

Kamei J, Iwamoto Y, Misawa M. Cough-induced activity of (-)-(2S, 3aR, 7aS)-1-[(S)-N-[(S)-1-ethoxycarbonyl-3-phenylpropyl]alanyl] hexahydro-2-indolinecarboxylic acid (trandolapril) in guinea pigs. *Res Commun Mol Pathol Pharmacol* (1994) 85(1): 105-8.

Chronic treatment with enalapril significantly enhanced the number of capsaicin-induced coughs in guinea pigs. Chronic treatment with trandolapril, at doses of 1 and 3 mg/kg, p.o., slightly enhanced the number of capsaicin-induced coughs. There were no significant differences in the number of capsaicin-induced coughs between trandolapril-treated and vehicle-treated animals. Cough induced activity of trandolapril is relatively lower than that of enalapril.

Muller CA, Opie LH, Peisach M, Pineda CA. Chronic oral pretreatment with the angiotensin converting enzyme inhibitor, trandolapril decreases ventricular fibrillation in acute ischaemia and reperfusion. *Eur Heart J* (1994) 15(7): 988-96.

Guinea pigs pre-treated with trandolapril or placebo orally for 10 days had their left anterior descending coronary artery ligated for 20 min and reperfused for 45 min. Trandolapril decreased cardiac ACE activity and prevented the fall in the ventricular fibrillation threshold during ischaemia. Trandolapril also decreased the incidence of spontaneous ventricular fibrillation during reperfusion. During ischaemia, trandolapril increased blood flow in the non-ischemic zone, but decreased blood flow in the central ischemic zone.

Richer C, Fornes P, Vacher E, Bruneval P, Giudicelli JF. Trandolapril's protective effects in stroke-prone spontaneously hypertensive rats persist long after treatment withdrawal. *Am J Cardiol* (1994) 73(10): 26C-35C.

Trandolapril's effects on the occurrence of stroke and mortality in young salt-loaded stroke-prone spontaneously hypertensive rats were investigated (doses used: 0.01 mg/kg and 1 mg/kg). Only the higher dose limited the increase in blood pressure. However, both doses of trandolapril prevented stroke and mortality and prevented (lower dose) or abolished (higher dose) vascular fibrinoid necrosis formation in the brain, kidney, and heart. Eight weeks after treatment withdrawal, the antihypertensive effect of the higher dose had disappeared, but stroke-related mortality and fibrinoid necrosis remained completely suppressed.

Secchi J, Lecaque D, Dunn FW, Jouquey S, Auberval UD, Benzoni F, Sirugue O, Hamon G. Effects of subchronic treatment with trandolapril and enalapril on cardiovascular morphologic alterations in the aged spontaneously hypertensive rat with heart failure. *J Cardiovasc Pharmacol* (1994) 23 Suppl 4: S30-7.

The effects of 3 months treatment with trandolapril or enalapril on hemodynamics, cardiac hypertrophy, and vascular structures were assessed in old spontaneously hypertensive rats with congestive heart failure. The mortality rate was lower in the two treated groups

than in the control group. Serum ACE activity was inhibited by 63% and 33% by trandolapril and enalapril, respectively, and the ventricular hypertrophy was greatly decreased by both compounds (-24% by trandolapril and -26% by enalapril). In the aorta, nuclear density was increased and the surface of cell nuclei was decreased by trandolapril. Enalapril appeared much less potent on these parameters.

Sekiguchi N, Ishii Y, Fujikura H, Hasegawa Y, Takayanagi I. Pharmacological action of (-)-(2S,3aR,7aS)-1-[(S)-N-[(S)-1-carbonyl-3-phenylpropyl]alanyl]hexahydro-2-indolinecarboxylic acid (trandolaprilat) in isolated smooth muscle preparations. *Gen Pharmacol* (1993) 24(3): 585-90.

Trandolaprilat was found to inhibit angiotensin I-induced contraction of the rat thoracic aorta, and to augment bradykinin-induced contraction of the guinea pig ileum. Trandolaprilat had no effect on contractions induced by norepinephrine, PGF_{2α}, 5-HT or CaCl₂ in the thoracic aorta of rats. Trandolaprilat also produced endothelium-dependent relaxation.

Vidal M, Joly G, Mombouli JV, Boulanger CM, Vanhoutte PM. The endothelium and vascular effects of the ACE inhibitor trandolaprilat. *J Cardiovasc Pharmacol* (1994) 23 Suppl 4: S1-5.

Experiments on femoral or left circumflex coronary arteries of the dog demonstrated that in contractions induced by prostaglandin F_{2α}, trandolaprilat did not cause direct endothelium-dependent or -independent relaxation. However, when given to preparations incubated with angiotensin I or bradykinin, trandolaprilat evoked significant endothelium-dependent relaxation. Trandolaprilat failed to cause any change in tension when given in the presence of acetylcholine. These experiments suggest that trandolaprilat does not directly release endothelium-derived relaxing factor from the endothelial cells, and augments the endothelium-dependent responses to bradykinin and angiotensin I by direct interaction with converting enzyme.

Vienet R, Grognet JM, Ezan E, Lecaque D, Hamon G, Corman B. Effect of chronic converting-enzyme inhibition on kidney function of senescent hypertensive rats. *J Cardiovasc Pharmacol* (1994) 23 Suppl 4: S19-25.

Senescent, genetically hypertensive rats, some with hypertrophy of the glomeruli and glomerulosclerosis, all with reduced renal blood flow, glomerular filtration rate, and filtration fraction, were chronically administered trandolapril from 18 to 21 months. Life expectancy of the animals was increased without affecting mean blood pressure. Filtration fraction was restored in many of the animals, and age-related albuminuria and rise in cGMP excretion were prevented.

Wei L, Clauser E, Alhenc-Gelas F, Corvol P. The two homologous domains of human angiotensin I-converting enzyme interact differently with competitive inhibitors. *J Biol Chem* (1992) 267(19): 13398-405.

This study attempted to characterize the two active sites of ACE, and investigated their interaction with four competitive ACE inhibitors, including trandolaprilat. This study showed that both the N and C domains of ACE contain a high affinity inhibitor binding site. The different inhibitor binding properties of the two domains gives strong evidence for the presence of structural differences between the two active sites of ACE. Amongst the ACE inhibitors, trandolaprilat was the most potent competitor for these binding

sites.

Williams TA, Corvol P, Soubrier F. Identification of two active site residues in human angiotensin I-converting enzyme. *J Biol Chem* (1994) 269(47): 29430-4.

ACE contains two zinc-dependent catalytic domains: N and C. Using trandolaprilat, the investigators demonstrated that Glu987 constitutes the third zinc ligand in the ACE C domain and suggest a role for Asp991 in positioning the C domain active site zinc ion.

C6. Reviews

Conen H, Brunner HR. Pharmacologic profile of trandolapril, a new angiotensin-converting enzyme inhibitor. *Am Heart J* (1993) 125(5 Pt 2): 1525-31.

This review article was notable only for a few statements. The effective plasma half-life of trandolaprilat at steady state is 24 hours whereas NDA studies showed a half-life is in the hundreds of hours. Secondly, this paper contains statements that may make it useful as a marketing tool: 1) that trandolaprilat has very high lipophilicity in comparison with other ACE inhibitors, which should contribute to improved tissue penetration; and 2) because of its long half-life, trandolapril, probably more than any other drug of its class, can be considered a true, once-a-day antihypertensive.

Duc LN, Brunner HR. Trandolapril in hypertension: overview of a new angiotensin-converting enzyme inhibitor. *Am J Cardiol* (1992) 70(12): 27D-34D.

This review is unremarkable other than it repeats that trandolaprilat's pharmacokinetic half-life is 24 hours and claims that trandolapril, more than any other drug of its class can be considered a true once-a-day antihypertensive.

Gaillard CA, de Leeuw PW. Clinical experiences with trandolapril. *Am Heart J* (1993) 125(5 Pt 2): 1542-6.

This review article echoes the themes of a long plasma half-life and high lipophilicity.

Linz W, Wiemer G, Gohlke P, Unger T, Scholkens BA. Contribution of kinins to the cardiovascular actions of angiotensin-converting enzyme inhibitors. *Pharmacological Reviews* (1995) 47(1): 25-49.

This paper reviews the evidence that the cardiovascular effects of ACE inhibitors are not only a result of a reduction of angiotensin II but also a result of the inhibition of the degradation of bradykinins.

Rendina V, Iaccarino G, Russo R, Enea J, Marchegiano R, Lembo G, Trimarco B. Trandolapril in patients with essential hypertension: effects on vascular and cardiac structural changes. *J Cardiovasc Pharmacol* (1994) 23 Suppl 1: S20-4.

This review article discusses trandolapril's effects on cardiac and vascular structures. After long-term antihypertensive treatment with trandolapril, there is a reversal of left ventricular hypertrophy which may contribute to the observed improved left ventricular diastolic function improvement. This improvement is detectable after 1-month withdrawal of trandolapril. Long-term trandolapril treatment also reverses structural changes in the forearm vascular bed. Lastly, trandolapril induces significant increases in brachial artery compliance and diameter.

Wiseman LR, McTavish D. Trandolapril. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in essential hypertension. *Drugs* (1994) 48(1): 71-90.

This is an unremarkable review other than it quotes a trandolaprilat half-life of 16 to 24 hours, and cites a fixed-dose study (Pauly and Safar, 1994; reviewed on page 349) as evidence that trandolapril may be superior to captopril.

Zannad F. Trandolapril. How does it differ from other angiotensin converting enzyme inhibitors? *Drugs* (1993) 46 Suppl 2: 172-81; discussion 182.

This review article appears destined to be a marketing tool for trandolapril. The article cites numerous in vitro or animal data that indicate trandolapril's superiority over other ACE inhibitors. The article repeats the oft-quoted 24-hour half-life for trandolaprilat. However, the center-piece of the article is a review of ABPM studies of ACE inhibitors where trandolapril is shown to have the highest trough:peak ratio (using diastolic blood pressure). This ABPM review drew from a total of 136 clinical trials of once-daily dosed ACE inhibitors. Based on the author's criteria, only 19 studies were included in the analysis. Of the two studies included on trandolapril, the one indicating the highest trough:peak ratio is a French abstract. The article concludes that trandolapril's greater than 24-hour effects make it ideal for those patients with questionable compliance.

Zannad F, Metzger A. Does trough-peak ratio of once daily ACE inhibitors and calcium antagonists comply with FDA recommendations? (abstract). *Am J Hypertens* (1994) 7(4 part 2): 27A.

Another potential marketing tool, this review contends that trough/peak ratio is the most relevant way to evaluate the therapeutic coverage of antihypertensive drugs. The authors performed a literature analysis (1988-92) of once daily ACE inhibitors and calcium antagonists evaluated by 24-hour ABPM. Of the 185 studies with ACE inhibitors, 18 complied with the criteria. The mean trough/peak ratios were computed for DBP and showed trandolapril to have the highest ratio (a French abstract is included as one of the studies for trandolapril). The article urges caution in the use of ACE inhibitors since many do not meet FDA standards of a trough/peak ratio >50%.

D. Dissolution

Dissolution data were provided for 1 mg (lot 131-0192) and 4 mg (lot 133-0192) tablets, but not the 2 mg tablet. These data are shown in Table 347 below.

Table 347. Dissolution data (%) for 1 and 4 mg tablets.

Tablet	1 mg				4 mg			
	10 min	20 min	30 min	45 min	10 min	20 min	30 min	45 min
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
Mean	52	83	103	105	50	85	102	104

Based upon these results, the sponsor proposed the method and specifications shown in Table 348 below.

Table 348. Proposed method and specifications for dissolution testing.

Dosage form, strength	Tablets: 1, 2, and 4 mg
Dissolution apparatus	
Dissolution medium	
Volume	
Speed of rotation	
Sampling time	
Analytical method	
Recommended dissolution specification	Q= at 45 minutes

The Biopharmaceutical reviewer recommends the dissolution specification be changed to Q= at 30 minutes.

The sponsor has requested a waiver of the requirement for an in vivo bioavailability study for the 2 mg tablet. In support of this request, the sponsor submitted comparative dissolution data for the 1, 2, and 4 mg tablets in water, simulated gastric fluid (without pepsin; pH=1.2), and simulated intestinal fluid (without pancreatin; pH=7.5), as shown in Table 349 below and Figure 151 below¹.

The comparative dissolution data submitted by the sponsor show that the 1, 2, and 4 mg tablets have similar dissolution profiles in the 3 media. Therefore, the request for a waiver of bioavailability testing for the 2 mg tablet should be granted.

¹ Tablets were lots 131-0192 (1 mg), 132-K54-P1-0294 (2 mg), and 133-0192 (4 mg). Study was performed with USP

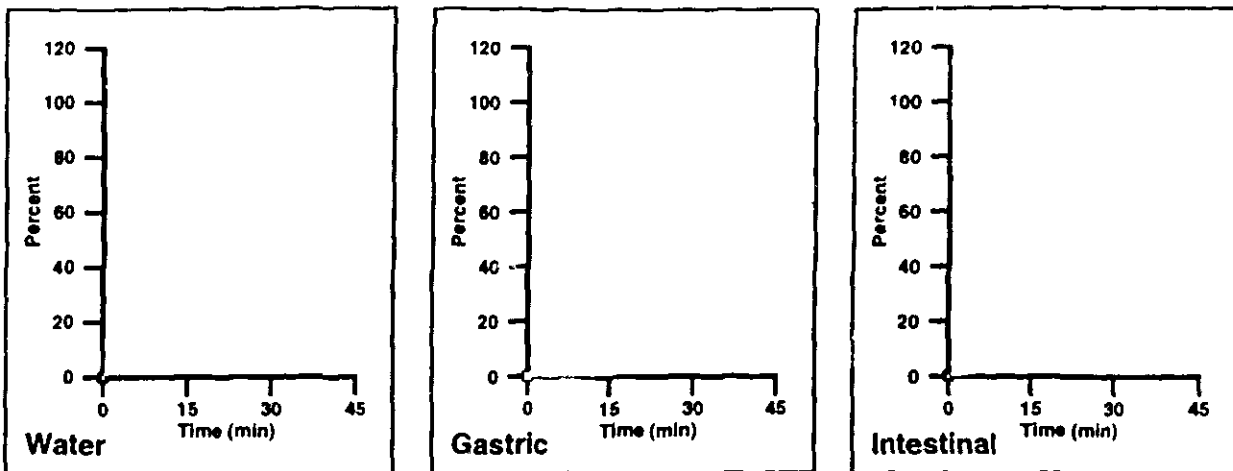


Figure 151. Comparative dissolution profiles for 1, 2, and 4 mg tablets.

Table 349. Comparative dissolution data for 1, 2, and 4 mg tablets.

Medium Tablet	1 mg			2 mg			4 mg		
	15 min	30 min	45 min	15 min	30 min	45 min	15 min	30 min	45 min
Water									
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
Mean	68	100	105	81	98	101	54	86	99
Gastric									
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
Mean	66	99	102	76	90	95	54	86	92

Table 349. Comparative dissolution data for 1, 2, and 4 mg tablets. (Continued)

Medium Tablet	1 mg			2 mg			4 mg		
	15 min	30 min	45 min	15 min	30 min	45 min	15 min	30 min	45 min
Intestinal									
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
Mean	47	85	102	69	94	99	45	81	94

Colophon

Several aspects of this review were novel, at least locally, and worthy of comment.

Three medical reviewers, a statistician, and a biopharmaceutics reviewer contributed material to this review. In addition, the review team acknowledges important contributions made by two project managers, Ms. Kathleen Bongiovanni and Mr. Gary Buehler, as well.

Rather than divide the work along lines of major sections (e.g., efficacy, safety, and clinical pharmacology) as has been done in the past, each medical reviewer went through an individual study at a time, wrote a draft review of that study, and then went on to the next study. When the individual studies were reviewed, these individuals went on to write the more integrative aspects of the review.

The biopharmaceutical and statistical reviewers generally added their comments to the individual study reviews as produced by the medical reviewers. At least, that was the plan. The idea was to avoid production of three separate descriptions of the trials.

The participants believe several advantages accrued from the organization of the review process. The medical reviewers were each, from time to time, engaged in activities other than the review of this NDA. Those who were available simply contributed more to some sections. By design, everyone finished work simultaneously.

The organization of the review document is independent of the way in which the work was divided; some sections of the review are the results of input from three or four persons. Instead, the organization reflects the idea that important things sometimes occur in 'unimportant' trials, and that, consequentially all the submitted trials merit at least a day's attention. What was worth reviewing was worth describing in the review, but not necessarily worth reading as part of a narrative whose intent was to describe the major findings. The result is a review in which the individual studies are all described, with a fair amount of detail, in an appendix that follows an integrative summary of the findings for the whole development program.

Although there were few review team meetings, there was quite a bit of pair-wise interaction between medical reviewers, between medical reviewers and the statistician, and between medical reviewers and the biopharmaceutical reviewer. These interactions gave the involved parties a better sense of what the others look for, in general, and what was important in this NDA. The quality of the final product is for other parties to judge, but it is almost certainly better than three or more partial reviews, and certainly better than what one reviewer from each discipline would have produced.

Individual reviewers produced documents in WordPerfect for DOS, WordPerfect for Windows, WordPerfect for Macintosh, and FrameMaker for Sun. One reviewer took these components and integrated them, with editorial and stylistic changes, into a single FrameMaker book, comprised of more than fifty documents and more than one hundred graphical files (produced in a variety of formats and with a variety of tools). Contributors had an opportunity first to re-edit their material as integrated into the review, and then to view and comment upon sections of the review they did not author.

The final document has a large number of cross references which ensure, for example, that study titles and cited page numbers are correct and consistent throughout. With little additional effort, many of these links would serve as hypertext links to take the reader of the electronic version to the cited material. A few of the graphs in the electronic version of the document 'remember' from whence they came; double-clicking them will launch the appropriate application with the graph and data.

Significant differences of opinion apparently did not arise during this review. Had they done so, the intent was to ensure the document captured those opinions in a way which permitted the reader to make informed decisions. How well that would have worked in practice remains to be seen.

Had all parties used the same word processor, the effort involved in integrating various contributions, particularly tables, would have been less, but this effort has to be weighed against the productivity of individuals who are used to different tools.

JAN 3 1995

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-528

CHEM.REVIEW #: 1

REVIEW DATE: 16 Dec 94

<u>SUBMISSION</u>	<u>TYPE</u>	<u>DOCUMENT DATE</u>	<u>CDER DATE</u>	<u>ASSIGNED DATE</u>
ORIGINAL		28 Sep 94	30 Sep 94	4 Oct 94

NAME & ADDRESS OF APPLICANT: Knoll Pharmaceutical Company
30 North Jefferson Road
Whippany, NJ 07981

DRUG PRODUCT NAME:

Proprietary:	Mavik
Nonproprietary/USAN:	Trandolapril
Code Name/#:	
Chem.Type/Ther.Class:	1 S

PATENT STATUS: Trandolapril is claimed in US 4,933,361 issued 12 Jun 90, and is assigned to Hoechst AG

PHARMACOL.CATEGORY/INDICATION: ACE inhibitor for treatment of hypertension

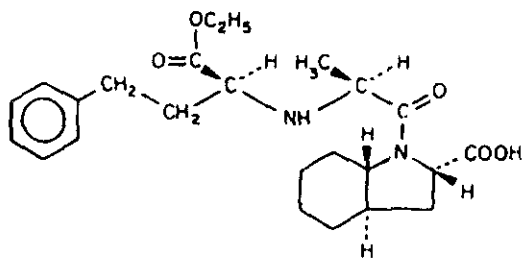
DOSAGE FORM: TCM

STRENGTHS: 1, 2, 4 mg

ROUTE OF ADMINISTRATION: Oral

DISPENSED: Rx OTC

STRUCTURAL FORMULA, CHEMICAL NAME, MOLECULAR FORMULA, MOLECULAR WEIGHT:



(2S-(1(R*(R*)),2a,3aα,7aβ))-1-(2-((1-(Ethoxycarbonyl)-3-phenyl-propyl)amino)-1-oxopropyl)octahydro-1H-indole-2-carboxylic Acid

C₂₄H₃₄N₂O₅

430.53

SUPPORTING DOCUMENTS:

RELATED DOCUMENTS (if applicable): None

CONSULTS: None

REMARKS/COMMENTS:

Trandolapril is a non-sulfhydryl angiotensin converting enzyme (ACE) inhibitor. It is a prodrug which is hydrolyzed in the liver to its active diacid, trandolaprilat.

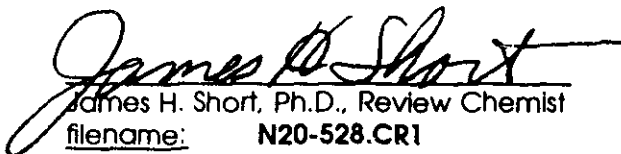
A Request for Trademark Review, dated 1 Dec 94, was sent to the Labeling and Nomenclature Committee. It was found acceptable.

CONCLUSIONS & RECOMMENDATIONS:

NOT APPROVABLE

The deficiencies noted during the review of this application are minor and should be easily correctable. None are of such a nature as to impede approval as far as the manufacturing and controls portion of the application is concerned.

cc:
Orig. NDA
HFD-110/Division File
HFD-110/JShort/10/5/94
HFD-110/CSO
District
HFD-102/CKumkumian
R/D Init by: RWolters/12/28/94


James H. Short, Ph.D., Review Chemist
filename: N20-528.CR1

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

APR 26 1995

NDA #: 20-528

CHEM.REVIEW #: 2

REVIEW DATE: 14 Apr 95

<u>SUBMISSION</u>	<u>TYPE</u>	<u>DOCUMENT DATE</u>	<u>CDER DATE</u>	<u>ASSIGNED DATE</u>
ORIGINAL		28 Sep 94		
AMENDMENT	BC	20 Mar 95	28 Mar 95	30 Mar 95

NAME & ADDRESS OF APPLICANT:

Knoll Pharmaceutical Company
 30 North Jefferson Road
 Whippany, NJ 07981

DRUG PRODUCT NAME:

Proprietary:	Mavik
Nonproprietary/USAN:	Trandolapril
Code Name/#:	
Chem.Type/Ther.Class:	1 S

PATENT STATUS:

Trandolapril is claimed in US 4,933,361 issued 12 Jun 90, and is assigned to Hoechst AG

PHARMACOL.CATEGORY/INDICATION:

ACE inhibitor for treatment of hypertension

DOSAGE FORM:

TCM

STRENGTHS:

1, 2, 4 mg

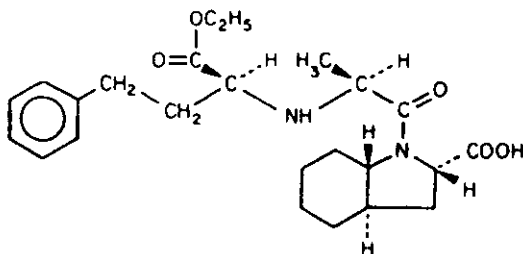
ROUTE OF ADMINISTRATION:

Oral

DISPENSED:

Rx OTC

STRUCTURAL FORMULA, CHEMICAL NAME, MOLECULAR FORMULA, MOLECULAR WEIGHT:



(2S-(1(R*(R*)),2a,3aα,7aβ))-1-[2-((1-(Ethoxycarbonyl)-3-phenylpropyl)amino)-1-oxopropyl]octahydro-1H-indole-2-carboxylic Acid

C₂₄H₃₄N₂O₅

430.53

SUPPORTING DOCUMENTS:

RELATED DOCUMENTS (if applicable): None

CONSULTS: Biopharm

REMARKS/COMMENTS:

The amendment of 20 Mar 95 provides answers to the deficiencies noted in the review of the CMC section of the original submission. The deficiencies are repeated, followed by the applicant's responses and my comments.

The name, Mavik, was reviewed by the Labeling and Nomenclature Committee, and found to be acceptable 1/11/95.

EER has not yet been returned.

The EA provided by the firm was not found acceptable, and additional information has been requested.

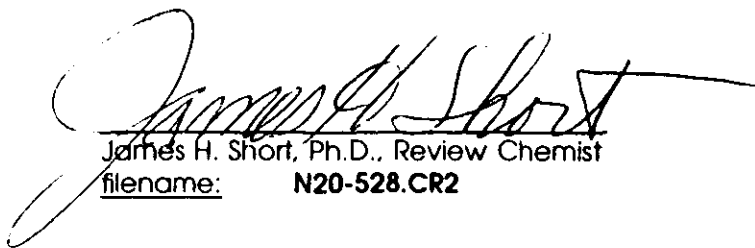
Methods validation has not yet been requested.

CONCLUSIONS & RECOMMENDATIONS:

NOT APPROVABLE

cc:
Orig. NDA
HFD-110/Division File
HFD-110/JShort/4/4/95
HFD-110/CSO
District

R/D Init by: RWalters/4/18/95


James H. Short, Ph.D., Review Chemist
filename: **N20-528.CR2**

AUG 31 1995

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-528

CHEM.REVIEW #: 3

REVIEW DATE: 23 Aug 95

<u>SUBMISSION</u>	<u>TYPE</u>	<u>DOCUMENT DATE</u>	<u>CDER DATE</u>	<u>ASSIGNED DATE</u>
ORIGINAL		28 Sep 94		
AMENDMENT	BC	26 Apr 95	27 Apr 95	2 May 95
AMENDMENT	BC	16 Aug 95	18 Aug 95	23 Aug 95

NAME & ADDRESS OF APPLICANT:

Knoll Pharmaceutical Company
30 North Jefferson Road
Whippany, NJ 07981

DRUG PRODUCT NAME:

Proprietary: Mavik
Nonproprietary/USAN: Trandolapril
Code Name/#:
Chem.Type/Ther.Class: 1 S

3'

PATENT STATUS:

Trandolapril is claimed in US 4,933,361 issued 12 Jun 90, and is assigned to Hoechst AG

PHARMACOL.CATEGORY/INDICATION:

ACE inhibitor for treatment of hypertension

DOSAGE FORM:

TCM

STRENGTHS:

1, 2, 4 mg

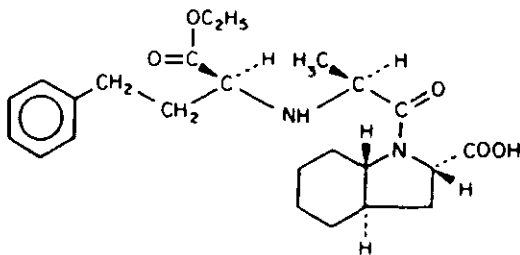
ROUTE OF ADMINISTRATION:

Oral

DISPENSED:

Rx OTC

STRUCTURAL FORMULA, CHEMICAL NAME, MOLECULAR FORMULA, MOLECULAR WEIGHT:



(2S-(1(R*(R*)),2a,3aα,7aβ))-1-(2-((1-(Ethoxycarbonyl)-3-phenylpropyl)amino)-1-oxopropyl)octahydro-1H-indole-2-carboxylic Acid

C₂₄H₃₄N₂O₅

430.53

SUPPORTING DOCUMENTS:**RELATED DOCUMENTS (if applicable):****CONSULTS:**

Division of Biopharmaceutics

REMARKS/COMMENTS:

The amendment of 26 Apr 95 provides responses to the Agency's letter of 9 Nov 95 requesting additional information concerning the Environmental Assessment section of the initial submission.

The amendment of 16 Aug 95 was in response to the Agency's deficiency letter of 23 May 95. This review is concerned with the applicant's responses to this letter.

The name, Mavik, was reviewed by the Labeling and Nomenclature Committee, and found to be acceptable 1/11/95.

EER was found acceptable as of 9 May 95. was found acceptable for manufacture of the drug substance. The applicant, US, was found acceptable for manufacture of the drug product. was found acceptable for packaging the drug product.

FUR, dated 31 Jul 95, was found acceptable 8 Aug 95.

Methods validation has not yet been requested.

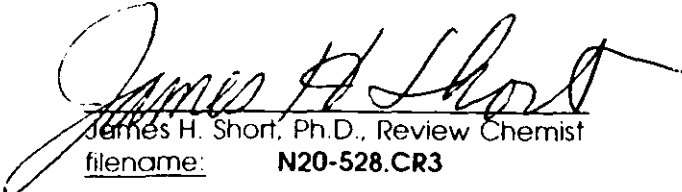
FONSI has not yet been written.

CONCLUSIONS & RECOMMENDATIONS:

NOT APPROVABLE

cc:
Orig. NDA
HFD-110/Division File
HFD-110/JShort/8/17/95
HFD-110/CSO
District

R/D Init by: RWolters/8/30/95


James H. Short, Ph.D., Review Chemist
filename: N20-528.CR3

Dongiavanni

MAR 22 1996

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-528**CHEM.REVIEW #:** 4**REVIEW DATE:** 20 Mar 96

<u>SUBMISSION</u>	<u>TYPE</u>	<u>DOCUMENT DATE</u>	<u>CDER DATE</u>	<u>ASSIGNED DATE</u>
ORIGINAL		28 Sep 94		
AMENDMENT	BC	4 Jan 96	5 Jan 96	16 Jan 96
	AF	15 Mar 96	18 Mar 96	20 Mar 96

NAME & ADDRESS OF APPLICANT:

Knoll Pharmaceutical Company
 300 Continental Drive - North
 Mt. Olive, NJ 07828-1234

DRUG PRODUCT NAME:

Proprietary:	Mavik
Nonproprietary/USAN:	Trandolapril
Code Name/#:	
Chem.Type/Ther.Class:	I S

PATENT STATUS:

Trandolapril is claimed in US 4,933,361 issued 12 Jun 90,
 and is assigned to Hoechst AG

PHARMACOL.CATEGORY/INDICATION:

ACE inhibitor for treatment of hypertension

DOSAGE FORM:

TCM

STRENGTHS:

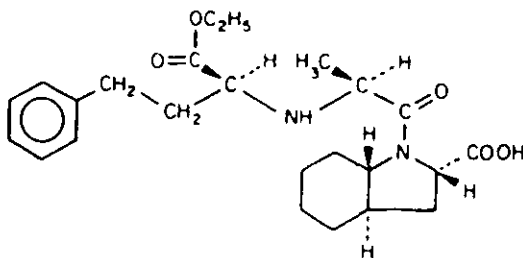
1, 2, 4 mg

ROUTE OF ADMINISTRATION:

Oral

DISPENSED:

Rx OTC

STRUCTURAL FORMULA, CHEMICAL NAME, MOLECULAR FORMULA, MOLECULAR WEIGHT:

[2S-(1(R*(R*)),2a,3aα,7aβ)]-1-[2-[[1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-
 octahydro-1H-indole-2-carboxylic Acid

C₂₄H₃₄N₂O₅

430.53

SUPPORTING DOCUMENTS:

*Packaging components which have previously been approved for other applications.

RELATED DOCUMENTS (if applicable):

CONSULTS:

Division of Biopharmaceutics

REMARKS/COMMENTS:

The amendment of 4 Jan 96 provides responses to the CMC deficiencies included in the Agency's APPROVABLE letter of 25 Sep 95.

The amendment of 15 Mar 96 provides a revised Package Insert (PI). The DESCRIPTION and HOW SUPPLIED sections were modified to comply with my requests in CR1. The PI is satisfactory with respect to the technical aspects of the labeling.

Methods validation will be requested as soon as the revised methods validation volumes are received from the applicant.

Trademark is acceptable.

FUR was requested on 25 Jan 96 and found acceptable on 21 Feb 96..

CONCLUSIONS & RECOMMENDATIONS:

APPROVAL is recommended in regard to the CMC portion of the application.

cc:

Orig. NDA

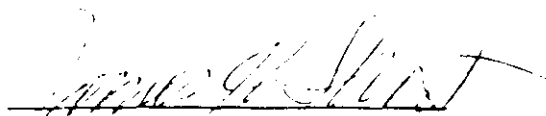
HFD-110/Division File

HFD-110/JShort/1/17/96

HFD-110/CSO

HFD-810/CHoberg

R/D Init by: RWolters/1/21/96


James H. Short, Ph.D., Review Chemist

filename: N20-528.CR4

APR 25 1996

DIVISION OF CARDIO-RENAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-528

CHEM.REVIEW #: 5

REVIEW DATE: 24 Apr 96

<u>SUBMISSION</u>	<u>TYPE</u>	<u>DOCUMENT DATE</u>	<u>CDER DATE</u>	<u>ASSIGNED DATE</u>
ORIGINAL		28 Sep 94		
AMENDMENT	BF	18 Apr 96	19 Apr 96	24 Apr 96

NAME & ADDRESS OF APPLICANT:

Knoll Pharmaceutical Company
 3000 Continental Drive North
 Mt. Olive, NJ 07828-1234

DRUG PRODUCT NAME:

Proprietary:	Mavik
Nonproprietary/USAN:	Trandolapril
Code Name/#:	
Chem.Type/Ther.Class:	I S

PATENT STATUS:

Trandolapril is claimed in US 4,933,361 issued 12 Jun 90, and is assigned to Hoechst AG

PHARMACOL.CATEGORY/INDICATION:

ACE inhibitor for treatment of hypertension

DOSAGE FORM:

TCM

STRENGTHS:

1, 2, 4 mg

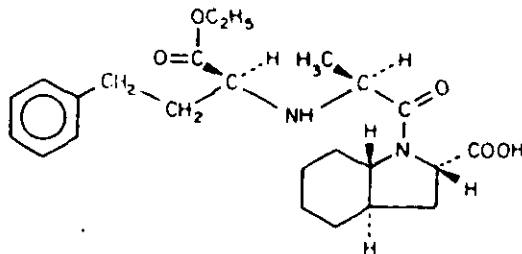
ROUTE OF ADMINISTRATION:

Oral

DISPENSED:

Rx OTC

STRUCTURAL FORMULA, CHEMICAL NAME, MOLECULAR FORMULA, MOLECULAR WEIGHT:



[2S-(1(R*(R*)),2a,3aα,7aB)]-1-[2-[[1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-octahydro-1H-indole-2-carboxylic Acid

C₂₄H₃₄N₂O₅

430.53

SUPPORTING DOCUMENTS:

RELATED DOCUMENTS (if applicable):

CONSULTS:

Division of Biopharmaceutics

REMARKS/COMMENTS:

The amendment of 18 Apr 96 provides final printed labels, as requested in the Agency's Approvable letter of 25 Sep 96, for Mavik Tablets, 1, 2 and 4 mg, packaged in bottles of 100s and in unit dose blister packages of 7s. The later are physicians' samples.

The bottle labels, as well as the unit dose carton labels, are in compliance with the technical aspects of the labeling, as required (or suggested) by 21 CFR 201, pertaining to the following: proprietary name, established name, strength, net contents, storage conditions, prescription legend, dosage recommendation, NDC number, and the applicant's name and address. There is a blank space for imprinting the lot number and expiration date on the labels, and provision is made for the lot number and expiration date on the carton labels. The storage statement reads "Store at controlled room temperature: 20-25°C (68-77°F) see USP." The bottle labels include the following statement "Dispense in well-closed container with safety closure." The carton labels include the following statement "This package is not child resistant."

The cards, which actually contain the tablets packaged in the cartons, contain the same information as do the cartons.

Methods validation requests, dated 15 Apr 96, have been sent to NWK-DO and to DDA.

CONCLUSIONS & RECOMMENDATIONS:

The technical aspects of the labels are satisfactory.

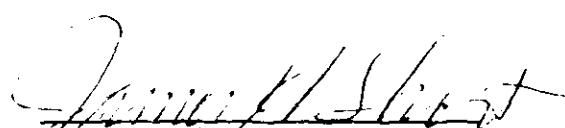
APPROVAL is recommended in regard to the CMC portion of the application.

cc:

Orig. NDA
HFD-110/Division File
HFD-110/JShort/4/24/96
HFD-110/CSO

R/D Int by: RWalters/

RWalters
9/12/96


James H. Short, Ph.D., Review Chemist
filename: N20-528.CR5

Kelani
Rec'd
6/13/9

Statistical Review and Evaluation

NDA #: 20-528

Date:

JUN - 7 1995

Applicant: Knoll Pharmaceutical Company

Name of Drug: Mavik (Trandolapril) Tablets

Documents Reviewed:

1. NDA submission volumes 1.45 to 1.48 "Potential Tumorigenic Effects to Mice by Oral Administration (Gavage) 78 Weeks", June 16, 1989, Date of Documents, Sept. 28, 1994.
2. NDA submission volumes 1.49 to 1.54 "Potential Tumorigenic Effects in long Term Administration (Gavage) to Rats for 104 Weeks", June 19, 1989, Date of Documents, Sept. 28, 1994.
3. NDA Original Amendment, statistical analyses of tumor data, April 14, 1995, Date of Documents, April 24, 1995. " "

I. Background

Two animal carcinogenicity studies (one in rats and one in mice) were included in this NDA submission. The purpose of these studies was to assess the potential tumorigenicity of the test material, Mavik (Trandolapril) tablets, when administered orally (gavage) to rats and mice for 104 and 78 weeks, respectively. Dr. ~~Thomas Papoian~~, Ernest DeBor HFD-110, who is the reviewing pharmacologist of this NDA has requested the Division of Biometrics to perform the statistical review and evaluation of these two studies.

II. The Rat Study

II. a. Design

In this study, 240 male and 240 female Sprague Dawley Cr1 CD(SD)BR rats were randomly distributed into four groups of 60 animals each. Three groups of rats received the test substance RU 44570 by oral route (gavage) at dose levels of 0.25, 2, and 8 mg/kg/day, respectively, for 104 weeks. From the beginning of the treatment until the week 17, the highest dose level of 16 mg/kg/day was used, then this dose was decreased and fixed at 8 mg/kg/day at the request of the sponsor. A fourth group of rats receiving the vehicle (0.5% methylcellulose solution in distilled water) by the same route acted as control. Body weights and food consumption were measured at intervals during the study. Clinical examination was performed at least once daily. A complete gross examination was performed on all animals including those that died during the experiment or were killed in moribund conditions. Microscopical examinations were performed on all tissues of animals in the high dose and control groups, and animals that died or were sacrificed prematurely. Microscopical examinations were also performed on all tissues of

animals showing macroscopical abnormalities in the medium and low dose groups.

II. b. Sponsor's Analyses

No statistical analysis was performed on survival data. The percentages of the surviving animals were calculated every 4 weeks and graphed in Figures 1 and 2 for males and females, respectively. The sponsor indicated that the distribution of the factors contributing to death or morbidity was comparable among the control and treated animals and showed no disturbance as result of treatment. In addition, the major factor contributing to death or morbidity in all groups including the control group was a pituitary tumor.

The methods given in the paper of Peto et al. ("Guidelines for Simple, Sensitive Significance Tests for Carcinogenic Effects in Long-Term Animal Experiments", In Long-Term and Short-Term Screening Assays for Carcinogens: A Critical Appraisal, International Agency for Research on Cancer Monographs, Annex to Supplement 2, World Health Organization, 311-426, 1980) were used to test the linear trend in the tumor data. The ordinal scores, 1, 2, 3, and 4 were assigned to the control, low, medium, and high dose groups. The 'ad hoc runs' procedure described in the above paper was used to find time intervals for the Peto method. Table 1 listed the p-values of Peto trend test on neoplastic lesions. Table 2 listed the numbers of animals with neoplastic lesions by organ/group/sex. The results of the above analyses showed that there were significant (at 0.05 level) positive linear trends in the pituitary gland chromophobe cell adenoma ($p = 0.0375$), adrenal glands pheochromocytoma ($p = 0.0197$), and mammary gland adenofibroma ($p = 0.0367$) in female rats. In addition, there are marginally significantly positive linear trends in the brain astrocytoma ($p = 0.0548$), and skin malignant histiocytoma ($p = 0.0571$) in females.

The sponsor concluded that "the test substance had neither carcinogenic potential nor increase the incidence of spontaneously occurring tumors."

II.c. Reviewer's analyses and Comments

The intercurrent mortality rates for both male and female rats (see Table 3) were tested for linear trend according to the death rate method described in the paper of Peto et al. (1980) using the time intervals 0-52, 53-80, and 81-106 weeks. The actual dose levels 0, 0.25, 2, and 8 mg/kg/day were the scores assigned to the control, low, medium, and high dose groups, respectively. The results of the analyses showed that there was no significant (at 0.05 level) linear trend in the intercurrent mortality rate in female ($p = 0.4459$) and male ($p = 0.4693$) rats.

Since the tumor data are not available for analysis in FDA formats or STUDIES formats at the time of review, the following tumor data

analyses were based on the summary incidence table as shown in Table 2. The methods given in the paper of Peto et al. (1980) and the methods of exact permutation trend test were used to test the linear trend in the tumor data. Noted that some tissues were not microscopically examined on all of the animals. Due to the limitation of the data available for analysis, the age-unadjusted method was applied to all of the data of the tumor/organ combinations. The pairwise comparisons between the high dose and control groups were also performed. The results of the above analyses showed that there were significant (at 0.05 level) positive linear trends in the pituitary gland chromophobe cell adenoma (trend: $p = 0.0043$; pairwise: $p = 0.0816$) and adrenal glands pheochromocytoma (trend: $p = 0.0252$; pairwise: $p = 0.0287$) in female rats. In addition, there is a marginally significantly positive linear trend in the mammary gland adenofibroma in females (trend: $p = 0.0919$; pairwise: $p = 0.1769$).

The incidence rates of the above tumor types and the numbers of animals microscopically examined are listed as follows:

Tumor/organ	Incidence of Tumors				P-values	
					Trend	Pairwise
Females:						
Pituitary Gland:						
Chromophobe Cell Adenoma	(60)	(40)	(46)	(60)		
	8	2	4	15	0.0043*	0.0816
Adrenal Glands:						
Pheochromocytoma	(60)	(35)	(37)	(60)		
	0	2	1	5	0.0252	0.0287
Mammary Gland:						
Adenofibroma	(60)	(46)	(43)	(59)		
	1	1	3	4	0.0919	0.1769

In order to reduce the overall false positive rate, the following decision rule was used to adjust the effect of multiple testings. A positive linear trend is considered not to occur by chance of variation alone if the p-value is less than 0.005 for a common tumor, and 0.025 for a rare tumor.

On the basis of the above rule, the positive linear trends with p-values marked with an asterisk in the above table are considered to be statistically significant.

III. The Mouse Study

III. a. Design

In this study, the test substance was orally (gavage) administered to three groups of 60 male and 60 female Crl:CD-1 (-1CR)BR mice each at the dose levels of 1, 5, and 25 mg/kg/day for 78 weeks. A fourth group of 60 male and 60 female mice received the vehicle (0.5% metnylcellulose solution in distilled water) by the same route and the same volume and acted as control. A clinical examination was performed at least once daily. Body weights and food consumption were measured at intervals during the study. A complete gross examination was performed on all animals including those that died during the experiment or were killed in moribund conditions. Microscopical examinations were performed in all animals in the high dose and control groups that were sacrificed at the end of the treatment period, or died or were sacrificed prematurely. Microscopical examinations were also performed on all tissues of animals showing macroscopical abnormalities in the intermediate and low dose level groups.

III. b. Sponsor's Analyses

No statistical analysis was performed on survival data. The percentages of the surviving animals were calculated every 4 weeks and graphed in Figures 3 and 4 for males and females, respectively. The sponsor indicated that the rates of mortality evaluated on completion of the study revealed no dose-relationship and no marked difference between treated and control groups.

Similar to the rat study, Peto methods were used to test the linear trend in the tumor data. The ordinal scores, 1, 2, 3, and 4 are assigned to the control, low, medium, and high dose groups. The 'ad hoc runs' procedure described in the above paper was used to find time intervals applied to Peto method. Table 4 listed the numbers of animals with neoplastic lesions by organ/group/sex. Table 5 listed the p-values of Peto trend test on neoplastic lesions. The test results of the analyses showed that no dose-related trend was detected in the tumor data.

Based on the results of the above analyses, the sponsor concluded that "the test substance administered daily by oral route in mice over a period of 78 weeks at the dose levels of 1, 5, and 25 mg/kg/day induced an increased incidence of nephropathy at the intermediate and high dose level groups. However, it had neither carcinogenic potential, nor increased the frequency of spontaneously occurring tumors."

III.c. Reviewer's analyses and Comments

The intercurrent mortality rates for both male and female mice (see Table 6) were tested for linear trend according to the death rate method described in the paper of Peto et al. (1980) using the time intervals 0-52 and 53-80 weeks. The actual dose levels 0, 1, 5, and

25 mg/kg/day were the scores assigned to the control, low, medium, and high dose groups, respectively. The results of the analyses showed that there was no significant (at 0.05 level) linear trend in the intercurrent mortality rate in female ($p = 0.4319$) and male ($p = 0.1568$) mice.

Similar to the rat study, the tumor data are not available for analysis in FDA formats or STUDIES formats at the time of review, the age-unadjusted Peto and exact permutation trend tests were used to test the linear trend in the tumor data as shown in Table 4. The pairwise comparisons between the high dose and control groups were also performed. The test results of the analyses showed that no dose-related trend was detected in the tumor data.

The reviewer also evaluated the validity of the experiment. The following two issues are important in determining the validity of an experiment: (1) The numbers of animals alive over the course of the study to get an adequate exposure to the chemical and to be at risk of forming late-developing tumors. (2) If the doses are high enough to present a reasonable tumor challenge to the animals.

With regard to the first issue, the following criteria or rules of thumb have been proposed by some experts in the field:

(A) Haseman proposes (through personal communication with Dr. Karl Lin) that a 50% survival rate of the 50 initial animals in the high dose group between weeks 80-90 will be considered as a sufficient number and an adequate exposure. However, the percentage can be lower or higher if the number of animals used in each treatment/sex is larger or smaller than 50 as long as there will be between 20 to 30 animals still alive during these weeks.

(B) Chu, Ceuto, and Ward ("Factors in the Evaluation of 200 National Cancer Institute Carcinogen Bioassays", Journal of Toxicology and Environmental Health, 8, 1981, pp. 251-280) propose that an experiment that has not shown a chemical to be carcinogenic should have (high dose) groups of animals with greater than 50% survival at one year (52 weeks).

In this study, the survival rates of the high dose mice at one-year were 92% and 97% for males and females, respectively. These one-year survival rates satisfy the criterion of Chu et al. (1981). The survival rates of the control, low, medium, and high dose mice in the terminal sacrifice were 88%, 87%, 85%, and 82%, respectively, for males, and 72%, 77%, 80%, and 75%, respectively, for females. There were sufficient mice in the treated groups living long enough to get an adequate exposure to the chemical and to be at risk of forming late-developing tumors based on Haseman's proposition.

With regard to the second issue, in the paper of Chu, Ceuto and Ward (1981), the following criteria for dose adequacy are mentioned.

(A) "A dose is considered adequate if there is a detectable loss in weight gain of up to 10% in a dosed group relative to the controls."

(B) "The administered dose is also considered an MTD (Maximum Tolerated Dose) if dosed animals exhibit clinical signs or severe histopathologic toxic effects attributed to the chemical."

(C) "In addition, doses are considered adequate if the dosed animals show a slightly increased mortality compared to the controls."

Figures 5 and 6 plotted the mean body weight versus time in weeks for males and females, respectively. In Tables 7-8, summaries of mean body weight data of the male and female mice are given. The body weight gains in the male low, medium, and high dose groups are 8.3%, 16.66%, and 16.66%, respectively, less than that of the male control group. The female low and medium dose groups gain more weights (11.11%) than that of the female control group. The female high dose group gained the same weight as that of the female control group. It seems that the high dose is not close to MTD for females based on body weight gain data. However, before drawing the final conclusion on the appropriateness of the selected high dose, the clinical and toxic signs of the treated animals still need to be checked.

V. Summary

IV.a. The Rat Study

The sponsor applied Peto's method to test the linear trend in the tumor data. The results of the analyses showed that there were significant (at 0.05 level) positive linear trends in the pituitary gland chromophobe cell adenoma ($p = 0.0375$), adrenal glands pheochromocytoma ($p = 0.0197$), and mammary gland adenofibroma ($p = 0.0367$) in female rats.

The reviewer applied Peto's method to test the positive trend in intercurrent mortality rates, the results of the analyses showed that no significant (at 0.05 level) linear trend in the intercurrent mortality rate was detected in both sexes.

The age-unadjusted Peto and exact permutation trend tests were used to test the linear trend in the tumor data as shown in Table 2. The pairwise comparisons between the high dose and control groups were also performed. Noted that some tissues were not microscopically examined on all of the animals. Results of tumor data analyses showed a significantly positive linear trend in the pituitary gland chromophobe cell adenoma ($p = 0.0043$) in female rats. However, there was no significant difference ($p = 0.0816$) in the incidence rates of this tumor between high and control female rats.

IV.b. The Mouse Study

No dose-related trend was detected in the tumor data in the sponsor's

statistical analyses.

The reviewer's test results revealed that there was no significant (at 0.05 level) linear trend in the intercurrent mortality rate in both sexes. The age-unadjusted Peto and exact permutation trend tests were used to test the linear trend in the tumor data as shown in Table 4. The pairwise comparisons between the high dose and control groups were also performed. The test results showed that no dose-related trend was detected in the tumor data.

The reviewer also evaluated the validity of the experiment. The following two issues are important in determining the validity of an experiment: (1) The numbers of animals alive over the course of the study to get an adequate exposure to the chemical and to be at risk of forming late-developing tumors. (2) If the doses are high enough to present a reasonable tumor challenge to the animals.

With regard to the first issue, in this study, there were sufficient mice in the treated groups living long enough to get an adequate exposure to the chemical and to be at risk of forming late-developing tumors based on Haseman's proposition. With regard to the second issue, it seems that the high dose is not close to MTD for females based on body weight gain data. However, before drawing the final conclusion on the appropriateness of the selected high dose, the clinical and toxic signs of the treated animals still need to be checked.

Daphne Lin

Daphne Lin, Ph.D.
Mathematical Statistician

Concur:

Karl K. Lin 6/7/95

Karl K. Lin, Ph.D., Group Leader, SARB

cc: Original NDA 20-528
HFD-110/Lipicky
HFD-110/Dr. Belair,
HFD-110/Dr. Papoian
HFD-110/Ms. Bongiovanni
HFD-710/Chron
HFD-715/Dr. Karl Lin
HFD-715/Dr. Daphne Lin
HFD-715/Chron (SARB)
HFD-502/Assistant Director (Pharmacology)
HFD-715/DRU 2.1.1, Mavik, Knoll Pharm. Company

Figure 1

SURVIVAL RATE - MALE

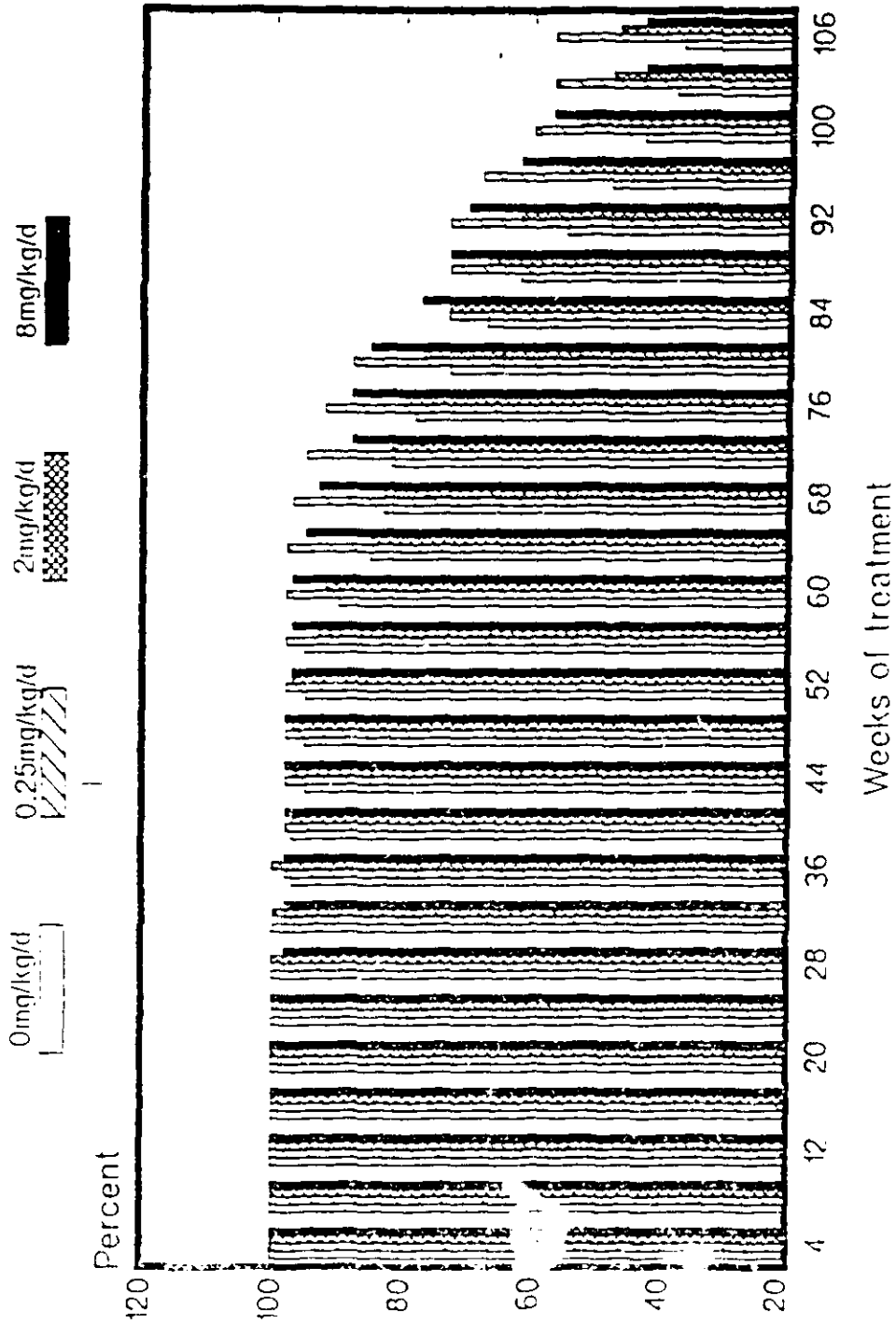
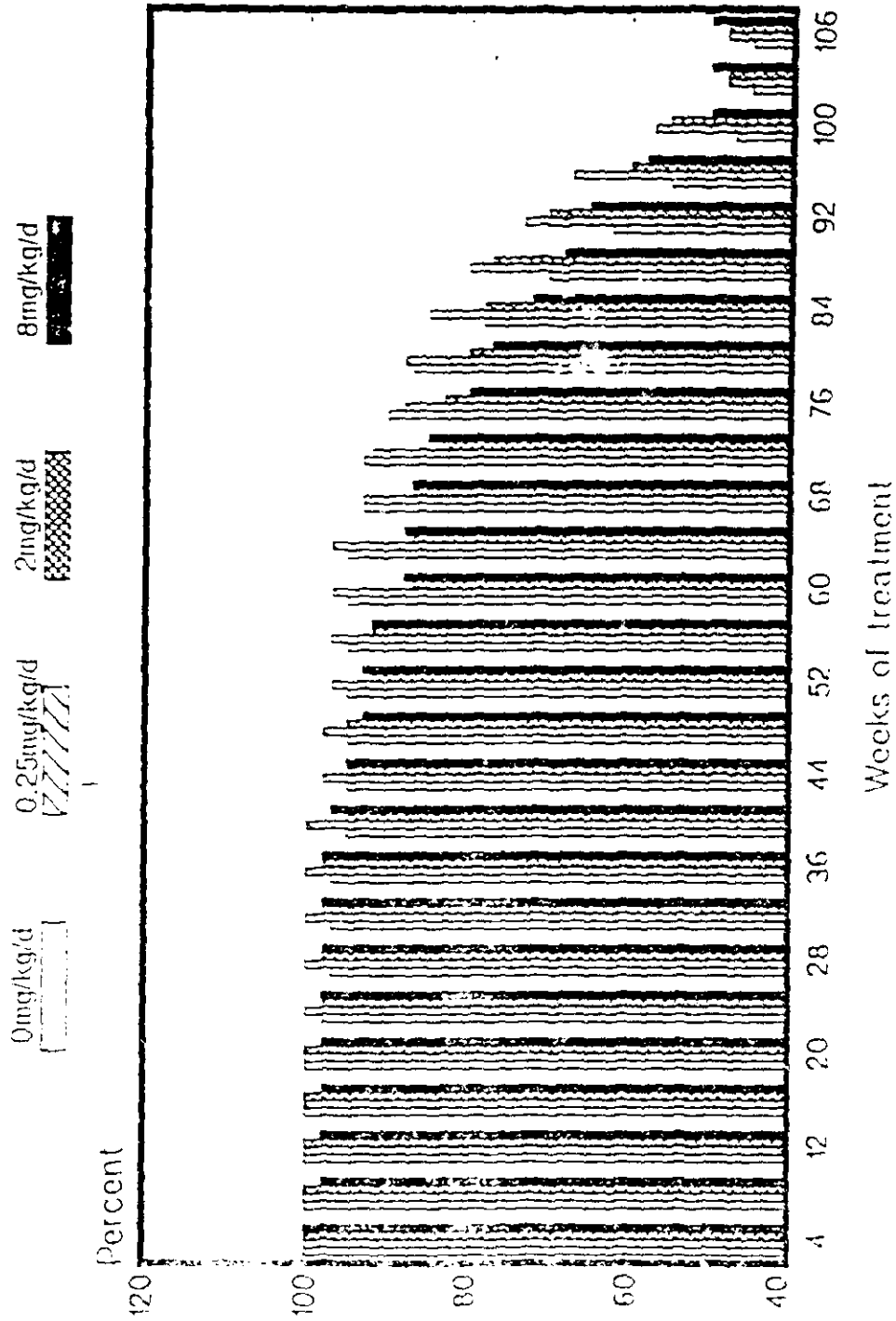


Figure 2

SURVIVAL RATE - FEMALE



SURVIVAL RATE - MALE

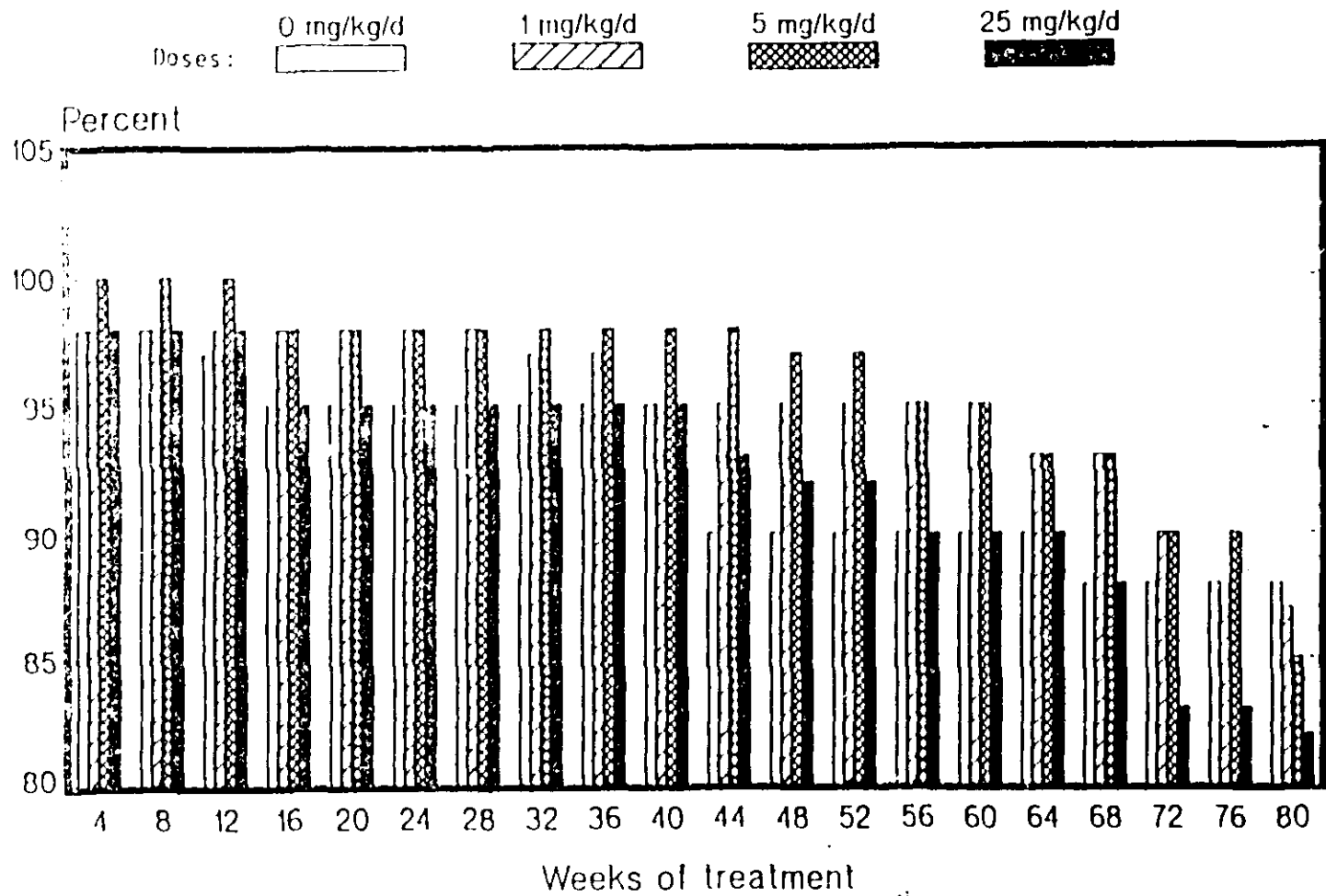


Figure 3

SURVIVAL RATE - FEMALE

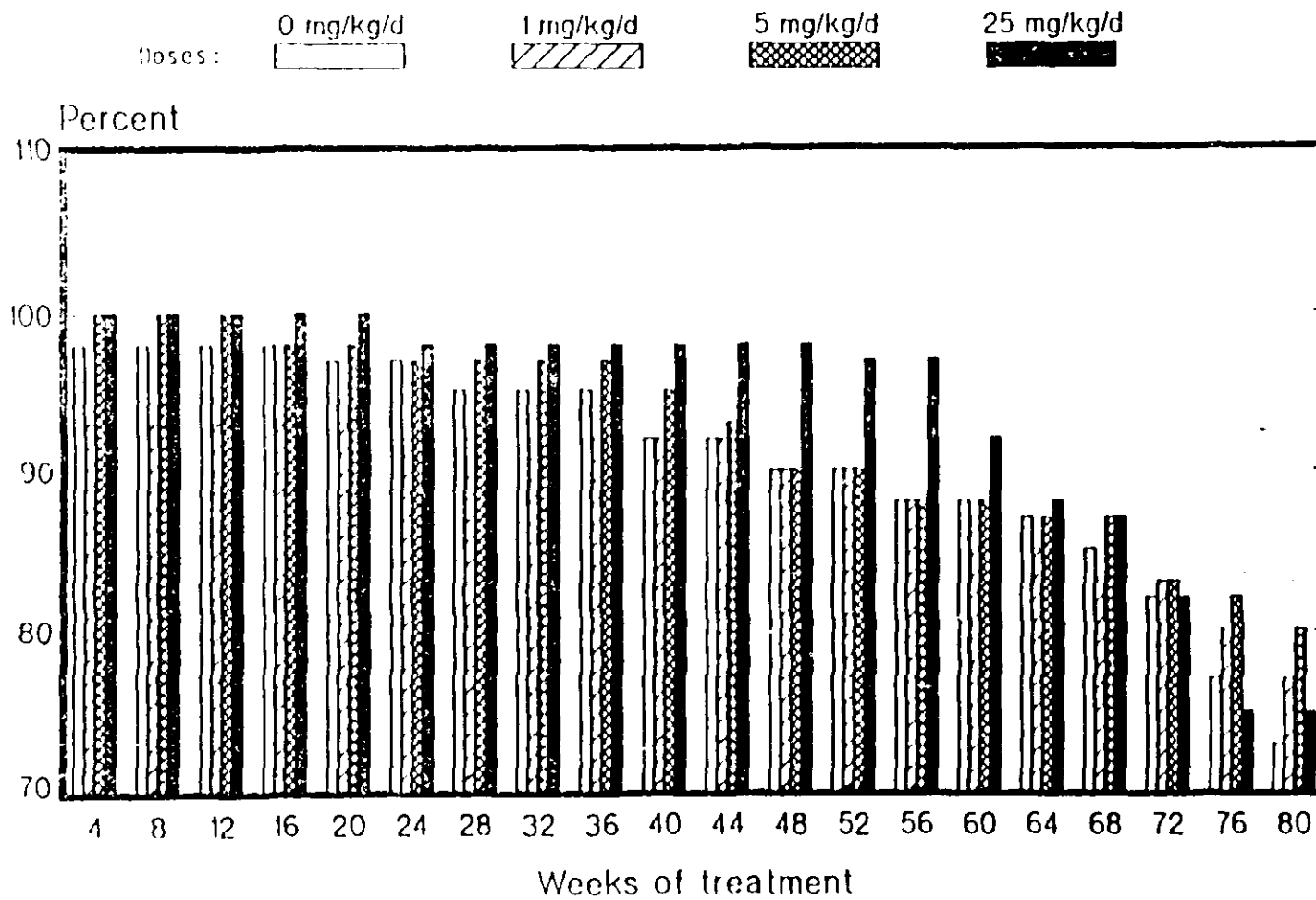


Figure 4

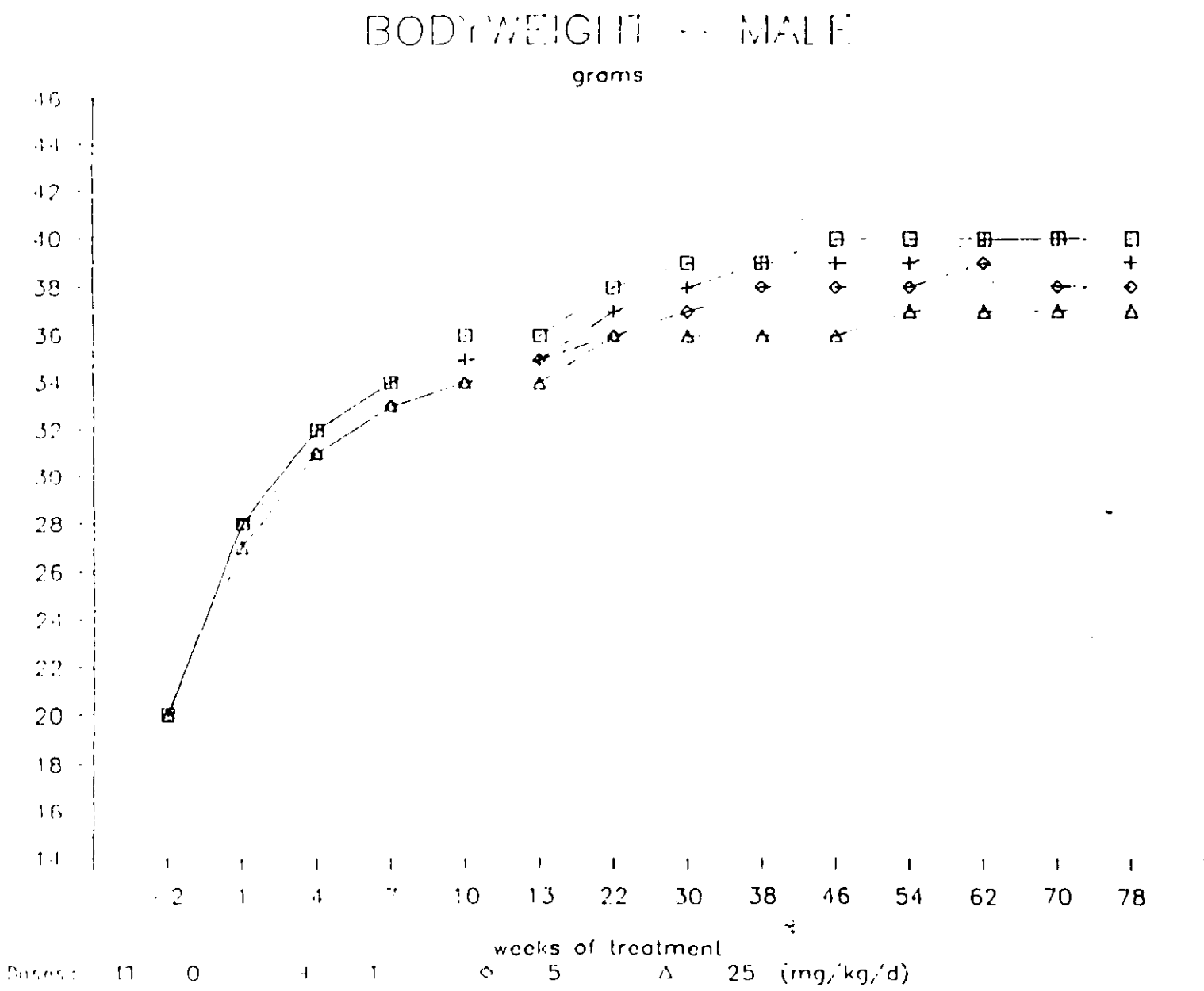


Figure 5

BODYWEIGHT -- FEMALE

grams

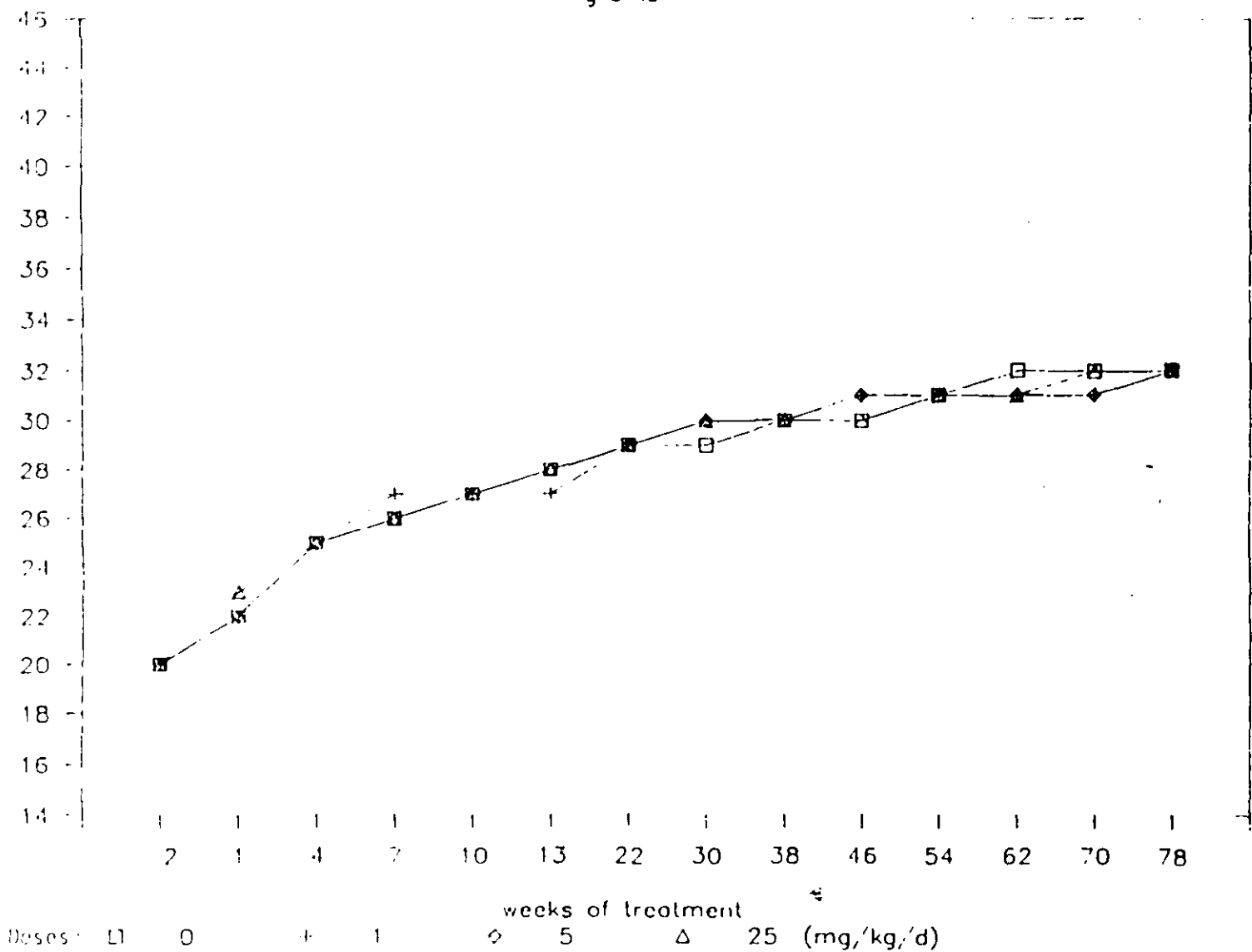


Figure 6

Table 1

PATHOLOGY REPORT (DRAFT)
STATISTICAL EVALUATION

PAGE :
CIT PROJECT: *102026

TEST ARTICLE :
TEST SYSTEM : RAT, 104 weeks, oral
SPONSOR :

PATHOL. NO.: 92026
DATE : 14-APR
PATHDATA SYSTEM V.

TREND TEST STATISTICS ON NEOPLASTIC LESIONS
COMBINED PREVALENCE AND DEATH RATE METHOD (PETO ET AL., 1980)

DOSE GROUPS : T,A,B,C
STATUS AT NECROPSY: K0 INCL. +
TIME INTERVAL FOR INCIDENTAL ANALYSIS: AD HOC RUN
SEX: F

ORGAN/TISSUE	TYPE OF NEOPLASM	TREND	P-VAL
HEART	HAEMANGIOENDOTHELIO.	1.479+	p = 0.08
LUNGS	BRONCHIO-ALVE. CARCI.	0.539-	
LUNGS	FIBROUS HISTIOCYTOMA	0.535-	
DUODENUM	LEOMYOMA.	0.929+	p = 0.22
LIVER	HEPATOCELLULAR ADEN.	4.153-	
PANCREAS	ACINAR CELL ADENOMA.	0.481+	p = 0.45
PANCREAS	ISLET CELL ADENOMA.	3.192-	
KIDNEYS	TUBULAR CELL ADENOMA	1.473-	p = 0.08
KIDNEYS	NEPHROBLASTOMA.	0.473+	p = 0.32
TESTES	INTERSTIT. CEL. TUMOUR	2.981+	p = 0.24
PITUITARY GLAND	ACIDO. CHROMO. CEL. AD.	6.368+	p = 0.25
PITUITARY GLAND	CHROMOPHOBE CEL. ADE.	8.387-	
PITUITARY GLAND	ACIDOPHIL CELL ADEN.	11.768-	
PITUITARY GLAND	ADEN. OF PARS INTERM.	1.612-	
THYROID GLAND	FARAFOLL. CEL. ADENOMA	0.698-	
THYROID GLAND	FOLLIC. CEL. ADENOMA.	7.950-	
THYROID GLAND	FOLLIC. CEL. CARCINOMA	1.615-	
ADRENAL GLANDS	PHEOCHROMOCYTOMA.	3.624-	
HEMOLYMPHORET. SYS.	MYELOID LEUKEMIA.	0.601-	
HEMOLYMPHORET. SYS.	MALIGN. LYMPH., HET. T.	1.448+	p = 0.22
HEMOLYMPHORET. SYS.	MALIGN. LYMPH., LYM-B.	0.017-	
HEMOLYMPHORET. SYS.	MALIGN. LYMPH., IMMUN.	0.901-	p = 0.28
SPLEEN	CAVERNOUS HAEMANGIO.	0.600-	
MAMMARY GLAND	FIBROADENOMA.	1.286+	p = 0.14
SKIN	PILOMATRICOMA.	1.295+	p = 0.36
SKIN	FIBROUS HISTIOCYTOMA	1.407-	
SKIN	LIPOMA.	1.852-	
SKIN	FIBROMA.	1.709+	p = 0.33

Table 1 (continued)

PATHOLOGY REPORT (DRAFT) PAGE :
 STATISTICAL EVALUATION CIT PROJECT: *102026

 TEST ARTICLE : PATHOL. NO.: 92026
 TEST SYSTEM : RAT, 104 weeks, oral DATE : 14-AP
 SPONSOR : PATHDATA SYSTEM V

TREND TEST STATISTICS ON NEOPLASTIC LESIONS
 COMBINED PREVALENCE AND DEATH RATE METHOD (PETO ET AL., 1980)

DOSE GROUPS : T,A,B,C SEX: I
 STATUS AT NECROPSY: K0 INCL. +
 TIME INTERVAL FOR INCIDENTAL ANALYSIS: AD HOC RUN

ORGAN/TISSUE	TYPE OF NEOPLASM	TREND	P-VA
SKIN	TRICHOFOILICULOMA.	3.165-	
SKIN	BASAL CELL TUMOUR.	0.198-	
SKIN	HISTIOCYTOMA.	1.472-	
SKIN	FIBROSARCOMA.	1.425-	p = 0.11
SKIN	FIBROLOPOMA.	0.585-	
SKELFETAL MUSCLE	RHABDOMYOMA.	0.585-	
SKELFETAL MUSCLE	LIPOSARCOMA.	0.525-	
OTHER ORGANS/TISSUES	LIPOMA, ADIPOSE TIS.	1.186-	p = 0.29
OTHER ORGANS/TISSUES	MESOTHELIOMA.	0.000-	p = 0.50
OTHER ORGANS/TISSUES	PAPILLOMA, TONGUE.	0.900-	

DOSE GROUPS : T,A,B,C SEX: FE
 STATUS AT NECROPSY: K0 INCL. +
 TIME INTERVAL FOR INCIDENTAL ANALYSIS: AD HOC RUN

ORGAN/TISSUE	TYPE OF NEOPLASM	TREND	P-VA
BRAIN	EPENDYMOA.	0.988-	p = 0.29
✓ BRAIN	ASTROCYTOMA.	1.690-	p = 0.05
HEART	HAEMANGIOENDOTHELIO.	0.049-	
LIVER	HEPATOCELLULAR ADEN.	1.703-	
LIVER	HEPATOCELLULAR CARC.	0.667-	p = 0.26
PANCREAS	ACTINAR CELL ADENOMA.	1.450-	p = 0.16
PANCREAS	ISLET CELL ADENOMA.	2.954-	
UTERUS	ENDOMETR. STROM. POLYP	2.377-	p = 0.14
VAGINA	HAEMANGIOMA.	1.631-	p = 0.07

Table 1 (continued)

PATHOLOGY REPORT (DRAFT)	PAGE	:
STATISTICAL EVALUATION	CIT PROJECT:	*102026

TEST ARTICLE :	PATHOL. NO.:	92026
TEST SYSTEM : RAT, 104 weeks, oral	DATE :	14-AP
SPONSOR :	PATHDATA SYSTEM	V

TREND TEST STATISTICS ON NEOPLASTIC LESIONS
 COMBINED PREVALENCE AND DEATH RATE METHOD (PETO ET AL., 1980)

DOSE GROUPS : T,A,B,C SEX: FE
 STATUS AT NECROPSY: K0 INCL. +
 TIME INTERVAL FOR INCIDENTAL ANALYSIS: AD HOC RUN

ORGAN/TISSUE	TYPE OF NEOPLASM	TREND	P-VA
PITUITARY GLAND	ACIDO. CHROMO. CEL. AD.	9.090-	
✓ PITUITARY GLAND	CHROMOPHOBE CEL. ADE.	10.257+	p = 0.03
PITUITARY GLAND	ACTIDOPHIL CELL ADEN.	3.443-	
THYROID GLAND	PARAFOLL. CEL. ADENOMA	0.000+	p = 0.50
THYROID GLAND	FOLLIC. CEL. ADENOMA.	2.774+	p = 0.05
✓ ADRENAL GLANDS	PHEOCHROMOCYTOMA.	6.758+	p = 0.02
HEMOLYMPHORET. SYS.	MYELOID LEUKEMIA.	0.490+	p = 0.37
HEMOLYMPHORET. SYS.	MALIGN. LYMPH., HET. T.	0.000+	p = 0.50
HEMOLYMPHORET. SYS.	MALIGN. LYMPH., LYM-B.	0.500-	
HEMOLYMPHORET. SYS.	MALIGN. LYMPH., LYM-C.	0.488+	p = 0.32
MAMMARY GLAND	FIBROADENOMA.	0.781+	p = 0.45
MAMMARY GLAND	ADENOMA.	2.284-	
MAMMARY GLAND	CYSTADENOMA.	6.920-	
✓ MAMMARY GLAND	ADENOFIBROMA.	6.097+	p = 0.03
MAMMARY GLAND	ADENOCARCINOMA.	3.607-	
MAMMARY GLAND	DUCTULAR ADENOCARCI.	0.561-	
SKIN	FIBROUS HISTIOCYTOMA	1.679+	p = 0.14
SKIN	LIPOMA.	0.714-	
SKIN	FIBROMA.	1.571-	
SKIN	BASAL CELL TUMOUR.	2.029-	
SKIN	HISTIOCYTOMA.	0.705+	p = 0.37
SKIN	FIBROSARCOMA.	1.552+	p = 0.08
✓ SKIN	MALIGN. HISTIOCYTOMA.	1.711+	p = 0.05
OTHER ORGANS/TISSUES	LIPOMA, ADIPOSE TIS.	1.083-	
OTHER ORGANS/TISSUES	HAEMANGIOMA, MESENT.	1.667-	
OTHER ORGANS/TISSUES	HAEMNAGIOPERIC, MES.	0.000+	p = 0.50

END OF REPORT SECTION

PATHOLOGY REPORT
 SUMMARY TABLES

 PAGE : 138
 CIT PROJECT : 2025 TCR

 TEST ARTICLE
 TEST SYSTEM : RAT, 104 weeks, oral
 SPONSOR

 PATHOL. NO.: 92025 MAA
 DATE : 01-MAR-89
 PDS PATHDATA SYSTEM TM

 NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
 STATUS AT NECROPSY: KO, INCL. +

ORGAN/FINDING	DOSE GROUP: SEX NO. ANIMALS:	T		A		B		C	
		M	F	M	F	M	F	M	F
		60	60	60	60	60	60	60	60
HEMOLYMPHORET. SYS.	NO. EXAM.:	1		3	1	4	2	2	1
- MYELOID LEUKEMIA.		1		1		1	1	1	
- MALIGN. LYMPH., HET. T.				1		1		1	1
- MALIGN. LYMPH., LYM-B.				1	1	1			
- MALIGN. LYMPH., IMMUN.						2			
- MALIGN. LYMPH., LYM-C.							1		
HEART	NO. EXAM.:	60	60	26	31	32	32	60	60
- HAEMANGIOENDOTHELIO.					1		1		
BRAIN	NO. EXAM.:	60	60	26	31	32	31	60	60
- EPENDYMOMA.			1				1		1
- ASTROCYTOMA.									1
DUODENUM	NO. EXAM.:	51	60	23	28	29	25	55	60
- LEOMYOMA.									1
LIVER	NO. EXAM.:	60	60	41	39	39	40	60	60
- HEPATOCELLULAR ADEN.		2		5	3	1		1	
- DUCTUL. ADENOC., META.					1				
- HEPATOCELLULAR CARC.							1		
PITUITARY GLAND	NO. EXAM.:	60	60	33	40	43	46	60	60
- ACIDO. CHROMO. CEL. AD.		18	31	14	27	24	24	21	27
- CHROMOPHOBE CEL. ADE.		15	8	6	2	3	4	10	15
- ACIDOPHIL CELL ADEN.		15	15	6	6	3	11	10	13
- ADEN. OF PARS INTERM.		1							
SKELETAL MUSCLE	NO. EXAM.:	60	60	29	31	32	31	60	60
- RABDOMYOMA.				1					
- LIPOSARCOMA.				1					

Table 2 (continued)

PATHOLOGY REPORT .
SUMMARY TABLES

PAGE . : 139
CIT PROJECT : 2026 TCR

TEST ARTICLE :
TEST SYSTEM : RAT, 104 weeks, oral
SPONSOR :

PATHOL. NO.: 92026 MAA
DATE : 01-MAR-89
PDS PATHDATA SYSTEM TM

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. +

ORGAN/FINDING	DOSE GROUP: SEX : NO. ANIMALS:	T		A		B		C	
		M	F	M	F	M	F	M	F
		60	60	60	60	60	60	60	60
SKIN	NO. EXAM.:	59	60	37	34	35	34	59	59
- FILOMATRICOMA.		2		2		3		3	
- FIBROUS HISTIOCYTOMA		2		3	1	2	1	2	1
- LIPOMA.		2			2		1	1	
- FIBROMA.		1	1	3		5		2	
- TRICHOFFOLLICULOMA.		3				1		1	
- BASAL CELL TUMOUR.		1	1	1	1	1		1	
- HISTIOCYTOMA.		1	1				1		1
- FIBROSARCOMA.								1	1
- MALIGN. HISTIOCYTOMA.								1	1
- FIBROLOPOMA.				1					
MAMMARY GLAND	NO. EXAM.:	59	60	25	46	32	43	60	59
- FIBROADENOMA.			14		19		20	1	13
- ADENOMA.			6		8		1		6
- CYSTADENOMA.			7		6		6		2
- ADENOFIBROMA.			1		1		3		4
- ADENOCARCINOMA.			2		1				
- DUCTULAR ADENOCARCI.					2				
ADRENAL GLANDS	NO. EXAM.:	59	60	30	35	33	37	60	60
- PHEOCHROMOCYTOMA.			8		2	2	3	1	6
THYROID GLAND	NO. EXAM.:	60	59	31	31	32	31	60	60
- PARAFOLL. CEL. ADENOMA			1		1		1	1	
- FOLLIC. CEL. ADENOMA.			4		3				2
- FOLLIC. CEL. CARCINOMA			1						
PANCREAS	NO. EXAM.:	59	60	28	33	30	31	60	60
- ACINAR CELL ADENOMA.			1		4		1	3	1
- ISLET CELL ADENOMA.			2	2					
SPLEEN	NO. EXAM.:	59	60	25	31	32	31	60	60
- CAVERNOUS HAEMANGIO.				1					
LUNGS	NO. EXAM.:	60	60	30	32	33	34	60	60
- MAMA. ADENOCARC. MET.			1						
- BRONCHIO-ALVE. CARCI.				1					
- FIBROUS HISTIOCYTOMA				1					

Table 2(continued)

PATHOLOGY REPORT .
SUMMARY TABLES

PAGE . . . : 140
CIT PROJECT : 2026 TCR

TEST ARTICLE :
TEST SYSTEM : RAT, 104 weeks, oral
SPONSOR :

PATHOL. NO.: 92026 MAA
DATE : 01-MAR-89
FDS PATHDATA SYSTEM TM

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. +

ORGAN/FINDING	DOSE GROUP: SEX :	T		A		B		C	
		M	F	M	F	M	F	M	F
		NO. ANIMALS:		60	60	60	60	60	60
KIDNEYS	NO. EXAM.:	53	60	39	36	46	36	60	60
- TUBULAR CELL ADENOMA								1	
- NEPHROBLASTOMA.						1			
TESTES	NO. EXAM.:	60		39		41		50	
- INTERSTIT. CEL. TUMOUR		2		3		5		4	
UTERUS	NO. EXAM.:		60		35		38		59
- ENDOMETR. STROM. POLYP					1				2
VAGINA	NO. EXAM.:		59		32		29		58
- HAEMANGIOMA.									1
SEMINAL VESICLES	NO. EXAM.:	60		32		35		60	
- BRON. ALVED. CARC., ME.				1					
LYMPH NODES	NO. EXAM.:	8	3	13	4	13	2	17	5
- MALIG. HISTIOCYT., ME.									1
- BRON. ALVED. CARC., ME.				1					
OTHER ORGANS/TISSUES	NO. EXAM.:	8	3	14	6	10	3	8	5
- LIPOMA, ADIPOSE TIS.				2	2	3		1	
- MESOTHELIOMA.								1	
- HAEMANGIOMA, MESENT.			1						
- HAEMNAGIOPERIC., MES.					1				
- PAPILLOMA, TONGUE.				1					

Table 3
Intercurrent Mortality Rates
Male Rats

<u>Weeks</u>	<u>Control</u>			<u>Low</u>			<u>Medium</u>			<u>High</u>		
	S	D	%	S	D	%	S	D	%	S	D	%
1-52	60	3	5	60	1	1.6	60	2	3.3	60	2	3.3
53-80	57	13	22.8	59	6	10.1	58	12	20.6	58	7	12.1
81-106	44	22	50	53	19	35.8	46	18	39.1	51	25	49
Term.	22			34			28			26		"

Peto Test: p-value = 0.4693

Female Rats

<u>Weeks</u>	<u>Control</u>			<u>Low</u>			<u>Medium</u>			<u>High</u>		
	S	D	%	S	D	%	S	D	%	S	D	%
1-52	60	3	5	60	2	3.3	60	5	8.3	60	4	6.6
53-80	57	5	8.7	58	5	8.6	55	7	12.7	56	10	17.8
81-106	52	25	48.1	53	24	45.3	48	19	39.6	46	16	34.7
Term.	27			29			29			30		

Peto Test: p-value = 0.4459

Notes: S: Number of animals starting during the period
D: Deaths
%: Percent of death during the period

Table 4

PATHOLOGY REPORT
SUMMARY TABLES

PAGE : 25
CIT PROJECT : 2035 TCS

EST ARTICLE :
TEST SYSTEM : MOUSE, 78 weeks, oral
SPONSOR :

PATHOL. NO.: 92035 MAA
DATE : 21-OCT-88
PDS PATHDATA SYSTEM TM

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. +
NEOPLASTIC FINDINGS

ORGAN/FINDING	DOSE GROUP: SEX NO. ANIMALS:	T		A		B		C	
		M	F	M	F	M	F	M	F
		60	60	60	60	60	60	60	60
HEMOLYMPHRET. SYS.	NO. EXAM.:	1	4	2	1	3	1	3	
- MALIGNANT LYMPH. P.D.		1	2	2	1	2	1	3	
- MALIGNANT LYMPH. H.T.			2						
- MYELOID LEUKEMIA.						1			
.....									
OVARIES	NO. EXAM.:		60	47		44		60	
- PAPILLARY CYSTADENO.			2			1			
- HAEMANGIOMA.			1						
- GRANULOSA CELL TUMO.			2						
- LUTEOMA.				1					
.....									
LIVER	NO. EXAM.:	60	60	23	16	16	15	60	60
- HEPATOCELLULAR ADEN.		6		8		4		2	
- HEPATOCELLULAR CARC.		1							
- HAEMANGIOMA.				1				1	
- ENDOMETR. SARCO., MET.			2		2		1		
.....									
SKELETAL MUSCLE	NO. EXAM.:	60	60	8	14	9	12	60	60
- HISTIOCYTOMA.			1						
.....									
URINARY BLADDER	NO. EXAM.:	60	57	8	14	9	12	60	59
- ENDOMETR. SARCO., META.					1				
.....									
PANCREAS	NO. EXAM.:	60	60	10	15	10	14	60	60
- ENDOMETR. SARCO., META.					1				
.....									
THYMUS	NO. EXAM.:	51	53	4	13	5	11	44	52
- THYMOMA.					1		1		1
.....									
LUNGS	NO. EXAM.:	60	60	14	17	13	16	60	60
- BRONCHI./ALVEO. ADEN.		6	3	4	2	3	3	1	1
- ALVEOLAR TYP. II ADE.		1		1				1	
.....									
KIDNEYS	NO. EXAM.:	60	60	18	22	14	24	60	60
- HISTIOSARCOMA.		1							
- ENDOMETR. SARCO., META.							1		

PATHOLOGY REPORT .
SUMMARY TABLES

PAGE : 26
CIT PROJECT : 2036 TCS

TEST ARTICLE :
TEST SYSTEM : MOUSE, 78 weeks, oral
SPONSOR :

PATHOL. NO.: 92036 MAA
DATE : 21-OCT-88
PDS PATHDATA SYSTEM TM

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. +
NEOPLASTIC FINDINGS

ORGAN/FINDING	DOSE GROUP:		T		A		B		C	
	SEX :		M	F	M	F	M	F	M	F
	NO. ANIMALS:		60	60	60	60	60	60	60	60
UTERUS	NO. EXAM.:		60		46		40		60	
- ENDOMETR. STRO. POLYP.				2						1
- LEIOMYOSARCOMA.			1		1		1			
- ENDOMETRIAL SARCOMA.				3		2		2		
- LEIOMYOMA.			1				1			
- HAEMANGIOMA.							1			1
- FIBROLEIOMYOMA.					1					
VAGINA	NO. EXAM.:		58		18		12		59	
- HAEMANGIOPERICYTOMA.										1
SUBCUTANEOUS TISSUE	NO. EXAM.:		7	1	1	4	2	4	1	
- HISTIOCYTOMA.			1	1		1		1		
- FIBROHISTIOCYTOMA.			3							
LYMPH NODES	NO. EXAM.:		5	7	2	4	3	5	2	3
- RENAL HISTIOSAR., ME.			1							
- ENDOMETR. SARC., META.						1				
OTHER ORGANS/TISSUES	NO. EXAM.:		3	2	1	3	5	2	9	4
- HAEMANGIOMA.			1				1			
- LIPOMA, ADIPOSE TIS.									1	

Table 5

PATHOLOGY REPORT (DRAFT)
 STATISTICAL EVALUATION

PAGE :
 CIT PROJECT: *102036

TEST ARTICLE :
 TEST SYSTEM : MOUSE, 78 weeks, oral
 SPONSOR :

PATHOL. NO.: 92036
 DATE : 14-AP
 PATHDATA SYSTEM V

TREND TEST STATISTICS ON NEOPLASTIC LESIONS
 COMBINED PREVALENCE AND DEATH RATE METHOD (PETO ET AL., 1980)

DOSE GROUPS : T,A,B,C
 STATUS AT NECROPSY: KO INCL. +
 TIME INTERVAL FOR INCIDENTAL ANALYSIS: AD HOC RUN

SEX:

ORGAN/TISSUE	TYPE OF NEOPLASM	TREND	P-VA
LUNGS	BRONCHI./ALVEO. ADEN.	7.438-	
LUNGS	ALVEOLAR TYP. II ADE.	0.313-	
LIVER	HEPATOCELLULAR ADEN.	9.226-	
LIVER	HEPATOCELLULAR CARC.	1.468-	
LIVER	HAEMANGIOMA.	1.063+	p = 0.24
KIDNEYS	HISTIOSARCOMA.	1.468-	
HEMOLYMPHORET. SYS.	MALIGNANT LYMPH.P.D.	0.991-	
OTHER ORGANS/TISSUES	HAEMANGIOMA.	2.218-	
OTHER ORGANS/TISSUES	LIPOMA, ADIPOSE TIS.	0.667+	p = 0.05
SUBCUTANEOUS TISSUE	HISTIOCYTOMA.	0.000+	p = 0.50
SUBCUTANEOUS TISSUE	FIBROHISTIOCYTOMA.	2.000-	

DOSE GROUPS : T,A,B,C
 STATUS AT NECROPSY: KO INCL. +
 TIME INTERVAL FOR INCIDENTAL ANALYSIS: AD HOC RUN

SEX: FF

ORGAN/TISSUE	TYPE OF NEOPLASM	TREND	P-VA
LUNGS	BRONCHI./ALVEO. ADEN.	2.638-	
OVARIES	PAPILLARY CYSTADENO.	2.484-	
OVARIES	HAEMANGIOMA.	1.513-	
OVARIES	GRANULOSA CELL TUMO.	3.024-	
OVARIES	LUTEOMA.	0.513-	
UTERUS	ENDOMETR. STRO. POLYP.	2.580-	
UTERUS	LEIOMYOSARCOMA.	1.393-	
UTERUS	ENDOMETRIAL SARCOMA.	4.881-	

Table 5 (continued)

PATHOLOGY REPORT (DRAFT) PAGE :
 STATISTICAL EVALUATION CIT PROJECT: *102036

TEST ARTICLE : PATHOL. NO.: 92036
 TEST SYSTEM : MOUSE, 78 weeks, oral DATE : 14-AP
 SPONSOR : PATHDATA SYSTEM V

TREND TEST STATISTICS ON NEOPLASTIC LESIONS
 COMBINED PREVALENCE AND DEATH RATE METHOD (PETO ET AL., 1980)

DOSE GROUPS : T, A, B, C SEX: FE:
 STATUS AT NECROPSY: KO INCL. +
 TIME INTERVAL FOR INCIDENTAL ANALYSIS: AD HOC RUN

ORGAN/TISSUE	TYPE OF NEOPLASM	TREND	P-VA
UTERUS	LEIOMYOMA.	1.014-	
UTERUS	HAEMANGIOMA.	2.065+	p = 0.10
UTERUS	FIBROLEIOMYOMA.	0.507-	
VAGINA	HAEMANGIOPERICYTOMA.	1.556+	p = 0.10
HEMOLYMPHORET. SYS.	MALIGNANT LYMPH. P. D.	1.073-	
HEMOLYMPHORET. SYS.	MALIGNANT LYMPH. H. T.	3.030-	
HEMOLYMPHORET. SYS.	MYELOID LEUKEMIA.	0.463+	p = 0.34
THYMUS	THYMOMA.	2.068+	p = 0.16
SKELETAL MUSCLE	HISTIOCYTOMA.	1.500-	
SUBCUTANEOUS TISSUE	HISTIOCYTOMA.	0.750-	

END OF REPORT SECTION

Table 6
Intercurrent Morcality Rates
Male Mice

Weeks	Control			Low			Medium			High		
	S	D	%	S	D	%	S	D	%	S	D	%
1-52	60	6	10	60	3	5	60	2	3.3	60	5	8.3
53-80	54	1	1.8	57	5	8.7	58	7	12.1	55	6	10.9
Term.	53			52			51			49		

Peto Test: p-value = 0.1568

Female Mice

"

Weeks	Control			Low			Medium			High		
	S	D	%	S	D	%	S	D	%	S	D	%
1-52	60	6	10	60	6	10	60	6	10	60	2	3.3
53-80	54	11	20.4	54	8	14.8	54	6	11.1	58	13	22.4
Term.	43			46			48			45		

Peto Test: p-value = 0.4319

Notes: S: Number of animals starting during the period
D: Deaths
%: Percent of death during the period

Table 7

Summary of Mean Body Weight (g) with (Standard Deviation)

The Male Mice Study

Dose	Week 1	Week 78	Gain in Wt.	Gain rel. to Control
Control	28 (2)	40 (4.4)	12	-
Low	28 (2)	39 (4.5)	11	- 8.3%
Medium	28 (1.8)	38 (3.8)	10	-16.66%
High	27 (2)	37 (3.9)	10	-16.66%

Table 8

Summary of Mean Body Weight (g) with (Standard Deviation)

The Female Mice Study

Dose	Week 1	Week 78	Gain in Wt.	Gain rel. to Control
Control	23 (1.3)	32 (2.9)	9	-
Low	22 (1.3)	32 (2.7)	10	11.11%
Medium	22 (1.2)	32 (3.1)	10	11.11%
High	23 (1.2)	32 (2.7)	9	0%

Note: A negative sign stands for decrease in weight gain relative to control.

**ENVIRONMENTAL ASSESSMENT
AND
FINDING OF NO SIGNIFICANT IMPACT
FOR
MAVIK TABLETS
(trandolapril)**

NDA 20-528

**FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND
RESEARCH**

**DIVISION OF
CARDIO-RENAL DRUG PRODUCTS
(HFD-110)**

FINDING OF NO SIGNIFICANT IMPACT

NDA 20-528

MAVIK TABLETS

[Trandolapril]

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. FDA is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process.

The Food and Drug Administration, Center for Drug Evaluation and Research has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

In support of their new drug application for Mavik Tablets, Knoll Pharmaceutical Company conducted a number of environmental studies and prepared an environmental assessment in accordance with 21 CFR 25.31a (a) (attached) which evaluates the potential environmental impacts of the manufacture, use and disposal of the product.

Trandolapril is a chemically synthesized angiotensin converting enzyme (ACE) inhibitor which is administered as an oral tablet for the treatment of hypertension. The drug substance is manufactured by The drug
product is manufactured and packaged by Knoll Pharmaceutical Company, Whippany, New Jersey. Tablets are packaged into blister packs by
The finished drug product will be used in hospitals, clinics and by patients in their homes.

The drug substance, trandolapril, is a pro-drug that is rapidly and extensively hydrolyzed in the liver to the diacid, trandolaprilat. The drug substance and its metabolites are excreted into the sewer system. Chemical and physical test results indicate that they will be restricted to the aquatic environment. The maximum expected environmental concentration (MEEC), based on production estimates for the 5th year after approval of the NDA, is well below 1 part per billion.

The Minimum Inhibitory Concentration (MIC) of trandolaprilat is 400 mg/L for Nostoc Sp. in the Microbial Growth Inhibition Test (TAD 4.02). The MIC is more than 1000 mg/L for molds, ascomycetes and bacteria. Based on structure activity relationships, both trandolapril and its diacid metabolite, trandolaprilat, are highly susceptible to biodegradation by hydrolytic enzymes and peptidases in domestic sewage and in the waste water treatment works.

Disposal includes out of specification lots, returned, unused or expired product, empty or partly used product and packaging. These will be disposed at licensed incineration facilities and landfills. Empty, or partially empty packages generated in American hospitals and clinics will be disposed according to their regulations. Empty or partially empty containers from home use will be disposed in the community solid waste management system which may include landfills, incineration and recycling. Minimal quantities of unused drug may be disposed in the sewer system.

The Center for Drug Evaluation and Research has concluded that the product may be manufactured, used and disposed without any expected adverse environmental effects. Precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release.

Adverse effects are not anticipated upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places.

April 17, 1996 Florian Zielinski

DATE PREPARED BY: Florian Zielinski,
Review Chemist, New Drug Chemistry I

4/17/96 Robert J. Wolters

DATE CONCURRENCE: Robert J Wolters,
Office of Drug Evaluation I, Center for Drug Evaluation and Research

4/17/96 Nancy B. Sager

DATE APPROVED: Nancy B. Sager, Team Leader
Environmental Assessment Team
Center for Drug Evaluation and Research

Attachments: Environmental Assessment
Material Safety Data Sheets (trandolapril and trandolaprilat)

Original: NDA 20-528
HFD-357 FONSI File [NDA # 20-528]
HFD-004 Docket File
HFD-205 FOI COPY
HFD-110 Division File
HFD-110 CSO, Kathleen Bongiovanni
HFD-110 Review Chemist, Florian Zielinski

NDA SUBSECTION 3.0

ENVIRONMENTAL ASSESSMENT

Trandolapril Tablets

Knoll Pharmaceutical Company
199 Cherry Hill Road
Parsippany, NJ 07054

The National Environmental Policy Act requires Environmental Assessments (EAs) to be public documents. Subsections 3.1 through 3.15 (i.e., Items 1 through 15 of this EA) and accompanying Appendix A and Appendix B are suitable for public disclosure. These nonconfidential subsections and appendices are complete with the exception of proprietary information. The proprietary information, which is contained in Appendices C (EA) and D (study reports) could be beneficial to competitors and therefore, must remain confidential. The text of the public document (Subsections 3.1 through 3.15 and Appendices A and B) is based on the text of Appendix C. Appendix C is intended for review as the confidential version of EA Items 1 through 15. Appendix A contains the Material Safety Data Sheets (MSDS). Appendix B contains the references which are cited.

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3.1 DATE

April 15, 1996

3.2 NAME OF APPLICANT/PETITIONER

Knoll Pharmaceutical Company

3.3 ADDRESS

199 Cherry Hill road

Parsippany, NJ 07054

3.4 DESCRIPTION OF THE PROPOSED ACTION

3.4.1 REQUESTED APPROVAL

Knoll Pharmaceuticals has filed an NDA pursuant to section 505 (b) of the Federal Food, Drug and Cosmetic act for Trandolapril (Mavik) tablets containing 1 mg (100 mg tablet), 2 mg (125 mg tablet), and 4 mg (175 mg tablet) of the active ingredient packaged in bottles and blister packaging of 100 tablets each to obtain an approval for marketing of this drug product in the United States. A five year production forecast for trandolapril is provided in Confidential Appendix C

An Environmental Assessment (EA) Report has been prepared pursuant to the requirements listed in 21 CFR Part 25.31a(a) and according to the guidelines provided in the Guidance for Industry for the Submission of an Environmental Assessment in Human Drug Applications and Supplements from the Center for Drug Evaluation Research (CDER) of U.S. FDA (FDA, 1995). Appendix A has Material Safety Data (MSDS) Information, Appendix B has references, and Appendix C is the confidential appendix of the EA document

and contains proprietary information that should not be disclosed. Appendix D is also a confidential document containing study reports and results.

3.4.2 NEED FOR ACTION

Trandolapril is used for the treatment of hypertension. It is assumed that the drug product will be prescribed for one percent of the hypertension patients in the United States.

3.4.3 PRODUCTION LOCATIONS

The manufacture of the bulk drug substance, the drug product, and the packaging operations are conducted at three different locations as stated below:

1. Drug Substance Manufacture:
2. Drug Product Manufacture: Knoll Pharmaceutical Company, 30 North Jefferson Road, Whippany, New Jersey 07981, USA
3. Packaging Operations: 1) Bottles - Knoll Pharmaceutical Company, 30 North Jefferson Road, Whippany, New Jersey 07981, USA; and 2) Blisters

Figure 3.4-1 provides a schematic for the manufacture of trandolapril tablets.

3.4.3.1 Environmental Setting of Roussel-Uclaf Facilities

The drug substance trandolapril is produced at _____ located

The plant is in the vicinity of a _____

small river, and the surroundings of the plant are predominantly agricultural, with few minor industries.

3.4.3.2 Environmental Setting of Knoll Pharmaceutical Manufacturing Facilities

The drug substance is imported into the United States from Formulation of the drug substance and the manufacture of drug product, trandolapril tablets, and packaging of tablets into bottles is conducted at Knoll Pharmaceutical Company. Knoll Pharmaceutical company is located at 30 North Jefferson Road in Hanover Township, New Jersey. The Postal District is Whippany, New Jersey. The site consists of 22 acres of zoned Industrial Park (IP) and contains approximately a quarter acre of land that has been classified as Wetlands by the State of New Jersey. The site is bounded on the South by Stoney Brook. Approximately 5 acres are mixed forest. Jersey Central Power & Light have two right-of-ways on the property. One sits along the east property line. The second travels west to east directly over the employee parking lot. A natural gas pipe line, maintained by Columbia Gas co., also travels along the JCP&L right-of-way. Properties to the north and east are zoned residential. All sanitary sewer effluent flows directly to the POTW, Hanover Sewerage Authority. No waste treatment is performed on the sewer effluent outside of the facility as the site meets and exceeds all discharge limitations as stipulated in the current permit.

3.4.3.3 Environmental Setting of Ivers-Lee Manufacturing Facilities

The trandolapril tablets are packaged into blister packages at the Plant. The plant is located on approximately 15 acres of land in the township of . The plant is about 40 miles from

The 15-acre site is a level terrain covered by one building, driveways, walkways, parking areas. It is surrounded by light industry, commercial establishments, and residential communities. The main elevation above sea level is 190 ft.

3.4.4 LOCATIONS OF USE

Trandolapril tablets will be consumed by patients suffering from hypertension throughout the United States. Typical locations of use are by patients in hospitals, clinics, and by patients in their homes.

3.4.5 DISPOSAL SITES

At U.S. Hospitals, pharmacies, or clinics, empty or partially empty packages will be disposed of according to hospital, pharmacy or clinic procedures and that in the home, empty or partially empty containers will be typically disposed of by a community's solid waste management system which may include landfills, incineration and recycling. Minimal quantities of unused drug may be disposed of in the sewer system. The methods of disposal of rejected, expired, returned drug product or drug substance, solid or aqueous wastes by the manufacturers are described in Section 6.0.

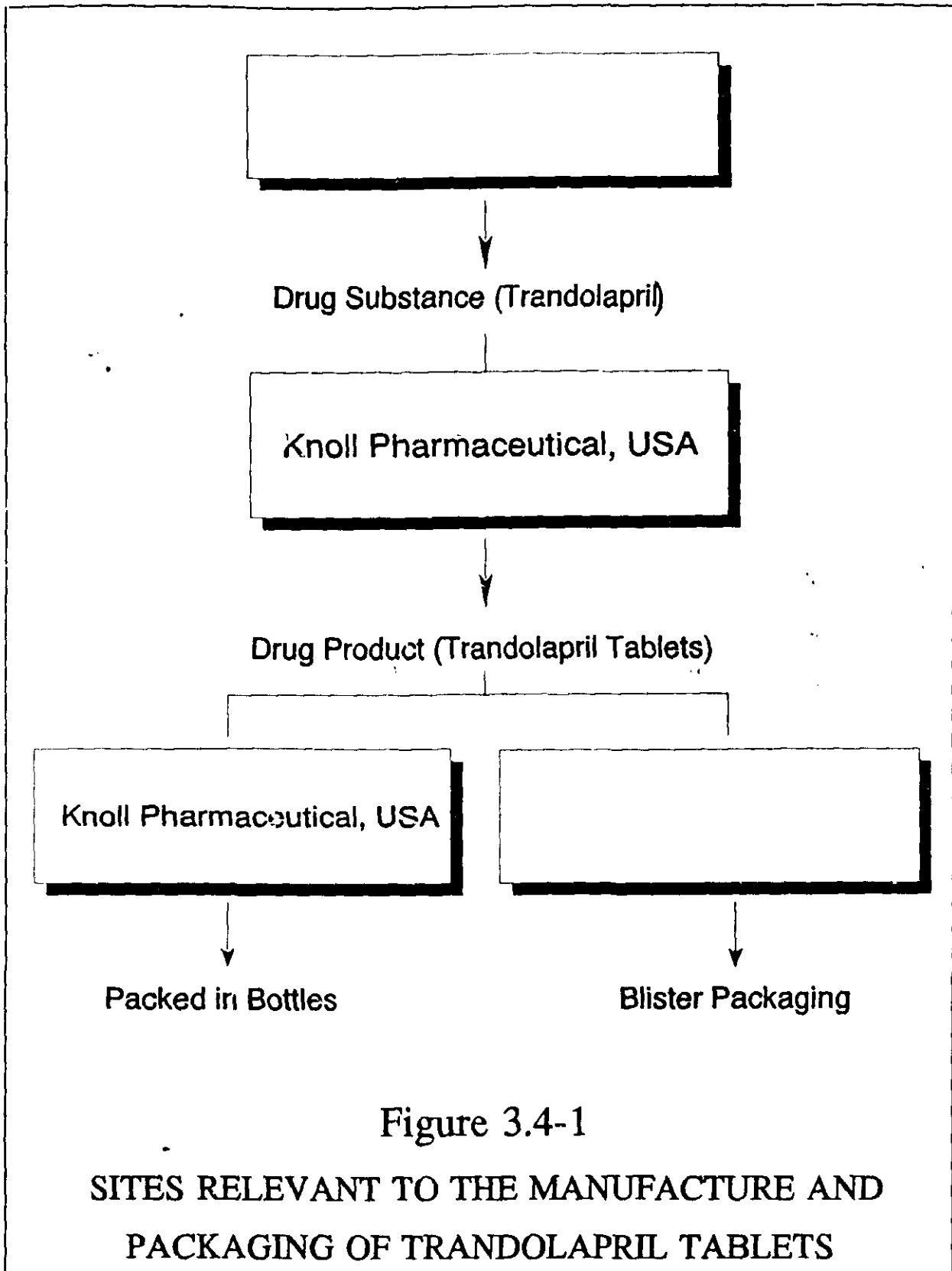
3.5 IDENTIFICATION OF CHEMICAL SUBSTANCES THAT ARE THE SUBJECTS OF PROPOSED ACTION

3.5.1 DRUG SUBSTANCE

3.5.1.1 Nomenclature

3.5.1.1.1 Established Name (United States Adopted Name - USAN)

Trandolapril



3.5.1.1.2 Brand or Proprietary Name

Mavik

3.5.1.1.3 Chemical Abstracts (CA) Index Name

[2S-(1(R*(R*)), 2 α , 3 $\alpha\alpha$, 7 $\alpha\beta$)]-1-[2[[1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-octahydro-1 H-indole-2-carboxylic acid

3.5.1.1.4 IUPAC Chemical Name

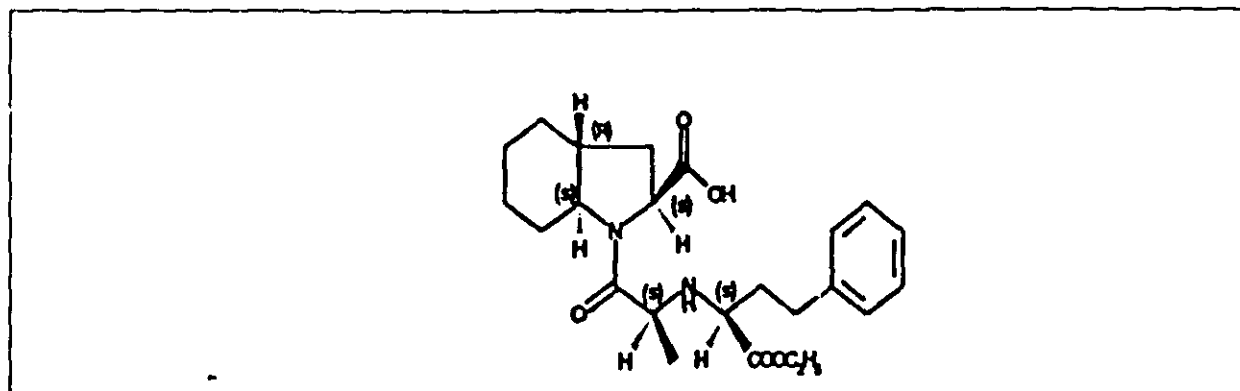
(2S,3aR,7aS)-1-[(S)-N-[(S)-1-(ethoxycarbonyl)-3-phenylpropyl]alanyl]-hexahydro-2-indolinecarboxylic acid

3.5.1.2 Chemical Abstracts Service (CAS) Registry Number

87679-37-6

3.5.1.3 Molecular Formula $C_{24}H_{34}N_2O_5$ 3.5.1.4 Molecular Weight

430.5

3.5.1.5 Structural (Graphic) Formula

3.5.1.6 Physical Description

White Crystalline Powder

3.5.2 ADDITIVES

There are a number of additives added to the drug substance during the formulation and manufacture of drug product, trandolapril tablets. The active ingredient, trandolapril, content constitutes 1 mg, 2 mg, and 4 mg, in the 100 mg, 125 mg, and 175 mg tablets, respectively. The specifications for the tablets call for Trandolapril to be present at no less than and no more than of the labeled amount. The quantities of excipients present in these tablets and their Chemical Abstracts Service Registry Numbers (CASRN) are presented in confidential Appendix C. A number of these excipients are naturally occurring, biodegradable and should have no environmental impact.

3.5.3 IMPURITIES

The impurities in the trandolapril bulk drug substance may not exceed 1% of related substances according the established acceptance criteria. The related substances exceeding 1% have not been identified. Other impurities such as water; and residual solvents such as ethyl alcohol, toluene, and dichloromethane; and Palladium and sulfate ash also do not exceed 1% and, therefore, have not been identified. This is consistent with the requirement of FDA, CDER guidelines (FDA, 1995). Solvents and their disposal will be addressed in Section 3.6.

3.6 INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

In this section, introduction of substances into the environment are discussed for both the manufacturing sites in the United States: 1) Knoll Pharmaceuticals Company,

Whippany, New Jersey, site of drug product, trandolapril tablet manufacture and bottle packaging; and 2) . site of blister packaging of the tablets produced at Knoll Pharmaceuticals. A brief description of the site of manufacture of the bulk drug, trandolapril, is also provided along with the certifications required for the foreign site as required in FDA, CDER guideline (FDA, 1995).

3.6.1 MANUFACTURE OF BULK DRUG, TRANDOLAPRIL, AT

is the site of the production of bulk drug, trandolapril. The plant operates under a technical plan to protect, preserve, and enhance the quality of the environment as specified by the Law in the field of Environmental Protection and Occupational Hygiene. A letter from the Regional Director of Industry and Research, Ministry of Industry, and National and Regional Development has confirmed that the plant is in conformity with the prescriptions intended to ensure the public health and safety and consequently, the protection of the environment. The original letter in and its English translation are provided in Attachment 3.15-1. A letter (in and its English translation) from the staff responsible for the Environmental Protection at indicating the compliance of the facility with the environmental regulations is provided in Attachment 3.15-2. At this facility the effluents are controlled and submitted to specific treatment, if necessary, before discharging into the atmosphere or river, The solvents are either recycled or destroyed in facilities agreed to by the Government Authorities. The aqueous effluents are transferred either to a biological treatment station or

to the incineration facilities. The solid wastes are disposed of at the licensed waste disposal sites. The Plant and its personnel operate in conformity with the rules prescribed by the Ministry of Labor of governing occupational Hygiene and Safety.

3.6.2 PREPARATION OF TRANDOLAPRIL TABLETS AT KNOLL
PHARMACEUTICAL COMPANY, WHIPPANY, NEW JERSEY, USA

3.6.2.1 Substance Expected to be Emitted

The manufacturing process steps for the tablets containing 1 mg of active ingredient, trandolapril, are summarized in confidential Appendix C. The manufacturing process for the 2 mg and 4 mg tablets is basically the same as for the 1 mg tablet and is also summarized in confidential Appendix C.

Atmospheric Emissions

No volatile organic emissions are expected to be released into the atmosphere due to the absence of these materials in the tablet manufacturing process. Particulate emissions from the tableting process are not expected as the dust collectors exhaust within the manufacturing facility. The filtered air is then discharged into the atmosphere.

Aqueous Wastes

Release of the drug substance as aqueous waste is not anticipated during the manufacture of the drug product. Upon completion of process step 1 (confidential Appendix C) approximately half of the (hydroxypropyl methylcellulose) suspension is used in the manufacture of tablets while the other half is sewerd into the municipal sewage system as aqueous waste. No prohibited or hazardous compounds are used

in the manufacture of Trandolapril tablets. No other releases are anticipated as a result of tableting.

Solid Waste

Solid wastes from the tableting and from the dust collectors will be collected and disposed of by incineration by approved waste disposal contractors. Trandolapril and the excipients used to make the tablets are not controlled substances.

3.6.2.2 . Controls Exercised

Standard safety precautions are used for all the chemicals used in the manufacture of Trandolapril tablets. Release of particulate material from the tableting facility will be controlled by dust collectors. The air emission equipment used in the tableting facility are permitted by the State of New Jersey, Department of Environmental Protection (NJDEP).

The following table lists the equipment and NJDEP permits for the air emission control equipment:

NDA 20528

6 OF 9

Table 3.6-1

Air Emission Control Equipment and Permits

<u>NJDEP Permit No.</u>	<u>Expiration Date</u>	<u>Equipment Permitted</u>
080796	9/8/98	Central Vacuum
080798	9/8/98	Production Dust Controller
079511	11/3/98	Despatch Tray Dryer
107443	8/17/2000	Packaging Line #1, Dust Collector
108106	10/5/95*	Packaging Line #2, and #4 Dust Collector

*Temporary permit, renewing automatically every three months pending the issue of permanent permit by the NJDEP.

Aqueous wastes that will be sewerd do not contain hazardous or prohibited components as a result of tableting. Thetrandolapril tableting facility process sewers are discharged into the local Hanover Sewerage Authority's Wastewater Treatment Plant (WTP). The Whippany, New Jersey facility has been issued a permit by the Hanover Sewerage Authority (Industrial Discharge Permit #D-9, expiration date, March 31, 1996; due for renewal after this date), to discharge materials within permissible limits.

The Knoll Pharmaceutical facility is designated as a solid waste generator by EPA (EPA ID # NJD058118308). Solid wastes will be disposed of or incinerated by the following permitted waste disposal contractors:

*In operation under temporary approval by the NJDEP

**In operation under temporary approval by the NYDEC

Other permitted waste disposal contractors may be used as necessary.

3.6.2.3 Citation of and Statement of compliance with Applicable Emission Requirements

A schematic of manufacturing process and the excipients used in formulation are provided in confidential Appendix C. Particulate emissions from the tableting facilities at Whippany, NJ are regulated by permits issued by the New Jersey Department of Environmental Protection. Wastewater from manufacturing must meet General Pretreatment Standards in 40 CFR Part 403 and the Effluent Guidelines and Standards for Pharmaceutical Manufacturing in 40 CFR Part 439. The prohibitions and limitations for discharge into the local WTP are set forth by the Hanover Sewage Authority Industrial discharge Permits. Solid wastes will be disposed of or incinerated by permitted waste contractors (e.g.,). Certification of compliance with application emission requirements by the facility manager is provided in Attachment 3.15-3. Environmental permits are reviewed in the above sections and also detailed in Attachment 3.15-4.

3.6.2.4 Effect of Approval on Compliance with Current Emission Requirements

Emissions and releases from the tableting of trandolapril will not exceed the limitations of current permits. Manufacturing of this product will be scheduled to fit within the existing framework of activities for which current emission requirements are applicable.

3.6.3 PACKAGING OF TRANDOLAPRIL TABLETS AT KNOLL PHARMACEUTICAL COMPANY, WHIPPANY, NEW JERSEY

Tablets are packaged into bottles at Knoll Pharmaceutical Company. Emissions during packaging are expected to be negligible. Emission controls in place are similar to those described in Section 3.6.2.

3.6.4 PACKAGING OF TRANDOLAPRIL TABLETS AT

At this facility, tablets are packaged into blister packs. Substances expected to be emitted and emission controls are described below.

3.6.4.1 Substances Expected to be Emitted

Air emissions due to packaging of the drug product will be negligible. All equipment used for packaging are fitted with filters. These filters provide greater than 90% control efficiency for particulates. No volatile organic emissions are expected as no organic solvents are used to package the drug product. A light mist of a dilute alcohol/water solution is applied onto the packaging equipment to sanitize the equipment prior to use. Air emissions from this are negligible.

Aqueous Waste

Equipment used in packaging the drug product is hand scraped to remove residual solids before being washed with water. Equipment is washed with water and detergent after each batch. This washout water is sewerred into process drains which discharge into the Sewage Authority system. Emissions of the drug product are minimal due to the efficiency of product residue removal.

Solid Waste

Solid wastes from packaging, such as product filters and loose product residues obtained from equipment cleaning are collected and disposed of by a permitted incinerator. Tyvek suits and other product contaminated wastes are also collected, drummed, and incinerated. Non-hazardous solid wastes (i.e., paper, plastic, drums, etc.) are either recycled or are disposed of at a state operated landfill.

3.6.4.2 Controls Exercised

Air Emissions are controlled by product filters on the equipment used in the packaging process. Release of particulates from the tabling facility cannot exceed 50 lbs per hour. The packaging facility is permitted by the New Jersey Department of Environmental Protection (Air Pollution Control ID Number 05193). Aqueous wastes from process drains are released to the Sewage Authority sewer system. Solid waste is disposed of by permitted solid waste contracts. The facility is recognized by NJDEP as a waste generator (NJDEP ID -). The site is not governed by any solid or hazardous waste permits.

3.6.4.3 Citation of and Statement of Compliance with Applicable Emission Requirements

Particulate emissions from the packaging facility are regulated by and in compliance with NJDEP. Waste water effluents is regulated and in compliance with the Sewage Authority. Solid waste from packaging operations will be either recycled or incinerated by permitted waste disposal contractors. A statement of compliance with Good Manufacturing Practices (GMPs) and with other requirements by the Director of Quality Assurance at is provided as Attachment 3.15-5. A statement of compliance with all applicable emission requirement is provided by the facility manager (Attachment 3.15-6).

3.6.4.4 Effect of Approval on Compliance with Current Emission Requirements

Emissions and releases from packaging operations of trandolapril tablets will not exceed the limitations of current permits. The packaging operations at this facility will fit within the existing framework of activities for which current emission requirements are applicable.

3.6.5 OCCUPATIONAL SAFETY

Employee education on health and safety is provided through lectures and hands-on training at Knoll Pharmaceuticals and facilities. Existing work procedures (including the use of uniforms, respirators, gloves, safety shoes, and eye protection) and engineering controls designed for the equipment (e.g., exhausts to remove dust) are adequate to ensure employee safety.

All employees will be trained in the proper operation of equipment in order to

minimize potential safety, health and environmental risks. Extensive safety training is mandated at these facilities, and information on safe handling is available to personnel for chemicals used in the manufacturing process. Industrial hygiene monitoring of exposure to hazardous materials is routinely conducted. Personnel participate in periodic physical evaluations.

The safe transport of all drug-related materials is ensured by following the protocols, which include formal qualification of vendors, training of personnel, and rigid specification of containers and materials. Access to drug substances and products is restricted to authorized personnel.

3.6.6 EXPECTED INTRODUCTION CONCENTRATIONS

Human drugs find their way into the environmental compartments (eg. soil, air, water) through manufacture, use, disposal and accidental spills. The two major sources of environmental exposure of the drug are: 1) the elimination of the drug or its metabolites from the patients who are using the drug product through their excreta and the consequent release of unchanged drugs and/or metabolites into the environment; and 2) release of the drug or its precursors through wastewater from the manufacturing plants. In either case, the municipal sewage could be the main recipient of these contaminant sources. The expected introduction concentrations (EIC) estimated in the subsections below do not take into consideration any degradation of the drug or its metabolites during transport in the municipal sewage to the waste water treatment plant (WWTP), and, therefore, are worst case scenarios.

3.6.6.1 Expected Introduction Concentration From Use

Trandolapril may be regarded chemically as derivative of a trans-octahydroindole-2-carboxylic acid. The alanyl residue is linked to the nitrogen atom by an amido (peptide) linkage and the nitrogen atom of alanine bears a 1-carboxy-3-phenylpropyl residue. Trandolapril is extensively metabolized in the body of the patients using the drug. There is one major metabolic pathway in the human body which is the conversion of trandolapril to trandolaprilate, a diacid metabolite, which is formed by the hydrolysis of carboxylic ester (Figure 3.6-2). Animal studies with rodents have demonstrated that after dosing with trandolapril, about 33% of the parent drug and its metabolites are eliminated in the urine and about 66% in the feces. Unmetabolized trandolapril amounted to <1.0% of the dosage amount in urine and 10% in feces. The metabolite trandolaprilate occurred at 14% and 30% of the dosed amount in urine and feces, respectively. No other metabolites are excreted in the feces at a level >4% of the dosage amount. The metabolic pathway of trandolapril is presented in Figure 3.6-1.

For a worst case estimate of the amount of trandolapril entering a typical wastewater treatment plant (WTP) through human use, it is assumed that all the drug forecasted for production in the United States in the fifth year of production (year 2000) will be ingested and eliminated by the U.S. population. The worst case estimate assumes that there will be no metabolism of trandolapril in the human body and that there will be no degradation in the domestic sewage receiving human excreta containing the drug products.

The 1990 Census gives the population of the United States as 250,378,000. Typical minimum and maximum flow rates for wastewater treatment systems are set by

Federal and State agencies to range from 280 to 1,500 L/person/day (Metcalf & Eddy, Inc., 1979). The concentration of the drug is estimated from the dilution of the total drug produced in a year in the total wastewater produced in the United States.

The Expected Introduction Concentration (EIC) from use at the WTP can be estimated from the following equation:

$$EIC = \frac{\text{Total drug produced (fifth year production)}}{\text{Total waste water in the United States}}$$

An equivalent method for calculating the concentration of drug that would be expected at the WTP is given in Interim Guidance to the Pharmaceutical Industry for Environmental Assessment Compliance Requirements for the FDA (PMA, 199i) which estimates the EIC in ppm as follows:

$$ppm = (A) (B) (C) (D) (E) (F)$$

A = pounds/year production

B = year/365 days

C = day person/280 L (74 gallons)

D = 1/250 million persons

E = gallons/8.34 pounds

F = one million

Another method for calculating the expected introduction concentration (EIC) of the drug at the WTP is given in Guidance for Industry for the Submission of an Environmental Assessment in Human Drug Applications and Supplements published by the Center for Drug Evaluation Research (CDER), FDA, (FDA 1995). The estimate of the EIC in ppm based on

this method is as follows.

$$\text{EIC-Aquatic (ppm)} = (A) (B) (C) (D)$$

A = Kg/year production

B = 1\Liters per day entering WTP

C = years/365 days

D = 10^6 mg/Kg (conversion factor)

3.6.6.2 Expected Introduction Concentration from Disposal

Synthesis of the drug substance at _____ in France conforms to all applicable regulations of the French government. No releases of trandolapril are anticipated due to disposal from this facility. No releases are expected due to disposal during drug product manufacture (Knoll Pharmaceutical Company, NJ, USA) or during packaging _____ and Knoll Pharmaceutical, NJ, USA). Release of effluent from these facilities would not be expected to introduce trandolapril to publicly owned wastewater treatment plants.

3.7 FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

As described in section 3.6.6.1, trandolapril is the minor component ($\leq 10\%$) expected to be found in the excreta of the patients using trandolapril. The major component is the diacid metabolite, trandolaprilate. When domestic sewage enters the wastewater

Figure 3.6-1
Metabolic Pathway of Trandolapril

treatment plant (WTP), these components become part of the WTP and will be further subjected to degradation, depletion and partitioning as described below in the section 3.7.2.

3.7.1 AIR

Little or no emissions of the trandolapril to the atmosphere occur during the synthesis of the bulk drug, manufacture of the drug product, use of the drug by patients or the disposal of the drug. Trandolapril is a solid at ambient temperature and has very low volatility being a high molecular weight (430.54) compound, with a melting temperature of 125°C. The vapor pressure of trandolapril has been determined to be $\leq 4 \times 10^{-6}$ Pa at 80°C (Appendix D-1-A; Table 3.7-1). Dust from the manufacturing facilities will be trapped by vent filters that are collected for landfilling or incineration.

3.7.2 WATER

Trandolapril and its metabolite trandolaprilate (diacid) excreted by the patients using the drug will be introduced into the domestic sewage which will enter the WTPs throughout the United States. The EIC from use estimated in Section 3.6.6.1, assumes: 1) that all of the drug produced during the fifth year production will be consumed by the patients in the United States; 2) the drug is excreted unchanged; and 3) no biodegradation occurs in the domestic sewage. Assuming that no depletion mechanisms for the drug exist in the WTP and all the drug will enter the wastewater effluent, it can be assumed that * * * * * will enter the surface water. Typical dilution factor for the WTP effluents entering the rivers is 10^{-3} (Metcalf and Eddy, 1979), and therefore, the worst case concentration of the drug entering the surface water is expected to be * * * * * below the detection limit (Table 3.7-2). The concentration of the

drug would further decrease downstream of the river as the drug would get diluted with additional water further from the effluent out fall.

The potential depletion mechanisms that could occur in the WTP and the partitioning of the drug into wastewater effluents and sludge solids is described below. The consequent fate of the drug and resulting concentrations in the aquatic (surface water) and terrestrial (soil) environmental compartments are estimated in Table 3.7-2.

3.7.2.1 . Biodegradation

Trandolapril and its diacid metabolite, trandolaprilate are highly susceptible to biodegradation in the domestic sewage and in the WTP, based on the structure-activity relationship. The peptide link and the carboxylic acid ester function are susceptible to biological degradation since hydrolytic enzymes and peptidases are abundant in microbial systems (EPA, 1979, 1987). The aryl ring of the phenylpropyl moiety would be subject to microbial (oxidative) degradation and hydroxylated to a phenol. This would undergo further oxidation with complete degradation of the aromatic ring to a single carboxylic acid residue.

3.7.2.2 Chemical Degradation

Hydrolysis, and photolysis could be the potential degradation pathways for trandolapril and trandolaprilate in the WTP. However, none of these three processes could contribute to significant depletion during the residence time of the drug in the WTP (≤ 48 hours), as explained below.

Trandolapril is quickly degraded to trandolaprilate under alkaline conditions (Appendix D-3). Hydrolysis studies have shown some depletion ($\sim 30\%$) of trandolapril by 72 hours, especially at pH 7 and 9, but no depletion was noticed for trandolaprilate at any pH

(5, 7 or 9) at 50°C (Appendix D-1-B; Tables 3.7-3 and 3.7-4). Since trandolaprilate is the main component in the WTP, hydrolysis is not a depletion mechanism.

Compounds with absorbance in the range of 290 to 800 nm are known to photodegrade extensively. However, trandolapril showed no absorption in the wavelength range of >250 nm (Appendix D-1-B). Furthermore, exposure of neutral buffers containing trandolapril showed negligible degradation. Direct photodegradation, during the short residence time (<48 hours) of the drug in the WTP, therefore, is not expected to be a depletion pathway. However, many compounds with absorbance of <250 nm are known to photodegrade extensively under indirect photodegradation conditions in the presence of naturally occurring sensitizers suggesting that indirect photodegradation could be a depletion pathway for trandolapril and trandolaprilate in the WTP and surface waters.

3.7.2.3 Sorption/Desorption

Log octanol/water partition coefficient ($\log K_{ow}$) and log sorption coefficient (K_{oc}) data can be used to predict sorption/desorption of organic chemical to sludge and soils. Chemicals with $\log K_{ow}$ of 4 or greater and with $\log K_{oc}$ with 3 are considered highly sorbed to organic matter in sludge and soil (FDA, 1987). $\log K_{ow}$ and $\log K_{oc}$ values for trandolapril and trandolaprilate were estimated based on experimentally derived water solubility values (Appendix D-2; Tables 3.7-5 and 3.7-6), and were ≤ 1.51 and ≤ 2 , respectively (Table 3.7-7). These results suggested both trandolapril and trandolaprilate are unlikely to adsorb to sludge and will partition into wastewater effluents in the WTP.

3.7.2.4 Bioaccumulation/Bioconcentration

Bioaccumulation of chemicals generally refers to their introduction into animals by ingestion, while the bioconcentration of chemicals refers to their absorption from water by aquatic organisms. Bioaccumulation of trandolapril and trandolaprilate in aquatic animals is unlikely, because the solubility at the pH range of surface water [pH 5 - 843 mg/L (trandolapril), 7,450 mg/L (trandolaprilate); pH 7 = 8,150 mg/L (trandolapril), 10,400 (trandolaprilate)] would facilitate fecal elimination or transport via gills to the surrounding water (Tables 3.7-5 and 3.7-6). Thus even if trandolaprilate or trandolapril partition into surface water, bioaccumulation in surface water is not a concern and no environmental impacts are expected.

Based on the experimentally derived water solubility values, bioconcentration factor (BCF) can be calculated for trandolapril and trandolaprilate from the following equation (FDA, 1987):

$$\log \text{BCF} = 2.791 - 0.564 \text{ Log } S, \text{ where } S \text{ is water solubility in mg/L (ppm)}$$

The estimated log BCF for trandolapril was ≤ 1.2 and log BCF for trandolaprilate was ≤ 0.61 (Table 3.7-7) at all the three pHs tested (5, 7, 9), and neither of these chemicals are expected to be found in the animal tissues.

If terrestrial organisms come into contact with sediment to which trandolapril or trandolaprilate is adsorbed, bioaccumulation (via ingestion) is not expected. Trabalka and Garten (1982) have reported that chemicals with log K_{ow} values of less than 3.5, or solubilities greater than 10 mg/L, do not accumulate in mammals or birds. These cutoff

values for log K_{ow} and solubility were based respectively on a frequency analysis of 123 paired observations for 68 chemicals and 90 paired observations for 47 chemicals.

3.7.2.5 Summary of Fate of Trandolapril and Trandolaprilate in Aquatic Environmental Compartment

The worst case expected EIC of trandolapril through human use is estimated at *****. As stated in the previous sections, biodegradation is expected to be the major depletion mechanism for trandolapril in the sewers and in the WTP. Also based on the water solubility, log K_{ow} and log K_{oc} values for trandolapril and its major metabolite, trandolaprilate, both of these compounds will partition into wastewater effluent and no adsorption to the sludges is expected. Therefore, aquatic environmental compartment is the main exposure pathway. The maximum expected environmental concentration (MEEC) in the surface water based on 10^{-3} dilution (Metcalf and Eddy, 1979) of the WTP effluent, is expected to be ***** through human use (Table 3.7-2). In surface water and sediment, these compounds are expected to further undergo biodegradation and photodegradation as well as dilution away from the effluent out fall facilitating the reduction of this concentration further. According to the recent guidance document from FDA, CDER (FDA, 1995) compounds with EIC of >1 ppb are not of significant environmental concern. The EIC of trandolapril ** ***** *** ***** is several orders of magnitude lower than the cutoff (>1 ppb) limit and, therefore, should not have any environmental impact.

3.7.3 SOIL

As described above, the compounds will not partition into sludge and therefore, soil is not expected to be exposed, and is not of any concern in terms of environmental exposure.

3.8 ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES

The worst case concentration of trandolapril and its metabolite combined in surface water are estimated to be ** *****. No environmental impacts are expected at this concentration. However, a microbial growth inhibition study has been conducted to see if trandolapril or trandolaprilate have any effect on bacteria, alga, or fungi, microorganisms that contribute to environmental processes.

3.8.1 Microbial Growth Inhibition (Appendix D-4; Table 3.8-1)

The metabolite, trandolaprilate, was evaluated for potential inhibitory effects on the growth of representative microbial species. Widespread inhibition may result in ecosystem-level effects, which may include, depending on the organisms inhibited, reduction in plant growth or quality through nutritional disturbances (i.e., interruption of nutrient cycling), and interference of the natural degradative functions of microorganisms that play a dominant role in transformations of biotic and manufactured chemical wastes. Inhibition may also be deleterious to normal microbial processes that occur in the WTP.

No inhibition was observed for the test organisms *Aspergillus niger*, *Trichoderma viride*, *Clostridium perfringens*, and *Bacillus subtilis* at any concentration of trandolapril tested up to and including 1,000 mg/L which is also the No Observed Effect

Concentration (NOEC). Inhibition was observed for the *Nostoc sp* at 400 mg/L of trandolaprilate.

The MEEC of * **** * in the surface water is several orders of magnitude lower than NOEC for bacteria and fungi (1000 ppm) and for *Nostoc* (200 ppm) suggesting no impact of trandolapril on microbial processes in aquatic environment.

3.9 USE OF RESOURCES AND ENERGY

The proposed action does not require a large commitment of resources. Moreover, no irreversible or irretrievable commitment of limited national resources will be involved. Total estimated 1994 consumption of water at Knoll Pharmaceutical Company was 15,800 000 gallons, electricity was 7,50,000 KWH.

As discussed in Item 3.8, the environmental effects of releases from manufacturing of trandolapril is negligible on the bacterial, fungal and algal species tested. Therefore, it is unlikely that threatened or endangered species could be affected.

Knoll Pharmaceuticals or sites are considered to have no historical or archaeological importance based on the State of New Jersey listings. Moreover, no expansion of facilities is necessary for this action.

3.10 MITIGATION MEASURES

Controls on emissions at Knoll Pharmaceutical and facilities have been described in Section 3.6. Since the maximum expected environmental concentrations (MEEC) is several orders of magnitude lower than no observed effect concentration (NOEC) for microbiological species (Tables 3.7-2), no environmental impacts are anticipated.

TABLE 3.7-1. Trandolapril - Determination of Vapor Pressure
ONE PAGE SUMMARY

TEST CHEMICAL: TRANDOLAPRIL
TITLE OF STUDY: TRANDOLAPRIL - DETERMINATION OF VAPOR PRESSURE
NATURE OF STUDY: PHYSICAL, CHEMICAL PROPERTIES
STUDY PERFORMED BY:
<u>SUMMARY OF RESULTS</u>
The vapor pressure study was performed using the gas saturation method suggested in FDA, TAD guideline 3.03, Vapor Pressure (FDA, 1987). Experiments were carried at flow rates between 10 mL/min to 50 mL/min and a temperature of 80°C for 11 days. No volatilization of the compound was noticed because no quantifiable test chemical was noticed in volatile traps. Based on the limit of quantitation of (0.5 µg/mL), the vapor pressure at 80°C can be calculated to be $\leq 4 \times 10^{-6}$ Pa.
<u>ENVIRONMENTAL SIGNIFICANCE</u>
Because of the extremely low vapor pressure, high molecular weight (430) and melting temperature of 125°C, the compound is not likely to volatilize into the atmosphere and will have no impact in the environment.

TABLE 3.7-2

Summary of Environmental Impact of Trandolapril Through Human Use (Year 2000 Production Forecast)

Expected Introduction Concentration (EIC) in WTP as a Result of Human Use* =	
Maximum Expected Environmental Concentration (MEEC) in WTP EIC - Unknown Degradation** = MEEC	
Trandolapril Adsorbed to Metropolitan Sewage Sludge = 0%	0%
Trandolapril Remaining in Aqueous Phase = 100% ($K_{ow} < 2$)	100%
MEEC of Trandolapril in WTP Wastewater Effluent	
MEEC of Trandolapril in Surface Water after the Release of Wastewater Effluent from WTP Assuming a Thousand Fold Dilution*** in the River or Stream =	
NOEC for Bacteria, Fungi, and Alga =	1000 ppm (except for nitrogen fixing alga, <i>Nostoc</i> sp. 400 ppm)
35 CONCLUSION: No environmental impacts due to trandolapril are anticipated since NOEC for microorganisms is several orders of magnitude higher than MEEC for the aquatic environmental compartments.	

*Section 3.6.5.1 EIC estimations through human use.

**Data shows hydrolysis and photolysis are not depletion mechanism (Section 3.7.2.2). Biodegradation (3.7.2.1) may be feasible but data is not available; hence no depletion mechanisms in the WTP are suggested.

***Metcalf & Eddy, 1979 (Appendix C, Section 7.2).

**TABLE 3.7-3. Trandolapril - Determination of the Abiotic Degradation
ONE PAGE SUMMARY**

TEST CHEMICAL:	TRANDOLAPRIL
TITLE OF STUDY:	DETERMINATION OF THE ABIOTIC DEGRADATION
NATURE OF STUDY:	ENVIRONMENTAL FATE - CHEMICAL DEGRADATION
STUDY PERFORMED BY:	
<u>SUMMARY OF RESULTS</u>	
The abiotic degradation (hydrolysis) of trandolapril was determined at pH 5, 7 and 9 using the method provided in FDA, TAD 3.09, Hydrolysis. The study was conducted at 50°C for 5 days (120 h). Based on the preliminary study results, percent hydrolyzed results were estimated.	
<u>Percent Hydrolyzed at Hours</u>	
<u>pH & Temperature</u>	<u>0</u> <u>24</u> <u>72</u> <u>120</u>
pH 5 at 50°C	0 1.4 11.4 19.4
pH 7 at 50°C	0 3.7 29.4 40
pH 9 at 50°C	0 2 35.7 44.4
<u>ENVIRONMENTAL SIGNIFICANCE</u>	
Hydrolysis of trandolapril was noticed at pH 7 and pH 9. Since the residence time of drugs in WTP is ≤48 hours, hydrolysis may not be a depletion mechanisms for the drug in WTP.	

**TABLE 3.7-4. Trandolaprilate - Determination of the Abiotic Degradation
ONE PAGE SUMMARY**

TEST CHEMICAL:	TRANDOLAPRILATE			
TITLE OF STUDY:	DETERMINATION OF THE ABIOTIC DEGRADATION			
NATURE OF STUDY:	ENVIRONMENTAL FATE AND EFFECTS			
STUDY PERFORMED BY:				
<u>SUMMARY OF RESULTS</u>				
The abiotic degradation (hydrolysis) of trandolapril was determined at pH 5, 7 and 9 using the method provided in FDA, TAD 3.09, Hydrolysis. The study was conducted at 50°C for 5 days (120 h). Based on the preliminary study results, percent hydrolyzed results were estimated.				
<u>Percent Hydrolyzed at Hours</u>				
<u>pH & Temperature</u>	<u>0</u>	<u>24</u>	<u>72</u>	<u>120</u>
pH 5 at 50°C	0	0	0	0
pH 7 at 50°C	0	0	0	0
pH 9 at 50°C	0	0	0	0
<u>ENVIRONMENTAL SIGNIFICANCE</u>				
Trandolaprilate, the acid metabolite of trandolapril, is the major component excreted by the patients using trandolapril. Since no hydrolysis was found at 50°C in any pH, hydrolysis will not be a depleting mechanism for the drug in the WTP.				

**TABLE 3.7-5. Trandolapril - Water Solubility
ONE PAGE SUMMARY**

TEST CHEMICAL: TRANDOLAPRIL												
TITLE OF STUDY: TRANDOLAPRIL - WATER SOLUBILITY												
NATURE OF STUDY: PHYSICAL CHEMICAL PROPERTIES												
STUDY PERFORMED BY: FRANCE												
<u>SUMMARY OF RESULTS</u>												
Water solubility of trandolapril was studied in pH 5, 7 and 9 buffers as specified in FDA, TAD 3.01, Water Solubility guidelines (FDA, 1987). Based on the water solubility results, the octanol/water partition coefficient (K_{ow}), the sorption coefficient (K_{oc}) and the bioconcentration factor (BCF) have also been estimated as seen in Table 3.7-3. The water solubility study was conducted at $20 \pm 5^\circ\text{C}$. Preliminary results are tabulated as follows:												
<table border="1"> <thead> <tr> <th><u>pH</u></th> <th><u>Solubility (g/L)</u></th> <th><u>Solubility (ppm)</u></th> </tr> </thead> <tbody> <tr> <td>5</td> <td>0.843</td> <td>843</td> </tr> <tr> <td>7</td> <td>8.15</td> <td>8,150</td> </tr> <tr> <td>9</td> <td>10.1</td> <td>10,100</td> </tr> </tbody> </table>	<u>pH</u>	<u>Solubility (g/L)</u>	<u>Solubility (ppm)</u>	5	0.843	843	7	8.15	8,150	9	10.1	10,100
<u>pH</u>	<u>Solubility (g/L)</u>	<u>Solubility (ppm)</u>										
5	0.843	843										
7	8.15	8,150										
9	10.1	10,100										
<u>ENVIRONMENTAL SIGNIFICANCE</u>												
Trandolapril is reasonably water soluble as stated above and as a result will not bioaccumulate in fish and other aquatic species. Therefore, trandolapril will have no environmental impact.												

TABLE 3.7-6. Trandolaprilate - Water Solubility
ONE PAGE SUMMARY

TEST CHEMICAL: TRANDOLAPRILATE

TITLE OF STUDY: TRANDOLAPRILATE - WATER SOLUBILITY

NATURE OF STUDY: PHYSICAL CHEMICAL PROPERTIES

STUDY PERFORMED BY:

SUMMARY OF RESULTS

Water solubility of trandolaprilate was studied in pH 5, 7 and 9 buffers as specified in FDA, TAD 3.01, Water Solubility guidelines (FDA, 1987). Based on the water solubility results, the octanol/water partition coefficient (K_{ow}), the sorption coefficient (K_{oc}) and the bioconcentration factor (BCF) have also been estimated as seen in Table 3.7-3. The water solubility study was conducted at $20 \pm 5^\circ$. Preliminary results are tabulated as follows:

<u>pH</u>	<u>Solubility (g/L)</u>	<u>Solubility (ppm)</u>
5	7.45	7,450
7	10.4	10,400
9	7.5	7,500

ENVIRONMENTAL SIGNIFICANCE

Trandolapril is reasonably water soluble as stated above and as a result will not bioaccumulate in fish and other aquatic species. Therefore, trandolapril will have no environmental impact.

TABLE 3.7-7. K_{ow} , K_{oc} and BCF of Trandolapril and Trandolaprilate
ONE PAGE SUMMARY

TEST CHEMICAL: TRANDOLAPRIL AND TRANDOLAPRILATE						
TITLE OF STUDY: KOW, KOC AND BCF ESTIMATIONS						
NATURE OF STUDY: ENVIRONMENTAL FATE AND EFFECTS						
STUDY PERFORMED BY:						
<u>SUMMARY OF RESULTS</u>						
Estimations of octanol/water partition coefficient (K_{ow}), adsorption coefficient (K_{oc}) and bioconcentration factor (BCF) are derived from preliminary experimental water solubility values determined at 3.I.T., France. $\text{Log } K_{ow} = 5.00 - 0.670 \log S$, where S is water solubility in $\mu\text{g/L}$ (ppb) $\text{Log } K_{oc} = 3.64 - 0.55 \log S$, where S is water solubility in mg/L (ppm) $\text{Log BCF} = 2.791 - 0.564 \log S$ is water solubility in mg/L (ppm)						
<u>pH</u>	<u>Trandolapril</u>			<u>Trandolaprilate</u>		
	<u>Log K_{ow}</u>	<u>Log K_{oc}</u>	<u>Log BCF</u>	<u>Log K_{oc}</u>	<u>Log K_{ow}</u>	<u>Log BCF</u>
pH 5	1.03	2.093	1.138	0.40	1.151	0.608
pH 7	0.37	1.490	0.585	0.30	1.429	0.524
pH 9	0.31	1.440	0.535	0.39	1.506	0.535
<u>ENVIRONMENTAL SIGNIFICANCE</u>						
The K_{ow} and K_{oc} values suggest that neither trandolapril nor trandolaprilate would strongly bind to organic matter present in sludge or soils, and therefore are likely to partition into wastewater effluent and eventually reach surface water, the aquatic environmental compartment. The BCF values suggest that neither of them will be found in the tissues of aquatic species.						

**TABLE 3.8-1. Trandolaprilate - Determination of Microbial Growth Inhibition
ONE PAGE SUMMARY**

TEST CHEMICAL: TRANDOLAPRILATE

TITLE OF STUDY: TRANDOLAPRILATE - DETERMINATION OF MICROBIAL GROWTH INHIBITION

NATURE OF STUDY: ENVIRONMENTAL FATE

STUDY PERFORMED BY:

SUMMARY OF RESULTS

A microbial growth inhibition study was conducted with trandolaprilate, a diacid metabolite of the drug trandolapril. The study was conducted according to FDA, TAD 4.02 guidelines, Microbial Growth Inhibition. The minimum inhibitory concentration (MIC) results are reported as follows:

<u>Species</u>	<u>MIC (mg/L)</u>
<i>Aspergillus niger</i>	≥ 1000
<i>Trichoderma vivide</i>	≥ 1000
<i>Clostridium perfringes</i>	≥ 1000
<i>Bacillus subtilis</i>	≥ 1000
<i>Nostoc sp.</i>	400

ENVIRONMENTAL SIGNIFICANCE

The MIC of trandolaprilate described above are several orders of magnitude higher than the MEEC of ***. Since trandolaprilate at the environmental concentrations shows no toxicity, there will be no environmental impact.

Because of the implementation of emission, safety and waste mitigation controls and no anticipated environmental impacts, no mitigation measures are planned.

3.11 ALTERNATIVES TO THE PROPOSED ACTION

No potential adverse environmental impacts have been identified for the proposed action. Because no adverse environmental impact is expected, alternatives to the proposed action are not being considered. If this product were not manufactured (as a no-action alternative), trandolapril to control hypertension in patients would not be available

3.12 LIST OF PREPAREPS

The undersigned certify that the information presented is true, accurate, and complete for preparation of the Environmental Assessment Report in accordance with 21 CFR 25.31(a).

Signature _____

Date April 15, 1996

Title Director, Environmental Fate and Assessment

Signature _____

Date April 15, 1996

Title Program Manager, Environmental Fate and Assessment

3.13 CERTIFICATION

The undersigned official certifies that the information presented herein and provided to _____ by Knoll Pharmaceuticals (applicant) is true, accurate, and complete to the best of our knowledge.

The undersigned official certifies that while Appendix C (the confidential EA) and Appendix D (study reports) are confidential, the EA summary document (this document) and Appendices A and B contain non-confidential information and acknowledges that the non-confidential information will be made available to the public in accordance with 40 CFR part 1506.6.

Signature Robert P. Kelly Date 7/16/96

Title ASSOCIATE DIRECTOR, REGULATORY AFFAIRS

3.14

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- B-7. U.S. Food and Drug Administration. 1995. Guidance for the Industry for the Submission of an Environmental Assessment in Human Drug Applications and Supplements. FDA, Washington, D.3. *Reference not included.

3.15 ATTACHMENTS

- 3.15-1 Certificate of Compliance with the Environmental Regulations for the the Site of Bulk Drug Manufacture, from the Ministry of Industry and National and Regional Development, Regional Director of Industry and Research,
- 3.15-2 Certificate of Compliance from the Facility Manager at The Site of Bulk Drug Trandolapril Manufacture
- 3.15-3 Certificate of Compliance from the Facility Manager, at Knoll Pharmaceutical Company, Whippany, New Jersey, The Site of Drug Product Manufacture.

- 3.15-4 Knoll Pharmaceutical Internal Memos (September 26, 1995 and October 6, 1995) Detailing Environmental Permits.
- 3.15-5 Certificate of Compliance from the Director of Quality Assurance at The Site of Packaging from the Trandolapril Tablets.
- 3.15-6 Environmental Settings and Emission Controls at Site of Packaging for the Trandolapril Tablets.

ATTACHMENT 3.15-1

**Certificate of Compliance with the Environmental Regulations for the
the Site of Bulk Drug Manufacture, from the Ministry of Industry and National
and Regional Development, Regional Director of Industry and Research,**

4 pages

PURGED

ATTACHMENT 3.15-2

**Certificate of Compliance from the Facility Manager at
The Site of Bulk Drug Trandolapril Manufacture**

2 PAGES

PURGED

ATTACHMENT 3.15-3

**Certificate of Compliance from the Facility Manager, at Knoll Pharmaceutical
Company, Whippany, New Jersey, The Site of Drug Product Manufacture**



0055

BASP Pharma

TELEFAX

Date: April 1, 1998 Pages: Ten (w/cover) FAX No: 201-331-3842

To: Richard Nelson

From: Dave Hardy

Rich,

As discussed, attached are copies of the Environmental Permits that pertain to the production of Mavik. The documents are as follows:

- a. NJDEP Permit #080798: Tablet Manufacturing Central Dust Collector
- b. NJDEP Permit #080798: Tablet Manufacturing Dept. Process Dust Collector
- c. NJDEP Permit #079511: Despatch Tray Dryer (assumes Mavik is tray dried)
- d. NJDEP Permit #107443: Packaging Line Dust Collector #1
- e. NJDEP Permit #108108: Packaging Line Dust Collector #2/4
- f. NJDEP Permit #069099: 48" Coating Pan Dust Collector
- g. NJDEP Permit #116128: 60" Coating Pan Dust Collector
- h. NJDEP Permit #119280: 48" Coating Pan Solution Prep Dust Collector

i. Hanover Sewerage Permit (HSA) # D-9

Notes:

- 1. Items #f, g & h apply only if Mavik is film coated.
- 2. The HSA Permit is currently being renewed. The new expiration date will be March 31, 2001.

If you have any further questions, please do not hesitate to contact me. I will E-Mail a brief site summary (per ABC Lab letter) later today.

DIVISION OF ENVIRONMENTAL QUALITY
AIR POLLUTION CONTROL PROGRAM

0056

A/C Correspondence must include your A/C PLANT ID NUMBER

Certificate Number 080796 APC PLANT ID 25238

(Mailing Address)

(Plant Location)

KNOLL PHARMACEUTICALS A UNIT OF BASF KDF
30 NO. JEFFERSON ROAD
WHIPPANY NJ 07981

KNOLL PHARMACEUTICALS
30 NO. JEFFERSON ROAD
HANOVER TWP

Applicant's Designation of Equipment D.C. CY802 (CENTRAL VACUUM)
N.J. Stack No. 017 No. of Stacks 003 No. of Sources 32
Approval 12/14/87 Effective 12/14/87 09/08/98

• CERTIFICATE TO OPERATE CONTROL APPARATUS OR EQUIPMENT •

• FIVE YEAR RENEWAL •

THIS RENEWED FIVE YEAR CERTIFICATE IS BEING ISSUED UNDER THE AUTHORITY OF CHAPTER 106, P.L. 1967 (N.J.S.A. 26:2C-9.2). THE POSSESSION OF THIS DOCUMENT DOES NOT RELIEVE YOU FROM THE OBLIGATION OF COMPLYING WITH ALL PROVISIONS OF THE NEW JERSEY ADMINISTRATIVE CODE, TITLE 7, CHAPTER 27.

THE EQUIPMENT COVERED BY THIS CERTIFICATE MAY BE SUBJECT TO AT LEAST TWO PERIODIC COMPLIANCE INSPECTIONS, PURSUANT TO N.J.A.C. 7:27-8.8(C). PURSUANT TO N.J.A.C. 7:27-8.11, YOU WILL BE INVOICED FOR A \$200 FEE AFTER EACH PERIODIC INSPECTION THAT IS CONDUCTED. YOU MAY ALSO BE SUBJECT TO FEES FOR SERVICES THAT ARE PERFORMED BY THE DEPARTMENT IN ACCORDANCE WITH THE CONDITIONS OF APPROVAL OF THIS DOCUMENT. IF YOU FAIL TO PAY A FEE, THE DEPARTMENT MAY ASSESS CIVIL ADMINISTRATIVE PENALTIES AND/OR REVOKE THIS CERTIFICATE.

PURSUANT TO N.J.A.C. 7:27-8.7(F), THE DEPARTMENT MAY MODIFY THE CONDITIONS OF APPROVAL OF THIS CERTIFICATE AT THE TIME OF RENEWAL OR AT ANY TIME WHEN THE CERTIFICATE IS IN FORCE, IF DEEMED NECESSARY TO PROTECT HUMAN HEALTH, WELFARE OR THE ENVIRONMENT.

IN ACCORDANCE WITH N.J.S.A. 54:4-3.56 TO 3.58, YOU MAY BE ENTITLED TO AN EXEMPTION FROM TAXATION IF YOUR EQUIPMENT IS TAXED AND IS CONSIDERED TO BE AN AIR POLLUTION CONTROL DEVICE. A TAX EXEMPTION APPLICATION MAY BE OBTAINED FROM THE BUREAU OF NEW SOURCE REVIEW (SEE OTHER SIDE).

IN ACCORDANCE WITH N.J.A.C. 7:27-8.3(D), YOU SHALL MAKE THIS CERTIFICATE READILY AVAILABLE FOR INSPECTION ON THE OPERATING PREMISES.

Approved by:

Donald Patterson

01/02/90

NEW JERSEY STATE DEPARTMENT OF ENVIRONMENTAL PROTECTION

DIVISION OF ENVIRONMENTAL QUALITY
AIR POLLUTION CONTROL PROGRAM

0057

All Correspondence must include your APC PLAN ID NUMBER

Certificate Number 080798 APC PLANT ID 25238

(Mailing Address)

(Plant Location)

KNOLL PHARMACEUTICALS A UNIT OF BASF KDF
30 NO. JEFFERSON ROAD
WHIPPANY NJ 07981

KNOLL PHARMACEUTICALS
30 NO. JEFFERSON ROAD
HANOVER TWP

Production Dust Collector

Applicant's Designation of Equipment

D.C.-CY801 (RM AIR EXHAUST)

N.J. Stack No. 019

No. of Stacks 001

No. of Sources 53

Approval 12/14/87

Effective 12/14/87

09/08/98

CERTIFICATE TO OPERATE CONTROL APPARATUS OR EQUIPMENT

FIVE YEAR RENEWAL

THIS RENEWED FIVE YEAR CERTIFICATE IS BEING ISSUED UNDER THE AUTHORITY OF CHAPTER 106, P.L. 1967 (N.J.S.A. 26:2C-9.2). THE POSSESSION OF THIS DOCUMENT DOES NOT RELIEVE YOU FROM THE OBLIGATION OF COMPLYING WITH ALL PROVISIONS OF THE NEW JERSEY ADMINISTRATIVE CODE, TITLE 7, CHAPTER 27.

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PURSUANT TO N.J.A.C. 7:27-8.7(F), THE DEPARTMENT MAY MODIFY THE CONDITIONS OF APPROVAL OF THIS CERTIFICATE AT THE TIME OF RENEWAL OR AT ANY TIME WHEN THE CERTIFICATE IS IN FORCE, IF DEEMED NECESSARY TO PROTECT HUMAN HEALTH, WELFARE OR THE ENVIRONMENT.

IN ACCORDANCE WITH N.J.S.A. 54:4-3.56 TO 3.58, YOU MAY BE ENTITLED TO AN EXEMPTION FROM TAXATION IF YOUR EQUIPMENT IS TAXED AND IS CONSIDERED TO BE AN AIR POLLUTION CONTROL DEVICE. A TAX EXEMPTION APPLICATION MAY BE OBTAINED FROM THE BUREAU OF NEW SOURCE REVIEW (SEE OTHER SIDE).

IN ACCORDANCE WITH N.J.A.C. 7:27-8.9(D), YOU SHALL MAKE THIS CERTIFICATE READILY AVAILABLE FOR INSPECTION ON THE OPERATING PREMISES.

Approved by:

Donald Patterson

07/01/92

DIVISION OF ENVIRONMENTAL QUALITY
AIR POLLUTION CONTROL PROGRAM

0058

All Correspondence must indicate your APC PLANT ID NUMBER

Certificate Number 079511 APC PLANT ID 25288

(Mailing Address)

(Plant Location)

KNOLL PHARMACEUTICALS A UNIT OF BASF KDF
30 NO. JEFFERSON ROAD
WHIPPANY NJ 07981

KNOLL PHARMACEUTICALS
30 NO. JEFFERSON ROAD
HANDVER TWP

Applicant's Designation of Equipment: DESPATCH TRAY DRYER (Z-200)
N.J. Stack No. 015 No. of Stacks 001 No. of Sources 01
Approval 08/12/87 Effective 11/10/87 ~~11/03/98~~ 11/03/98

• CERTIFICATE TO OPERATE CONTROL APPARATUS OR EQUIPMENT •
• FIVE YEAR RENEWAL •

THIS RENEWED FIVE YEAR CERTIFICATE IS BEING ISSUED UNDER THE AUTHORITY OF CHAPTER 106, P.L. 1967(N.J.S.A.26:2C-9.2). THE POSSESSION OF THIS DOCUMENT DOES NOT RELIEVE YOU FROM THE OBLIGATION OF COMPLYING WITH ALL PROVISIONS OF THE NEW JERSEY ADMINISTRATIVE CODE, TITLE 7, CHAPTER 27.

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PURSUANT TO N.J.A.C. 7:27-8.7(P), THE DEPARTMENT MAY MODIFY THE CONDITIONS OF APPROVAL OF THIS CERTIFICATE AT THE TIME OF RENEWAL OR AT ANY TIME WHEN THE CERTIFICATE IS IN FORCE, IF DEEMED NECESSARY TO PROTECT HUMAN HEALTH, WELFARE OR THE ENVIRONMENT.

IN ACCORDANCE WITH N.J.S.A. 54:4-3.56 TO 3.58, YOU MAY BE ENTITLED TO AN EXEMPTION FROM TAXATION IF YOUR EQUIPMENT IS TAXED AND IS CONSIDERED TO BE AN AIR POLLUTION CONTROL DEVICE. A TAX EXEMPTION APPLICATION MAY BE OBTAINED FROM THE BUREAU OF NEW SOURCE REVIEW (SEE OTHER SIDE).

IN ACCORDANCE WITH N.J.A.C. 7:27-8.3(D), YOU SHALL MAKE THIS CERTIFICATE READILY AVAILABLE FOR INSPECTION ON THE OPERATING PREMISES.

Bonnie Peterson

Approved by: _____

08/20/87-2

DIVISION OF ENVIRONMENTAL QUALITY
AIR POLLUTION CONTROL PROGRAM

0059

All Correspondence must include your APC PLANT ID NUMBER

Certificate Number 107443

APC PLANT ID 25238

(Mailing Address)

(Plant Location)

KNOLL PHARMACEUTICALS A UNIT OF BASF KDF
30 NO. JEFFERSON ROAD
WHIPPANY NJ 07961

30 NO. JEFFERSON ROAD
HANOVER TWP

Applicant's Designation of Equipment

PACKAGING LINES DUST COLLECTOR

N.J. Stack No. 026

No. of Stacks 001

No. of Sources 04

Approval 06/04/92

Effective 06/04/92

ISSUED 08/17/00

CERTIFICATE TO OPERATE CONTROL APPARATUS OR EQUIPMENT

FIVE YEAR

THIS FIVE YEAR CERTIFICATE IS BEING ISSUED UNDER THE AUTHORITY OF CHAPTER 106/P.L. 1967 (N.J.S.A. 26:2C-9.2). THE POSSESSION OF THIS DOCUMENT DOES NOT RELIEVE YOU OF THE OBLIGATION TO COMPLY WITH ALL PROVISIONS OF THE NEW JERSEY ADMINISTRATIVE CODE, TITLE 7, CHAPTER 27.

THE EQUIPMENT COVERED BY THIS CERTIFICATE MAY BE SUBJECT TO AT LEAST ONE PERIODIC COMPLIANCE INSPECTION, PURSUANT TO N.J.A.C. 7:27-8.8(C). YOU WILL BE NOTIFIED BY LETTER WITHIN 60 DAYS OF THE EFFECTIVE DATE OF THIS DOCUMENT OF THE MAXIMUM NUMBER OF THESE INSPECTIONS (IF GREATER THAN ONE). PURSUANT TO N.J.A.C. 7:27-8.11, YOU WILL BE INVOICED FOR A \$200 FEE AFTER EACH PERIODIC INSPECTION THAT IS CONDUCTED. YOU MAY ALSO BE SUBJECT TO FEES FOR SERVICES THAT ARE PERFORMED BY THE DEPARTMENT IN ACCORDANCE WITH THE CONDITIONS OF APPROVAL OF THIS DOCUMENT. IF YOU FAIL TO PAY A FEE, THE DEPARTMENT MAY ASSESS CIVIL ADMINISTRATIVE PENALTIES AND/OR REVOKE THIS CERTIFICATE.

PURSUANT TO N.J.A.C. 7:27-8.7(F), THE DEPARTMENT MAY MODIFY THE CONDITIONS OF APPROVAL OF THIS CERTIFICATE AT THE TIME OF RENEWAL OR AT ANY TIME WHEN THE CERTIFICATE IS IN FORCE, IF DEEMED NECESSARY TO PROTECT HUMAN HEALTH, WELFARE OR THE ENVIRONMENT.

IN ACCORDANCE WITH N.J.S.A. 54:14-3.56 TO 3.58, YOU MAY BE ENTITLED TO AN EXEMPTION FROM TAXATION IF YOUR EQUIPMENT IS TAXED AND IS CONSIDERED TO BE AN AIR POLLUTION CONTROL DEVICE. A TAX EXEMPTION APPLICATION MAY BE OBTAINED FROM THE BUREAU OF NEW SOURCE REVIEW (SEE OTHER SIDE).

IN ACCORDANCE WITH N.J.A.C. 7:27-8.3(D), YOU SHALL MAKE THIS CERTIFICATE READILY AVAILABLE FOR INSPECTION ON THE OPERATING PREMISES.

Approved by:

Donald Patters

NRG

06/04/92-04



DIVISION OF ENVIRONMENTAL QUALITY
AIR POLLUTION CONTROL PROGRAM

0060

All Correspondence must indicate your APC PLANT ID NUMBER

Certificate Number 104106

APC PLANT ID 25238

(Mailing Address)

(Plant Location)

KNOLL PHARMACEUTICALS A UNIT OF EAST KOD
30 NO. JEFFERSON ROAD
WHIPPARY NJ 07981

30 NO. JEFFERSON ROAD
HANOVER TWP

Series 2+4

Applicant's Designation of Equipment
N.J. Stack No. 027
Approval 07/23/92

PACKAGING DUST COLLECTOR
No. of Stacks 002
Effective 07/23/92

No. of Sources 04
07/01/96

• TEMPORARY CERTIFICATE TO OPERATE CONTROL APPARATUS OR EQUIPMENT •
• CONDITIONAL 90 DAY EXTENSION •

THIS TEMPORARY CERTIFICATE IS BEING EXTENDED TO ALLOW FOR FURTHER FIELD/OFFICE EVALUATION.

THIS EXTENSION SHALL NOT BE CONSTRUED TO EXTEND THE COMPLIANCE DATES(S) OF ANY ORDER ISSUED BY OR ENTERED INTO WITH THE DEPARTMENT AS THE RESULT OF AN ADMINISTRATIVE OR JUDICIAL ACTION.

THE EQUIPMENT COVERED BY THIS CERTIFICATE MAY BE SUBJECT TO PERIODIC COMPLIANCE INSPECTIONS, PURSUANT TO N.J.A.C. 7:27-8.8(C). YOU WILL BE INVOICED FOR A \$200 FEE AFTER EACH PERIODIC COMPLIANCE INSPECTION. YOU WILL NOT BE INVOICED FOR AN INSPECTION DURING WHICH A 5 YEAR APPROVAL DETERMINATION IS MADE. YOU MAY BE INVOICED FOR FEES FOR OTHER SERVICES THAT ARE PERFORMED BY THE DEPARTMENT PURSUANT TO CONDITIONS OF APPROVAL.

IF THE DEPARTMENT IS SOLELY RESPONSIBLE FOR BEING UNABLE TO INSPECT THIS EQUIPMENT IN OPERATION AS PERMITTED DURING THIS 90-DAY PERIOD, THIS TEMPORARY CERTIFICATE WILL BE EXTENDED AUTOMATICALLY. HOWEVER, IF YOU ARE RESPONSIBLE FOR THE DEPARTMENT'S BEING UNABLE TO INSPECT, E.G., NOT NOTIFYING THE DEPARTMENT WHEN THIS EQUIPMENT OR PROCESS IS IN OPERATION, THIS CERTIFICATE MAY NOT BE EXTENDED AND YOU WILL BE NOTIFIED BY THE DEPARTMENT THAT YOU MUST APPLY FOR AND OBTAIN AN EXTENSION AUTHORIZING YOU TO CONTINUE TO OPERATE THE EQUIPMENT. THE DEPARTMENT RESERVES THE RIGHT TO WITHHOLD ANY EXTENSION OF THIS TEMPORARY CERTIFICATE, IN WHICH EVENT YOU WILL BE NOTIFIED THAT YOU MUST APPLY FOR AND OBTAIN AN EXTENSION AUTHORIZING YOU TO CONTINUE TO OPERATE AFTER THE EXPIRATION DATE OF THIS CERTIFICATE.

IN ACCORDANCE WITH N.J.A.C. 7:27-8.2(b), THIS CERTIFICATE MUST BE READILY AVAILABLE FOR INSPECTION ON THE OPERATING PREMISES.

PLEASE REFER TO YOUR INITIAL PERMIT APPROVAL FOR OPERATING CONDITIONS.

Approved by: Donald P. Petter

DIVISION OF ENVIRONMENTAL QUALITY
AIR POLLUTION CONTROL PROGRAM

0061

~~All Certificates issued under this program are UNLAWFUL~~
Certificate Number 069099 APC PLANT ID 25230

(Mailing Address)

(Plant Location)

KNOLL PHARMACEUTICALS A UNIT OF BASF KOP
30 NO. JEFFERSON ROAD
MIDDLETOWN NJ 07941

30 NO. JEFFERSON ROAD
MIDDLETOWN NJ

Applicant's Designation of Equipment: TORIT-B-C (CARBON CONTAINER)
N.J. Stack No. 009 No. of Stacks 001 No. of Sources 01
Approval: 08/29/84 Effective: 08/29/84 Issued: 08/29/84

10-CERTIFICATE TO OPERATE CONTROL APPARATUS OR EQUIPMENT
FIVE YEAR RENEWAL

THIS RENEWED FIVE YEAR CERTIFICATE IS BEING ISSUED UNDER THE AUTHORITY OF CHAPTER 106, P.L. 1967 (N.J.S.A. 26:2C-9, 2). THE POSSESSION OF THIS DOCUMENT DOES NOT RELIEVE YOU FROM THE OBLIGATION OF COMPLYING WITH ALL PROVISIONS OF THE NEW JERSEY ADMINISTRATIVE CODE, TITLE 7, CHAPTER 27.

THE EQUIPMENT COVERED BY THIS CERTIFICATE MAY BE SUBJECT TO AT LEAST TWO PERIODIC COMPLIANCE INSPECTIONS, PURSUANT TO N.J.A.C. 7:27-8.3(C). PURSUANT TO N.J.A.C. 7:27-8.3, YOU WILL BE INVOICED FOR A \$200 FEE AFTER EACH PERIODIC INSPECTION THAT IS CONDUCTED. YOU MAY ALSO BE SUBJECT TO FEES FOR SERVICES THAT ARE PERFORMED BY THE DEPARTMENT IN ACCORDANCE WITH THE CONDITIONS OF APPROVAL OF THIS DOCUMENT. IF YOU FAIL TO PAY A FEE, THE DEPARTMENT MAY ASSESS CIVIL ADMINISTRATIVE PENALTIES AND/OR REVOKE THIS CERTIFICATE.

PURSUANT TO N.J.A.C. 7:27-8.7(F), THE DEPARTMENT MAY MODIFY THE CONDITIONS OF APPROVAL OF THIS CERTIFICATE AT THE TIME OF RENEWAL OR AT ANY TIME WHEN THE CERTIFICATE IS IN FORCE, IF DEEMED NECESSARY TO PROTECT HUMAN HEALTH, WELFARE OR THE ENVIRONMENT.

IN ACCORDANCE WITH N.J.S.A. 54:4-2.56 TO 2.58, YOU MAY BE ENTITLED TO AN EXEMPTION FROM TAXATION IF YOUR EQUIPMENT IS TAXED AND IS CONSIDERED TO BE AN AIR POLLUTION CONTROL DEVICE. A TAX EXEMPTION APPLICATION MAY BE OBTAINED FROM THE BUREAU OF NEW SOURCE REVIEW (SEE OTHER SIDE).

IN ACCORDANCE WITH N.J.A.C. 7:27-8.3(D), YOU SHALL MAKE THIS CERTIFICATE READILY AVAILABLE FOR INSPECTION ON THE OPERATING PREMISES.

Donald Patterson

Approved by: _____

NRD

06/13/94-17

DIVISION OF ENVIRONMENTAL QUALITY
AIR POLLUTION CONTROL PROGRAM

0062

~~All Control, permit, and other information available from the State Department of Environmental Protection~~
Certificate Number: 214129... LOG NUMBER: 940474A APC PLANT ID: 25238

(Mailing Address)

(Plant Location)

KNOLL PHARMACEUTICALS A UNIT OF BASF KDR
30 NO. JEFFERSON ROAD
WHIPPANY NJ 07981
Took photo of ID

30 NO. JEFFERSON ROAD
HANOVER TWP

Applicant's Designation of Equipment: CY803 (4000) CFM TOREX DUST COLLEC.
N.J. Stack No. 018 No. of Stacks: 001 No. of Sources: 01
Approval Effective: 02/22/94 Expiration: 02/22/99

CERTIFICATE TO OPERATE CONTROL APPARATUS OR EQUIPMENT
CONDITIONAL FIVE YEAR DIRECT

THIS CONDITIONAL FIVE YEAR CERTIFICATE IS BEING ISSUED UNDER THE AUTHORITY OF CHAPTER 196 OF P.L. 1967 (N.J.S.A. 17:27-9.2) WITHOUT A FIELD INSPECTION. HOWEVER, FIELD INSPECTIONS ARE SCHEDULED FOR THE FUTURE AND APPROPRIATE ACTIONS WILL BE TAKEN IF SUCH INSPECTIONS DISCLOSE DEVIATIONS FROM YOUR APPROVED PERMIT.

THE EQUIPMENT COVERED BY THIS CERTIFICATE MAY BE SUBJECT TO AT LEAST TWO PERIODIC COMPLIANCE INSPECTIONS, PURSUANT TO N.J.A.C. 7:27-9.3(C). PURSUANT TO N.J.A.C. 7:27-9.3(D), YOU WILL BE INVOICED FOR A \$200 FEE AFTER EACH PERIODIC INSPECTION THAT IS CONDUCTED. YOU MAY ALSO BE SUBJECT TO FEES FOR SERVICES THAT ARE PERFORMED BY THE DEPARTMENT IN ACCORDANCE WITH THE CONDITIONS OF APPROVAL OF THIS DOCUMENT. IF YOU FAIL TO PAY A FEE, THE DEPARTMENT MAY ASSESS CIVIL ADMINISTRATIVE PENALTIES AND/OR REVOKE THIS CERTIFICATE.

IN ACCORDANCE WITH N.J.S.A. 17:54A-3.56 TO 3.58, YOU MAY BE ENTITLED TO AN EXEMPTION OF TAXATION IF YOUR EQUIPMENT IS TAXED AND IS CONSIDERED TO BE AN AIR POLLUTION CONTROL DEVICE. A TAX EXEMPTION APPLICATION MAY BE OBTAINED FROM THE BUREAU OF NEW SOURCE REVIEW. (SEE OTHER SIDE)

IF IT IS NECESSARY TO amend your EMERGENCY STANDBY PLANS, PLEASE CONSULT WITH THE APPROPRIATE REGIONAL OFFICE. (SEE OTHER SIDE)

IN ACCORDANCE WITH N.J.A.C. 7:27-9.3(D), THIS CERTIFICATE MUST BE READILY AVAILABLE FOR INSPECTION ON THE OPERATING PREMISES. PLEASE REFER TO YOUR INITIAL PERMIT APPROVAL FOR OPERATING CONDITIONS.

Approved by: Donald Patterson

NRO

05/16/94-55

DIVISION OF ENVIRONMENTAL QUALITY
AIR POLLUTION CONTROL PROGRAM

0063

All Certificates are subject to the AIR PLANT ID NUMBER

Certificate Number 119280

APC PLANT ID 25238

(Mailing Address)

(Plant Location)

KNOLL PHARMACEUTICALS A UNIT OF BASF KDF
30 NO. JEFFERSON ROAD
MIDDLETOWN NJ 07941

30 NO. JEFFERSON ROAD
HANOVER TWP

Form 64

Applicant's Designation of Equipment
N.J. Stock No. 029
Approval

88" PAN SOL-PREP DUST COLLECTOR
No. of Stacks 001
Effective 12/19/94

No. of Sources 01
06/16/00

CERTIFICATE TO OPERATE CONTROL APPARATUS OR EQUIPMENT

FIVE YEAR

THIS FIVE YEAR CERTIFICATE IS BEING ISSUED UNDER THE AUTHORITY OF CHAPTER 106, P.L. 1967 (N.J.S.A. 26:2C-9.2). THE POSSESSION OF THIS DOCUMENT DOES NOT RELIEVE YOU OF THE OBLIGATION TO COMPLY WITH ALL PROVISIONS OF THE NEW JERSEY ADMINISTRATIVE CODE, TITLE 7, CHAPTER 27.

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IN ACCORDANCE WITH N.J.A.C. 7:27-8.3(D), YOU SHALL MAKE THIS CERTIFICATE READILY AVAILABLE FOR INSPECTION ON THE OPERATING PREMISES.

Approved by:

Donald Patterson

NR0

06/09/95-04

HANOVER SEWERAGE AUTHORITY
1000 ROUTE 10, P.O. BOX 250, WHIPPANY, NEW JERSEY 07981

INDUSTRIAL SEWER DISCHARGE PERMIT

PERMIT NO. 1-A SIC NO. 2834
In compliance with the provisions of the Federal Water Pollution Control Act of 1972 (PL 92-500) and its amendments, The Clean Water Act of 1977 and the Regulations and Specifications concerning Discharges to the Hanover Sewerage Authority.

WELL PHARMACEUTICAL COMPANY
30 NORTH JEFFERSON ROAD, WHIPPANY, NEW JERSEY 07981

is authorized to discharge industrial wastewater from the facility located at:
BLOCK 9102 LOT 05 into the Hanover Sewerage Authority Wastewater Treatment System in accordance with discharge limitations, monitoring requirements and other conditions as set forth therein.

Effective Date: April 1, 1991
Expiration Date: MARCH 31, 1996

Pete W. Parks
Industrial Director

ATTEST: *Michael C. Squire*
ADMINISTRATOR

ATTACHMENT 3.15-4

Knoll Pharmaceutical Internal Memos Detailing Environmental Permits

6 pages

PURGED

ATTACHMENT 3.15-6

**Environmental Settings and Emission Controls at
Site for Packaging for the Trandolapril Tablets**

FACSIMILE TRANSMISSION COVER SHEET

Please deliver immediately to:

Name	
Location	
Telephone Number	
FAX Number	

From:

Name	
Department	
FAX Number	
Telephone Number	
NOTE	
Number of pages (including cover sheet)	5

4 pages

PURGED

APPENDIX A

Material Safety Data Sheets

- A-1 Material Safety Data Sheets (MSDS) for Trandolapril**
- A-2 Material Safety Data Sheets (MSDS) for Trandolaprilat**

APPENDIX A-1

Material Safety Data Sheets (MSDS) for Trandolapril

**MATERIAL SAFETY
DATA SHEET**

NO : 1741

NFI :

Date of issue : 10/12/89

Date of revision : 07/03/90

Version :

0080
000113

**NOTA : THE LATEST PERTINENT MODIFICATIONS INTRODUCED IN THE SAFETY DATA SHEET ARE
SIGNALIZED BY A LETTER PLACED IN FRONT OF THE CONCERNED HEADING.
INDUSTRIAL HYGIENE DELEGATION - TEL**

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY

IDENTIFICATION OF THE SUBSTANCE OR PREPARATION

TRADE NAME : TRANDOLAPRIL
CHEMICAL FAMILY : antihypertensive
FORMULA : C24 H33 N2 O5
MOLECULAR MASS : 429.7
KIND OF USE : Medical use

COMPANY/UNDERTAKING IDENTIFICATION

SUPPLIER
ADDRESS

EMERGENCY PHONE NUMBER

2. COMPOSITION/INFORMATION ON INGREDIENTS

SUBSTANCE

SYNONYMS
CAS NUMBER
CHEMICAL NAME

3. HAZARDS IDENTIFICATION

MAIN HAZARDS : HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED.

4. FIRST-AID MEASURES IN CASE OF EXPOSURE

INHALATION : Make the victim blow his nose
SKIN CONTACT : Rinse with plenty of water
EYES CONTACT : Rinse immediately with plenty of water for at least 15 minutes.
INGESTION : Do not make the victim vomit.
Alert a physician.

5. FIRE-FIGHTING MEASURES

- SUITABLE EXTINGUISHING MEDIA : Usual means .
- UNSUITABLE EXTINGUISHING MEDIA : Jet of water from a fire hose
- FIRE AND EXPLOSION HAZARDS : In case of fire, the product emits toxic fumes.
- SPECIAL PROTECTIVE EQUIPMENT : Wear a self-contained respiratory apparatus.
- OTHER RECOMMENDATIONS : Avoid rejection of extinguishing water in the environment .

6. ACCIDENTAL RELEASE MEASURES

- MEASURES AFTER LEAKING OR SPIL : Collect thoroughly into plastic bag.
Rinse the polluted area with plenty of water.

7. HANDLING AND STORAGE

HANDLING

- TECHNICAL MEASURES : Mechanical sucking ventilation at source of formation of dust
- SPECIAL PROTECTION MEASURES : None.

STORAGE

- SENSITIVITY TO DAMP : Not observed.
- SENSITIVITY TO LIGHT : Not observed.
- SENSITIVITY TO OXIDATION : Not observed.
- SPECIAL STORAGE REQUIREMENTS : Room temperature (<86 F)
- STORAGE CONDITIONS : Usual conditions (T <= 86 F, away from light)
- SHELF LIFE : 3 years.
- STORAGE FACILITIES : Harmful products.
- INCOMPATIBLE SUBSTANCES : Unknown.
- RECOMMENDED PACKAGING MATERIAL : Glass, polythene.
- SUPPLIER PACKAGING : polythene bag in aluminium or cardboard packaging.
- PACKAGING MATERIAL TO BE AVOIDED : Unknown.
- OTHER RECOMMENDATIONS : None.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

CONTROL OF EXPOSURE

- TWA : ND
- STEL : ND
- IDLH : ND
- OLFACTORY THRESHOLD : ND

PERSONAL PROTECTION/WORKSHOP

- RESPIRATORY TRACT PROTECTION : Wear a dust respirator.
- PROTECTIVE GLOVES : Wear suitable gloves
- EYES PROTECTION : Wear glasses

PERSONAL PROTECTION/LABORATORY

- RESPIRATORY TRACT PROTECTION : Wear a dust respirator.
- PROTECTIVE GLOVES : Wear suitable gloves
- EYES PROTECTION : Wear glasses

9. PHYSICAL AND CHEMICAL PROPERTIES

- APPEARANCE : white powder
- pH : Not determined.
- CHARACTERISTIC TEMPERATURES : Not determined.
- FLASH POINT : Not applicable.
- AUTOINFLAMMABILITY : Not determined.
- EXPLOSIVITY CHARACTERISTICS : ND
- EXPLOSIVE PROPERTIES/KST : 125 (dP/dT max : 468 mbar)
- MINIMUM INFLAMMATION ENERGY : 20 mJ
- COMMENTS ON FLAMMABILITY : little explosive.
Very low speed of inflammation propagation.
- VAPOUR PRESSURE : Not determined.
- VOLUMIC MASS : Not determined.
- SOLUBILITY : Insoluble in water Soluble in : chloroform, methanol,
methylene chloride
- OTHER DATA : alpha 20D : -17 to -19° (2%, pure ethanol)

10. STABILITY AND REACTIVITY

- HAZARDOUS REACTIONS : None to our knowledge
- HAZARDOUS DECOMPOSITION PRODUCTS : Unknown.

11. TOXICOLOGICAL INFORMATION

- ACUTE TOXICITY : LD 50 oral route/rat : > 5000 mg/kg LD 50 oral route
/mouse : 3990(F)-4875 mg/kg(M). LD50 IP/rat : 1420(M)-1435
mg/kg(F).
No observable or systemic effects were noted per oral route
in rat and dogs during chronic studies.
- PHYSIOLOGICAL ACTIVITY : Substance physiologically active at low dose.,
antihypertive (3 mg/kg per os) and angiotensine conversion
enzyme inhibitor (0.3 mg/kg per os).
Possible adverse effects in case of treatment :
hypotension, cough, angioneurotic oedema.
- SPECIFIC EFFECTS : Carcinogenicity : no carcinogenic potentiality by oral
route in rat.
- COMMENTS/SYMPTOMS : HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF
SWALLOWED., Remark : when handling the product, nausea,
headache, tremors were noted.

12. ECOLOGICAL INFORMATION

- ENVIRONMENT : Biodegradable in natural media

13. DISPOSAL CONSIDERATIONS

- NEUTRALIZATION OF THE PRODUCT : Incineration in accordance with laws.
- DESTRUCTION SOILED PACKAGING : Incineration in accordance with laws.

14. TRANSPORT INFORMATION

INTERNATIONAL REGULATIONS

LAND WAYS : 6.1.90°c,6.1A
 MARITIME WAYS : 6.1.gr III,p6236.6 nocrl
 AERIAL WAYS : 6.1.gr III,,6 nocrl
 ONU : 2811

OTHER REGULATIONS

LAND WAYS - FRANCE : 6.1.90°c,6.1A

SPECIAL PRECAUTIONS

15. REGULATORY INFORMATION

COMMUNITY REGULATIONS

LABELLING (EEC NUMBER) : Labelling according to EEC regulations
 SYMBOLS : Xn
 PHRASES :
 SPECIAL RISKS : HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED.
 SAFETY ADVICES : Wear suitable gloves and eye/face protection.

16. OTHER INFORMATION

FURTHER INFORMATIONS : None.

All the regulatory instructions mentioned are intended to help the addressee to comply with his obligations when using the product. This list cannot be considered as exhaustive and does not discharge the addressee from his duty to enquire about all other regulatory provisions which may apply to the possession and handling of the product for which he bears sole liability.

The information given in this data sheet has been introduced in accordance with the guidelines established by article 10 of EEC directive, dated March 5, 1991.
 This data sheet complements the user's instructions, but does not replace them.
 The information it contains is based on the knowledge available about the product concerned at the time it was compiled. Users are further reminded of the possible risks of using a product for purposes other than those for which it was intended.
 The required information complies with current EEC legislation; addressees are requested to apply any additional national requirements.

APPENDIX A-2

Material Safety Data Sheets (MSDS) for Trandolaprilat

**MATERIAL SAFETY
DATA SHEET**

NO : 1754
NFI :

Date of Issue : 10/12/89
Date of revision : 06/20/91
Version :

0085

**NOTA : THE LATEST PERTINENT MODIFICATIONS INTRODUCED IN THE SAFETY DATA SHEET ARE
SIGNALIZED BY A LETTER PLACED IN FRONT OF THE CONCERNED HEADING.
INDUSTRIAL HYGIENE DELEGATION - TEL**

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY

IDENTIFICATION OF THE SUBSTANCE OR PREPARATION

TRADE NAME :
CHEMICAL FAMILY : Antihypertensive **TRANDCLOARILAT**
FORMULA : C22 H30 N2 O5
MOLECULAR MASS : 402.5
KIND OF USE : Medical use

COMPANY/UNDERTAKING IDENTIFICATION

SUPPLIER :
ADDRESS :

EMERGENCY PHONE NUMBER

2. COMPOSITION/INFORMATION ON INGREDIENTS

SUBSTANCE

3. HAZARDS IDENTIFICATION

MAIN HAZARDS : HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED.

4. FIRST-AID MEASURES IN CASE OF EXPOSURE

INHALATION : Make the victim blow his nose
SKIN CONTACT : Rinse with plenty of water
EYES CONTACT : Rinse immediately with plenty of water for at least 15 minutes.
INGESTION : Do not make the victim vomit.
Alert a physician.

5. FIRE-FIGHTING MEASURES

SUITABLE EXTINGUISHING MEDIA : Usual means .
UNSUITABLE EXTINGUISHING MEDIA : Jet of water from a fire hose
FIRE AND EXPLOSION HAZARDS : In case of fire, the product emits toxic fumes.
SPECIAL PROTECTIVE EQUIPMENT : Wear a self-contained respiratory apparatus.
OTHER RECOMMENDATIONS : Avoid rejection of extinguishing water in the environment.

6. ACCIDENTAL RELEASE MEASURES

MEASURES AFTER LEAKING OR SPIL

- : Collect thoroughly into plastic bag.
Rinse the polluted area with plenty of water.

0086

7. HANDLING AND STORAGE

HANDLING

TECHNICAL MEASURES

- : Mechanical sucking ventilation at source of formation of dust.

SPECIAL PROTECTION MEASURES

- : None.

STORAGE

SENSITIVITY TO DAMP

- : Hygroscopic, (for anhydrous product)

SENSITIVITY TO LIGHT

- : Sensitive to light

SENSITIVITY TO OXIDATION

- : Not observed.

SPECIAL STORAGE REQUIREMENTS

- : Refrigerator or cold place (36 to 46 F)

STORAGE CONDITIONS

- : Keep at temperature below : +8°C Keep protected from light

SHELF LIFE

- : 1 year (provisional)

STORAGE FACILITIES

- : Harmful products

INCOMPATIBLE SUBSTANCES

- : Unknown.

RECOMMENDED PACKAGING MATERIAL

- : glass, polythene.

SUPPLIER PACKAGING

- : glass, polythene

PACKAGING MATERIAL TO BE AVOIDED

- : Unknown.

OTHER RECOMMENDATIONS

- : None.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

CONTROL OF EXPOSURE

TWA

- : ND

STEL

- : ND

IDLH

- : ND

OLFACTORY THRESHOLD

- : ND

PERSONAL PROTECTION/WORKSHOP

RESPIRATORY TRACT PROTECTION

- : Wear a dust respirator.

PROTECTIVE GLOVES

- : Wear suitable gloves

EYES PROTECTION

- : Wear glasses

PERSONAL PROTECTION/LABORATORY

RESPIRATORY TRACT PROTECTION

- : Wear a dust respirator.

PROTECTIVE GLOVES

- : Wear suitable gloves

EYES PROTECTION

- : Wear glasses

9. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE

- : white powder

pH

- : Not concerned.

CHARACTERISTIC TEMPERATURES

- : Not determined.

FLASH POINT

- : Not applicable.

AUTOINFLAMMABILITY

- : Not determined.

EXPLOSIVITY CHARACTERISTICS

- : ND

VAPOUR PRESSURE : Not determined.
 VOLUMIC MASS : Not determined.
 SOLUBILITY : Soluble in : DMF.
 Slightly soluble in water, chlorinated solvents, acetone,
 alcohols.

OTHER DATA : None.

10. STABILITY AND REACTIVITY

HAZARDOUS REACTIONS : None to our knowledge
 HAZARDOUS DECOMPOSITION PRODUCTS : Unknown.

11. TOXICOLOGICAL INFORMATION

ACUTE TOXICITY : LD 50 oral route /mouse : > 5 g/kg .
 LD50 IP/rat : 5700 mg/kg .LD50 IP/souris : > 3000 mg/kg.
 PHYSIOLOGICAL ACTIVITY : Substance physiologically very active., anolytic,
 angiotensine conversion enzyme inhibitor (0,3 mg/kg per os)
 and antihypertensive (3 mg/kg per os).
 COMMENTS/SYMPTOMS : HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF
 SWALLOWED.
 Safety evaluation in progress Toxicity data being
 insufficient at this date, it is necessary to take
 effective measures in order to avoid any accidental
 exposure

12. ECOLOGICAL INFORMATION

ENVIRONMENT : Biodegradable in natural media

13. DISPOSAL CONSIDERATIONS

NEUTRALIZATION OF THE PRODUCT : Set in specialized and approved recuperator service
 DESTRUCTION SOILED PACKAGING : Set in specialized and approved recuperator service

14. TRANSPORT INFORMATION

INTERNATIONAL REGULATIONS

LAND WAYS : NDT
 MARITIME WAYS : NDT
 AERIAL WAYS : NDT

OTHER REGULATIONS

LAND WAYS - FRANCE : Not regulated

SPECIAL PRECAUTIONS

15. REGULATORY INFORMATION

COMMUNITY REGULATIONS

LABELLING (EEC NUMBER) : Labelling according to EEC regulations
 SYMBOLS : Xn
 PHRASES :

SPECIAL RISKS : HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED.

SAFETY ADVICES : Keep container tightly closed .
Keep at temperature not exceeding (46°F).

ADDITIONAL LABELLING : Refrigerator or cold place (36 to 48 F)

16. OTHER INFORMATION

FURTHER INFORMATIONS : None.

All the regulatory instructions mentioned are intended to help the addressee to comply with his obligations when using the product. This list cannot be considered as exhaustive and does not discharge the addressee from his duty to enquire about all other regulatory provisions which may apply to the possession and handling of the product for which he bears sole liability.

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The information it contains is based on the knowledge available about the product concerned at the time it was compiled. Users are further reminded of the possible risks of using a product for purposes other than those for which it was intended.
The required information complies with current EEC legislation; addressees are requested to apply any additional national requirements.

NDA #20-528

AUG 31 1995

**REVIEW AND EVALUATION OF PHARMACOLOGY
AND TOXICOLOGY DATA**

TRANDOLAPRIL (MAVIK®)

Knoll Pharmaceutical Company

Whippany, NJ

**Ernest J. Belair, Ph.D.
Thomas Papoian, Ph.D.
D.G. Patel, Ph.D.**

8/24/95

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**REVIEW AND EVALUATION OF PHARMACOLOGY
AND TOXICOLOGY DATA**

Ernest J. Belair, Ph.D.
Thomas Papoian, Ph.D.
D.G. Patel, Ph.D.
8/24/95

SUBMISSION DATE: 9/28/94
CENTER RECEIPT DATE: 9/29/94
REVIEWERS ASSIGNMENT DATE: 10/4/94

SPONSOR: Knoll Pharmaceutical Company
30 North Jefferson Road
Whippany, NJ 07981

DRUG:

Code Name:

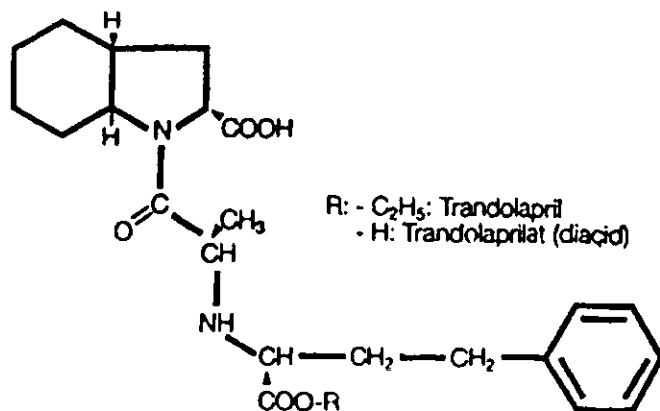
Generic Name: Trandolapril

Trade Name: Mavik®

Chemical Name: (2S,3aR,7aS)-1-[(S)-N](S)-1-carboxy-3-phenylpropyl [alanyl] hexahydro-2-indolinecarboxylic acid, 1-ethyl ester

Molecular Formula: C₂₄H₃₄N₂O₅

Molecular Weight: 430.54

Structure:

Formulation: 1, 2, and 4 mg tablets for oral administration

CATEGORY:

Pharmacologic Class: Angiotensin converting enzyme (ACE) inhibitor

Proposed Clinical Indication: Hypertension

RELATED DRUGS/INDs/NDAs/DMFs:

7

RATIONALE:

Angiotensin converting enzyme (ACE) catalyzes the conversion of angiotensin I to angiotensin II, which, in turn, functions to increase blood pressure by inducing vasoconstriction of vascular smooth muscle and aldosterone secretion by the adrenal cortex facilitating renal sodium and fluid reabsorption. In addition, angiotensin II possesses trophic effects on the heart resulting in cardiac and vascular hypertrophy. Inhibition of circulating (plasma) and tissue ACE activity results in reduced angiotensin II formation. The subsequent reduction in angiotensin II levels leads to peripheral vasodilation, reduced blood pressure and total peripheral resistance, and decreased renal sodium and fluid retention.

Trandolapril is an inhibitor of angiotensin converting enzyme (ACE) activity and is intended for use as an antihypertensive agent. The diacid, trandolaprilat, is the active metabolite and possesses high lipophilicity when compared to the diacids of other ACE inhibitors. This property may contribute to good penetration into various tissues resulting in more efficient ACE inhibition in both tissue and in plasma, and may provide additional beneficial effects on reversing cardiac and vascular hypertrophy.

I. PHARMACODYNAMICS

(Note: In addition to their location with their respective studies, all Tables and Figures for this section were also located in Volumes 1.9 and 1.10, respectively.)

I.A. PRIMARY EFFECTS

I.A.1. *In Vivo* Studies

I.A.1.a. Trandolapril, Special Pharmacology (Part 1)

Note: Trandolapril () is the ethyl ester prodrug of the angiotensin converting enzyme (ACE) inhibitor trandolaprilat (). These studies were conducted to determine: (1) certain aspects of cardiovascular pharmacology, (2) effect on the pressor response to angiotensin I after oral and intravenous treatment, (3) antihypertensive activity in the genetically hypertensive rat with normal or raised renin levels, and (4) the hemodynamic effect in the anesthetized dog pretreated with a diazide.

I.A.1.a.(1). In vivo inhibition of angiotensin-converting enzyme (ACE) activity (Vol. 1.8 pp. 153-155 and Vol. 1.12 pp. 16-21):

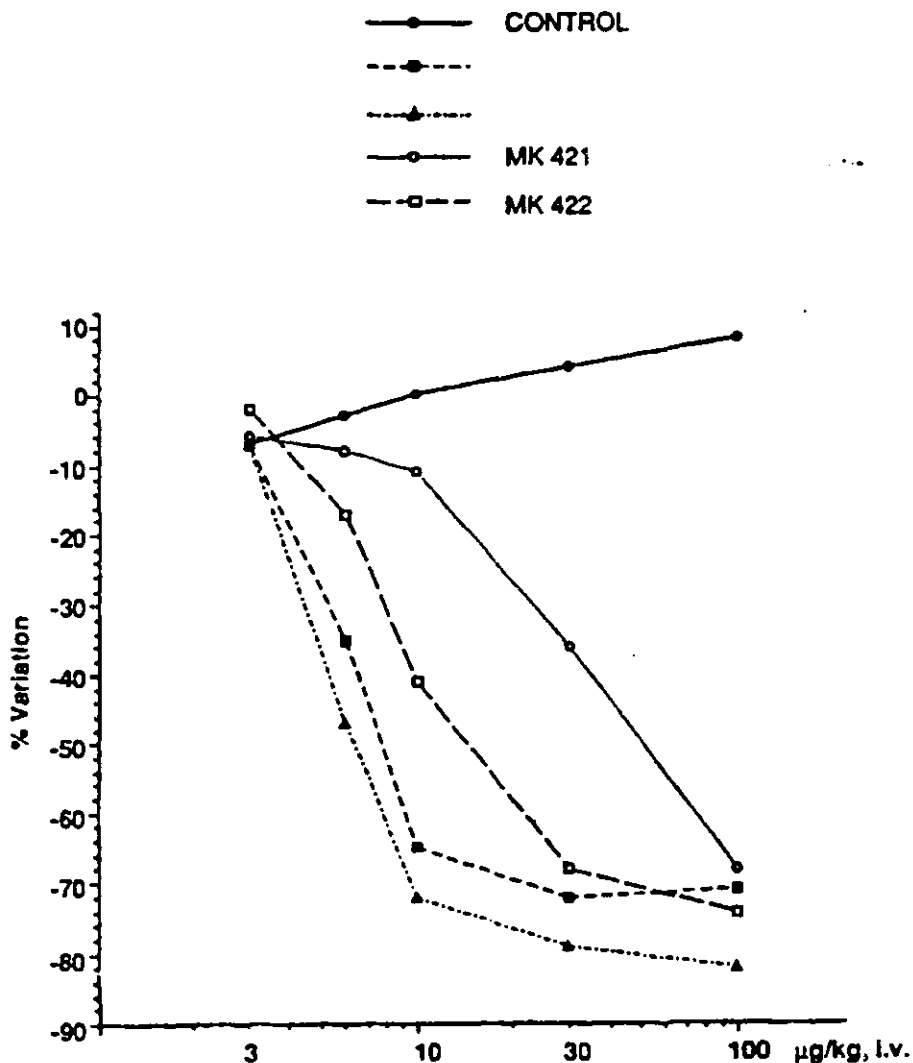
a. This study determined the ability of (trandolapril) administered IV to inhibit the pressor effect of angiotensin I (Ang I) in ganglion-blocked rats.

Sprague-Dawley rats (6 test and 6 control) were ganglion-blocked with chlorisondamine to stabilize blood pressure (BP) and heart rate (HR) and to avoid reflex activity. After 30 min, rats were given 500 ng/kg Ang I IV followed 20 min later with a second injection of Ang I. Ten min later, test rats received the first of 5 successive doses of (3, 6, 10, 30, and 100 µg/kg) or saline (controls). Ten minutes after each dose of , rats received a dose of Ang I. After the last injection of Ang I, rats were given 500 ng/kg angiotensin II (Ang II) to test the specificity of the inhibition produced by . In addition to , the same groups of rats were used to test (the diacid of , MK 421 (enalapril), and MK 422 (enalaprilat, the diacid of MK 421) (b and c below).

Results are summarized in Figure 1 (Sponsor's Figure 1). Control rats showed a stable pressor response to Ang I. Animals treated with showed a dose-dependent inhibition of the pressor response to Ang I. The ID₅₀ was calculated from the dose-effect curve to be 13.1 ± 1.3 µg/kg. The diacid was equipotent as did not affect the pressor response to Ang II verifying the specificity of for inhibiting the conversion of Ang I.

Figure 1

Effect of ACE Inhibitors on Pressor Response to Angiotensin I in the Anesthetized Ganglion-Blocked Rat



b. This study determined the ability of [redacted] administered IV to inhibit the pressor effect of angiotensin I (Ang I) in ganglion-blocked rats.

Rat treatments and doses were the same as described above for (a)

Results showed a similar [redacted]-mediated dose-dependent inhibition of the pressor response to Ang I as that found for [redacted] with an ID₅₀ of 9.9 ± 0.7 µg/kg.

c. This study determined the ability of MK 421 (enalapril) and MK 422 (enalaprilat, the diacid of MK 421) administered IV to inhibit the pressor effect of angiotensin I (Ang I) in ganglion-blocked rats.

Rat treatments and doses were the same as described above for

Results showed that MK 421 caused a dose-dependent inhibition of the Ang I-mediated pressor effect with an ID_{50} of $83.0 \pm 7.1 \mu\text{g/kg}$. MK 422 (enalaprilat) was about 3.2X more potent than MK 421 (enalapril) with an ID_{50} of $25.6 \pm 2.4 \mu\text{g/kg}$.

I.A.1.a.(2). ACE inhibition after oral administration in the rat (Vol. 1,8 pp. 155-159 and Vol. 1,12 pp. 21-31):

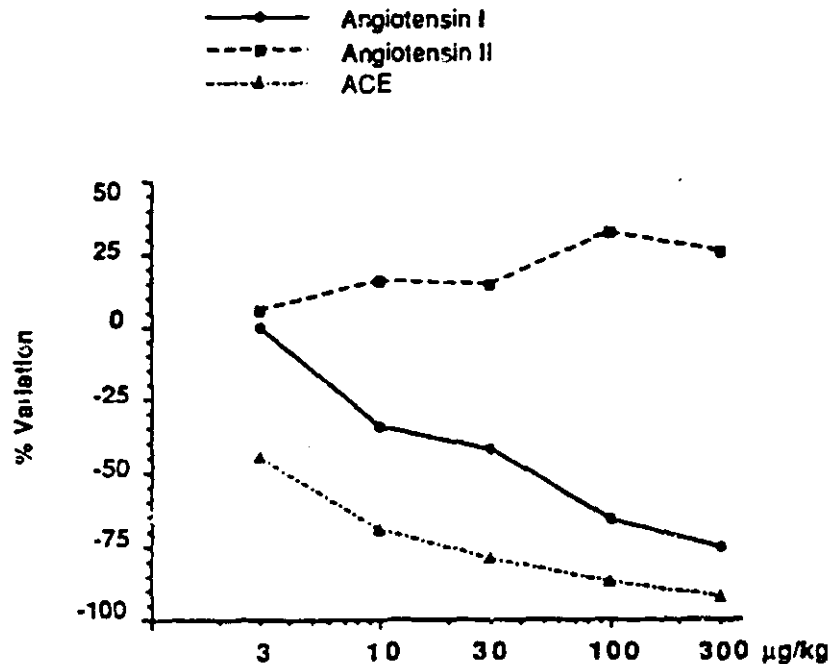
a. This study determined the ability of orally administered to inhibit the pressor effect of angiotensin I (Ang I) and serum ACE activity in ganglion-blocked rats. In addition to the same groups of rats were used to test (the diacid of and MK 421 (enalapril) (b and c below).

Male Sprague-Dawley rats (4-9 per group) were given by gavage at 3, 10, 30, 100, and 300 $\mu\text{g/kg}$. After 30 min, rats received 500 ng/kg Ang II IV followed 10 min later with two doses of 500 ng/kg Ang I IV 10 min apart. After blood pressure returned to normal, blood was drawn for assay of ACE activity.

Results (Figure 2; Sponsor's Figure 2) showed a dose-dependent inhibitory effect of oral on the pressor response induced by Ang I. The effect was significant at doses $\geq 10 \mu\text{g/kg}$. The ID_{50} was 35 $\mu\text{g/kg}$. Serum ACE activity was also inhibited dose-dependently with an ID_{50} at 3.6 $\mu\text{g/kg}$. The dose of RU 44 570 to inhibit ACE was 10X lower than the dose necessary to inhibit the pressor response to Ang I. Since the dose-response curves were parallel, the interpretation was that no other mechanism was involved in the inhibition of the pressor response to Ang I. caused slight potentiation of the pressor effect of Ang II which was significant at the higher doses (100-300 $\mu\text{g/kg}$).

Figure 2

Effect of Oral [redacted] on the Pressor Effects of Angiotensin I and II and on Serum ACE Activity in the Rat



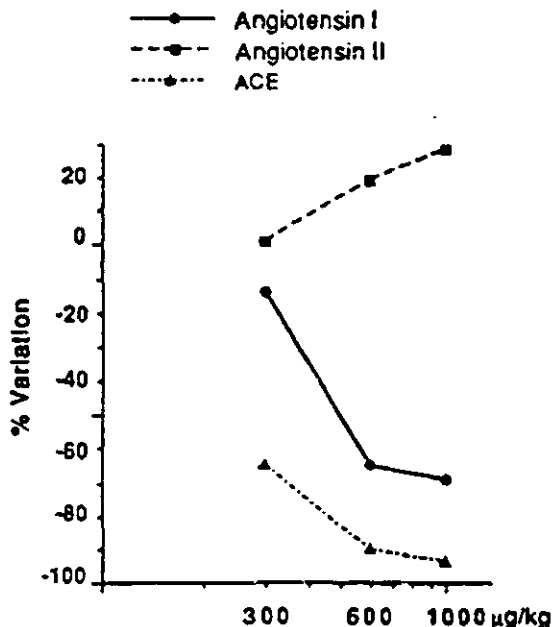
b. This study determined the ability of orally administered [redacted] (the diacid) to inhibit the pressor effect of angiotensin I and II (Ang I and II) and serum ACE activity in ganglion-blocked rats.

Rats were treated as (a) above except that [redacted] was given at 300, 600, and 1000 µg/kg.

Results (Figure 3; Sponsor's Figure 3) showed an inhibitory effect of oral [redacted] on the Ang I-induced pressor response which was significant at ≥ 600 µg/kg, but not at 300 µg/kg. The ID_{50} was 500 µg/kg. Serum ACE activity was also significantly inhibited at ≥ 300 µg/kg showing a greater effect on ACE activity than on the pressor effect of Ang I. Similar to [redacted] above, the interpretation was that no other mechanism was involved in the inhibition of the pressor response to Ang I since the dose-response curves were parallel. [redacted] caused an increase in the pressor effect of Ang II which was significant at ≥ 600 µg/kg.

Figure 3

Effect of Oral on Pressor Effects of
Angiotensin I and II and on Serum ACE Activity in the Rat



c. This study determined the ability of orally administered MK 421 (enalapril) to inhibit the pressor effect of angiotensin I (Ang I) and serum ACE activity in ganglion-blocked rats.

Rats were treated as (a) above except that MK 421 was given at 10, 30, 100, and 300 µg/kg.

Results showed a dose-dependent inhibition of the pressor response induced by Ang 1 which was significant at ≥ 100 µg/kg with an ID₅₀ of 90 µg/kg. Serum ACE activity was also similarly inhibited which was significant at ≥ 30 µg/kg with an ID₅₀ of 69 µg/kg. There was a slight increase in the pressor response to Ang II.

i.A.1.a.(3). ACE inhibition on the anesthetized dog (Vol. 1,8 pp. 159-160 and Vol. 1,12 pp. 31-35):

This study determined the ability of administered IV to inhibit the pressor effect of Ang I in dogs.

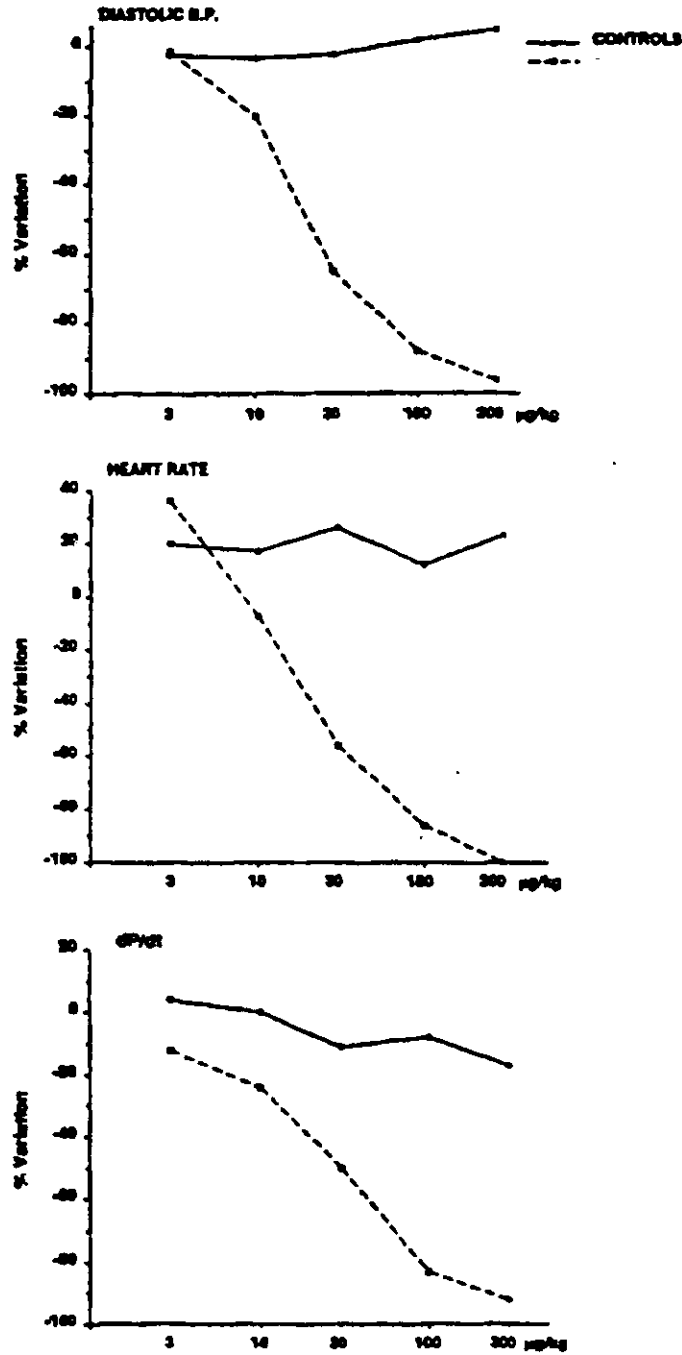
Adult anesthetized dogs (n=8) were ganglion blocked with chlorisondamine (dose not given). Muscarinic receptors were blocked with methylatropine and beta-adrenergic receptors were blocked with propranolol (doses not given). After 30 min, two injections of Ang 1 were given IV 20 min apart (doses not given). After another 10

min, the first dose of _____ (or solvent) was given IV followed by a dose of Ang I 10 min later. This was repeated for a total of 5 doses of _____ (3, 10, 30, 100, and 300 $\mu\text{g}/\text{kg}$). Blood pressure (BP), heart rate (HR), left ventricular pressure (LVP), and the first derivative of LVP (dP/dt) were measured.

Results (Figure 4; Sponsor's Figure 5) showed a dose-dependent inhibition of the pressor response to Ang I for diastolic BP ($IC_{50} = 22.8 \pm 11.5 \mu\text{g}/\text{kg}$), HR ($IC_{50} = 28.0 \pm 20.4 \mu\text{g}/\text{kg}$), and dP/dt ($ID_{50} = 27.5 \pm 21.2 \mu\text{g}/\text{kg}$).

Figure 4

Effects of [Drug Name] and Solvent (Controls) on Angiotensin I-Induced Increases in Diastolic Blood Pressure, Heart Rate, and dP/dt in the Anesthetized Dog



I.A.1.a.(4). Inhibition of the pressor effect of Ang I in the conscious dog (Vol. 1,8 pp. 160-163 and Vol. 1,12 pp. 35-43):

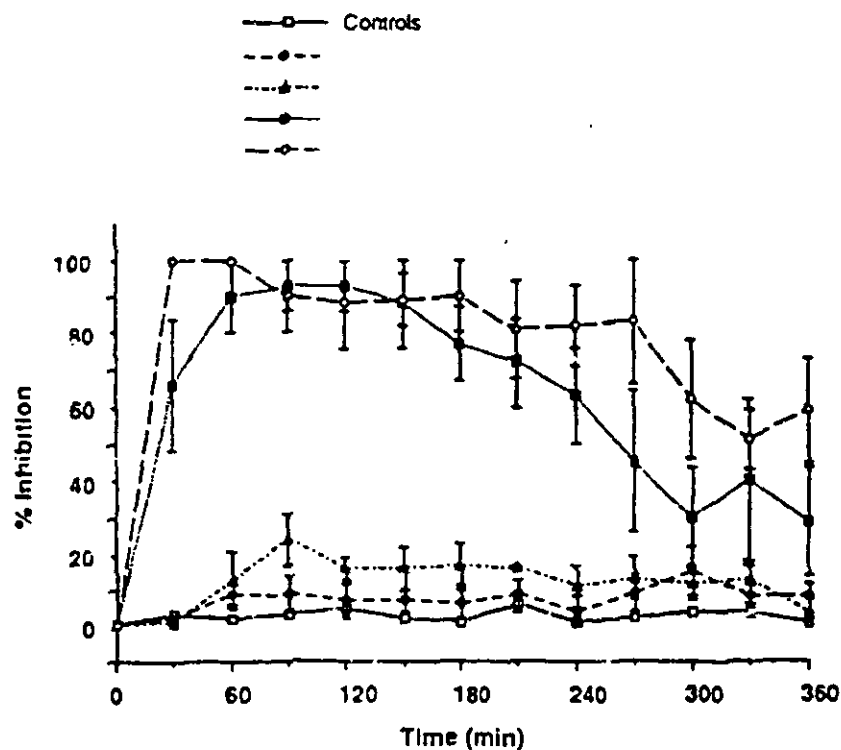
a. This study determined the ability of orally administered [redacted] to inhibit the pressor effect of Ang I in dogs. In addition to [redacted] the same groups of dogs were used to test [redacted] (the diacid of [redacted]), and MK 421 (enalapril) (b and c below).

Adult dogs (n=24) were given two injections of 150 ng/kg Ang I 10 min apart which caused a 50 mmHg increase in diastolic BP without inducing major bradycardia (data not provided). Dogs were then given oral doses of [redacted] at 30, 100, 300, and 1000 µg/kg in gelatin capsules (controls received empty capsules). The dogs then received Ang I every 30 min for 6 hours and the BP measured.

Results (Figure 5; Sponsor's Figure 6) showed a 90-100% inhibition of the Ang I-mediated pressor response at doses ≥ 300 µg/kg which was still very strong for up to 4-6 hours. The 100 µg/kg dose showed a modest effect (24%) at 90 min.

Figure 5

Inhibition of the Pressor Response to Angiotensin I After Oral Administration of [redacted] in the Conscious Dog



b. This study determined the ability of orally administered (the diacid) to inhibit the pressor effect of Ang I in dogs.

Animals were treated as (a) above, except that was given orally at 100, 300, and 1000 µg/kg.

Results showed a dose-dependent inhibition of the pressor response to Ang I. The peak effect was 67-78% for 300-1000 µg/kg, respectively which lasted for 2-3 hours with considerable activity (37-47%) at 6 hours.

c. This study determined the ability of orally administered MK 421 (enalapril) to inhibit the pressor effect of Ang I in dogs.

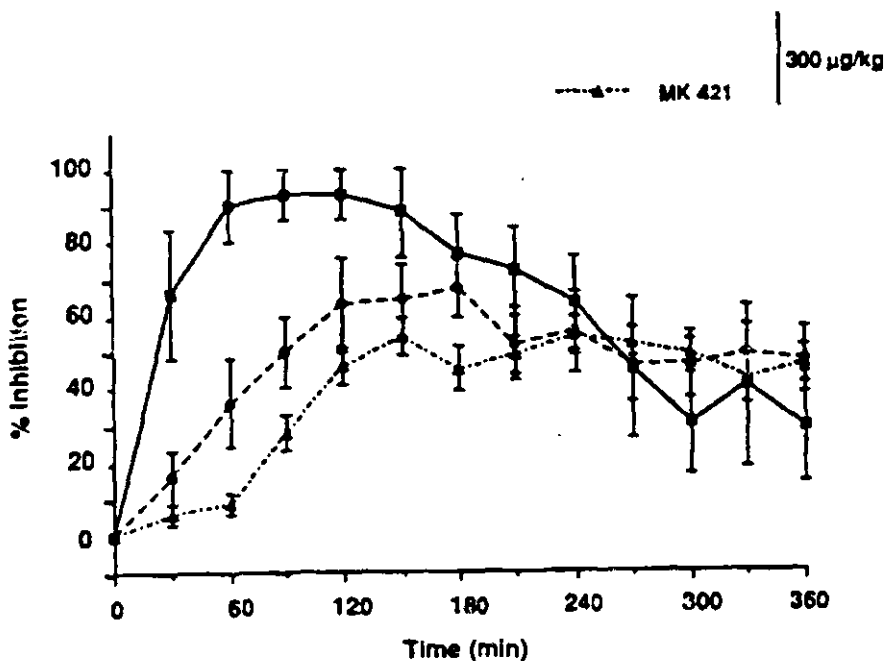
Animals were treated as (a) above, except that MK 421 was given at 100, 300, and 1000 µg/kg.

Results showed a dose-dependent inhibition of the pressor response to Ang I which was similar in magnitude and time-course as in (b) above.

The comparative effects of (a) , and (c) MK 421 on the inhibition of the Ang I-induced pressor response at the comparable oral dose of 300 µg/kg are shown in Figure 6 (Sponsor's Figure 9).

Figure 6

Inhibition of the Pressor Response to Angiotensin I in the Conscious Dog. Comparisor. and MK 421 (Oral Route)



I.A.1.a.(5). Antihypertensive effects of _____ (Vol 1.8 pp. 163-166 and Vol. 1.12 pp. 43-64):

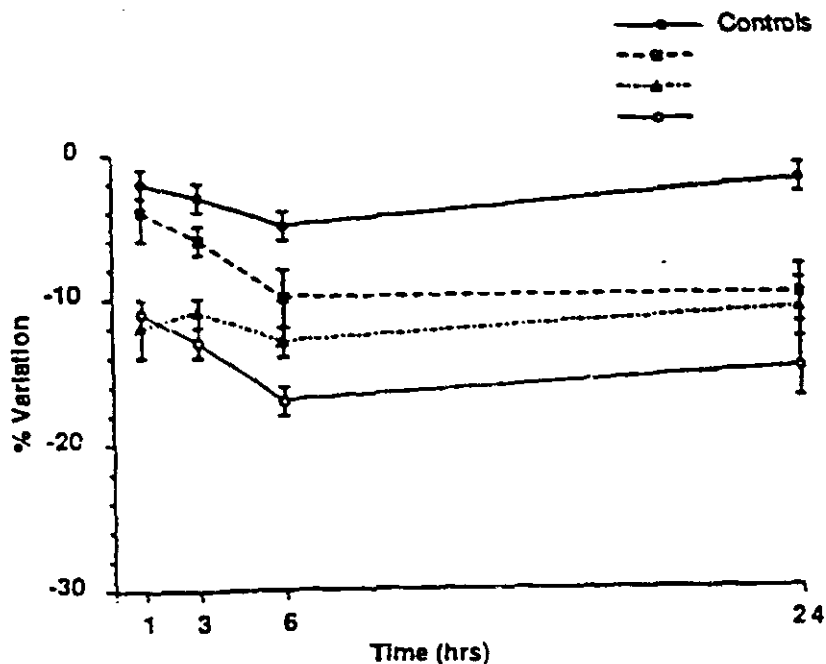
a. This study determined the effect of orally administered _____ on blood pressure (BP) and heart rate (HR) in the genetically hypertensive (GH) rat.

Male GH rats (n=67) were administered _____ by oral gavage at 0.3, 3, and 30 mg/kg. Controls received solvent. BP and HR were measured before and 1, 3, 6, and 24 hours after drug administration.

Results showed dose-dependent decreases in systolic, diastolic, and mean BP. The maximum effects on systolic BP occurred after 6 hours at doses of 0.3, 3, and 30 mg/kg with reductions of 9, 13 and 14%, respectively. The effects on diastolic and mean BP were similar. The maximum effects on mean BP were at 6 hours with reductions of 10, 13, and 17% at doses of 0.3, 3, and 30 mg/kg, respectively (Figure 7; Sponsor's Figure 12). HR was not significantly different from controls, except for the 3 mg/kg dose which produced a slight increase (+7%) in HR at 6 hours.

Figure 7

Effect of Oral Administration of RU 44 570 on Mean Blood Pressure in the Conscious Genetically Hypertensive Rat (Mean Blood Pressure)



b. This study determined the effect of orally administered MK 421 on BP and HR in the GH rat.

Male GH rats (n=45) were treated as (a) above, except that MK 421 was given at 3 and 30 mg/kg.

Results showed decreases of 10-16% in BP at the 30 mg/kg dose with a slight increase (+11%) in HR at 6 hours.

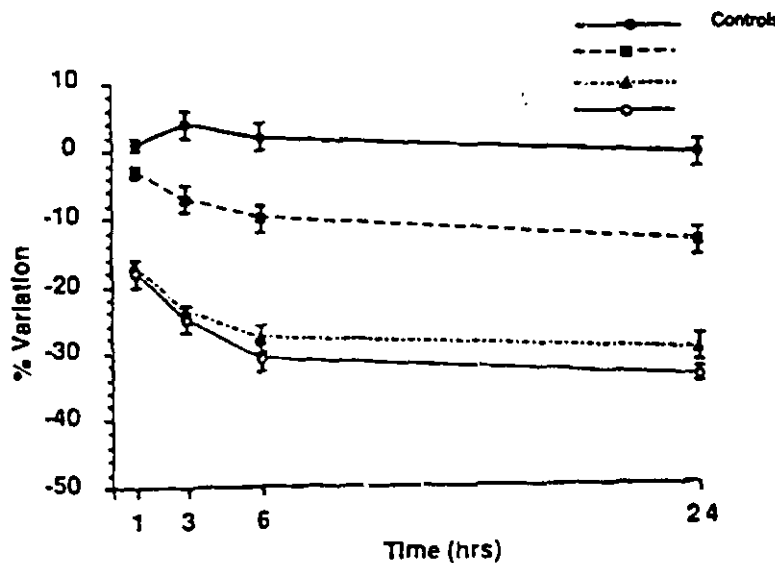
c. This study determined the effect of orally administered on BP and HR in the hydrochlorothiazide-pretreated GH rat.

Male GH rats (n=75) were pretreated with an undisclosed dose of hydrochlorothiazide twice daily for 2 days prior and on the third day 1 hour prior to oral dosing with RU 44 570 at 0.3, 3, and 30 mg/kg. Controls received solvent.

Results (Figure 8; Sponsor's Figure 20) showed dose-dependent decreases in systolic, diastolic, and mean blood pressures which were maximal at 24 hours. Mean BP was reduced 14, 30, and 34% for 0.3, 3, and 30 mg/kg, respectively. Heart rates were essentially unaffected. Although not stated, the reduction in mean BP by treatment with hydrochlorothiazide alone was only 2-9% when compared to the previous experiments above (5)(a) and (b).

Figure 8

Effect of Oral Administration of on Mean Blood Pressure in the Conscious Genetically Hypertensive Rat Pretreated with Hydrochlorothiazide (Mean Blood Pressure)



d. This study determined the effect of orally administered MK 421 on BP and HR in the hydrochlorothiazide-pretreated GH rat.

Male GH rats (n=45) were treated as in (c) above, except that MK 421 was given orally at 3 and 30 mg/kg.

Results showed a dose-dependent effect on systolic, diastolic, and mean BP which peaked at 6 hours and was sustained for 24 hours. The reduction in mean BP was 15 and 25% for the 3 and 30 mg/kg doses, respectively.

I.A.1.a.(6). Hemodynamic effects of _____ in the hydrochlorothiazide-pretreated anesthetized dog (Vol 1.8 pp. 166-169 and Vol. 1.12 pp. 64-83):

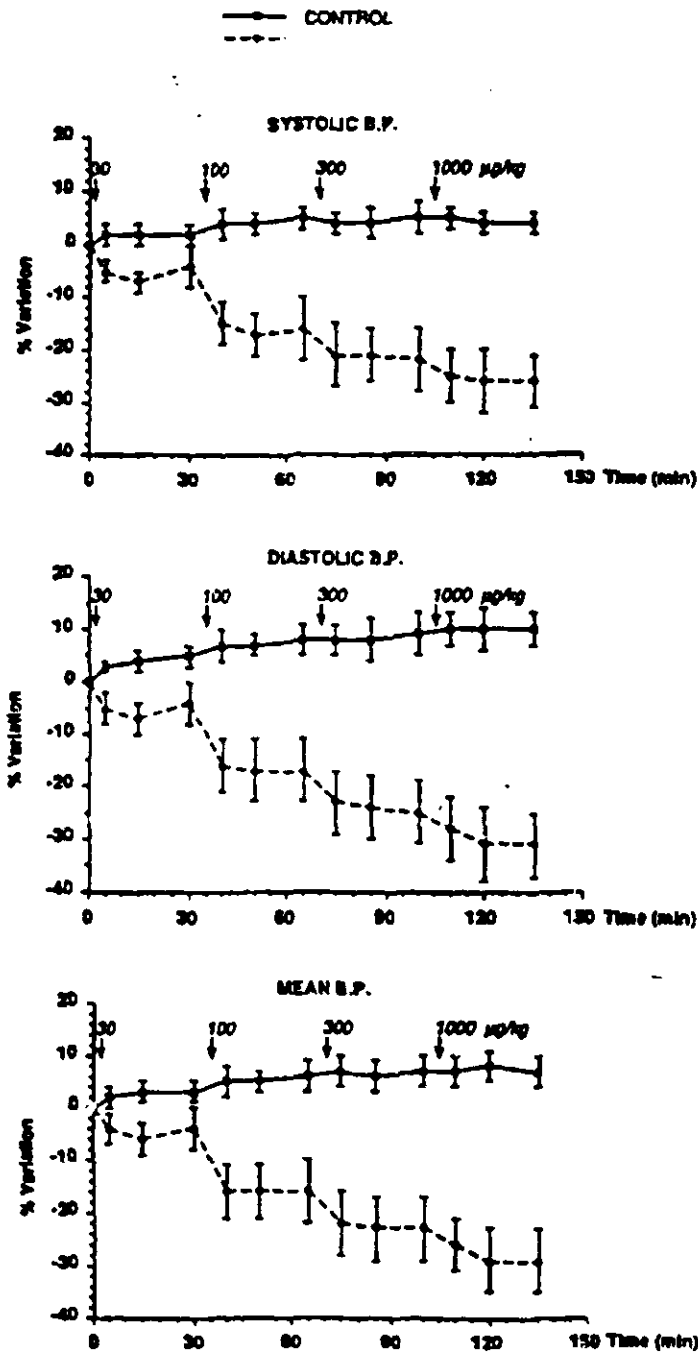
This study examined the hemodynamic effects (systolic, diastolic, and mean BP, HR, dP/dt, cardiac output, stroke volume, total peripheral resistance, left ventricular work, and stroke work) of _____ in the hydrochlorothiazide-pretreated anesthetized dog.

Dogs (n=9) were pretreated with an undisclosed dose of hydrochlorothiazide twice daily for 3 days, and 30 min before administration of anesthesia. After cardiac output stabilized, dogs were infused IV with 30 µg/kg _____. Hemodynamic parameters were measured for 5, 15, and 30 min at which time _____ was infused again to a total cumulative dose of 100 µg/kg. This process was repeated to total cumulative doses of 300 and 1000 µg/kg.

Results showed dose-dependent decreases in systolic, diastolic, and mean BP which were significant at doses of ≥ 100 µg/kg (Figure 9; Sponsor's Figures 26-28). The maximum effects on mean BP were decreases of 6, 16, 23, and 29% at doses of 30, 100, 300, and 1000 µg/kg, respectively. The effect of hydrochlorothiazide alone on reducing BP was not provided.

Figure 9

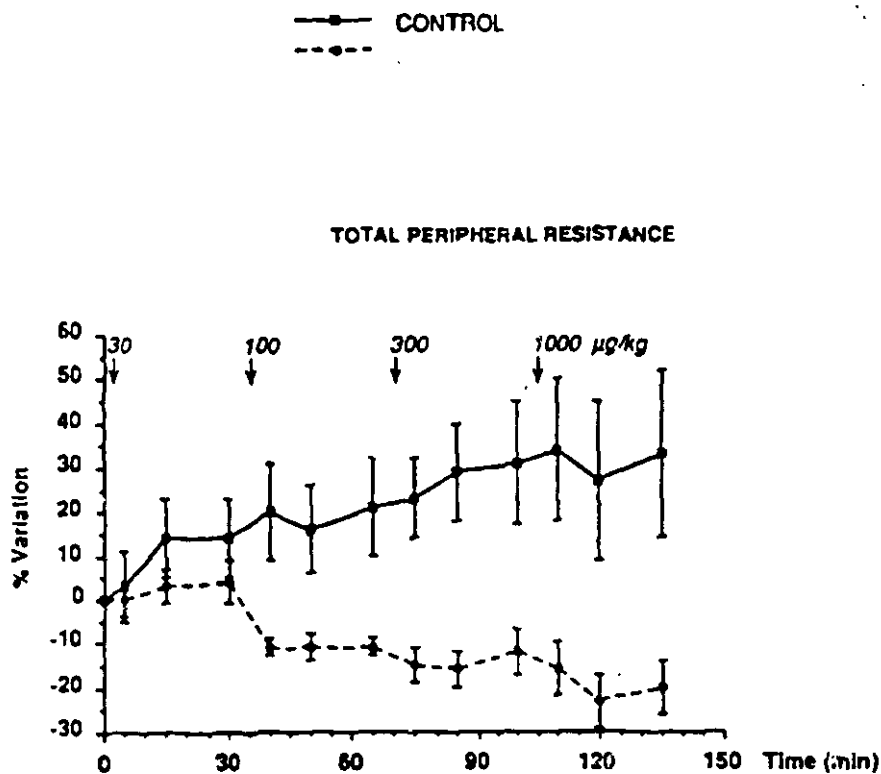
Hemodynamic Study of Intravenous [redacted] in the Hydrochlorothiazide-Pretreated Anesthetized Dog (Systolic, Diastolic, and Mean Blood Pressure)



There was a slight affect on HR (about +12%) when compared to controls. Left ventricular dP/dt was progressively decreased in both control (-27 ± 2% maximum) and -treated animals (-16 ± 12% maximum), but due to the large variability in the test group, results were not significant. Cardiac output tended to decrease in both groups (-12 to -17%) with no significant difference between the groups or from initial values. Stroke volume also decreased in both groups (-15 to -17%) with no significant difference between the groups. Total peripheral resistance decreased (-11 to -23%) at doses beginning with 100 µg/kg (Figure 10; Sponsor's Figure 33). Control animals showed an increase (+34%) in resistance over time.

Figure 10

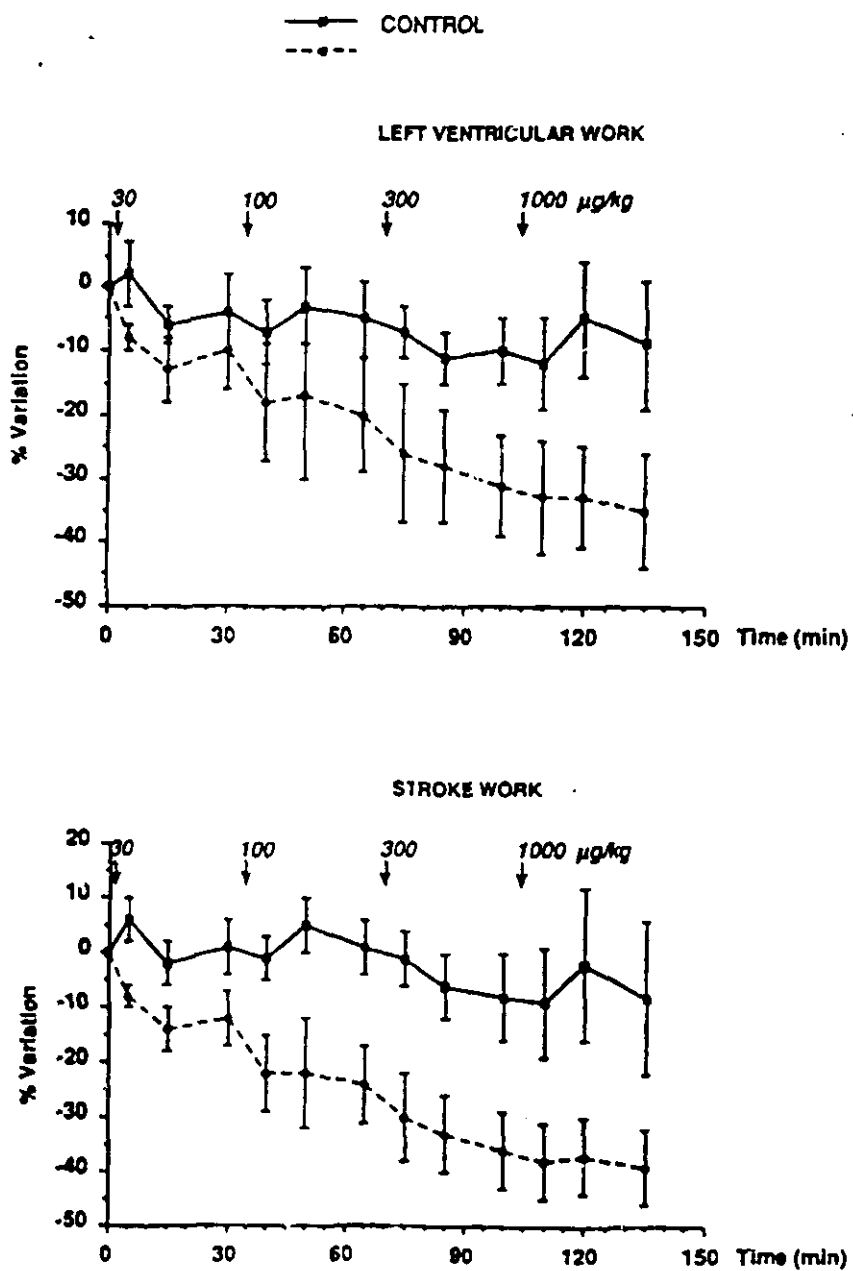
Hemodynamic Study of Intravenous in the Hydrochlorothiazide-Pretreated Anesthetized Dog (Total Peripheral Resistance)



dose-dependently reduced heart work, expressed as left ventricular work or stroke work, with the major effect occurring at doses ≥ 100 µg/kg (Figure 11; Sponsor's Figures 34-35).

Figure 11

Hemodynamic Study of Intravenous in the Hydrochlorothiazide-Pretreated Anesthetized Dog (Left Ventricular Work and Stroke Work)



I.A.1.a.(7). Conclusions:

was shown to have activity as an ACE inhibitor when given either by the intravenous or oral routes. In the rat it selectively inhibited the pressor effects of Ang I, but not Ang II. It showed about 6 times more activity on a mg/kg basis in reducing an Ang I-mediated pressor response (diastolic BP) than a known ACE inhibitor enalapril (MK 421) when given IV (-72% vs -11% reduction in pressor effect to Ang I at 10 mg/kg and MK 421, respectively), and was about 3 times more active when given orally (-42% vs -13% reduction in pressor effect to Ang I for 30 mg/kg and MK 421, respectively). Inhibition of serum ACE activity was more sensitive to both ACE inhibitors, and MK 421, than was inhibition of the Ang I-induced pressor response. However, the difference in inhibition was greater for (9.7X) than for MK 421 (1.3X) (Table 1). The reason for this lack of correlation between inhibition of ACE activity and inhibition of the Ang I-mediated pressor effect by was not known, but was thought to be linked to the importance of circulating ACE and Ang I levels when compared to their levels in tissues.

Table 1

ID₅₀ in the Rat (Oral)

	<u>Inhibition of Ang I-Mediated Pressor Effect</u>	<u>ACE Inhibition</u>
MK 421	35.0 µg/kg 90.0 µg/kg	3.6 µg/kg 69.0 µg/kg

In the conscious dog, after oral administration was more active and the effect more rapid to the pressor effect of Ang I than MK 421 (93% peak reduction at 90 min vs 54% peak reduction after 150 min for and MK 421, respectively). The diacid of showed similar activity to MK 421, which is in contrast to the diacid of MK 421, MK 422, which, according to the Sponsor, is not easily absorbed after oral administration.

Pretreatment with a thiazide has been shown to increase the antihypertensive activity of ACE inhibitors which may be due to the greater activity of the renin-angiotensin system following thiazide-induced sodium depletion. In the hydrochlorothiazide-pretreated GH rat, was shown to have greater antihypertensive activity than in non-pretreated rats (Table 2). This effect was also observed with MK 421.

Table 2

Effect at 6 Hours of Oral and MK 421 on
Mean Blood Pressure in Hydrochlorothiazide-Pretreated Rats

	<u>Dose</u> <u>(µg/kg)</u>	<u>Thiazide</u> <u>Pretreatment</u>	<u>% Reduction</u> <u>in Mean BP</u>
	3	-	-13
		+	-28
	30	-	-17
		+	-31
MK 421	3	-	-7
		+	-15
	30	-	-15
		+	-25

In the dog, preliminary studies (not provided) showed that the hemodynamic effects of were transitory. Therefore, pretreatment with a thiazide was chosen as the experimental model. Results showed that caused a dose-dependent fall in BP, total peripheral resistance, and heart work. This was interpreted to mean that the compound acted like a vasodilator with no baroreflex activation accompanying the decreased BP.

These studies demonstrated that possessed significant antihypertensive activity which was active after oral administration, and with a long duration of action (up to 24 hours in the rat). Its mechanism of action was postulated to be via a decrease in Ang II production following ACE inhibition. Its most important effect is a vasodilation not accompanied by a baroreflex activation. In the dog studies, the activation state of the renin-angiotensin system was important for the final effect of the compound.

I.A.1.b. Effect of (Trandolapril) on angiotensin I converting enzyme activity in different rat tissues. Comparison with MK 421 (Vol 1.8 pp. 170-172 and Vol. 1.12 pp. 156-247):

ACE activity has been found to be present in several tissues, such as endothelium, lung, kidney, and heart. Different ACE inhibitors may vary in their antihypertensive effects due to differences in tissue specific enzymes or in the drug's liposolubility in a particular tissue. This study (conducted in from 6/85 to 7/85) compared the effects of two ACE inhibitors, (trandolapril) and MK 421 (enalapril), on the inhibition of converting enzyme activity in different tissues to determine the potential site of antihypertensive action.

Sprague-Dawley rats were given either (0.003, 0.01, 0.03, and 0.1

mg/kg) or MK 421 (0.01, 0.03, 0.1. and 0.3 mg/kg) by gastric intubation. The rats were sacrificed 0.5, 2, 6, and 24 hours after dosing, and the following tissues assayed for ACE activity: heart, kidneys, brain, adrenal glands, part of one lung, abdominal aorta, and serum.

The inhibition of ACE activity produced by _____ reached its peak by 30 min in all tissues examined, whereas that induced by MK 421 reached its peak at 2 hours after dosing in most tissues, except the adrenal glands where it peaked after 30 min. ACE activity remained inhibited 24 hours after dosing with _____, but was beginning to return to normal by 24 hours after MK 421 administration. ACE activity in the striatum (basal ganglia) was not affected by either inhibitor. Comparison of the dose-responses between the two inhibitors showed that _____ was about 6-12X more potent than MK 421. _____ showed more inhibitory activity than MK 421 in the heart and adrenals suggesting that the activities of dipeptidyl carboxypeptidase activities in these tissues were affected differently by the two inhibitors.

I.A.1.c. Trandolapril, Special Pharmacology (Part 2):

These studies (conducted in _____ from 2/84 to 11/90) determined the antihypertensive activity of _____ after either pretreatment with indomethacin, bilateral nephrectomy, or after chronic administration. In addition, the hypotensive activity and hemodynamic profile of _____ were also studied.

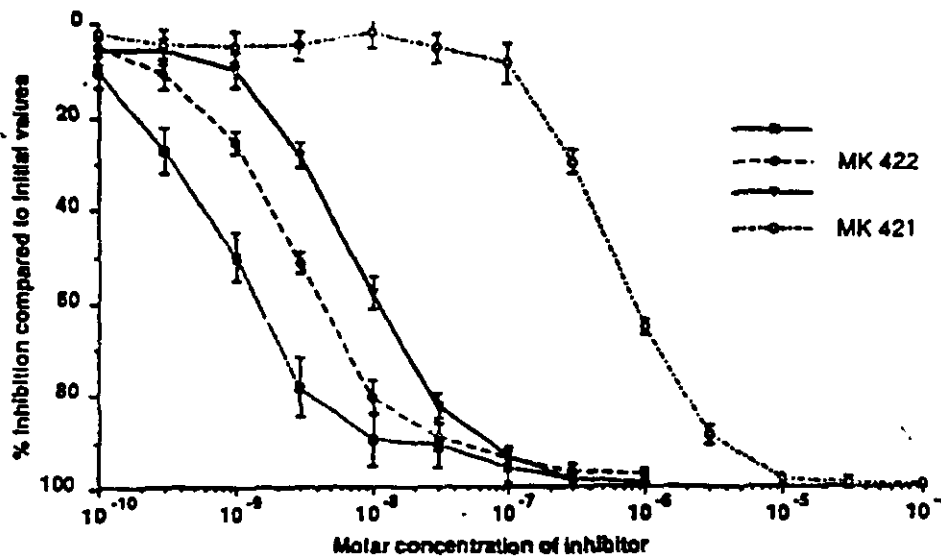
I.A.1.c.(1) Inhibition of angiotensin converting enzyme (ACE) *in vitro* (Vol. 1.8 pp. 172-173 and Vol. 1.12 pp. 10-20):

This study determined the ability of _____ to inhibit ACE in human, dog, and rat sera, and compared that activity to _____ (the diacid), MK 421, and MK 422 (the diacid of MK 421).

Results showed that all four drugs inhibited ACE in all three species examined. In human sera, the IC₅₀ of _____ MK 422, _____ and MK 421 were 0.93 ± 0.35 nM, 2.85 ± 1.40 nM, 7.06 ± 2.11 nM, and 593 ± 208 nM, respectively (Figure 12; Sponsor's Figure 40). For both drugs, the diacid form was more potent than their respective parent drugs, except in the rat where they were equipotent. _____ was shown to be stable in human blood and plasma, and was not transformed into its diacid. _____ was about 3X more potent than MK 422 in its ability to inhibit ACE activity in human serum.

Figure 12

In Vitro Inhibitory Activity of MK 422,
and MK 421 on Human Serum ACE



I.A.1.c.(2) Inhibition of ACE *In vivo* (Vol. 1.8 pp. 173-174 and Vol. 1.12 pp. 20-25):

This study compared the effect of MK 421, and MK 422 on bradykinin-induced hypotension in the rat.

Sprague-Dawley rats were ganglion-blocked with chlorisondamine, then injected with phenylephrine to prevent hypotension. Once the blood pressure (BP) had stabilized, bradykinin was injected twice IV at 250 ng/kg 20 min apart. After 10 min, test drug (or solvent) was given IV, followed 10 min later with another injection of bradykinin. This cycle was repeated with 5 increasing doses of inhibitor at 3, 6, 10, 30, and 100 µg/kg.

Results showed that all compounds caused a dose-dependent potentiation of the hypotensive action of bradykinin, although the BP of the controls remained stable throughout the experimental period ($EC_{50} = 4.9 \pm 0.3$ and 4.1 ± 0.6 µg/kg for

respectively). The order of efficacy of the compounds was: MK 422, and MK 421.

I.A.1.c.(3) Inhibition of the responses induced by intravenous administration of angiotensin I (Ang I) in the anesthetized dog (Vol 1.8 pp. 174-176 and Vol. 1.12 pp. 25-32):

This study compared the effects of

MK 421, and MK 422

on the response to angiotensin I in the dog.

Adult beagle dogs (n=12) were injected with the following: chlorisondamine to block the ganglion receptors, methylatropine to block the muscarinic receptors, and propranolol to block the beta-adrenergic receptors. Ang I was then given twice at 250 ng/kg IV 20 min apart. Ten min after the last injection, the test compounds (or solvent) were given IV followed 10 min later with another injection of Ang I. This cycle was repeated 5 times to study 5 increasing doses of drug.

Effects of test drugs on heart rate increases were not studied due to the propranolol. All drugs caused a dose-dependent inhibition of the Ang I-induced increase in diastolic BP and dP/dt. Results are summarized in Table 3:

Table 3

Effect of _____, MK 421, and MK 422
on the Response to Angiotensin I in the Dog

Drug	ED ₅₀ (BP) (µg/kg)	ED ₅₀ (dP/dt) (µg/kg)
_____	22.8 ± 11.5	27.5 ± 21.2
_____	7.2 ± 2.3	5.5 ± 1.7
MK 421	209.3 ± 43.2	111.2 ± 9.5
MK 422	6.1 ± 0.9	16.4 ± 5.4

I.A.1.c.(4) Antihypertensive effect of _____ in the spontaneously hypertensive rat (SHR) (Vol. 1.8 pp. 176-177 and Vol. 1.12 pp. 32-36):

This study determined the effect of the inhibition of prostaglandin synthesis by indomethacin on the antihypertensive activity of _____ in SH rats.

SH rats (n=21) were given indomethacin by gastric intubation at 5 mg/kg (or vehicle), then given _____ orally at 3 mg/kg. BP and HR were measured at 1, 3, 6, and 24 hours after treatment.

Results showed that there was no difference in the ability of _____ to reduce blood pressure in animals treated with either indomethacin or vehicle (-16 ± 3% vs -17 ± 2% decrease in blood pressure, respectively). Heart rates were not affected.

It was concluded that inhibition of prostaglandins by indomethacin had no effect on the ability of _____ to reduce blood pressure in the SH rat.

I.A.1.c.(5) Effect of bilateral nephrectomy on the antihypertensive activity of _____ (Vol. 1.8 pp. 177-178 and Vol. 1.12 pp. 36-42):

This study examined the role of renal renin in the antihypertensive activity of _____ in SH rats.

SH rats (n=21) were bilaterally nephrectomized (or sham operated). Rats were then given orally at 3 mg/kg by gastric intubation. BP and HR were measured at 1, 3, 6, and 24 hours after treatment.

Results showed that nephrectomy had no effect on blood pressure in untreated animals. treatment lowered mean blood pressure ($-15 \pm 2\%$ at 6 hours) in sham-operated animals. However, the drug had little antihypertensive activity in nephrectomized rats ($-4 \pm 1\%$ at 6 hours). Heart rates in these animals were slightly lower ($-7-8\%$) in these animals as well.

It was concluded that the antihypertensive activity of is dependent upon the presence of kidneys and renin production.

I.A.1.c.(6) Antihypertensive activity of after 14 days of oral treatment (Vol. 1.8 pp. 178-180 and Vol. 1.12 pp. 42-53):

This study determined the antihypertensive activity of and MK 421 in SH rats after 14 days of oral treatment.

SH rats (n=36) were given or MK 421 for 14 days by adding the drugs to the drinking water at 3 mg/kg. After 14 days, blood was assayed for ACE activity, Ang I, and renin activity, and the hearts excised for ventricular weight.

Results showed that reduced the ventricular weight/ body weight ratios (an index of ventricular hypertrophy) more than MK 421 (-19 vs -9% respectively). Serum ACE activity was significantly reduced (-68%) with treatment after 14 days. However, MK 421 treatment caused a significant rise in serum ACE ($+69\%$) when measured one day after discontinuation of treatment, which may have been due to a rebound phenomenon. However, no time course data was provided. Plasma renin activity and plasma Ang I levels were increased in parallel by ($+487\%$ and $+247\%$, respectively). reduced mean blood pressure by 33% , whereas MK 421 had little effect (-2%). Heart rates were not affected by either drug.

I.A.1.c.(7) Antihypertensive activity of after acute oral administration (Vol 1.8 pp. 180-181 and Vol. 1.12 pp. 53-58):

This study examined the antihypertensive activity of (diacid) after a single oral dose in SH rats.

Male SH rats (n=25) were given orally at 30 mg/kg (or solvent). BP and HR was measured at 1, 3, 6, and 24 hours after treatment.

had a slight effect on mean blood pressure ($-4 \pm 2\%$ at 6 hours), but the effect was relatively stable for up to 24 hours. No other significant changes were observed.

I.A.1.c.(8) Hypotensive action of _____ (Vol. 1.8 pp. 181-182 and Vol. 1.12 pp. 58-66):

This study examined the hypotensive effects of _____ and MK 421 in normotensive rats.

Male Sprague-Dawley rats (n=29) were given either _____ at 3 mg/kg or MK 421 at 30 mg/kg by oral gavage. BP and HR were measured at 1, 3, 6, and 24 hours after treatment. A sample of arterial blood was taken 24 hours post-dose for measurement of ACE activity.

Results showed that both _____ and MK 421 reduced mean blood pressure* (-13 ± 1% vs -12 ± 2%, respectively at 6 hours), and the effects lasted up to 24 hours. Heart rates were not affected by either drug. Serum ACE activity was significantly reduced by _____ (-66%) when compared to control animals, whereas MK 421 had little effect (-7%).

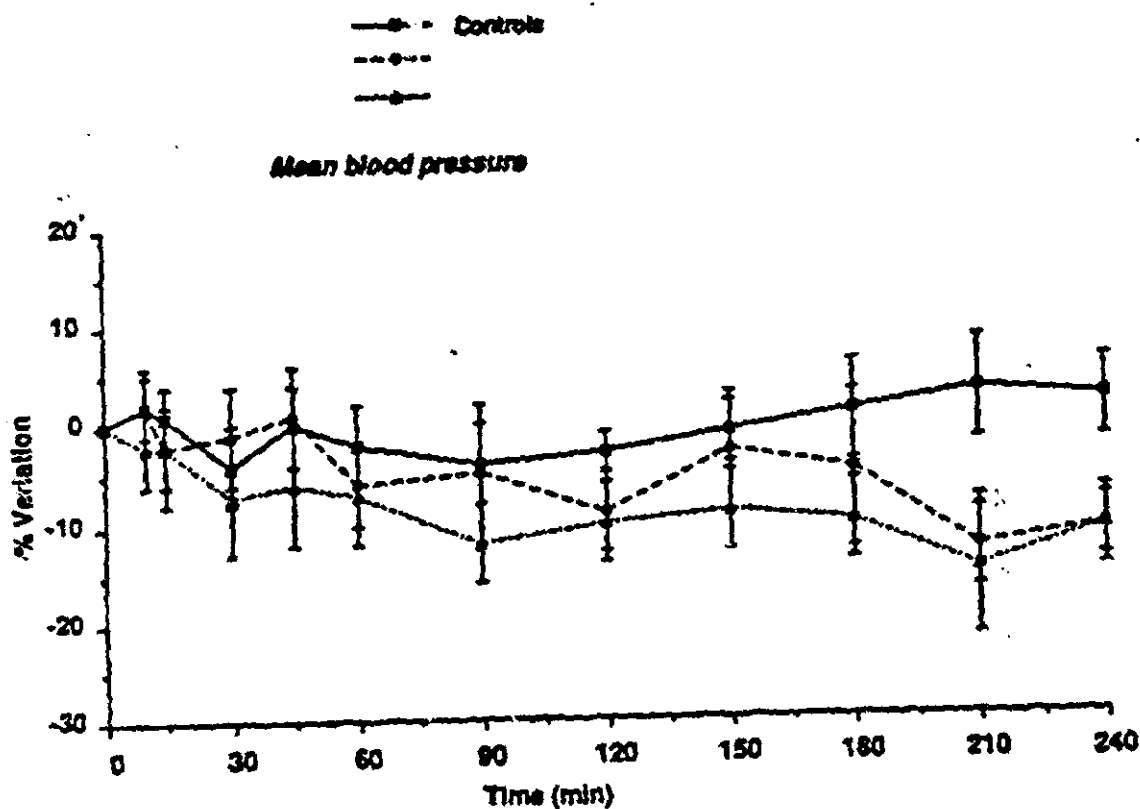
I.A.1.c.(9) Hemodynamic effect of _____ in the conscious normotensive dog (Vol. 1.8 pp. 182-184 and Vol. 1.12 pp. 66-77):

Male beagle dogs (n=16) were given _____ orally in capsule form at 3 mg/kg or 10 mg/kg. Controls received empty capsules. Hemodynamic parameters (BP, left ventricular dP/dt, HR, and ECG) were measured for up to 4 hours.

The largest reduction in mean blood pressure occurred at 3.5 hours (-13 ± 4% and -15 ± 7% at 3 and 10 mg/kg, respectively) (Figure 13; Sponsor's Figure 65). No other parameters were significantly affected.

Figure 13

Effect of Oral _____ on Mean Blood Pressure in the Conscious Dog



I.A.1.c.(10) Hemodynamic effects of _____ in the anesthetized normotensive dog
(Vol. 1.8 pp. 184-187 and Vol. 1.12 pp. 77-95):

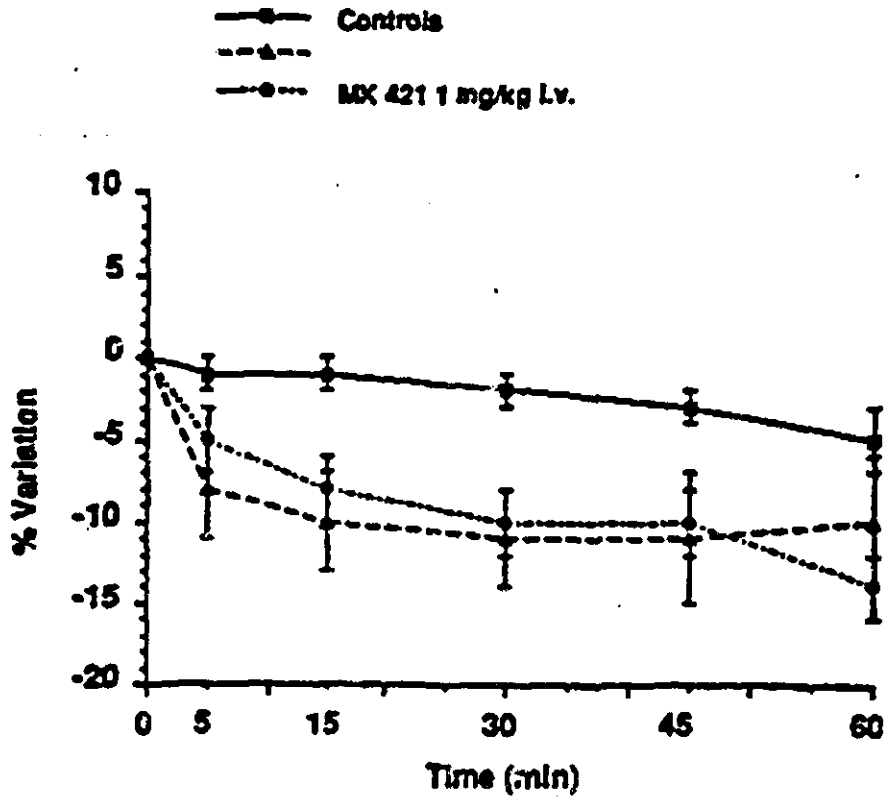
This study compared the hemodynamic effects of _____ and MK 421 given IV to anesthetized dogs.

_____ or MK 421 was given IV to anesthetized dogs at a rate of 2 ml/min for 5 min to a total dose of 1 mg/kg. Control animals received solvent. Hemodynamic parameters (BP, LVP, dP/dt, HR, CO, and ECG) were measured for up to 60 min.

There was a slight increase in heart rate caused by _____ administration (+5%) when compared to controls, which was 1% above baseline values. The effect of MK 421 on heart rate was similar. _____ caused a significant reduction in blood pressure (diastolic, systolic, and mean) with a maximum reduction in MAP of $-11 \pm 3\%$ at 30 min (Figure 14; Sponsor's Figure 72). The reduction in blood pressure was not significantly different from controls by 60 min. MK 421 reduced blood pressure with a maximum effect on MAP of $-14 \pm 2\%$ at 60 min.

Figure 14

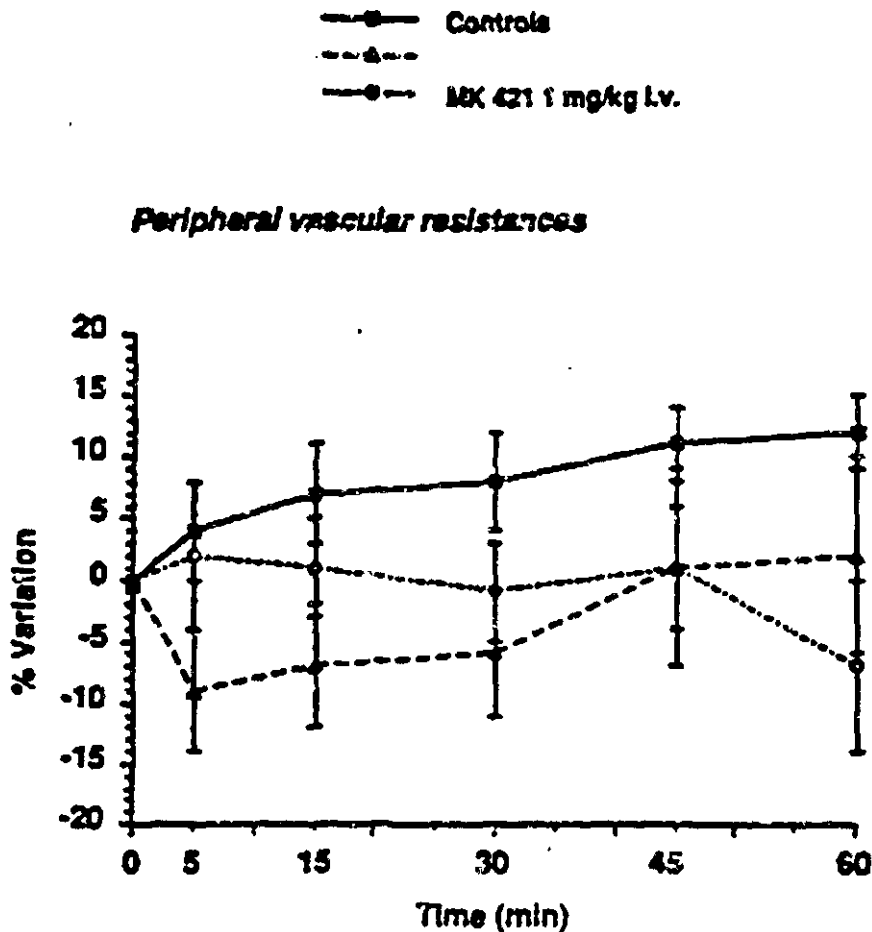
Effect of Solvent, and MK 421 on Mean Blood Pressure by the Intravenous Route in the Anesthetized Dog



Although control animals showed decreases in cardiac output and heart work, there were no significant differences in left ventricular dP/dt, cardiac output, stroke volume, or heart work (stroke work) between the three groups, mostly because of the large variation between animals. MK 421 did significantly inhibit the increase in peripheral vascular resistance that resulted in response to the reduction in cardiac output observed in control dogs (Figure 15; Sponsor's Figure 75).

Figure 15

Effect of Solvent, _____ and MK 421 on Peripheral Vascular Resistance by the Intravenous Route in the Anesthetized Dog



I.A.1.c.(11) Hemodynamic effects of _____ intravenously in the hydrochlorothiazide-pretreated dog (Vol. 1.8 pp. 187-193 and Vol. 1.12 pp. 95-117):

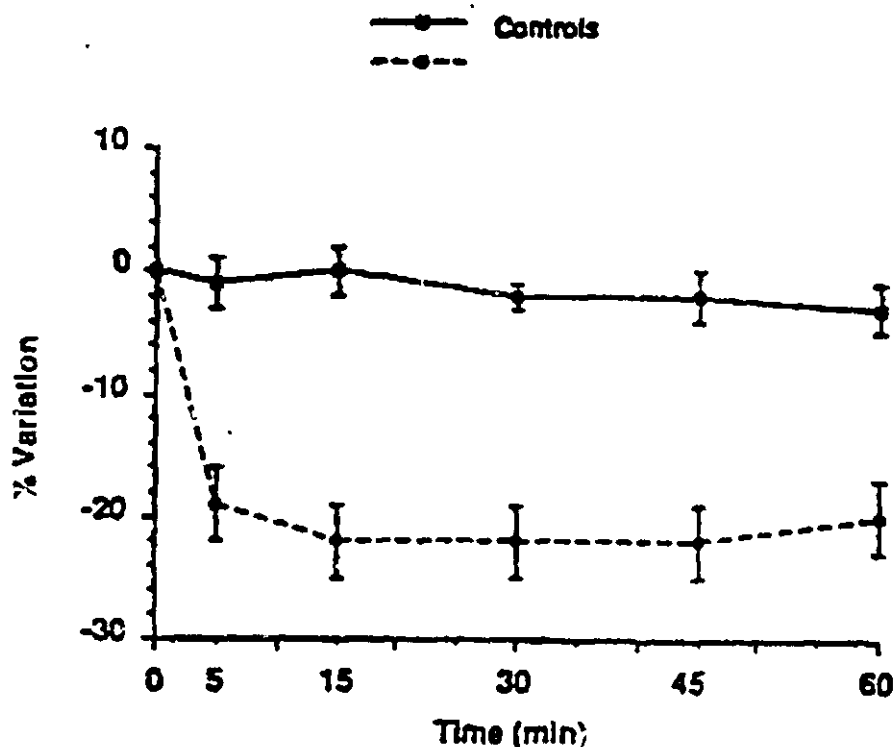
Adult beagle dogs (n=10) were treated with hydrochlorothiazide at 5 mg/kg twice daily for 2 days. The animals were then anesthetized and treated with

IV to a total dose of 1 mg/kg. Control animals received solvent. Hemodynamic parameters (BP, LVP, dP/dt, HR, CO, and ECG) were measured for up to 60 min.

There were no significant differences in heart rates between _____-treated animals and controls. The effect of _____ on mean blood pressure is shown in Figure 16 (Sponsor's Figure 81). There was a rapid and sustained reduction in mean blood pressure with a maximum reduction of $22 \pm 3\%$ in mean blood pressure at 30 min.

Figure 16

Effect of Solvent and on Mean Blood Pressure
by the Intravenous Route in the Hydrochlorothiazide-Pretreated Anesthetized Dog



There were no differences in dP/dt between the two groups ($-12 \pm 3\%$ vs $-18 \pm 6\%$ at 60 min for controls and -treated animals, respectively). decreased cardiac output $-18 \pm 5\%$ vs $-4 \pm 4\%$ for controls animals at 30 min, but this difference was not considered significant. significantly decreased peripheral vascular resistance at 5-15 min compared to controls ($-18 \pm 3\%$ vs $+5 \pm 2\%$ for and controls at 15 min, respectively). The decreased vascular resistance returned to control values by 30 min. There was a significant reduction in stroke volume (cardiac output) in -treated animals 30 min after treatment ($-17 \pm 5\%$) when compared to controls and initial values. This effect remained for up to 60 min, although it was not considered significant after 30 min. induced a large and sustained reduction in heart work (stroke work) which was maximal at 30 min ($-36 \pm 4\%$). Left ventricular work was similarly reduced (-30% at 30 min) when compared to controls. induced a significant reduction in femoral blood flow that potentiated the effect found in control animals ($-29 \pm 4\%$ vs $-13 \pm 9\%$ for

and controls, respectively). There was a gradual increase in femoral vascular resistance in both groups ($+16 \pm 12\%$ vs $+13 \pm 6\%$ at 60 min for controls and treated animals, respectively).

I.A.1.c.(12) Conclusions:

These studies showed the inhibitory effect of [redacted] and its diacid 403 against ACE. In the *in vitro* studies, [redacted] (the diacid) inhibited human, dog, and rat serum ACE at nanomolar concentrations (IC_{50} of 0.93 ± 0.35 nM and 7.06 ± 2.11 nM for [redacted], respectively in human serum). In general, the diacid was the more active form of the ACE inhibitors tested, except in rat plasma where they were equal. This was thought to be due to the rapid hydrolysis of [redacted] in rat plasma. Although in human plasma, [redacted] was not hydrolyzed to its diacid, it was still active. (Note: In the human, this conversion occurs in the liver).

In vivo, [redacted] given IV to rats potentiated the hypotensive effects of bradykinin ($EC_{50} = 4.9 \pm 0.3$). It was not clear what role, if any, bradykinin played in the hemodynamic effects of [redacted]. In the anesthetized dog, [redacted] was about 9X more active than MK 421 in inhibiting the Ang I-induced increases in blood pressure and about 4X more active in inhibiting the increase in dP/dt. The activities of the two diacids ([redacted] and MK 422) were comparable. It was thought that the differences in activity between the two parent molecules ([redacted] and MK 421) were possibly due to differences in their capacity to be hydrolyzed, since the activity of the diacids was similar both *in vitro* and *in vivo*. The abolishment of the drug's antihypertensive effect with bilateral nephrectomy in SH rats demonstrated that its activity is dependent upon the presence of renin which converts angiotensinogen to the ACE substrate angiotensin I. Also in SH rats, the hypotensive effect of [redacted] was not modified by indomethacin, suggesting that prostaglandins were not involved in the antihypertensive activity of [redacted] or other ACE inhibitors (MK 421). The drug had moderate effects on reducing blood pressure in rats and dogs with little effect on heart rates. In dogs the drug inhibited the increase in peripheral vascular resistance that resulted in response to the reduction in cardiac output. In hydrochlorothiazide-pretreated dogs, [redacted] had a greater effect on decreasing blood pressure, peripheral vascular resistance, and cardiac output than in control animals. This indicated that these antihypertensive effects may have been related to the increased plasma renin and angiotensin I concentrations induced by hydrochlorothiazide. The main effect of [redacted] is vasodilation without an associated reflex tachycardia. The magnitude of the response appears to be dependent upon the activation state of the renin-angiotensin system, since the antihypertensive effect is low in animals with low (nephrectomized) renin levels, and higher in animals with increased (hydrochlorothiazide-pretreated) renin levels.

I.A.1.4. Comparison of the effects of chronic administration of trandolapril and enalapril on angiotensin converting enzyme activity in different organs of the spontaneously hypertensive rat (Vol. 1.8 pp. 193-194 and Vol. 1.13 pp. 1-28):

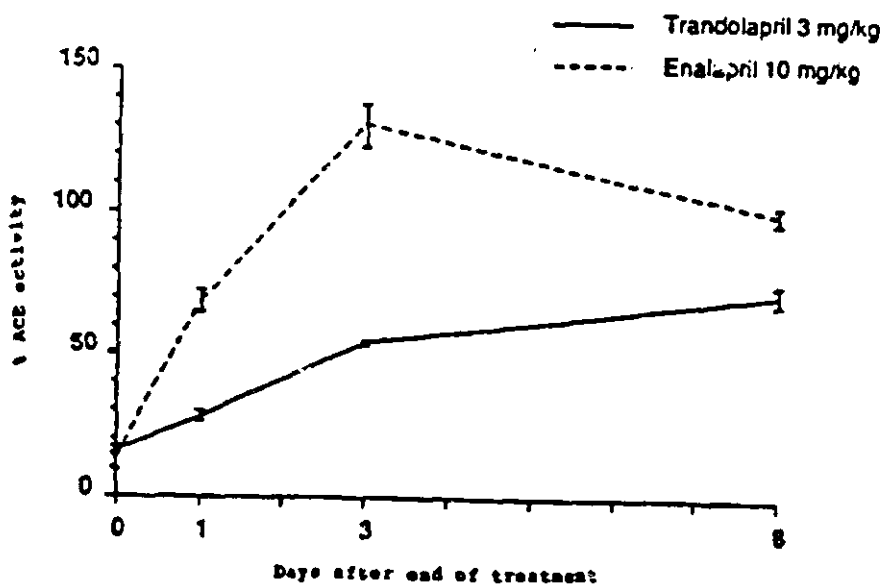
This study compared the effects of trandolapril and enalapril on blood pressure and tissue ACE activity in spontaneously hypertensive rats.

Male spontaneously hypertensive rats (Okamoto strain) were given free access to either trandolapril (3 mg/kg/day) or enalapril (10 mg/kg/day) in their drinking water for 14 days. Controls were given water only. Rats were then sacrificed either on the last day of treatment, or 1, 3, and 8 days after treatment. Homogenates of various tissues (heart atrium and ventricle, kidneys, and abdominal aorta) and serum were assayed for ACE activity.

Results showed that both trandolapril and enalapril at the doses used reduced serum ACE activity to a similar extent (about 90%). However, the persistence of inhibition varied between the two drugs. In the serum of animals treated with trandolapril, the inhibition persisted for up to 8 days after the end of treatment (72% of controls at day 8), whereas in animals treated with enalapril, serum ACE activity was at control values 3 days after the end of treatment (Figure 17; Sponsor's Figure 90). Similar differences in the persistence of inhibition were obtained in tissue ACE activity (aorta, heart atrium and left ventricle), except in the kidney (internal cortex).

Figure 17

Effect of 14 Days of Chronic Oral Treatment with Trandolapril or Enalapril on Angiotensin Converting Enzyme in Spontaneously Hypertensive Rats



It was concluded that trandolapril and enalapril were equipotent in reducing serum and tissue ACE activities (at the doses tested), except in the kidney. The activity of trandolapril, however, had a longer duration than enalapril.

I.A.1.5. Comparison of the effects on blood pressure of _____
_____ in the anesthetized rat (Vol. 1,8 pp. 195-196 and Vol. 1,13 pp. 29-61):

This study determined the effects of _____ (trandolapril), _____ (the diacid trandolaprilat), and _____ (a diketopiperazine derivative which is a degradation product of _____ present in pharmaceutical preparations) on blood pressure in anesthetized normotensive rats.

Male rats were anesthetized and given the following test compounds intravenously through the jugular vein:

_____ at 0.1, 0.3, 1.0, 3.0, and 10.0 mg/kg

_____ at 0.1, 0.3, 1.0, 3.0, and 10.0 mg/kg

_____ at 3.0 and 10.0 mg/kg

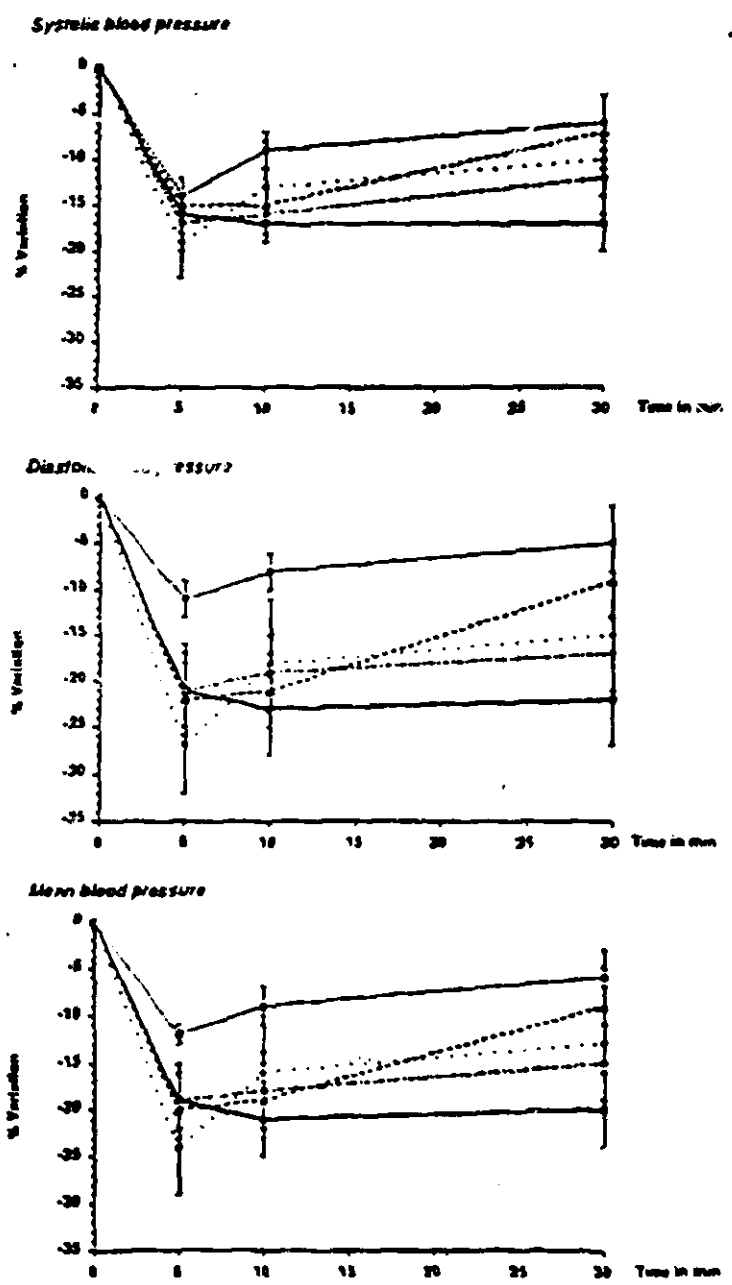
Systolic, diastolic and mean blood pressures were recorded 5, 10, and 30 min after injection of test compound.

Results showed that _____ significantly reduced blood pressure at doses of 0.1 mg/kg and above with the greatest effect on diastolic blood pressure (Figure 18; Sponsor's Figure 95). Increasing doses increased the effect only slightly. _____ significantly reduced blood pressure at doses of 0.3 mg/kg and above. _____ produced a transient (10 min) decrease in blood pressure only at the 10 mg/kg dose.

Figure 18

Effect of _____ by the Intravenous Route on Blood Pressure in the Anesthetized Normotensive Rat

— 0.1 mg/kg - - - 0.3 mg/kg - · - · 1 mg/kg - - - 3 mg/kg — 10 mg/kg



It was concluded that both _____ and the diacid _____ had hypotensive activity, particularly on diastolic blood pressure, in anesthetized normotensive rats at doses from 0.1 and 0.3 mg/kg, respectively. The degradation product _____ was inactive at 3 mg/kg.

I.A.1.6. Study of the effects of (trandolapril) _____ in the ageing spontaneously hypertensive rat; pharmacological study; blood pressure and cardiac hypertrophy; histological study of the cardiovascular apparatus (Vol. 1, 8 pp. 196-199 and Vol. 1, 13 pp. 62-157):

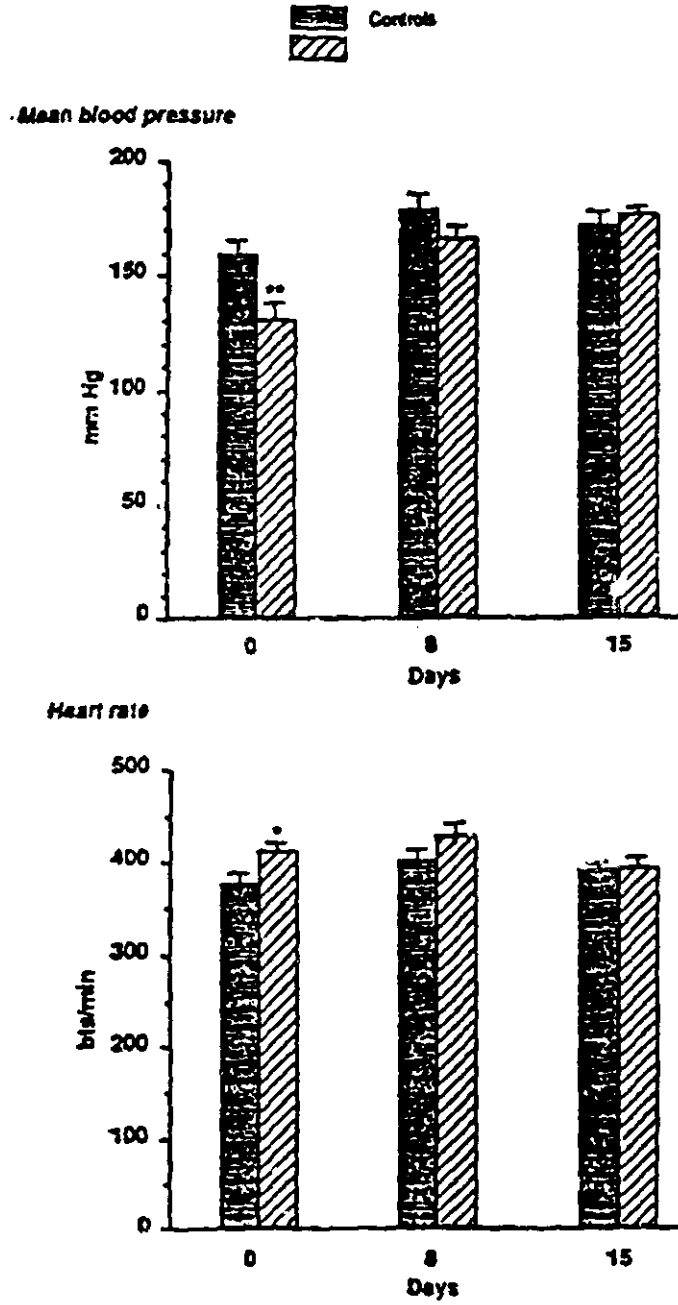
This study examined the effects of trandolapril _____ on cardiac hypertrophy in 20 month old spontaneously hypertensive (SH) rats with established hypertension and cardiac hypertrophy.

Male SH rats were given _____ at 3 mg/kg/day in their drinking water for 4 weeks. Control rats received drinking water only. On the day of sacrifice, blood pressure measurements were taken. Afterwards, cardiac reactivity (increases in heart rate) was measured in response to an IV injection of increasing doses of isoprenaline (isoproterenol). Animals were then sacrificed and evaluated for cardiac hypertrophy (ratio of ventricular weight to body weight). Heart samples were then fixed for histological examination by light microscopy, transmission (TEM) and scanning (SEM) electron microscopy. Blood sera was used to measure ACE activity.

Results showed that treatment with _____ at 3 mg/kg for 4 weeks decreased mean blood pressure by 18% ($P < 0.01$) on the last day of treatment (Day 0) (Figure 19; Sponsor's Figure 98). There was no effect by Day 8. There was a slight increase in heart rate (+10%) on Day 0. Cardiac hypertrophy was reduced by 35% on Day 0, and remained 19% less than controls on Day 15. Serum ACE activity was reduced 84% at the end of treatment (Day 0), and remained 24% reduced two weeks after the end of treatment (Day 15). _____-treated animals when compared to untreated SH rats showed a slight improvement in the response to isoprenaline at the end of treatment (Day 0) which may have been related to the reduced cardiac hypertrophy. Histological examination of hearts from both treated and untreated rats showed degenerative lesions characteristic of hypertension and ageing with large intragroup variability. No morphological evidence was reported that reduced these lesions.

Figure 19

Effect of . on Blood Pressure and Heart Rate After 4 Weeks of Treatment (Day 0) and 8 and 15 Days After the End of Treatment in the Ageing SH Rat



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I.A.1.7. Compared activities of oral (trandolapril) in normotensive and spontaneously hypertensive rats (Vol. 1.8 pp. 199-201 and Vol. 1.13 pp. 158-172):

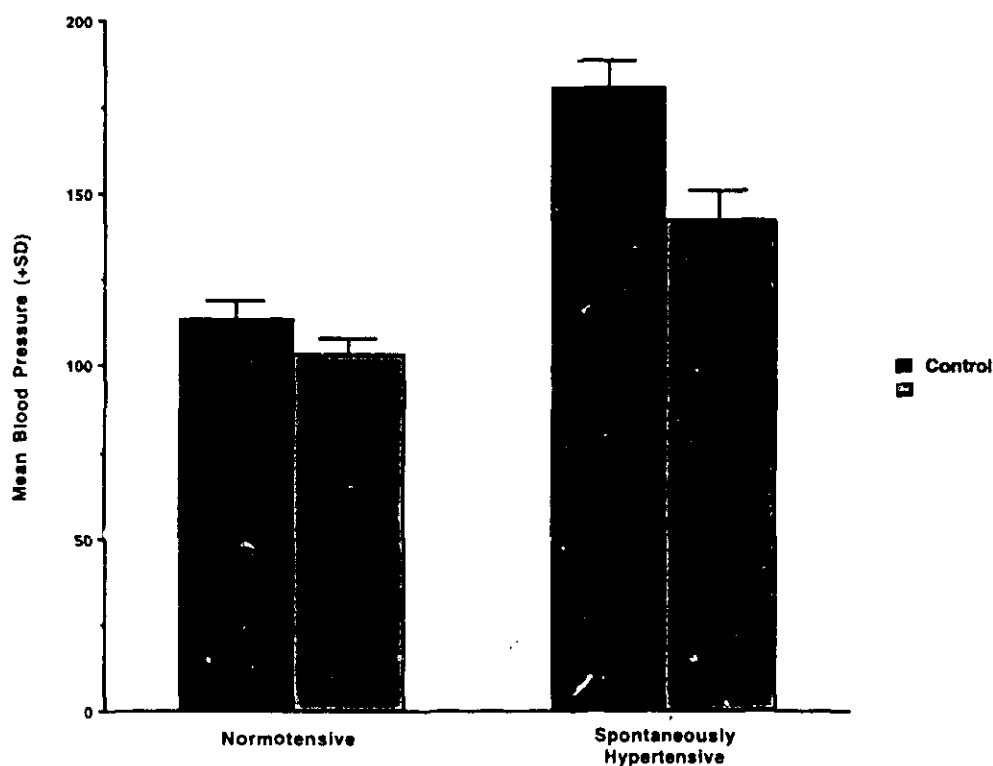
This study compared the effects of (trandolapril) treatment in normotensive and spontaneously hypertensive rats on blood pressure, heart rate, and ACE activity.

Groups of normotensive and SH rats received at 0.3 mg/kg/day for 15 days in the drinking water. Control animals from both groups received drinking water only. Rats were then anesthetized and blood pressure measurements taken. Arterial blood was collected for measurements of serum ACE activity and for assay of the diacid (trandolaprilat).

decreased serum ACE activity in both normotensive and spontaneously hypertensive rats to a similar extent (-67% vs -60%, respectively). However, there was a greater effect on reducing mean blood pressure in SH rats than in normotensive rats (-21% vs -9%, respectively) (Figure 20).

Figure 20

Effect of 15 Day Treatment with Given Orally (0.3 mg/kg/day) on
Mean Arterial Blood Pressure in Normotensive and Spontaneously Hypertensive Rats



Heart rates were minimally affected (+1% vs +4 % for SH and normotensive rats, respectively). The mean serum concentration of the diacid trandolaprilat was 78% greater in the SH rats than in the normotensive rats (17.32 ± 2.37 vs 9.71 ± 1.08 ng/ml, respectively). The reason for this difference was not clear.

These results showed that SH rats were more sensitive to the hypotensive effects of trandolapril than normotensive rats, demonstrating the importance of the renin-angiotensin system in maintenance of blood pressure in the SH rat model of hypertension.

I.A.1.8. Comparative effects of trandolapril and enalapril on the morphological and functional cardiovascular changes in genetic hypertension (Vol. 1.8 pp.201-204 and Vol. 1.13 pp. 173-284):

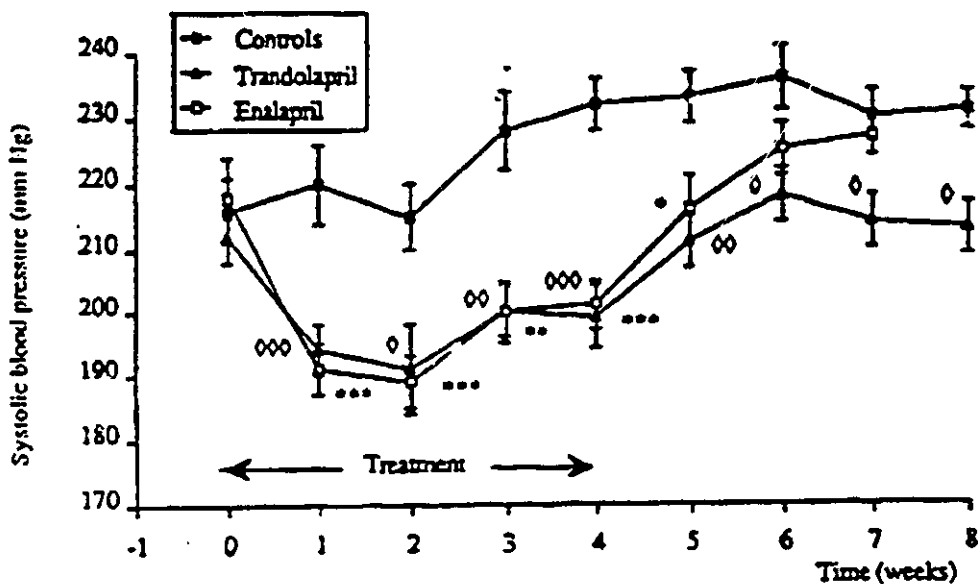
This study compared the effects of a 28 day oral treatment with trandolapril or enalapril on blood pressure, cardiac hypertrophy, and functional and morphological vascular changes in spontaneously hypertensive rats to try to establish a relationship between blood pressure effects and the degree of regression in hypertrophy.

Rats were given either trandolapril (0.4 mg/kg/day) or enalapril (10.0 mg/kg/day) by gavage for 4 weeks. Body weights, systolic blood pressure, and heart rates were monitored weekly. Specific parameters (intra-arterial blood pressures, reactivity to angiotensin I, heart weight, *in vitro* arterial compliance, reactivity to noradrenaline, and histological examination of vessel wall hypertrophy) were measured at three different times: (M1) Day +28 or end of treatment; (M2) when blood pressure reached half-way between those measured at the beginning and at the end of treatment (= Day +35 in enalapril and Day +42 in trandolapril rats); and (M3) when blood pressure was no longer significantly different between the treatment and control groups (= Day +49 in enalapril and Day +63 in trandolapril rats).

Results showed that both drugs reduced systolic blood pressure 11.2% and 14.2% at week 2 for trandolapril and enalapril, respectively when compared to controls with no difference between the drug-treated groups (Figure 21; Sponsor's Figure 103). The hypotensive effect of enalapril was not significantly different from controls by 2 weeks after the end of treatment (Week 6), whereas the effect of trandolapril was still significant at Week 8 (-7%). Heart Rates were unchanged.

Figure 21

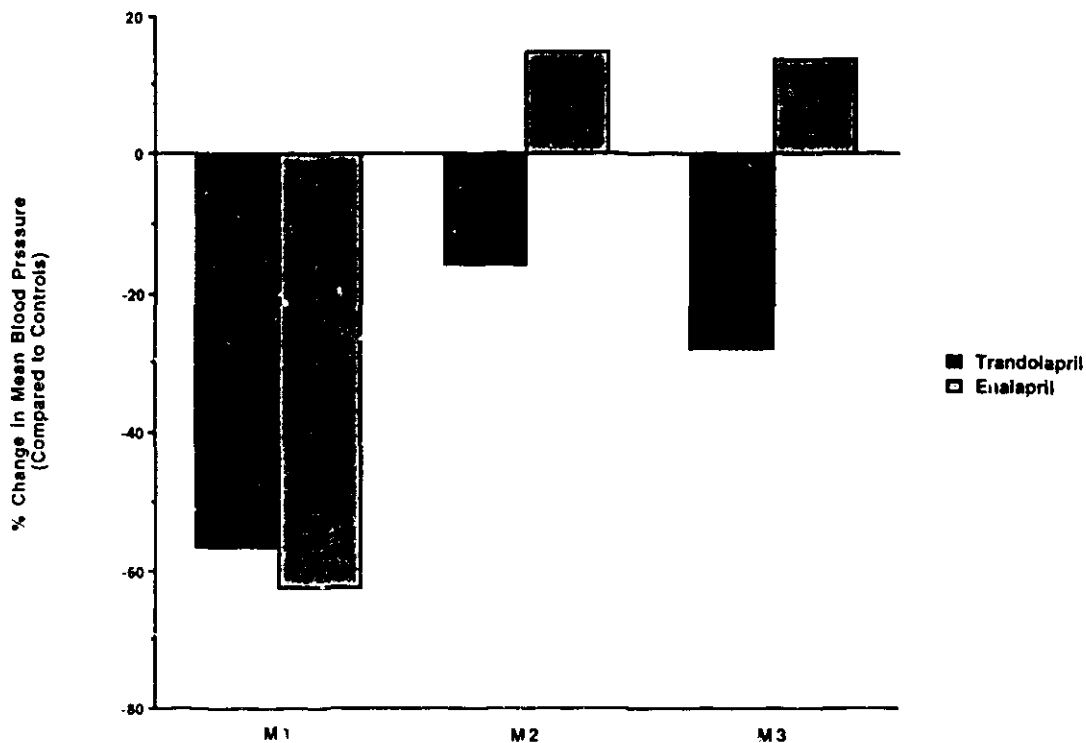
Effects of Orally Administered Trandolapril (0.4 mg/kg/day) and Enalapril (10 mg/kg/day) on Systolic Blood Pressure in Comparison to that of Control Animals (Conscious Rat)



Both drugs significantly ($P < 0.01$) reduced the rise in mean blood pressure to angiotensin I during the M1 period -56.5% vs -62.4% for trandolapril and enalapril, respectively (Figure 22). The changes at the other two measurement times (M1 and M2) were not significant.

Figure 22

Effect of Trandolapril and Enalapril on the Rise
in Mean Blood Pressure After an Injection of Angiotensin I



The ratio of heart weight to body weight (an index of cardiac hypertrophy) was significantly ($P < 0.05$) decreased in animals treated with both drugs (-8.7% and -7.3% for trandolapril and enalapril, respectively) at the M1 time period only. An increase in the compliance of mesenteric resistance arteries (decreased wall tension and increased internal diameter) was shown by a shift to the right in wall tension-internal diameter curves at the M1 time period only (+22.0% and +21.1% increase in internal diameter for trandolapril and enalapril, respectively) (Figure 23; Sponsor's Figure 111). At the M3 time period, wall tension was increased in relation to controls (-12.0% and -12.4% decrease in internal diameter for trandolapril and enalapril, respectively) (Figure 24; Sponsor's Figure 113). This indicates that at the M1 time period arterial wall tension was reduced by drug treatment (vessels from drug-treated animals that are equal in internal diameter to arteries from untreated animals have a lower wall

tension), but when blood pressure returns to normal (M3), there may be an increase in vessel wall tension (decreased internal diameter). The duration of this increase was not shown. These results are summarized in Figure 25 (Sponsor's Figure 114).

Figure 23

Effects of Orally Administered Trandolapril (0.4 mg/kg/day) and Enalapril (10 mg/kg/day) on Tension-Diameter Curves from Segments of Mesenteric Arteries Over the M1 Period ($\diamond$ and * = $P < 0.05$, Compared to Controls)

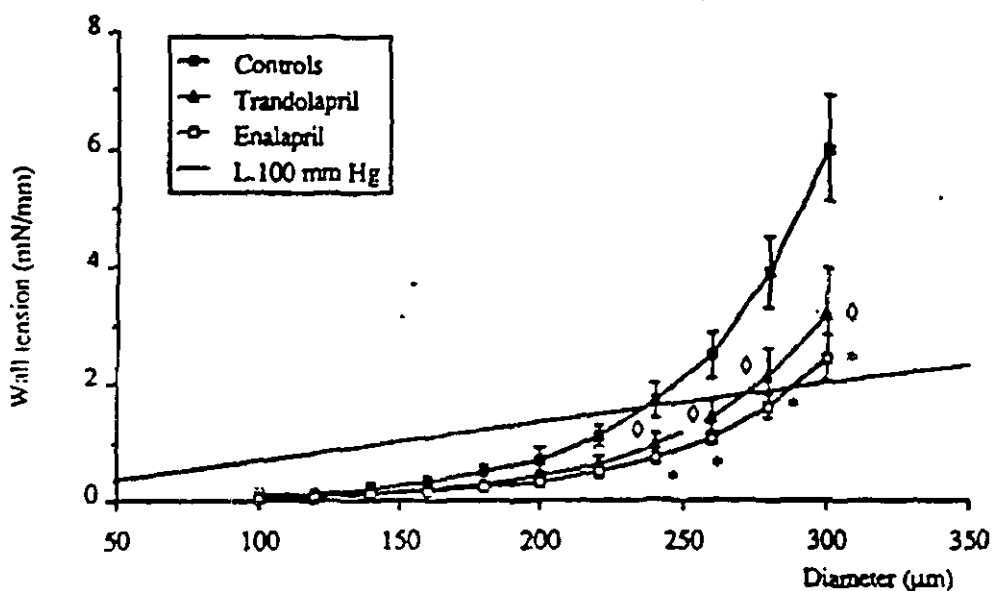


Figure 24

Effects of Orally Administered Trandolapril (0.4 mg/kg/day) and Enalapril (10 mg/kg/day) on Tension-Diameter Curves from Segments of Mesenteric Arteries Over the M3 Period ($\diamond$ and * = $P < 0.05$, Compared to Controls)

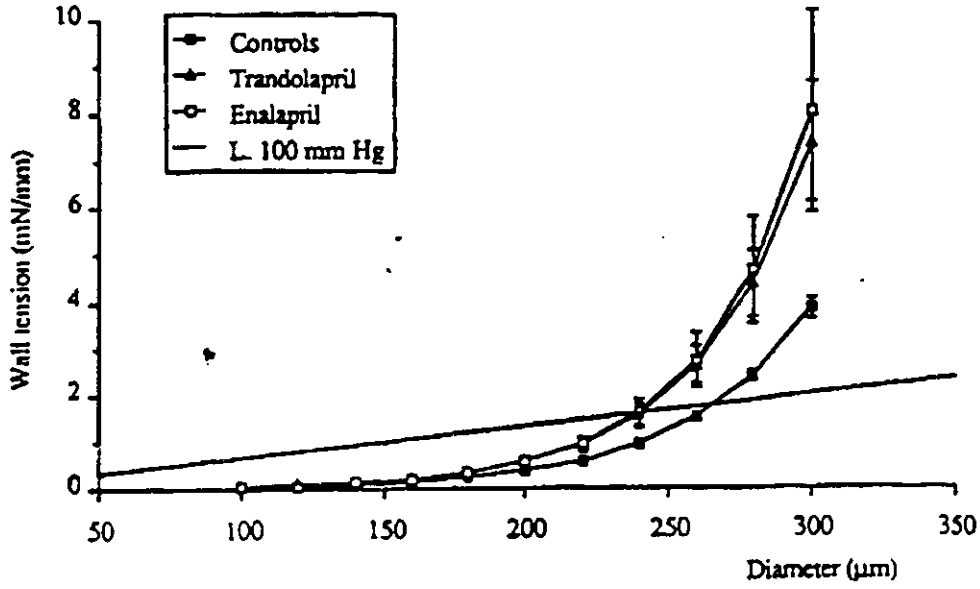
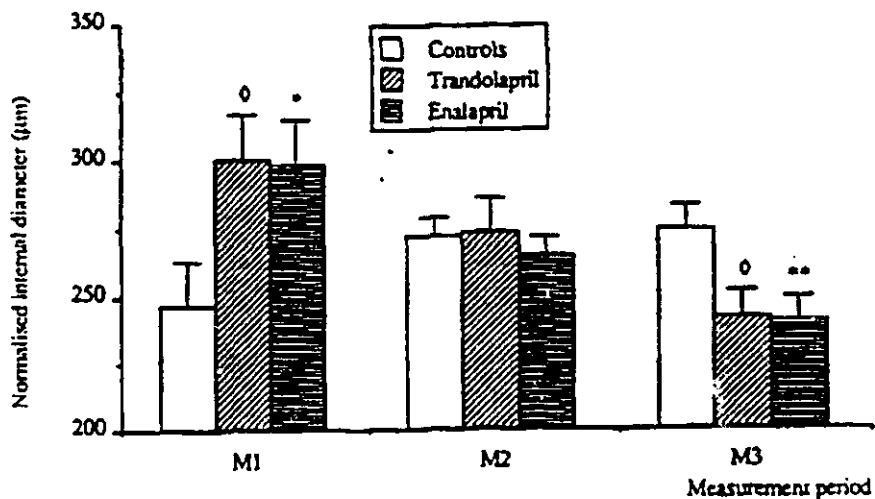


Figure 25

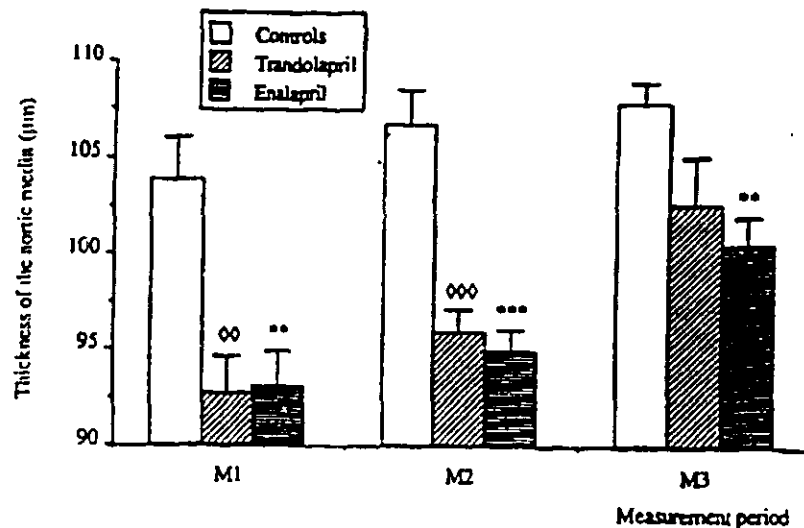
Effects of Orally Administered Trandolapril (0.4 mg/kg/day) and Enalapril (10 mg/kg/day) on Normalized Internal Diameter Values of Resistance Arteries Measured in Three Periods (M1, M2, and M3) ($\diamond$ and * = $P < 0.05$; ** = $P < 0.01$ Compared to Controls)



During the M1 and M2 time periods, the media thickness of the thoracic aorta was significantly reduced by both drugs (-10.0% to -10.7% and -10.4% to -11.0% for trandolapril and enalapril, respectively) (Figure 26; Sponsor's Figure 119). By the M3 time period, the effect was less pronounced. A similar effect was noted on the femoral artery with trandolapril.

Figure 26

Effects of Orally Administered Trandolapril (0.4 mg/kg/day) and Enalapril (10 mg/kg/day) on the Media Thickness of the Thoracic Aorta Determined Over the Three Periods (M1, M2, and M3) ($\diamond\diamond$ and $**$ = $P < 0.01$; $\diamond\diamond\diamond$ and $***$ = $P < 0.001$ Compared to Controls)



These studies showed that trandolapril at 0.4 mg/kg/day and enalapril at 10 mg/kg/day for 4 weeks lowered blood pressure, reduced cardiac hypertrophy, and reduced vessel wall tension during the course of treatment. Upon termination of treatment, the effects were found to be reversible within 2 to 4 weeks with vascular compliance (decreased wall tension) showing a more rapid reversibility. Trandolapril showed a longer duration of action on blood pressure after termination of treatment than enalapril.

I.A.1.9. Comparative effects of 14 day oral administration of trandolapril and enalapril on cardiac and aortic converting enzyme activity in the spontaneously hypertensive rat (Vol. 1.8 pp. 204-206 and Vol. 1.13 pp. 285-340):

This study examined the effects of different doses of trandolapril and enalapril given for 14 days to SH rats on vascular and cardiac ACE activity. In addition, the effects of the diacids, trandolaprilat and enalaprilat, on inhibition of tissue ACE activity were examined *in vitro*.

Groups (7-10 rats/ group) of male SH rats were given either trandolapril (0.1, 0.3, 1, 3, 10, 100, or 1000 µg/ml) or enalapril (10, 100, µg/ml and 1, 3, and 10 mg/ml) in the drinking water for 14 days. At the end of the experiment, animals were sacrificed, the thoracic aorta and heart removed, and homogenates of the tissues were

prepared for assay of ACE activity and protein content.

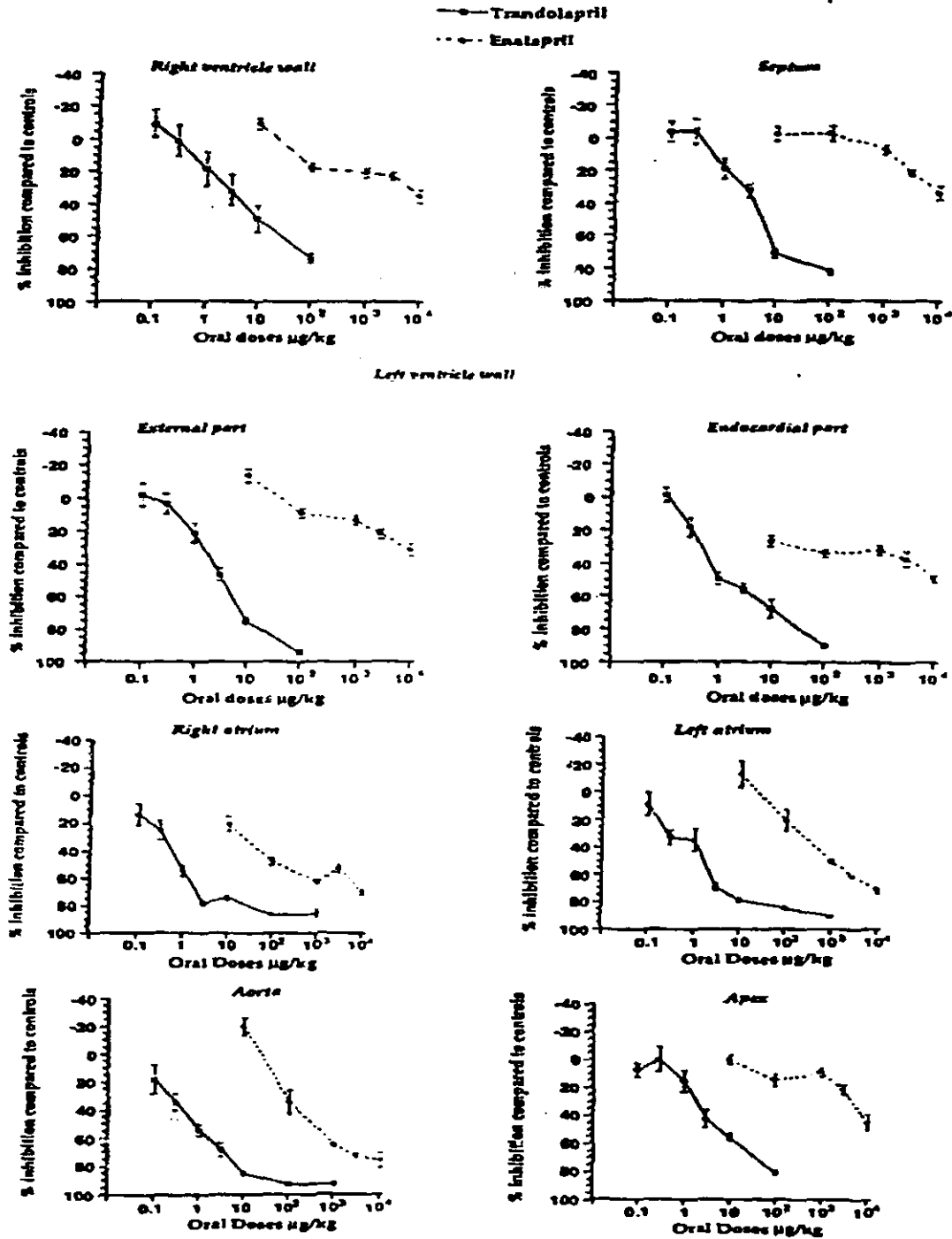
Results showed that both drugs caused a dose-dependent inhibition of ACE in all the various sections of the heart (right atrium, left atrium, apex, right ventricle wall, septum, and external and endocardial part of left ventricle wall) and aorta (the % inhibition/ dose-response curves were parallel (Figure 27; composite of Sponsor's Figures 121-122). Trandolapril was much more active than enalapril in reducing ACE in ventricular tissue. Significant inhibition was achieved with doses of trandolapril between 0.3 and 1.0 $\mu\text{g}/\text{kg}/\text{day}$ with near maximal activity at doses between 10 and 100 $\mu\text{g}/\text{kg}/\text{day}$. The minimum active dose of enalapril was between 10 and 100 $\mu\text{g}/\text{kg}/\text{day}$. Trandolapril was 250 to 1000X more active than enalapril on reducing ACE in these tissues.

The diacids, trandolaprilat and enalaprilat, caused dose-dependent inhibition of tissue ACE activity with trandolaprilat being 2.5 to 5.0X more active than enalaprilat.

It was concluded that trandolapril was more potent than enalapril in reducing ACE in the heart and aorta (minimum effective dose of between 0.3 and 1.0 $\mu\text{g}/\text{kg}/\text{day}$). This difference in potency between the two drugs was more pronounced on ventricular ACE activity where enalapril showed less activity.

Figure 27

Effects in Relation to the Dose of Trandolapril or Enalapril Administered Orally for 14 days in the SH Rat (% of ACE Inhibition)



I.A.1.10. Determination of the minimum effective dose of (trandolapril) in the spontaneously hypertensive rat after oral administration for 14 days - Comparison with enalapril (Vol. 1.8 pp. 206-209 and Vol. 1.13 pp. 341-379):

This study determined the minimal effective dose of trandolapril on blood pressure after oral treatment for 14 days in the SH rat. The effects were compared to that of enalapril.

Male SH rats were given either trandolapril () or enalapril (MK 421) in the drinking water for 14 days in three separate experiments (controls received water only):

- Experiment 1: Trandolapril at 0.3, 1, and 3 mg/kg/day
Enalapril at 1, 3, and 10 mg/kg/day
- Experiment 2: Trandolapril at 0.03, 0.1, and 0.3 mg/kg/day
- Experiment 3: Trandolapril at 0.003, 0.01, and 0.03 mg/kg/day

On day 15, blood pressure and heart rates were measured, blood was collected for assay of serum ACE activity, and the hearts were removed and weighed.

The effect of trandolapril on mean blood pressure as a function of dose is shown in Figure 28. Trandolapril caused a dose-dependent reduction in mean blood pressure with a minimal effective dose of 0.03 mg/kg/day (8.5% reduction in mean blood pressure). The effect of trandolapril on cardiac hypertrophy also showed a dose-dependent effect with the same minimal effective dose of 0.03 mg/kg/day (5.1% reduction in cardiac hypertrophy) (Figure 29). At this dose (0.03 mg/kg/day) trandolapril reduced serum ACE activity by 40.9% with a minimal effective dose of 0.003 mg/kg/day (Figure 30). When compared to the effects of trandolapril, a 10 mg/kg/day dose of enalapril was equivalent to a 0.3 mg/kg/day dose of trandolapril on mean blood pressure and cardiac hypertrophy, and to a 0.03 mg/kg/day dose of trandolapril on serum ACE activity.

Figure 28

Effect of Trandolapril on Mean Blood Pressure After 14 Days of Oral Treatment in the SH Rat

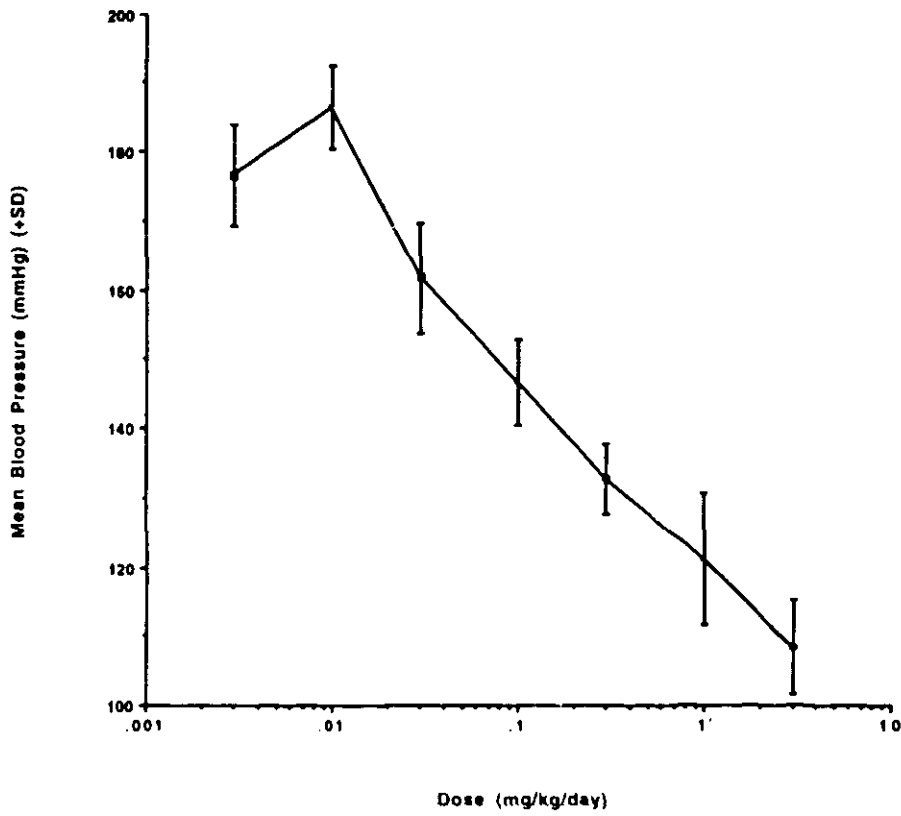


Figure 29

Effect of Trandolapril on Cardiac Hypertrophy After 14 Days of Oral Treatment in the SH Rat

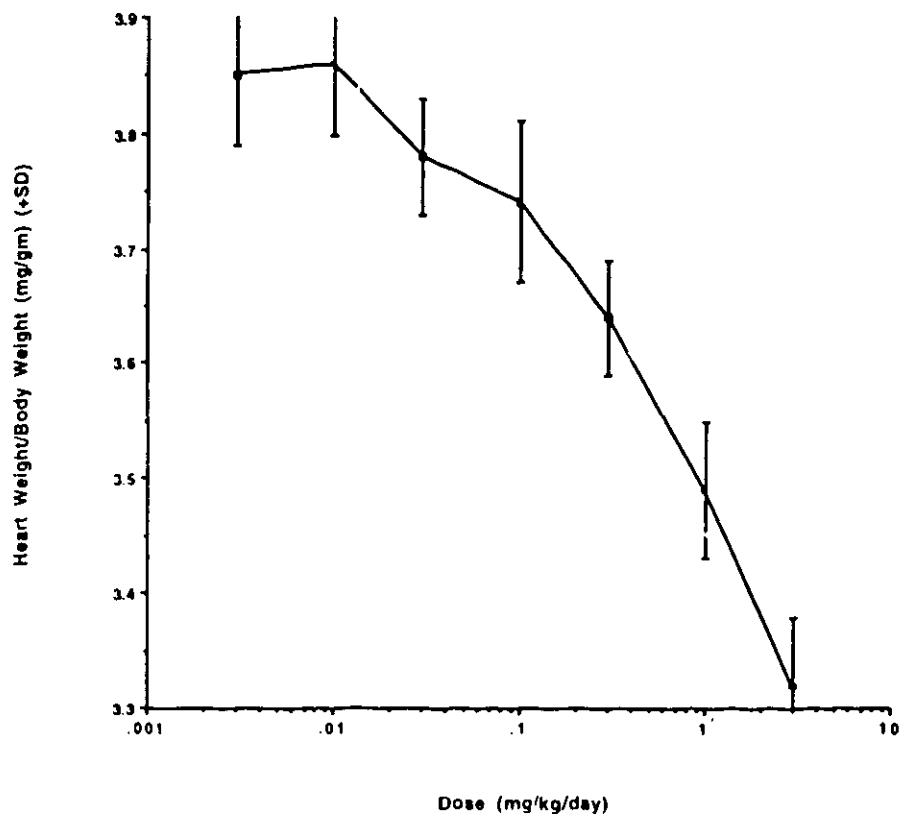
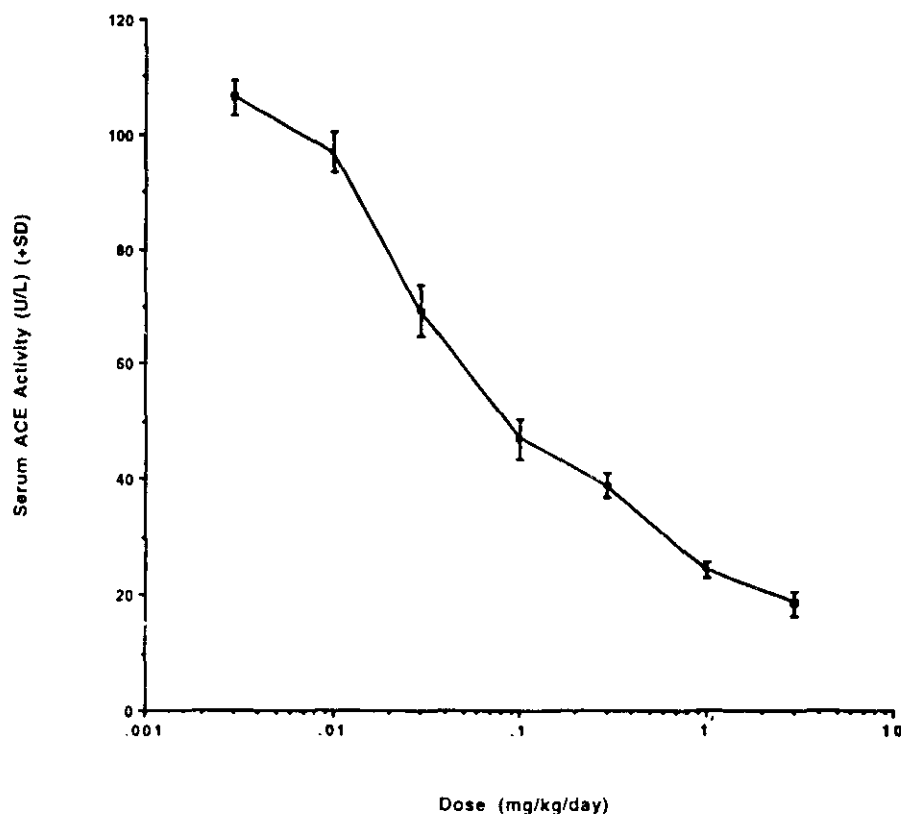


Figure 30

Effect of Trandolapril on Serum ACE Activity
After 14 Days of Oral Treatment in the SH Rat



I.A.1.11. Study of _____ on converting enzyme activity in different organs of the spontaneously hypertensive rat (Vol. 1.8 pp. 209-211 and Vol. 1.13 pp. 380-389):

This study examined the effect of treatment with trandolapril in drinking water for 15 days on SH rat serum and tissue (kidney, heart ventricle and atrium, and aorta) ACE activity. In addition, rebound ACE activity was determined at various times after the study.

Male SH rats (6/ group) were given trandolapril in the drinking water at 3 mg/kg for 15 days. Rats were sacrificed on Day 0 and 1, 3, and 8 days after the end of treatment. ACE activity was assayed in serum, and in homogenates of kidney, heart ventricle and atrium, and aorta.

Results showed that inhibition of ACE activity was organ specific (Table 4). At the end of treatment there was a > 90% inhibition of the ACE activity in serum, atrium, and aorta. In the left ventricle and renal inner cortex the activity was reduced by 60-70%. Reversibility of the inhibition 8 days after the end of treatment also varied with the specific organ. In serum and ventricles, ACE activity approached 60-80% of control values, while in the aorta, atrium, and kidney, levels were 10%, 20-30%, and 40% of the control values, respectively.

These results suggest that the inhibitory effects of trandolapril on ACE activity had a longer duration in specific organs (aorta, atrium, and kidney) than in serum.

Table 4

Inhibition of ACE Activity After 15 Days of Treatment with Trandolapril (a) and Reversion 8 Days After the End of Treatment (b)
(Results are Expressed as % Inhibition)

	<u>Serum</u>	<u>Left Ventricle</u>	<u>Atrium</u>	<u>Aorta</u>	<u>Renal Inner Cortex</u>
(a)	< 90%	60-70%	< 90%	< 90%	60%
(b)	20-40%	30%	70-80%	90%	60%

I.A.2. *In Vitro* Studies

I.A.2.a. Potentiation of the effects of bradykinin by (trandolapril)

(Vol. 1.8

pp. 211-212):

Certain inflammatory events, such as tissue damage, allergic reactions, and viral infections, produce a large number of inflammatory mediators, or kinins, in tissues and in plasma that can act locally to produce pain, vasodilatation, increased vascular permeability, and increased synthesis of prostaglandins. One such kinin is bradykinin, a nonapeptide formed from the liver-derived precursor kininogen by the enzymatic cleavage in the serum by kallikreins. The half-life of bradykinin is relatively short (about 15 seconds in plasma) due mostly to catabolism by angiotensin converting enzyme (ACE). This study determined the extent to which trandolapril, an angiotensin converting enzyme inhibitor, was capable of potentiating the effects of bradykinin both *in vitro* and *in vivo*.

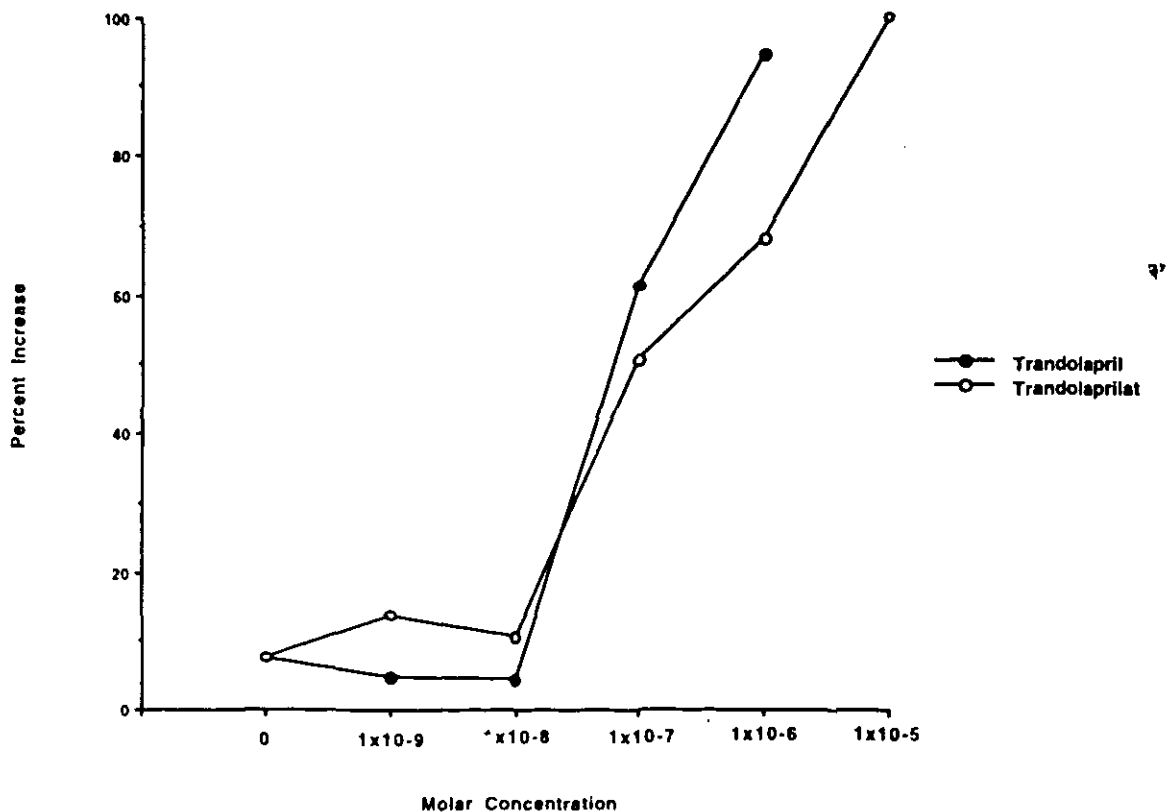
For the *in vitro* studies, segments of guinea pig ileum were used to study the potentiation of the effect of bradykinin by trandolapril and the diacid trandolaprilat on contraction force. As a positive control, antagonism by trandolapril to the effects of angiotensin I on smooth muscle contraction was also studied. For the *in vivo* studies,

rats were giventrandolapril or enalapril at 0, 0.03, 0.3, and 30.0 mg/kg orally 30 min before the induction of edema in rats by subplantar (into the sole) injections of carrageenin, a known inducer of bradykinin release *in vivo*. Measurements of paw volume were taken at baseline, and at 45 min, 90 min, and 3 hours after induction of edema. As a bradykinin-specificity control, another group of rats were given test drugs then injected with dextran, which causes edema independent of the release of bradykinin.

Results of the *in vitro* studies showed that bothtrandolapril andtrandolaprilat potentiated the response of bradykinin on guinea pig ileum smooth muscle in a concentration-dependent manner as measured by an increased force of contraction (Figure 31). The EC₅₀ values for both compounds were about 1 x 10⁻⁷ M. The EC₅₀ values for angiotensin I inhibition were 5 x 10⁻⁸ M fortrandolapril and 3 x 10⁻⁸ M fortrandolaprilat. The similarity in EC₅₀ values for bothtrandolapril and its diacid may indicate thattrandolapril is rapidly transformed into its diacid.

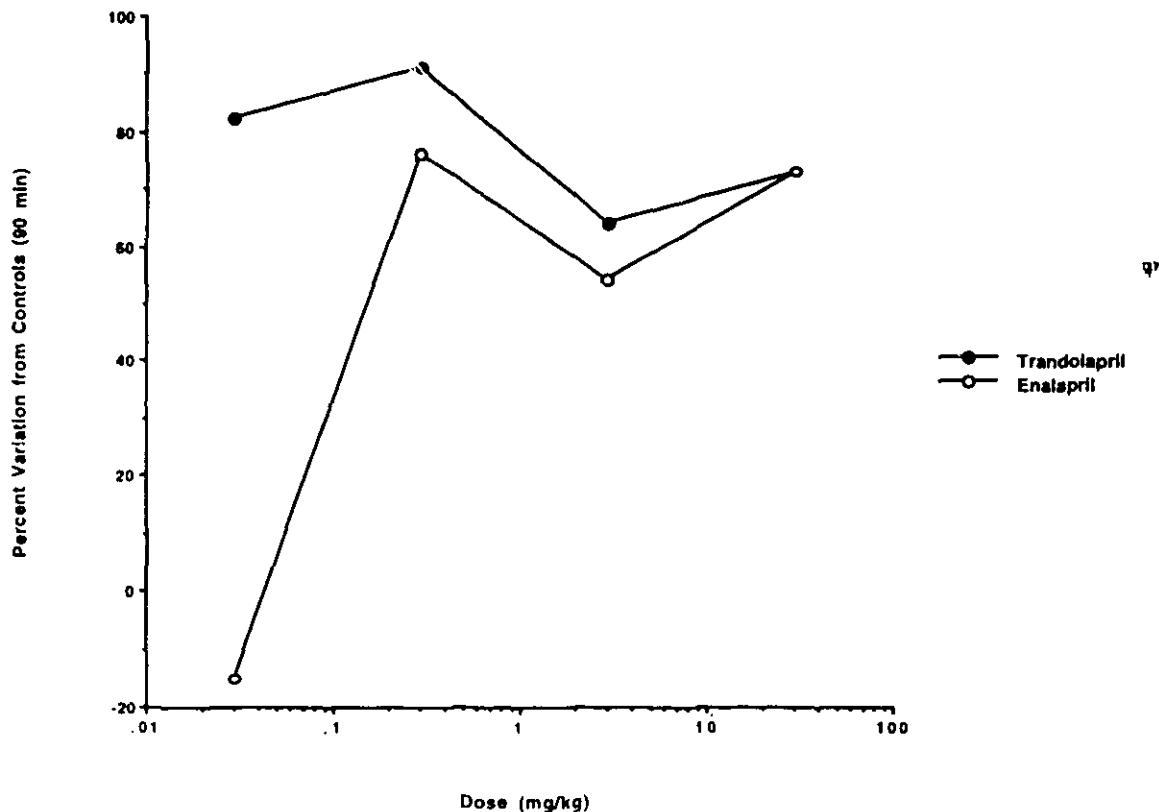
Figure 31

Potentialiation of the Action of Bradykinin on Guinea Pig Ileum Smooth Muscle by Trandolapril and Trandolaprilat



In the *in vivo* rat subplantar-edema study, both trandolapril and enalapril potentiated the response to bradykinin (Figure 32). Injection of carrageenin into control rats induced a 15% increase in swelling. In animals pretreated with trandolapril or enalapril, the swelling increased further by 91% and 76% for trandolapril (0.03 mg/kg) and enalapril (0.3 mg/kg), respectively, with the peak response occurring at 90 min after edema induction. In contrast, neither drug had any affect on the edema induced by dextran.

Figure 32

Potentiation of Carrageenin-Induced Subplantar Edema
by Trandolapril and Enalapril

It was concluded that *in vitro* trandolapril potentiated the response of guinea pig ileum smooth muscle to bradykinin (EC_{50} of 1×10^{-7}), and inhibited the response to angiotensin I (EC_{50} of 5×10^{-8}). Since angiotensin I is less than 1% as potent as angiotensin II on smooth muscle, it was not clear whether the inhibitory effect of trandolapril was on angiotensin I or on its conversion to angiotensin II. *In vivo* trandolapril potentiated the inflammatory reaction to carrageenin at doses shown in previous studies to inhibit ACE activity. Trandolapril had no effect on the edema caused by dextran which does not cause release of bradykinin. These results demonstrate that trandolapril had effects *in vitro* and *in vivo* consistent with inhibition of angiotensin converting enzyme activity.

I.A.2.b. Interaction of (trandolapril) and (trandolaprilat) with bradykinin-induced contractile responses in guinea pig ileum (Vol. 1.8 pp. 212-213 and Vol. 1.13 pp. 2-16):

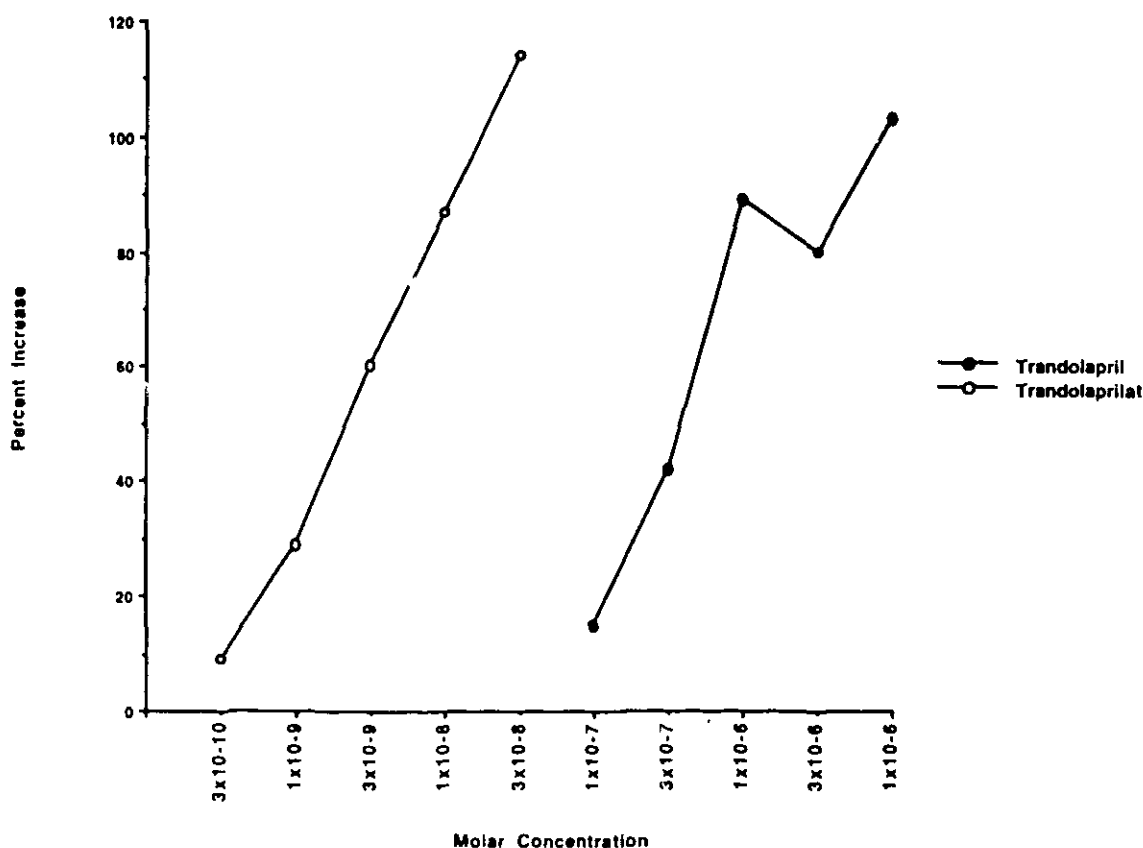
This study re-examined the extent to which trandolapril and its diacid trandolaprilat were capable of potentiating the effects of bradykinin on guinea pig ileum smooth muscle *in vitro*.

Test compounds (trandolapril at 1×10^{-7} M, 3×10^{-7} M, 1×10^{-6} M, 3×10^{-6} M, 1×10^{-5} M and trandolaprilat at 3×10^{-10} M, 1×10^{-9} M, 3×10^{-9} M, 1×10^{-8} M, 3×10^{-8} M) were put in contact with the ileum one minute before the addition of 5×10^{-9} M bradykinin to give a mean contraction force of 2 gms. The concentration which potentiated 50% of the bradykinin effect (EC_{50}) was determined.

Results (Figure 33) showed that the diacid trandolaprilat was about 200X more active than trandolapril (EC_{50} of 2.2×10^{-9} vs 4.4×10^{-7} , respectively). In the previous study above, both compounds had equal EC_{50} 's of about 1×10^{-7} M. The reason for the increased responsiveness of the diacid in this study was not clear. ¶

Figure 33

Potentiation of the Action of Bradykinin on Guinea Pig Ileum Smooth Muscle by Trandolapril and Trandolaprilat



I.A.2.c. Effects of the angiotensin converting enzyme inhibitor trandolaprilat on endothelium-dependent responses in isolated blood vessels (Vol. 1.8 pp. 214-215 and Vol. 1.14 pp. 17-39):

This study examined the effects of the diacid trandolaprilat (in the presence or absence of various agents) on the relaxation response of isolated segments of rat and dog arteries after contraction with prostaglandin.

The vessels used in this study were dog left circumflex coronary artery and femoral artery, and the rat thoracic aorta. Five mm length rings were prepared and incubated with various concentrations of trandolaprilat in the presence or absence of various relaxation agents (bradykinin, acetylcholine, angiotensin I, and adenosine

diphosphate) 45 min before contraction with prostaglandin $F_{2\alpha}$ (2×10^{-6} M). Changes in isometric force were measured using a strain gauge.

Results showed thattrandolaprilat did not cause direct vessel relaxation in the absence of additional agents. However, when given in the presence of bradykinin at 10^{-9} M (Figure 34; Sponsor's Figure 126), significant relaxation occurred. Since bradykinin is a vasorelaxant and is a substrate for ACE, inhibition of its degradation may be responsible for potentiation of the relaxation effects bytrandolaprilat. Significant relaxation also occurred whentrandolaprilat was given in the presence of angiotensin I at 10^{-8} to 10^{-9} M (Figure 35; Sponsor's Figure 129). In this case, inhibition of ACE activity may have prevented formation of angiotensin II, a vasoconstrictor. Similar results were obtained with adenosine diphosphate at 10^{-6} to 10^{-8} M (Figure 36; Sponsor's Figure 131). The relaxation observed in the presence of bradykinin, angiotensin I, and adenosine diphosphate was shown to be endothelium-dependent (no relaxation was observed when vessels free of endothelium were used). The relaxation observed in the presence of bradykinin was reversed by the addition of a B2 kinin receptor antagonist, indicating that the effect was bradykinin-dependent, and not due to a direct effect oftrandolaprilat.

Figure 34

Concentration-Response Curve to Trandolaprilat in the Presence of Bradykinin (10^{-9} M) in Canine Femoral Arteries With Endothelium Contracted with Prostaglandin $F_{2\alpha}$ (2×10^{-6} M) (* = $P < 0.05$)

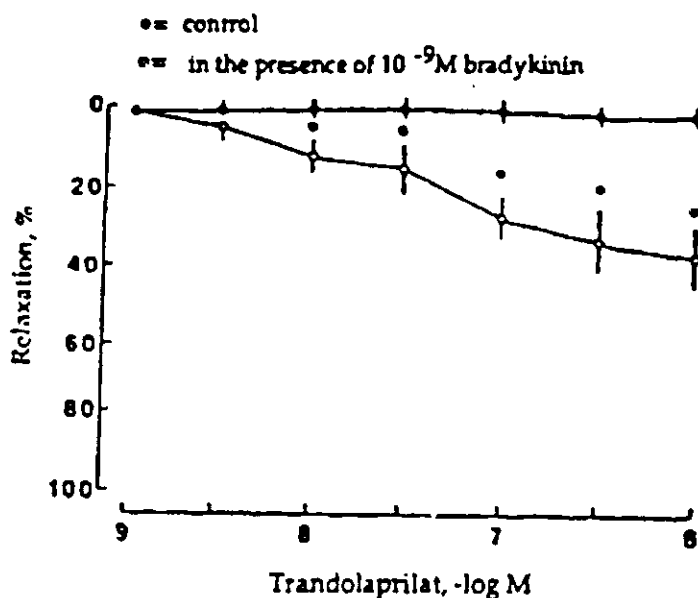


Figure 35

Concentration-Response Curve to Trandolaprilat in the Presence of Angiotensin I (10^{-8} to 10^{-9} M) in Normotensive Rat Aortas With and Without Endothelium Contracted with Prostaglandin $F_{2\alpha}$ (2×10^{-6} M) (* = $P < 0.05$)

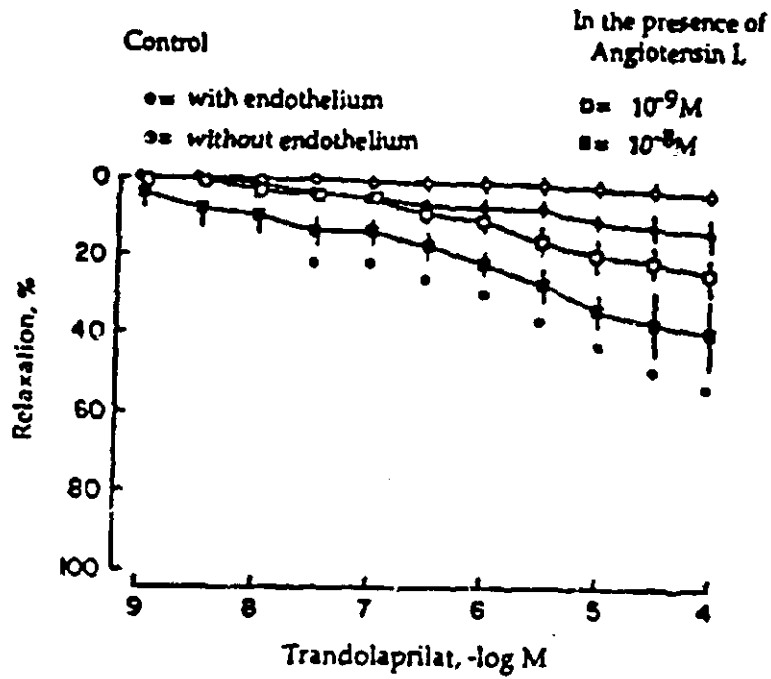
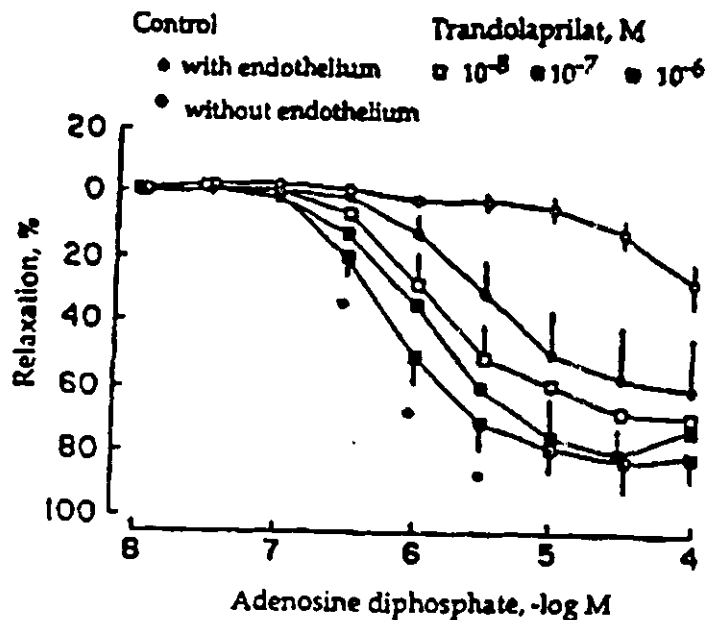


Figure 36

Effect of Increasing Concentrations of Trandolaprilat (10^{-8} to 10^{-6} M) on the Concentration-Response Curve to Adenosine Diphosphate in Rings of Canine Femoral Arteries With and Without Endothelium Contracted with Prostaglandin $F_{2\alpha}$ (2×10^{-6} M) (* = $P < 0.05$)



These results support the view that trandolaprilat reduces the degradation of bradykinin by ACE present on endothelial cells, resulting in potentiation of the relaxation response, and that this may be a major mechanism responsible for the vasodilator effects of ACE inhibitors. [Note: Bradykinin has been shown to induce the release of nitric oxide from endothelial cells resulting in vasodilation, Lancet 1994; 343:199-1206].

I.A.2.d. Study of the competition of trandolapril, trandolaprilat, and other angiotensin converting enzyme (ACE) inhibitors with a tritiated reference inhibitor (^3H -quinalaprilat) for binding to purified human renal ACE (Vol. 1.8 pp. 216-217 and Vol. 1.14 pp. 40-58):

This study determined the relative binding affinities of various ACE inhibitors for purified human renal ACE by measuring their ability to displace ^3H -quinalaprilat (a reference ACE inhibitor).

Microsomal ACE was purified from the cortex of fresh postmortem human kidneys, and incubated with ^3H -quinalaprilat with and without the various test inhibitors (lisinopril, captopril, the monoesters trandolapril and enalapril, and the

diacids trandolaprilat and enalaprilat). Affinity was determined by scintillation counting (molar concentration of inhibitor capable of reducing by 50% radioactive inhibitor binding to ACE).

Results showed that the diacid trandolaprilat had the highest binding affinity with an IC_{50} of 3.2×10^{-9} M (Table 5), while its monoester trandolapril had an IC_{50} 4.7X greater ($IC_{50} = 1.5 \times 10^{-8}$ M). The IC_{50} of enalapril (3.4×10^{-8} M) was 1470X higher than that of its diacid enalaprilat (5.0×10^{-5} M) indicating a relative weaker binding of the monoester form to its diacid when compared to that of trandolapril and its diacid trandolaprilat. When comparing the diacids, trandolaprilat had about 11X higher binding affinity than enalaprilat (IC_{50} of 3.2×10^{-9} M vs 3.4×10^{-8} M for trandolaprilat and enalaprilat, respectively).

Table 5

Inhibition of ^3H -Quinalaprilat Binding to Purified Human Renal ACE by Trandolaprilat and Trandolapril As Well As Various ACE Inhibitors ⁷

<u>Inhibitor</u>	<u>IC_{50}</u>
Trandolaprilat	3.2×10^{-9} M
Trandolapril	1.5×10^{-8} M
Lisinopril	4.6×10^{-9} M
Captopril	2.6×10^{-8} M
Enalaprilat	3.4×10^{-8} M
Enalapril	5.0×10^{-5} M

I.A.2.e. Determination of the inhibition constant of trandolapril and trandolaprilat in tissue ACE preparations. Comparative study with other converting enzyme inhibitors (Vol. 1.8 pp. 217-219 and Vol. 1.14 pp. 59-135):

This study determined the inhibition constants (IC_{50}) of trandolapril, trandolaprilat, and other ACE inhibitors (enalapril, enalaprilat, peridopril, peridoprilat, ramipril, and ramiprilat) by measuring their ability to displace 50% of the binding of a radioactive ligand (^3H -trandolaprilat) to extracts of various rat tissues (lung, heart ventricle, kidney cortex, brain, and adrenals).

Preliminary experiments with radiolabeled ligand and specific tissue protein concentrations determined the optimal time (45 min) and concentration of ligand (5×10^{-10} M) to reach equilibrium. Results using the various ACE inhibitors showed that

ACE had two binding sites for the diacid inhibitors (trandolaprilat, enalaprilat, peridoprilat, and ramiprilat), while the monoacid-monoester forms (trandolapril, enalapril, peridopril, and ramipril) bound to only one of the two. A summary of the relative affinities (IC_{50}) for the various tissues is shown in Table 6. As can be seen, the affinities of the various ACE inhibitors vary from one tissue to another. According to the Sponsor, however, none of the other inhibitors studies had significantly greater binding affinities than trandolaprilat or trandolapril when compared to their respective diacid and monoacid-monoester forms, respectively. Due to the variations in binding affinities between the various inhibitors and tissues studied, it was difficult to rank the inhibitors by overall affinity.

Table 6

Inhibition Constants (IC_{50}) of Several ACE Inhibitors
in Extracts of Different Rat Tissues

Tissue (IC_{50})		ACE Inhibitor								
		Trandol- april	Perind- opril	Enal- april	Ram- ipril	Capt- opril	Trandol- aprilat	Perind- oprilat	Enal- aprilat	Ram- iprilat
Lung	IC_{50} (10^{-7} M)	2.287	0.678	6.785	3.131	0.081	--	--	--	---
	$IC_{50(1)}$ (10^{-11} M)	--	--	--	--	--	1.458	0.199	0.251	0.825
	$IC_{50(2)}$ (10^{-9} M)	--	--	--	--	--	2.029	5.007	8.769	1.616
Heart	IC_{50} (10^{-7} M)	2.137	0.476	3.467	4.093	0.141	--	--	--	--
	$IC_{50(1)}$ (10^{-11} M)	--	--	--	--	--	0.487	2.182	2.677	2.715
	$IC_{50(2)}$ (10^{-9} M)	--	--	--	--	--	0.839	12.698	7.259	4.143
Kidney	IC_{50} (10^{-7} M)	0.198	0.776	0.467	0.253	0.153	--	--	--	--
	$IC_{50(1)}$ (10^{-11} M)	--	--	--	--	--	0.158	1.812	1.367	0.461
	$IC_{50(2)}$ (10^{-9} M)	--	--	--	--	--	0.437	6.558	11.211	3.244
Brain	IC_{50} (10^{-7} M)	1.819	0.707	3.869	1.493	0.137	--	--	--	--
	$IC_{50(1)}$ (10^{-11} M)	--	--	--	--	--	0.755	2.649	0.221	0.825
	$IC_{50(2)}$ (10^{-9} M)	--	--	--	--	--	0.939	3.520	5.626	1.334
Adrenals	IC_{50} (10^{-7} M)	3.771	0.703	4.458	1.875	0.116	--	--	--	--
	$IC_{50(1)}$ (10^{-11} M)	--	--	--	--	--	0.793	0.340	6.069	0.528
	$IC_{50(2)}$ (10^{-9} M)	--	--	--	--	--	1.955	3.869	11.437	2.610

I.A.2.f. Angiotensin converting enzyme inhibition bytrandolapril versus enalapril in human heart (Vol. 1,8 pp. 219-220 and Vol. 1,14 pp. 136-163):

This study compared the ACE activity found in the atria and ventricles of the postmortem human heart with that found in the pig and rat myocardium. In addition, the comparative binding affinities oftrandolaprilat and enalaprilat for ACE in human atrial tissues were determined.

Homogenates of human, pig, and rat myocardial samples were prepared and assayed for ACE using radioassay methods for detection of ACE inhibition.

Results showed that ACE activity in postmortem human heart (0.38 ± 0.05 mU/mg protein) was similar to the rat (0.34 ± 0.15 mU/mg protein), but was less than half of the activity found in pig (0.93 ± 0.14 mU/mg protein). The affinity oftrandolaprilat for ACE in human atrial tissue was about 6.8X higher than that of enalaprilat (1.32×10^{-10} M vs 9.04×10^{-10} M, respectively). Similarly, the IC_{50} oftrandolaprilat ($\sim 2.0 \times 10^{-9}$; Figure 37) was about 6X lower than that of enalaprilat ($\sim 1.2 \times 10^{-8}$ M; Figure 38).

Figure 37

IC₅₀ of Trandolaprilat for ACE from Human Heart

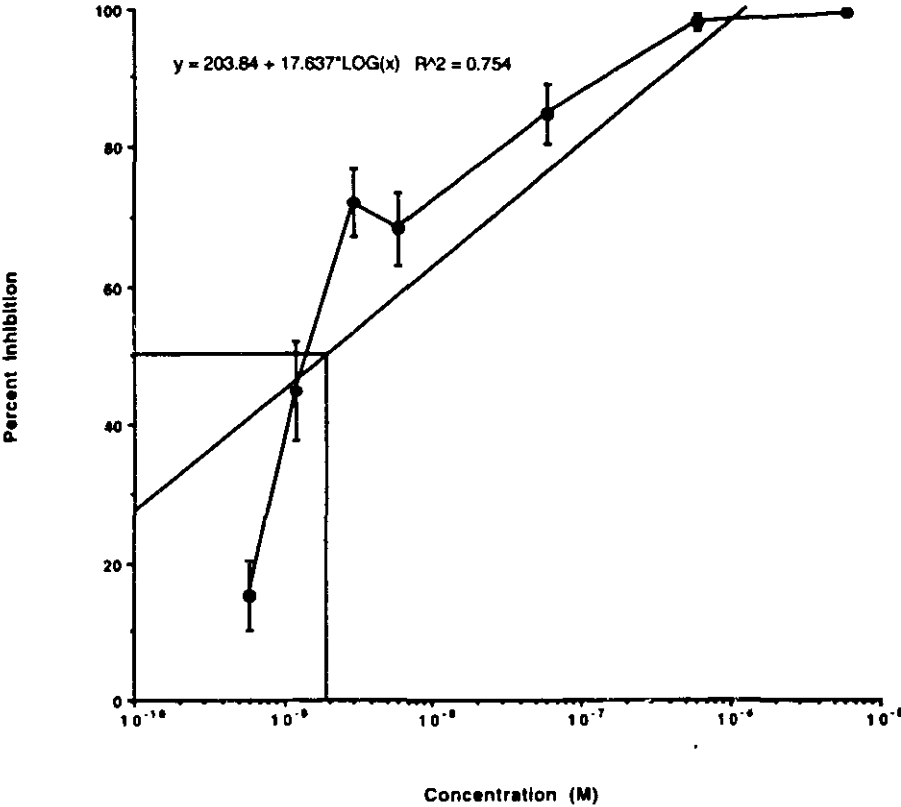
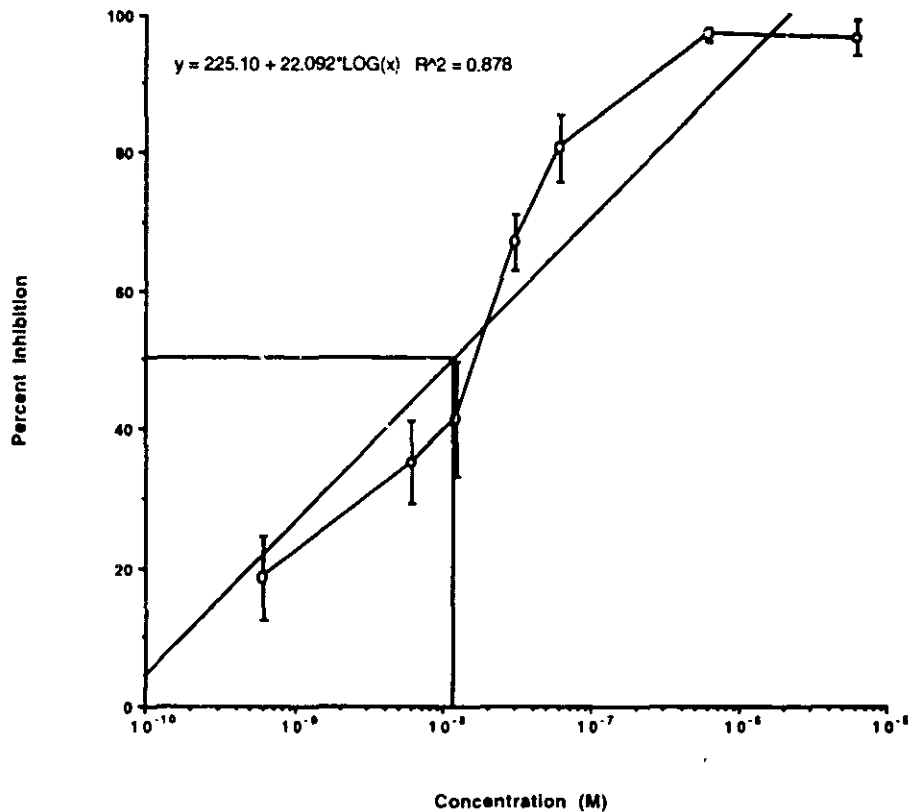


Figure 38

IC₅₀ of Enalaprilat for ACE from Human Heart

I.A.2.g. Study of the *in vitro* activation of _____ (trandolapril) and its diacid _____ to reactive metabolite(s) by measuring the covalent binding to hepatic microsomal proteins of humans and male rats (Vol. 1.8 pp. 220-222 and Vol. 1.14 pp. 164-198):

This study measured the covalent binding of radiolabeled trandolapril and trandolaprilat _____ to hepatic microsomal proteins from control and hepatic enzyme inducer (phenobarbital, dexamethasone, or 3-methylcholanthrene) pretreated male rats, and from human hepatic microsomes to investigate the possible formation of protein-reactive metabolites during biotransformation. In addition, the covalent binding of test agents to hepatic microsomal proteins was studied in the presence or absence of cofactors (unspecified NADPH generating factors necessary for effective biotransformation), piperonyl butoxide (a cytochrome P-450 inhibitor), and

reduced glutathione (GSH) (a free radical scavenger).

Results showed that the presence of cofactors were necessary for effective biotransformation and increased binding of both trandolapril and trandolaprilat (8.9X and 2.2X, respectively) to microsomal proteins from rats but not humans. The binding of trandolaprilat (the diacid) was 3.5-4.6X greater than trandolapril (the mono-ester) to human microsomal proteins, and 3.6X greater to rat proteins only in the absence of cofactors. Both piperonyl (4 mM) and GSH (4 mM) reduced the binding to rat microsomal proteins to that seen in the absence of cofactors. Binding was substantially increased to microsomal proteins from rats pretreated with phenobarbital or dexamethasone (3.4-4.0X and 1.3-1.6X for trandolapril and trandolaprilat, respectively).

These results demonstrated that both the prodrug (trandolapril) and the active compound (trandolaprilat) can effectively bind to rat and human hepatic microsomal proteins. In the absence of positive controls, the relative significance of these findings is difficult to interpret.

1.A.3. General Pharmacology and General Pharmacodynamics

1.A.3. a. General Pharmacology Study (Vol. 1,8 pp. 222-235 and Vol. 1,14 pp. 199-290):

This study examined the effects of trandolapril and trandolaprilat *in vivo*, *in vitro*, and in isolated organ systems in mice, rats, guinea pigs, and rabbits on physiological functions other than the cardiovascular system. The systems studied were: central nervous system, bronchorespiratory system, digestive system, diuresis, blood glucose, analgesic action, local anesthetic activity, platelet aggregation, coagulation, warfarin interaction, and isolated organs.

For the *in vivo* studies, animals were given single oral doses of trandolapril at 1, 3, 10, and 30 mg/kg, or IV doses of 0.1 to 10.0 mg/kg. Animals were examined for up to 48 hours. For the *in vitro* and isolated organ system studies, trandolaprilat was studied as well.

Central Nervous System: Trandolapril at oral doses of up to 30 mg/kg: (1) did not cause any CNS symptomatology (behavioral, neurological, or neurovegetative effects) to appear in rats; (2) did not modify pupillary diameter in mice; (3) did not change body temperature in mice; (4) did not have a motor incapacitating effect in mice (traction test); (5) prevented at doses ≥ 10 mg/kg the slight decrease (-1.30 C) in body temperature caused by reserpine in mice; (6) did not inhibit the cholinomimetic effects of oxotremorine in mice; (7) did not modify the characteristic head twitches and tremors associated with administration of the serotonin agonist 5-hydroxytryptophane (5-HTP) in mice; (8) had no effect on the stereotypic head movements associated with the post-synaptic dopaminergic receptor apomorphine in rats; (9) did not modify the sleeping time of hexobarbital and barbital in mice; (10) had no effect on the convulsant action of pentetrazole in mice; and (11) had no effect on the convulsant action of electroshock (ear electrodes) in mice.

Bronchorespiratory System: Trandolapril at doses of up to 10 mg/kg IV showed no antagonistic effect on acetylcholine- or histamine-induced bronchospasm in the anesthetized guinea pig.

Digestive System: Trandolapril was given orally to rats at doses from 1 to 30 mg/kg. Results showed that trandolapril: (1) did not effect the volume of gastric fluid or its acidity in pylorus-ligated rats when given at doses from 1-10 mg/kg, but a significant increase in gastric fluid volume and acidity occurred at the 30 mg/kg dose; (2) at doses of ≥ 1 mg/kg delayed gastric emptying after 3 hours as shown by a doubling in the number of amberlite beads remaining in the stomach; and (3) caused a small (13%) but significant decrease in the intestinal transit of animal charcoal in rats only at the 30 mg/kg dose.

Diuresis: Trandolapril was given orally to rats at doses from 1 to 30 mg/kg. Urine was collected 24 hours before and after drug administration and the following parameters measured: total volume, pH, sodium, potassium, chloride, calcium, magnesium, phosphate, creatinine, and urea. Results showed that a 35% increase in urinary volume occurred between 0-5 hours after a dose of 30 mg/kg. Urinary volume returned to control levels between 5-24 hours. No other parameters were altered during the time period studied.

Blood Glucose: Trandolapril did not alter blood glucose levels in fasting mice when given orally at doses of up to 30 mg/kg. The effect of trandolapril on blood glucose in fed mice was not determined.

Test for Analgesic Activity: Trandolapril when given orally to mice at doses of up to 30 mg/kg: (1) did not reduce the amount of writhing movements over 15 minutes caused by the injection of acetic acid (100 mg/kg); and (2) did not increase the reaction time to pain (licking of forepaws) when the mice were placed on a hot plate at 56° C.

Test for Local Anesthetic Activity: Trandolapril (30 μ l of a 5% solution) when instilled into the lacrimal sac of one eye in the guinea pig did not suppress the corneal reflex following stimulation with a hair for up to 60 minutes after drug administration. The positive control (a 1% lidocaine solution) caused a 100% disappearance of the corneal reflex.

Platelet Aggregation: Neither trandolapril nor the diacid trandolaprilat at 1×10^{-4} M interfered with the aggregation of rabbit plasma platelets induced by adenosine diphosphate (10 μ M), collagen (40 μ g/ml), sodium arachidonate (0.2 mM), or platelet activating factor (PAF; 0.05 mM).

Coagulation (Intrinsic Pathway): Trandolapril when given orally to rats at doses of up to 30 mg/kg did not alter the prothrombin time ratio (Quick's time in seconds) in blood taken from rats by cardiac puncture.

Warfarin Interaction: Rats were given 2 IP injections of warfarin (an anticoagulant) at 0.5 mg/kg 24 hours apart. Six hours after the last injection, rats were given trandolapril orally at doses up to 30 mg/kg. Blood samples were taken 18 hours after trandolapril administration for determination of prothrombin coagulation time ratios. Results showed that trandolapril did not affect the prothrombin time ratio. Also serum albumin-bound warfarin was not displaced by trandolapril (data not presented).

Isolated Organs: (1) Neither trandolapril nor trandolaprilat at concentrations from 1×10^{-6} M to 1×10^{-4} M antagonized the spasmogenic action (smooth muscle contraction) of guinea pig ileum *in vitro* to histamine (5-10 ng/ml), serotonin (40 ng/ml), or acetylcholine (10 ng/ml). When tested against the effect of angiotensin I on guinea pig ileum smooth muscle, it was shown that trandolaprilat was about 100X more potent than trandolapril in inhibiting angiotensin I-induced contraction (IC_{50} of 1.6×10^{-8} M vs 2.2×10^{-6} M, respectively). Neither trandolapril (1×10^{-5} M) nor trandolaprilat (1×10^{-7} M) showed any inhibitory effect on angiotensin II, indicating its mechanism of action as an inhibitor of ACE-mediated conversion of Ang I to Ang II. (2) Neither trandolapril nor trandolaprilat at concentrations from 1×10^{-6} M to 1×10^{-4} M interfered with the contracting action of adrenaline (concentration was given) on guinea pig seminal vesicles. (3) Neither trandolapril nor trandolaprilat at concentrations from 1×10^{-6} M to 1×10^{-4} M showed any effect on the contractile force or contraction rate of isolated guinea pig atria.

I.A.3. b. General Pharmacology (Vol. 1,8 pp. 235-245 and Vol. 1,14 pp. 291-331):

This study repeated some of the studies examined above (1.1.3.1.) with additional studies not conducted previously. In this study the effects of trandolapril were examined *in vivo*, *in vitro*, and in isolated organ systems in mice, rats, guinea pigs, rabbits and cats on physiological functions other than the cardiovascular system. The systems studied were: central nervous system, bronchorespiratory system, digestive system, diuresis, blood glucose, analgesic action, local anesthetic activity, platelet aggregation, coagulation, warfarin interaction, isolated organs, and nictitating membranes.

For the *in vivo* studies, animals were given single oral doses of trandolapril up to 30 mg/kg (up to 100 mg/kg for the effects on pupillary diameter), or IV doses of up to 10.0 mg/kg. Animals were examined for up to 48 hours.

Results of the effects of trandolapril are summarized as follows: (1) no effects of trandolapril were found when given orally on central nervous system [(a) no effects on pupillary diameter in mice at doses up to 100 mg/kg; (b) no change in rectal temperature in mice at doses up to 100 mg/kg in mice; (c) no effects on psychomotor incapacity in mice at doses up to 30 mg/kg; (d) no effects on barbiturate-induced sleeping time in mice at doses up to 30 mg/kg; (e) no effects on reserpine-induced ptosis at doses up to 30 mg/kg; (f) no effects on oxotremorine-induced decreases in rectal temperatures in mice at doses up to 30 mg/kg; (g) no change in the number of head twitches and tremors induced by 5-HTP in mice at doses up to 30 mg/kg; and (h) no effect on the anticonvulsant activity of electroshock or pentylenetetrazol administration]; (2) no effects when given at doses up to 10 mg/kg IV to guinea pigs on bronchospasm induced by acetylcholine or histamine; (3) no effects on gastric secretion or intestinal transit in rats when given orally at doses up to 10 mg/kg, but changes were found consisting of an increase in gastric secretion at the 30 mg/kg dose, delayed gastric transit at all doses tested (1 to 30 mg/kg), and decreased intestinal transit at 30 mg/kg; (4) decreased urine volume in rats at doses from 1 to 30

mg/kg; (5) no effect on blood glucose levels in rats at doses up to 30 mg/kg; (6) no effects on analgesic action (acetic acid-induced writhing and the hot-plate test) in mice at doses up to 30 mg/kg; (7) no effects on corneal reflex in guinea pigs when given 50 μ l of a 5% solution; (8) no effects on rabbit platelet aggregation at concentrations up to 1×10^{-3} M; (9) no effects on coagulation (prothrombin time) in rats at doses up to 30 mg/kg; (10) no displacement of albumin-bound warfarin in rats at doses up to 30 mg/kg; (11) no effect on the spasmogenic effect of adrenaline on guinea pig seminal vesicles at concentrations up to 1×10^{-4} M; and (12) no effects on the preganglionic sympathetic-induced stimulation or adrenaline-induced contractions of the cat nictitating membrane at doses up to 10 mg/kg.

I.A.3. c. Activity oftrandolapril on carrageenin- and dextran-induced paw edema in the rat (Vol. 1.8 pp. 245-247 and Vol. 1.14 pp. 332-352):

This study was a repeat of study I.A.2.a. to examine the effect oftrandolapril and enalapril on potentiating the effects of bradykinin *in vivo*.

Rats were giventrandolapril or enalapril at 0, 0.03, 0.3, and 30.0 mg/kg orally 30 min before the induction of edema in rats by subplantar (into the sole) injections of carrageenin, a known inducer of bradykinin release *in vivo*. Measurements of paw volume were taken at baseline, and at 45 min, 90 min, and 3 hours after induction of edema. ACE inhibitory activity of the test compounds would result in increased edema due to the inhibition of bradykinin catabolism by ACE. As a bradykinin-specificity control, another group of rats were given test drugs then injected with dextran, which causes edema independent of the release of bradykinin.

Results showed that carrageenin caused a 14% to 17% increase in edema (paw volume) at 3 hours. Pretreatment withtrandolapril (0.03 mg/kg) or enalapril (0.3 mg/kg) caused a 65% and 59% increase in edema when compared to control animals. The effect did not increase with increasing dosages. Neither drug had any affect on the edema induced by dextran indicating that the potentiating effect seen in the carrageenin-treated animals was due to drug-induced inhibition of the bradykinin catabolism by ACE. Similar results were reported in the earlier study (1.1.2.1.).

I.A.3. d. The effect oftrandolapril and enalapril on citric acid-induced cough in guinea pigs (Vol. 1.8 pp. 247-249 and Vol. 1.15 pp. 1-14):

This study examined the effect oftrandolapril and enalapril on the cough induced in antigen-sensitized guinea pigs after exposure to a mist of citric acid.

Guinea pigs, ovalbumin mist-sensitized or unsensitized, were exposed to a mist of citric acid before or after treatment withtrandolapril or enalapril at 1 mg/kg. The effect of drug treatment on the number of ACE-induced coughs were recorded.

Results showed that neither drug had any significant effect on the number of coughs recorded.

I.A.3. e. General pharmacology - trandolaprilat (Vol. 1.8 pp. 249-252 and Vol. 1.15 pp. 15-47):

This study examined the effects of trandolaprilat (the diacid) in several species (rats, guinea pigs, and rabbits) on the following physiologic systems: respiratory and cardiovascular systems, autonomic nervous system, digestive system, blood system, peripheral nervous system, and local action.

The effects of trandolaprilat are summarized as follows: (1) *Respiratory and cardiovascular system*: In rabbits there was a 11.6% decrease in systolic blood pressure at 0.3 mg/kg IV, a 25.6% decrease in diastolic blood pressure at 3 mg/kg IV, and a 28.2% decrease in carotid blood flow at 0.3 mg/kg IV. None of these decreases were dose-dependent. No significant changes were observed in heart rates, respiratory rates, or on ECGs. (2) *Autonomic nervous system and digestive system*: No effect on contractile force and beat rate on guinea pig isolated atrium when tested at concentrations of up to 1×10^{-4} M (acetylcholine and epinephrine were used as positive controls). (3) No effect when tested at concentrations of up to 1×10^{-4} M on the contractile response of guinea pig ileum to the spasmogens acetylcholine (1×10^{-7} M), histamine (3×10^{-7} M), serotonin (1×10^{-5} M), or BaCl_2 (1×10^{-3} M). (4) Enhanced and prolonged pilocarpine-induced stimulation of salivation in rats when given IV at doses ≥ 0.3 mg/kg. (5) Gastric lesions (stereoscopic microscope examination) in 2 out of 10 rats given oral doses of 30 mg/kg. (6) *Blood system*: No hemolytic activity (supernatant absorbance) on rabbit erythrocytes when tested at concentration of up to 1×10^{-4} M (saponin was used as the positive control). (7) *Peripheral nervous system*: No effect on the rat diaphragm contraction elicited by phrenic nerve stimulation (physostigmine was used as the positive control). (8) *Local action*: No effect on rabbit corneal reflexes (horse hair stimulation), and no eye irritant activity after instillation of 0.1 ml of 1% and 5% solutions into the conjunctival sacs.

I.A.3. f. General pharmacology - trandolapril (Vol. 1.8 pp. 253-255 and Vol. 1.15 pp. 48-77):

This study examined the effects of trandolapril in several species (mice, rats, and rabbits) on the following physiologic systems: respiratory and cardiovascular systems, digestive system, central nervous system, peripheral nervous system, and blood system.

The effects of trandolapril are summarized as follows: (1) *Respiratory and cardiovascular system*: Decreased systolic (Figure 39) and diastolic (Figure 40) blood pressure when given to rabbits IV (the effects of trandolapril at 0.3 mg/kg were comparable to enalapril at 3.0 mg/kg IV); decreased carotid arterial blood flow (maximum effect of -16.5% with 3 mg/kg IV at 180 min) with similar effects at all doses tested (0.3 to 10 mg/kg IV); minimal effects on respiration, heart rate, or ECG when tested in rabbits at concentrations of up to 10 mg/kg IV.

Figure 39

Effect of Trandolapril on Systolic Blood Pressure in Rabbits

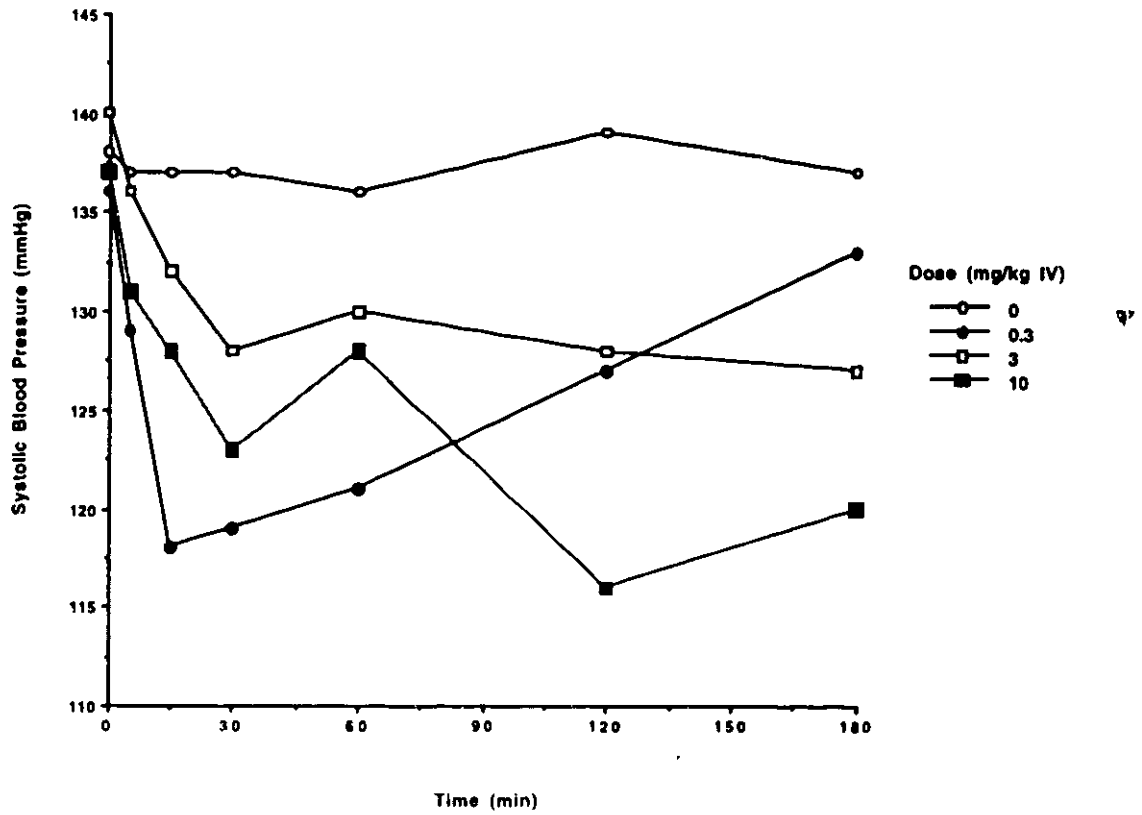
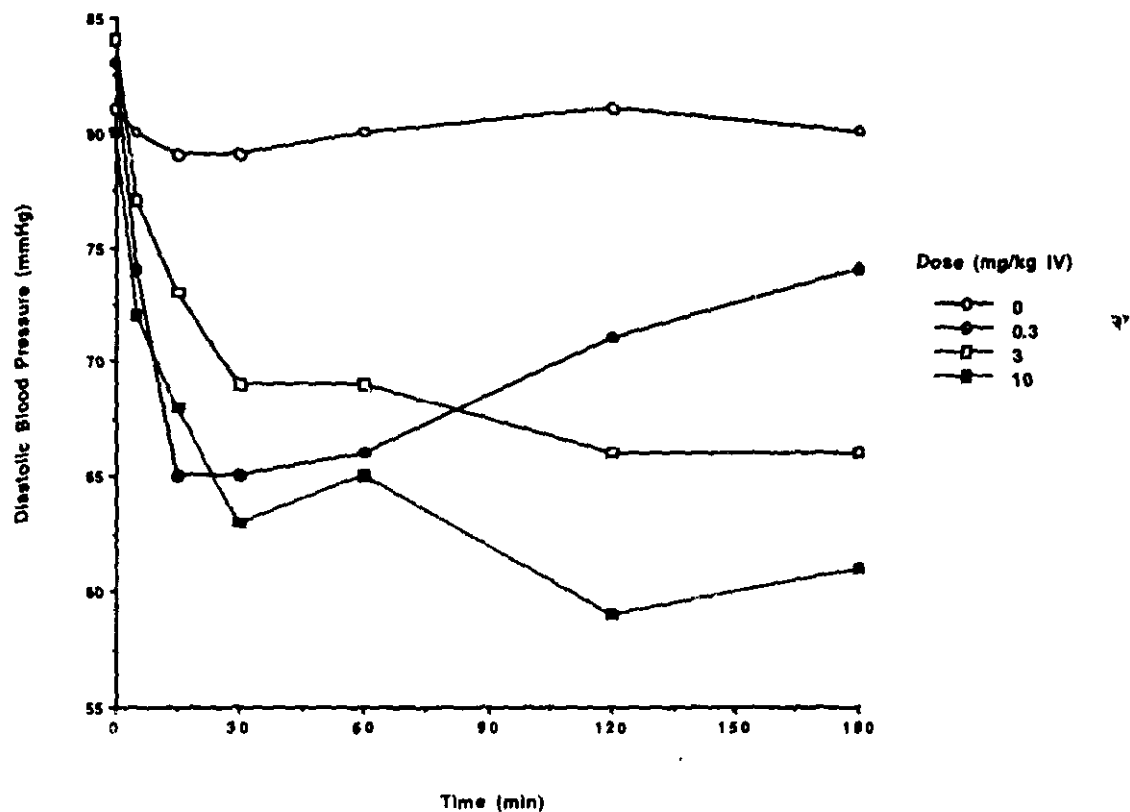


Figure 40

Effect of Trandolapril on Diastolic Blood Pressure in Rabbits



(2) *Digestive System*: Enhanced and prolonged pilocarpine-induced salivary secretion in rats at doses of ≥ 0.3 mg/kg IV (but not at 0.03 mg/kg IV); the effect of enalapril at 3.0 mg/kg IV on salivary secretion was comparable to trandolapril at 0.3 mg/kg IV; increased injury to gastric mucosa (lesions observed with a stereoscopic microscope) in 3/10 rats at an oral dose of 30 mg/kg (a similar effect occurred with enalapril in 2/10 rats at 10 mg/kg). (3) *Central nervous system*: no effects on spontaneous motor activity (number of turns in a cage wheel for 200 min) in mice when given orally at doses of up to 30 mg/kg. (4) *Peripheral nervous system*: no effect on rat diaphragm contraction by phrenic nerve stimulation (physostigmine was the positive control) when tested at concentrations of up to 1×10^{-4} M. (5) *Blood system*: no hemolytic effects (absorbance of supernatant) on rabbit erythrocytes when tested at concentrations of up to 1×10^{-4} M (saponin was the positive control).

I.B. SECONDARY EFFECTS

I.B.1. Cardiovascular

I.B.1.a. Rat

I.B.1.a.(1). Effects of (trandolapril) on general and regional hemodynamics in the genetically hypertensive rat (Vol. 1.8 pp. 256-258 and Vol. 1.15 pp. 78-121):

This study examined the vasodilator properties of trandolapril on different vascular regions in the spontaneously hypertensive (SH) rat by measuring changes in blood pressure, heart rate, regional vascular resistance, and regional blood flow.

Adult SH rats were given trandolapril by gavage at 5 mg/kg/day for 8 days. This dose (5 mg/kg) was chosen because it reduced blood pressure in SH rats to a similar extent as that observed with captopril (100 mg/kg) and enalapril (25 mg/kg). On the last day of treatment, rats were injected in the left ventricle with radioactive microspheres for dissemination into peripheral tissues. Rats were then sacrificed 4 hours later, and various tissues (kidneys, spleen, liver, skin, muscle, and brain) were removed and measured for radioactivity.

Results on general hemodynamics (Figure 41) showed that trandolapril (5 mg/kg/day for 8 days) given by gavage to SH rats decreased mean blood pressure by -30.5% with a minimal effect on heart rate (-5.8%). This decreased blood pressure was accompanied by a 37.2% decrease in total peripheral vascular resistance. There was also a decrease in cardiac hypertrophy (heart to body weight ratio of -8.0%) which may have resulted from the reduction in afterload in the heart. Examination of regional hemodynamics (Figure 42) showed decreased regional vascular resistance and increased regional blood flows in all tissues examined. The decreased vascular resistance in muscle and skin, as well as the increased blood flow in the liver and muscle were not statistically significant.

It was concluded that the fall in blood pressure after trandolapril administration was due to a decrease in peripheral vascular resistance (which was accompanied by increased regional blood flow), since there was minimal change in the cardiac index, heart rate, or stroke volume.

Figure 41

Effects of Trandolapril (5 mg/kg/day for 8 Days) on General Hemodynamics in the Spontaneously Hypertensive (SH) Rat

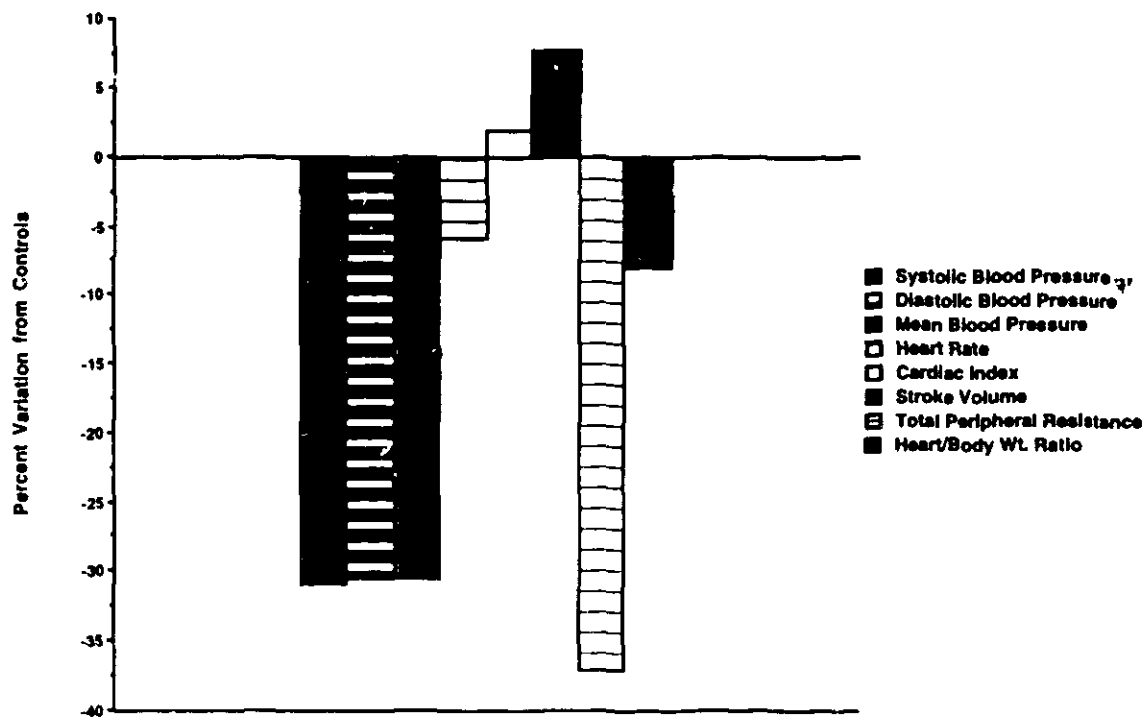
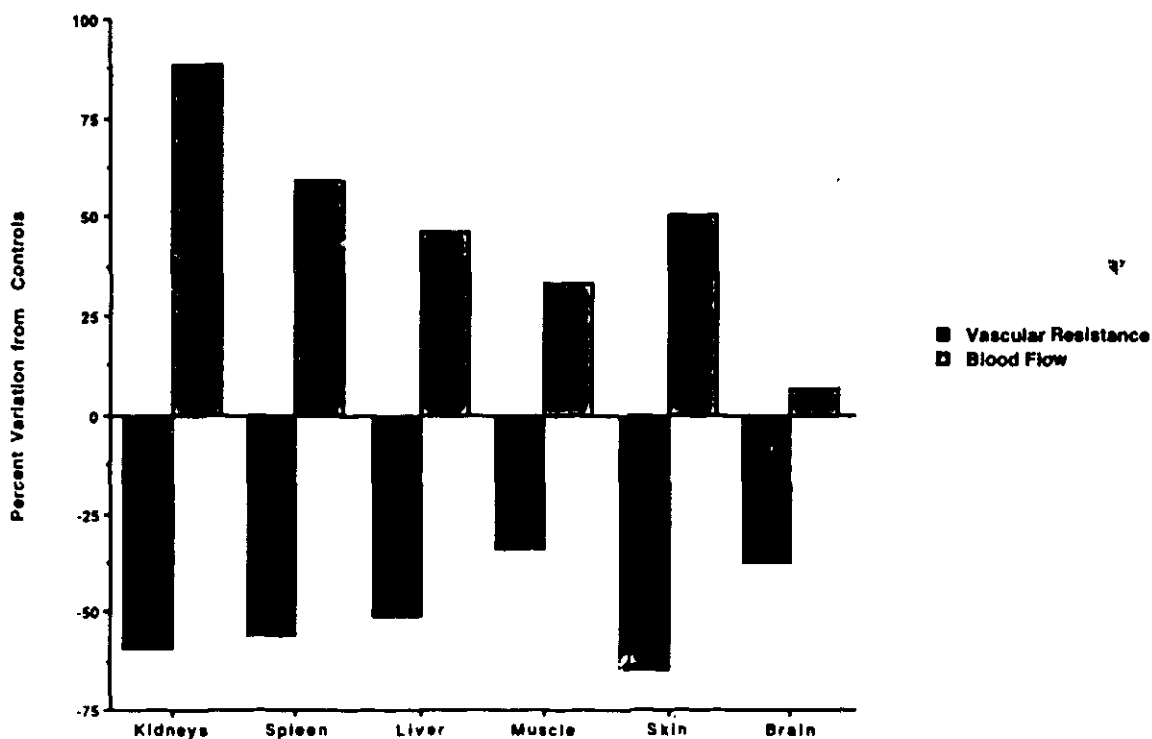


Figure 42

Effects of Trandolapril (5 mg/kg/day for 8 Days) on Regional Hemodynamics in the Spontaneously Hypertensive (SH) Rat



I.B.1.a.(2). Comparative study of the effects of (trandolapril) and enalapril after administration for 14 days on blood pressure, heart rate, and cardiac hypertrophy in the spontaneously hypertensive rat (Vol. 1,8 pp. 258-261 and Vol. 1,15 pp. 122-147):

This study examined the antihypertensive activities of trandolapril and enalapril and correlated the observations with effects of cardiac hypertrophy in the SH rat.

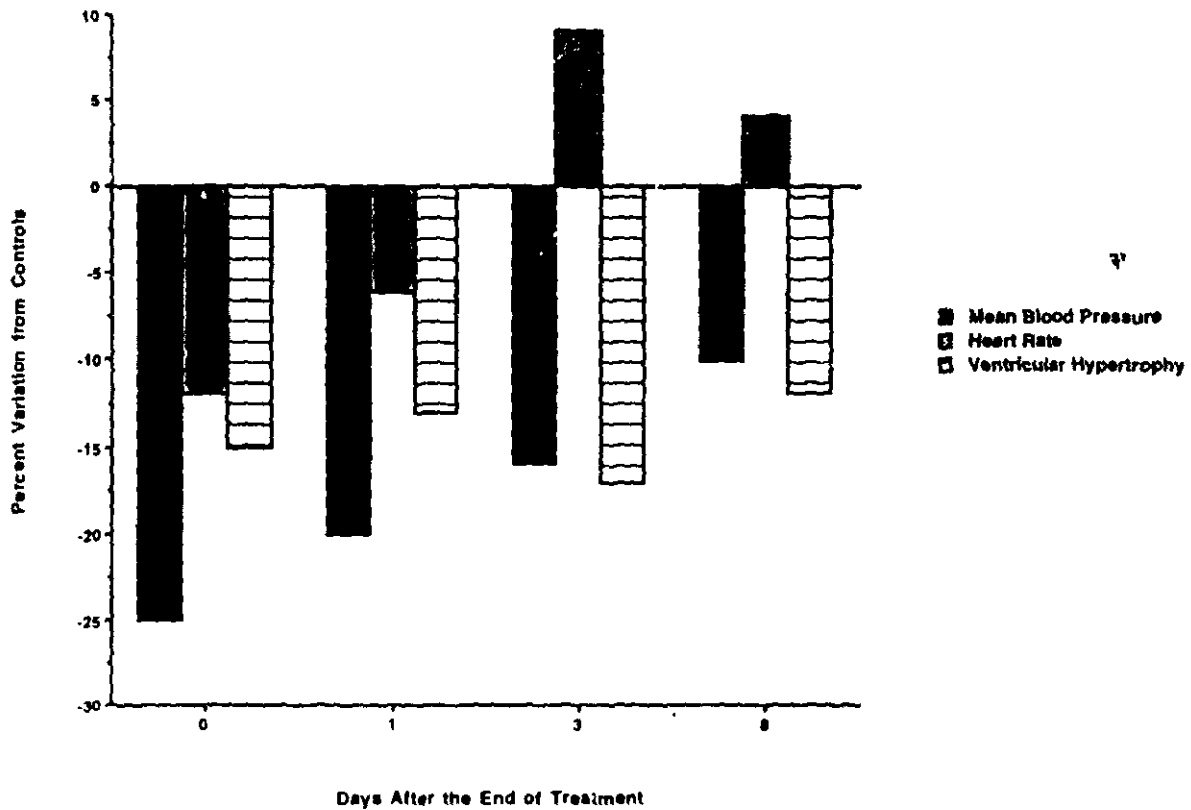
SH rats were given trandolapril (3 mg/kg/day) or enalapril (10 mg/kg/day) in their drinking water for 14 days. Blood pressures and heart rates were recorded on the last day of treatment, or on 1, 3, or 8 days after the end of treatment. In addition, rats were sacrificed for measurement of heart to body weight ratios.

Results (Figure 43) showed that trandolapril reduced systolic, diastolic, and mean blood pressures by 25% on the last day of treatment. The blood pressure was still decreased 10% 8 days later. Heart rates also decreased by 12% on the last day of treatment. However, heart rates increased to 9% above control values on day 3, but returned to near control levels by day 8 (+4%). Ventricular heart to body weight ratios were 15% less in trandolapril-treated animals than controls on the last day of treatment. This decrease was still significant (-12%) 8 days after the end of treatment.

Enalapril also decreased mean blood pressure (-10%) on the last day of treatment, but this effect returned to near normal levels (-3%) by 3 days after the end of treatment. Decreased heart rates (-12%) were observed 3 days after the end of treatment, but were near control values at the other time points examined. There was no effect on ventricular hypertrophy on the last day of treatment, but a 13% reduction was observed 3 days after the end of treatment.

Figure 43

Effect of Trandolapril (3 mg/kg/day for 14 days) on Mean Blood Pressure, Heart Rate, and Ventricular Hypertrophy in the Spontaneously Hypertensive (SH) Rat



It was concluded that trandolapril, when given at 3 mg/kg/day for 14 days, showed antihypertensive activity in the SH rat which was greater and more prolonged than enalapril at 10 mg/kg/day for 14 days (recall that previous comparative studies generally showed that a 10-fold higher dose of enalapril was required to reduce blood pressure to a similar extent as that for trandolapril). Also trandolapril induced a significant decrease (-15%) in ventricular hypertrophy that was sustained for up to 8 days after the end of treatment. The reason for the delayed effect of enalapril on ventricular hypertrophy (-13% on day 3) was not clear.

I.B.1.a.(3). Cardiovascular effects of chronic treatment with (trandolapril) and enalapril in the aged spontaneously hypertensive rat with heart failure - Pharmacological and histological study (Vol. 1,8 pp. 261-265 and Vol. 1,15 pp. 148-245):

This study examined the cardiovascular effects of 3 months of treatment with trandolapril or enalapril in spontaneously hypertensive (SH) rats.

SH rats (21 months old at the beginning of the study) were given trandolapril (0.3 mg/kg/day) or enalapril (10 mg/kg/day) in their drinking water for 3 months. Controls (SH and normotensive rats) received water only. A week before the end of treatment, a 24 hour urine sample was collected and assayed for creatinine, urea, chloride, sodium, potassium, and cGMP. On the last day of treatment, blood pressure and heart rates were measured. The rats were then sacrificed, and the blood collected for analysis of transaminases, bilirubin, creatinine, alkaline phosphatase, total and HDL cholesterol, triglycerides, serum ACE, and atrial natriuretic factor (ANF). Samples of the kidneys and hearts (for assay of ventricular isomyosins) were frozen. Segments of aorta and mesenteric artery were fixed for histology. A section of aorta was used to measure release of PGI₂.

Results showed that survival in the SH rats treated with either trandolapril (78%) or enalapril (82%) was better than untreated SH rats (44%) for the 3 months of the study. Terminal animals showed signs of heart failure including dyspnea and edema. Trandolapril decreased systolic and diastolic blood pressure -12% and -8%, respectively, while enalapril showed decreases of -18% and -20%, respectively. Heart rates were minimally affected. Serum ACE activity was decreased 63% by trandolapril and 33% by enalapril. Plasma ANF levels (which were about 10X higher in SH rats than in normotensive rats) was decreased 62% by trandolapril and 80% by enalapril. Urinary cGMP levels (which were about 3X higher in SH rats than in normotensive rats) were decreased 36% by trandolapril and 50% by enalapril. Trandolapril and enalapril reduced urine volume (45% and 37%, respectively), natriuresis (64% and 55%, respectively), and kaliuresis (27% and 26%, respectively) to values similar to those found in normotensive rats. Both trandolapril and enalapril reduced ventricular hypertrophy (-24% and -26%, respectively) and renal hypertrophy (-12% and -6%, respectively). Both drugs improved the mechanical properties (force developed as a function of baseline tension) of papillary muscle (+23-37% and +31-55% for trandolapril and enalapril, respectively). There was a shift in the isomyosins from V₃ (ββ homodimer with a slower shortening velocity characteristic of hypertrophied myocardium) to V₁ (αα homodimer with a faster shortening velocity) in both drug treated groups indicating decreased cardiac hypertrophy. However, since human myocardium is predominantly V₃ (ββ), the significance of these findings to the clinical situation is not clear. Trandolapril had a greater effect than enalapril on reducing the media thickness of mesenteric artery (9.8% vs 0.1%, respectively). Light and electron photomicrographs of arteries from drug-treated rats showing reduced media thicknesses were of poor quality due to photocopying.

It was concluded that trandolapril at a dose (0.3 mg/kg/day) that produced minimal antihypertensive effects (-10% decrease in mean blood pressure) had a significant effect of reducing cardiac hypertrophy and vessel resistance in aged SH rats. While enalapril showed similar activity (10 mg/kg/day), its effect on reducing media thickness was minimal.

I.B.1.a.(4). Effects of 4 weeks of chronic treatment with _____ on hypertension of renal origin and the associated vascular structural alterations in the rat (Vol. 1.8 pp. 265-268 and Vol. 1.15 pp. 246-280);

This study examined the effects of treatment with trandolapril for 4 weeks on blood pressure, cardiac hypertrophy, and artery histology in rats made hypertensive by renal artery occlusion.

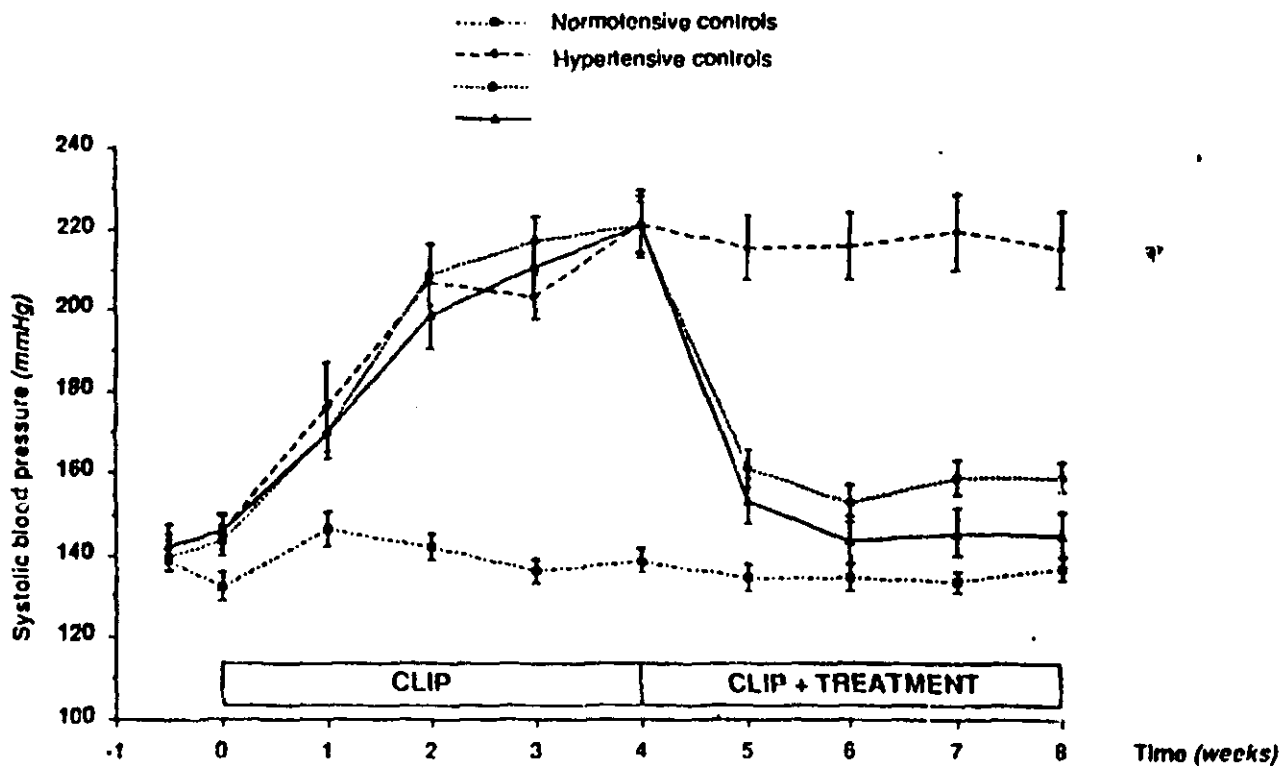
Rats were made hypertensive by clip occlusion of the left renal artery. Control rats received the same operation, but without the clip. Four weeks after occlusion and development of arterial hypertension, rats were given trandolapril at 0.3 and 1.0 mg/kg/day for 4 weeks by gavage. Hypertensive and normotensive controls received vehicle (sodium hydroxide). Systolic blood pressure and heart rates were recorded weekly one hour after gavage. At the end of the treatment period, rats were sacrificed. The hearts were removed, dried and weighed for determination of heart weight to body weight ratios (index of cardiac hypertrophy). Segments of the aorta and mesenteric arteries were removed and processed for histological examination (media thickness and lumen diameter).

Results showed that trandolapril at both doses (0.3 and 1.0 mg/kg/day) reduced systolic blood pressure to near normotensive levels within 1 to 2 weeks after the beginning of treatment (Figure 44; Sponsor's Figure 222). Heart rates were not affected.

Figure 44

Changes in Systolic Blood Pressure During the 8 Weeks Following Partial Occlusion of the Renal Artery of the Conscious Rat

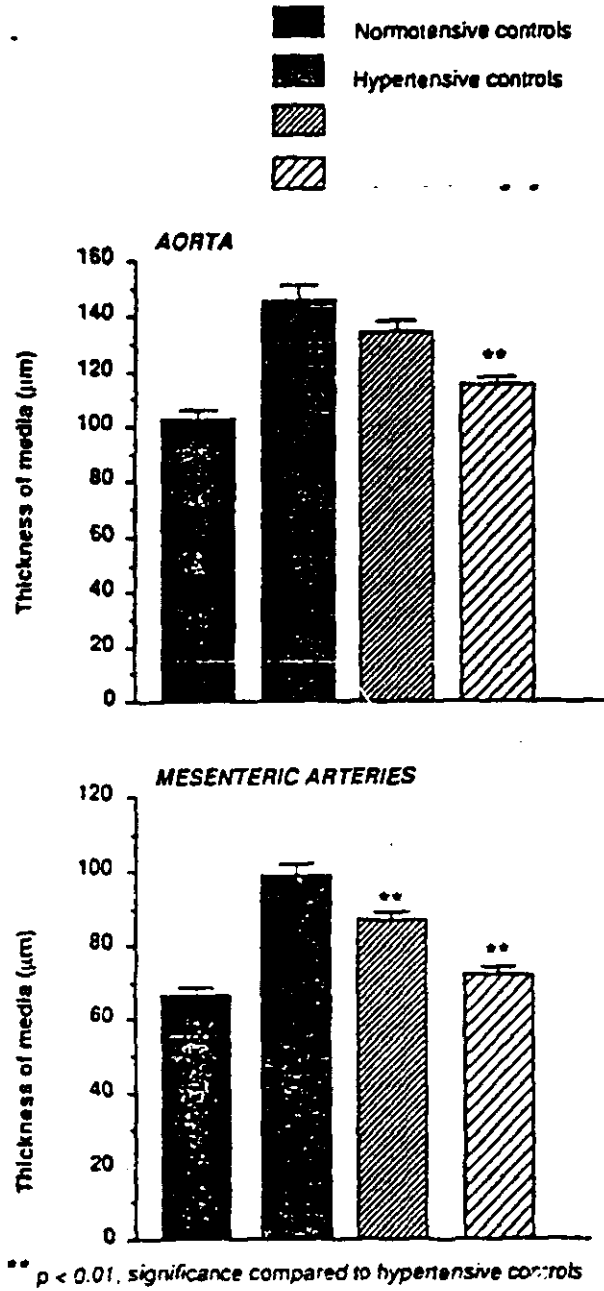
- from 0 to 4 weeks : development of arterial hypertension
- from 4 to 8 weeks: regression of hypertension under the effect of a treatment with



A dose-dependent regression in cardiac hypertrophy was observed with a 17% reduction in the heart weight to body weight ratio at the 0.3 mg/kg/day dose. At the 1.0 mg/kg/day dose, the reduction was 30% with the heart weight being near normal levels. A similar dose-dependent regression in the thickness of the aortic and mesenteric arteries was observed. The 1.0 mg/kg/day dose reduced media thickness in both arteries to near normal levels (Figure 45; Sponsor's Figure 225).

Figure 45

Thickness of Media of Aortae and Mesenteric Arteries in Normotensive Controls, Hypertensive Controls, and Hypertensive Rats Treated with _____ for 4 Weeks



It was concluded that oral administration of trandolapril at 0.3 and 1.0 mg/kg/day for 4 weeks to rats with hypertension of renal origin reduced blood pressure and regressed cardiac hypertrophy, with the 1.0 mg/kg/day dose reducing blood pressure and cardiac hypertrophy to levels found in normotensive rats.

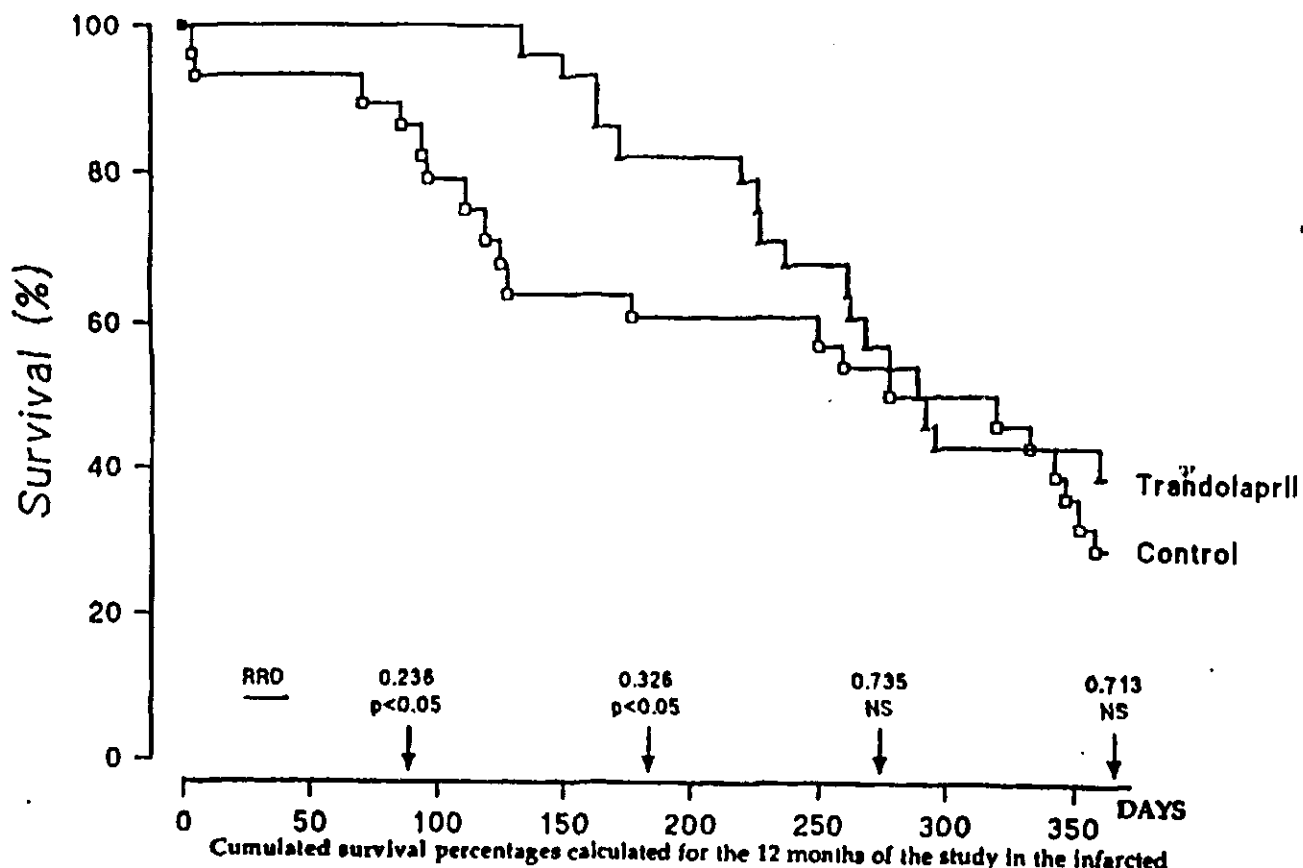
I.B.1.a.(5). Effects of trandolapril on survival in the rat with heart failure of ischemic origin. Analysis of the hemodynamic and morphological factors involved (Vol. 1.8 pp.268-272 and Vol. 1.15 pp. 281-392):

This study examined the long-term effects (12 months) of trandolapril on survival, systemic and regional hemodynamics, and cardiac and vascular morphology in rats made ischemic by coronary artery ligation (model of heart failure).

The left coronary artery of rats was ligated to induce ischemia and ultimately heart failure as shown by electrical signs of myocardial infarction of the ECG. Eight days later, rats were given trandolapril at 0.1 mg/kg/day in their drinking water for 365 days. Control rats (infarcted and sham-operated) received water only. At the end of the treatment period, diuresis, natriuresis, blood pressure, heart rates, cardiac output, and regional blood flows to the kidney, myocardium, spleen, liver, brain, stomach, muscle, and skin were determined (microsphere technique). Histological examination was performed on hearts (collagen staining, nuclear density, fibrosis, infarct size, and ventricular hypertrophy) and aortas (elastin and collagen densities, media thickness, and nuclear density) on any animals that died during the course of the experiment, and on all animals at sacrifice (end of experiment).

Results showed that trandolapril-treated animals had significantly better survival for the first 6 months of treatment when compared to untreated infarcted animals (Figure 46; Sponsor's Figure 228). After 6 months there was no difference.

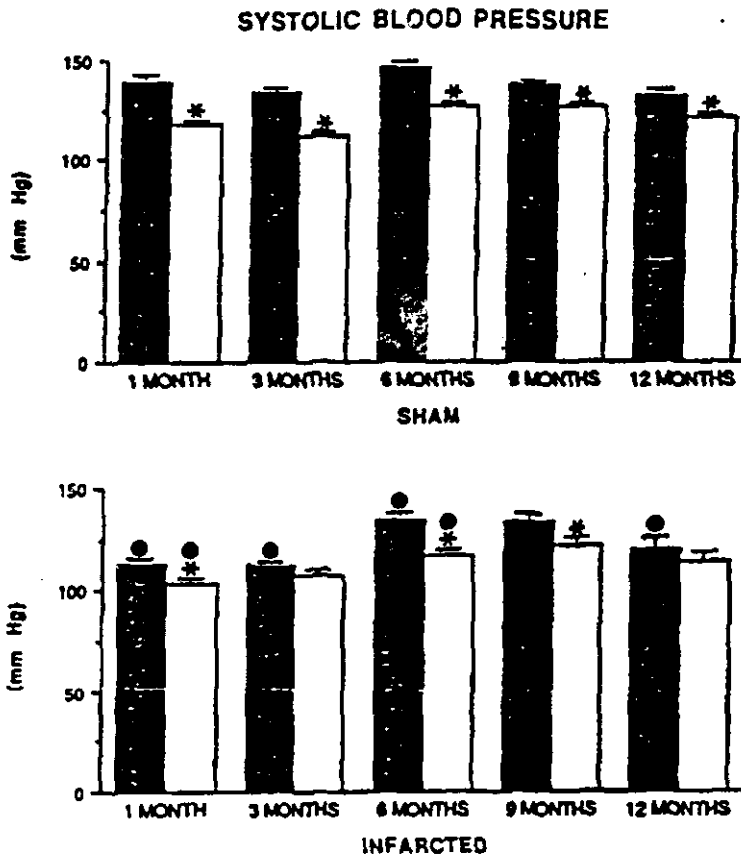
Figure 46



Cumulated survival percentages calculated for the 12 months of the study in the infarcted animals treated with trandolapril (Δ , n = 28) or untreated ($\square$, n = 28)
 RRD : relative risk of death calculated at 3, 6, 9 and 12 months.

The effects of trandolapril on systolic blood pressure were moderate (but significant), and the effects of drug treatment on sham or infarcted rats appeared to be similar (Figure 47; Sponsor's Figure 230). There was no effect on heart rates.

Figure 47



Mean values ($\pm$ sem) of systolic blood pressure determined at 1, 3, 6, 9 and 12 months in all the animals surviving at these dates in infarcted or sham animals treated with trandolapril ($\square$) or not ($\blacksquare$).

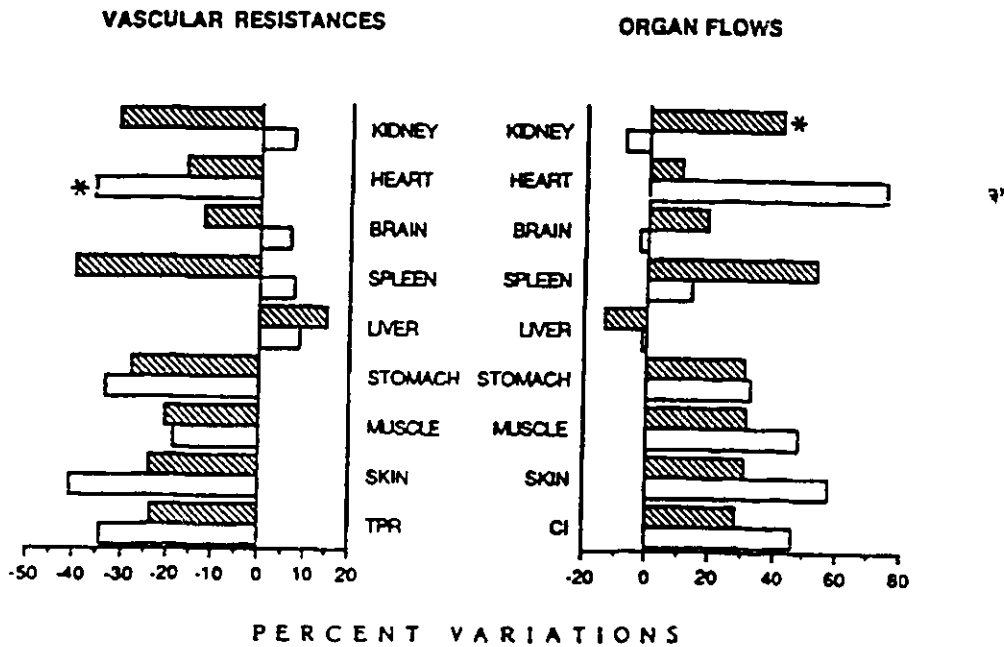
* Value significantly different from the corresponding untreated value : p at least < 0.05 .

• Value significantly different from the corresponding sham value : p at least < 0.05 .

There was an increase in urine output (diuresis) in trandolapril-treated animals. This effect was greater in the sham-operated animals (6.6X increase over controls at 6 months). In infarcted animals, the greatest effect was at 9 months (2.6X over controls). Minor effects were noted on excretion of sodium (natriuresis), urinary creatinine, and urinary cGMP. Infarct size was not reduced in trandolapril-treated animals. In fact,

there was a moderate (54%) increase in infarct size at 9-12 months. However, there were reductions in left ventricular hypertrophy (27% at 6-9 months) and fibrosis (49.8%, 35.2%, and 49.1% reduction in subendocardial collagen, subepicardial collagen, and aortic collagen density, respectively, at 6-9 months). Decreases in vascular resistance were found in most tissues examined. Conversely, regional (organ) blood flows were increased in trandolapril-treated animals (Figure 48: Sponsor's Figure 250).

Figure 48



Percentages of variation of the various local vascular resistances (or systemic TPR) and the corresponding regional flows (or cardiac CI) calculated in sham (■) or infarcted (□) animals between those treated with trandolapril or not

$$\left(\frac{\text{treated} - \text{untreated}}{\text{untreated}} \times 100 \right)$$

* : Significant variation : p at least < 0.05

It was concluded that the increased survival of infarcted rats treated with 0.1 mg/kg/day trandolapril for 12 months could be explained by the decreased cardiac hypertrophy (due mostly from reduced collagen synthesis) and reduced vascular resistances with corresponding increases in regional (organ) blood flows.

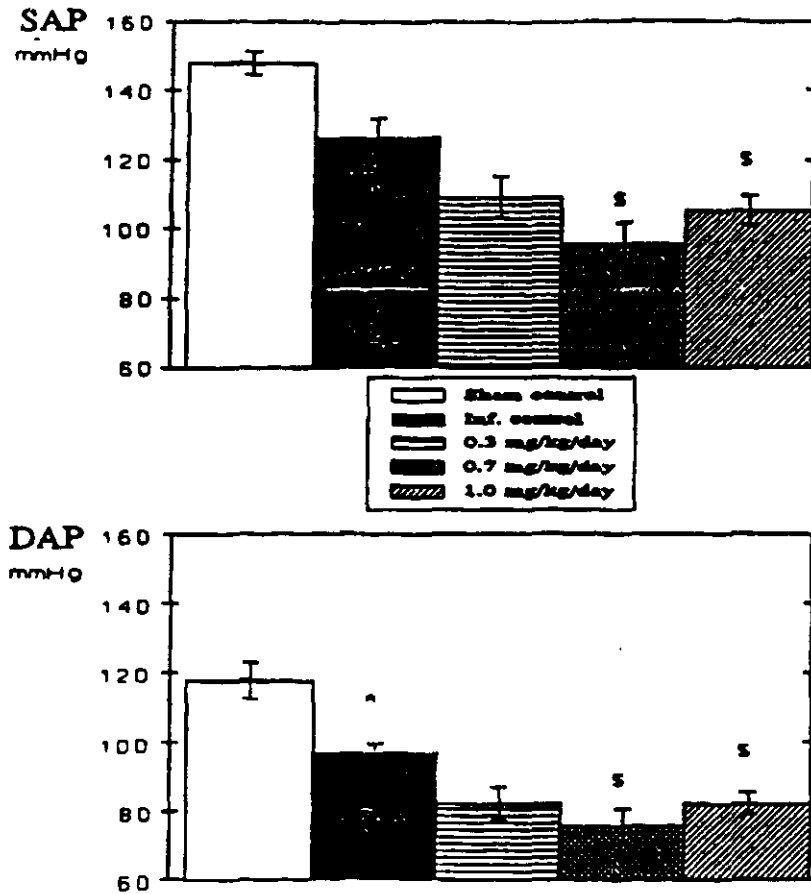
I.B.1.a.(6). Effect of chronic oral trandolapril treatment on hemodynamics and ventricular function in rats with healed myocardial infarction (Vol. 1.8 pp. 272-274 and Vol. 1.15 pp. 393-423):

This study examined the effects of trandolapril given orally for 24 weeks on systemic hemodynamics, ventricular function, and coronary blood flow in rats surviving myocardial infarction due to coronary artery occlusion.

Myocardial infarction was produced in rats by pulling on an occluder loop of a thread placed around the left coronary artery. Two weeks later surviving rats were given trandolapril in their drinking water at 0.3, 0.7, and 1.0 mg/kg/day for 24 weeks. Sham-operated and infarcted animals receiving water only served as controls. At the end of the treatment period, the following parameters were measured: systemic hemodynamics (systolic and diastolic aortic blood pressure, left ventricular systolic pressure, maximal rate of left ventricular pressure development, and left ventricular end diastolic pressure), clinical chemistry on heparinized blood, coronary reserve (Langendorf perfused hearts), cardiac hypertrophy due to congestive heart failure (heart weight/body weight ratio), and infarct size.

Results showed that survival in rats receiving trandolapril at 0.7 and 1.0 mg/kg/day increased from 68% (untreated controls) to 100% and 92%, respectively. In untreated controls, cardiac hypertrophy due to congestive heart failure (heart weight/body weight ratio) increased 46% when compared to sham-operated controls. Trandolapril at 0.3, 0.7, and 1.0 mg/kg/day reduced cardiac hypertrophy to 27%, 15%, and 12% increases over sham-operated controls, respectively. Heart rates were significantly increased (+9%) in only the 1.0 mg/kg/day dose group when compared to untreated infarcted controls where they approached the values seen in the sham-operated controls. Likewise, the PQ-intervals were normalized (decreased 13%) in only the 1.0 mg/kg/day group when compared to untreated controls where they approached the values seen in the sham-operated controls. Systolic and diastolic blood pressures were significantly reduced in the 0.7 and 1.0 mg/kg/day treatment groups when compared to the infarcted controls (Figure 49; Sponsor's Figure 257). Left ventricular end diastolic pressure (LVEDP) in the 0.3 and 1.0 mg/kg/day dose groups was significantly reduced when compared to infarcted controls and approached the values seen in sham-operated controls (Figure 50; Sponsor's Figure 260). Coronary reserve in infarcted controls decreased 33%. Trandolapril treatment at 0.7 and 1.0 mg/kg/day increased coronary reserve to values seen in sham-operated controls (Figure 51; Sponsor's Figure 261). Cardiovascular parameters are summarized in Table 7 (Sponsor's Table 306).

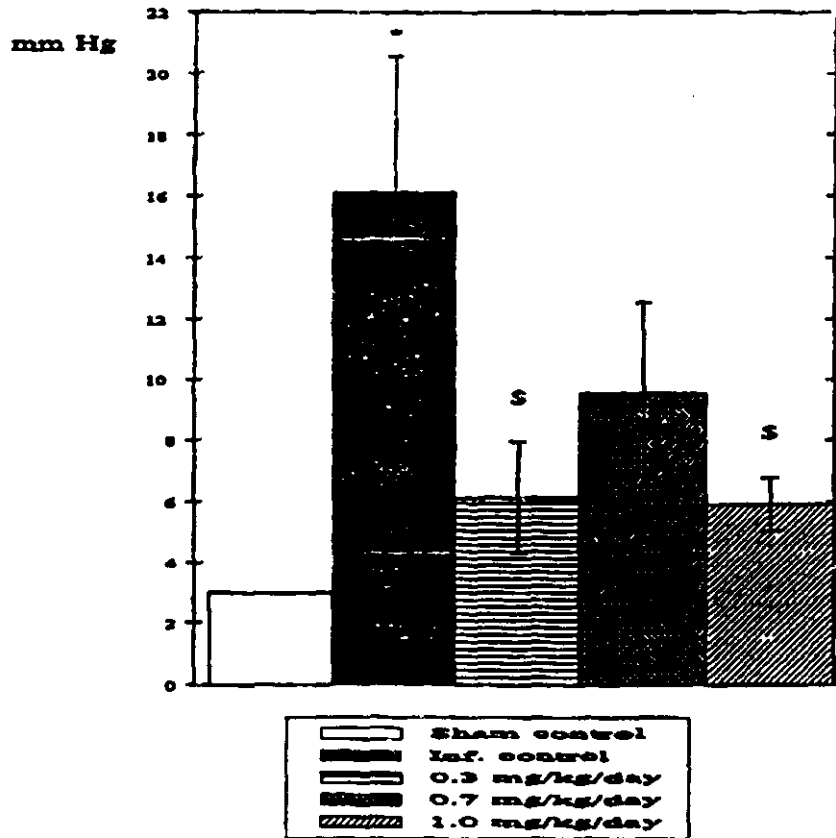
Figure 49



Systolic (SAP) and diastolic (DAP) blood pressure of sham operated and chronically infarcted rats with or without trandolapril treatment over 24 weeks

Mean $\pm$ SEM, * significant to sham operated control
 § significant to infarct control

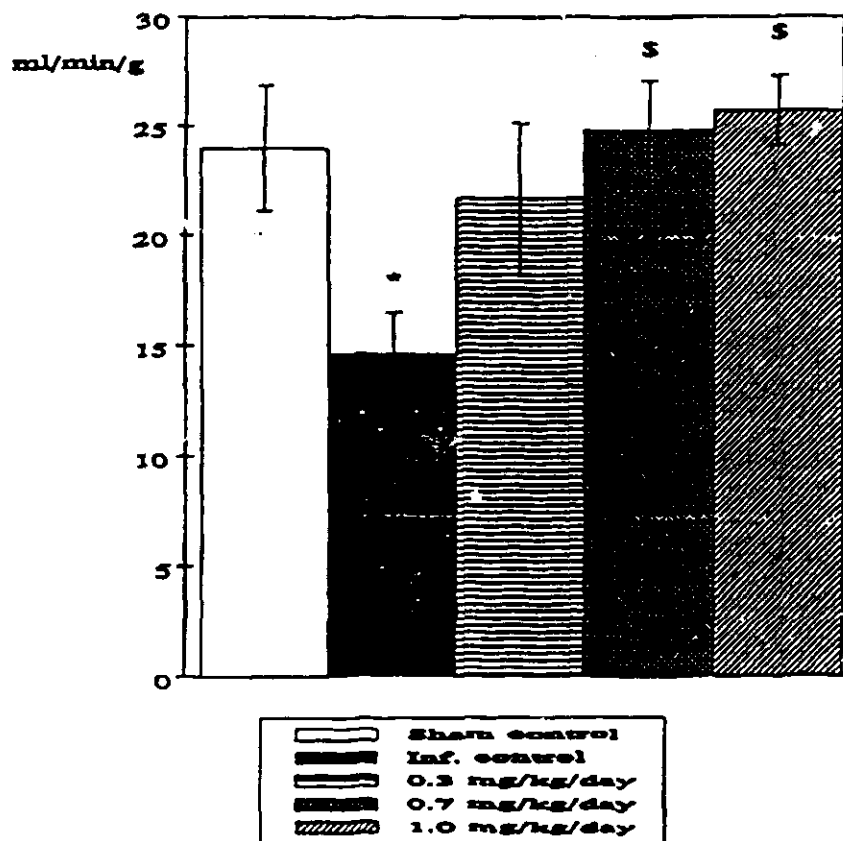
Figure 50



Left ventricular enddiastolic pressure (LVEDP) in sham operated and chronically infarcted rats with or without trandolapril treatment over 24 weeks

Mean $\pm$ SEM, * significant to sham operated control
§ significant to infarct control

Figure 51



Coronary Reserve under 100 mm Hg perfusion pressure in sham operated and chronically infarcted rats with or withouttrandolapril treatment over 24 weeks

Mean $\pm$ SEM, * significant to sham operated control
\$ significant to infarct control

Table 7

Summary of all cardiovascular parameters measured in sham operated and chronically infarcted rats with or without treatment over 24 weeks

Parameter	Sham control	Inf. control	Trandolapril		
			0.3 mg/kg/d	0.7 mg/kg/d	1.0 mg/kg/d
SAP (mmHg)	153 ± 6.2	129 ± 5.2 †	109 ± 6.1	96 ± 6.2*	105 ± 4.5
DAP (mmHg)	121 ± 5.4	99 ± 3.5 †	82 ± 4.9	76 ± 4.9*	82 ± 3.3
HR (min ⁻¹)	393 ± 7.7	343 ± 10 †	374 ± 14.8	352 ± 9.2	373 ± 5.0*
PQ (ms)	44 ± 0.5	52 ± 1.5 †	48 ± 2.3	49 ± 1.5	45 ± 1.1*
LVSP (mmHg)	157 ± 6.3	132 ± 4.6 †	112 ± 5.8	102 ± 5.4*	108 ± 4.5*
LVEDP (mmHg)	3.0 ± 0.2	14.7 ± 4.2 †	6.1 ± 1.8*	9.5 ± 3.0	5.9 ± 0.88*
LV dp/dt max (mmHg/s)	9019 ± 560	7384 ± 562 †	6944 ± 601	6040 ± 497	6763 ± 431
Coronary reserve (ml/min/g)	23.2 ± 2.6	15.5 ± 1.9 †	21.7 ± 3.4	24.8 ± 2.3*	25.7 ± 1.6*
Body weight (g)	447 ± 12.6	469 ± 13.0 †	454 ± 17.2	465 ± 12.2	463 ± 11.4
Heart weight (g)	1.14 ± 0.033	1.77 ± 0.09 †	1.53 ± 0.10	1.41 ± 0.091*	1.38 ± 0.045*
HW/BW	0.26 ± 0.06	0.38 ± 0.026 †	0.33 ± 0.036	0.30 ± 0.017*	0.29 ± 0.015*

Mean ± SEM

* p < 0.05 vs infarct control

† p < 0.05 vs sham operated control

These results suggest that trandolapril, when given orally at an optimum dose of 0.7 mg/kg/day for 24 weeks, increased survival and reduced cardiac hypertrophy resulting from congestive heart failure in rats surviving myocardial infarction due to coronary artery occlusion. These effects appeared to be related to its ability to reduce left ventricular end diastolic pressure and normalize coronary reserve (blood flow).

I.B.1.a.(7). Role of tissue angiotensin II in regression of isoproterenol-induced left ventricular hypertrophy by converting enzyme inhibitor in rats (Vol. 1,8 pp. 274-276 and Vol. 1,16 pp. 1-24):

This study examined the effect oftrandolapril on the cardiac hypertrophy induced in rats by isoproterenol, and correlated these effects with tissue (cardiac) angiotensin II levels to determine whether ACE inhibition can reduce isoproterenol-induced hypertrophy.

Rats were given either vehicle, isoproterenol (4 mg/kg/day for 7 days by subcutaneous infusion), or isoproterenol andtrandolapril (3.0 mg/kg/day for 10 days given orally in drinking water starting 3 days before isoproterenol administration). Another group of rats underwent bilateral nephrectomy to examine possible effects of renal renin on the system. At the end of the 7 day infusion, measurements were taken of: systolic blood pressure, heart rates, atrial and left and right ventricular weights, and plasma and cardiac (atria and ventricles) angiotensin II levels.

Hemodynamic effects are summarized in Figure 52. Heart rates were increased (+19%) by isoproterenol. Trandolapril, however, did not alter this effect (+21%). Systolic blood pressure was slightly decreased (-7%) by isoproterenol. Trandolapril further decreased blood pressure (-19%).

Figure 52

Effect of Isoproterenol with and without Concomitant Administration of Trandolapril on Heart Rate and Systolic Blood Pressure in Rats



Isoproterenol increased (+15%) left ventricular weights while trandolapril reduced this effect to a +9% increase (Figure 53; Sponsor's Figure 262). These changes in ventricular weights were associated with relatively similar changes in left ventricular angiotensin II concentrations (+176% increase by isoproterenol vs 1% increase with trandolapril) (Figure 54; Sponsor's Figure 263). However, similar correlations were not observed between right ventricular and atrial weights and their corresponding angiotensin II levels (Figure 55). Trandolapril had no effect on the isoproterenol-induced increase in plasma angiotensin II levels. No differences in tissue angiotensin II levels in the atria and ventricles were found after bilateral nephrectomy, indicating that renal renin was not involved in regulating their levels in cardiac tissues.

Figure 53

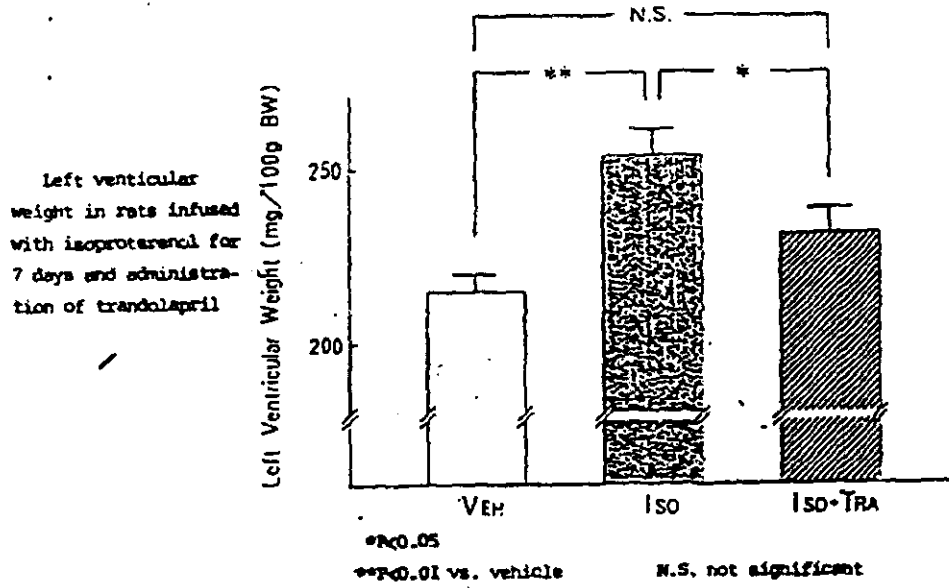


Figure 54

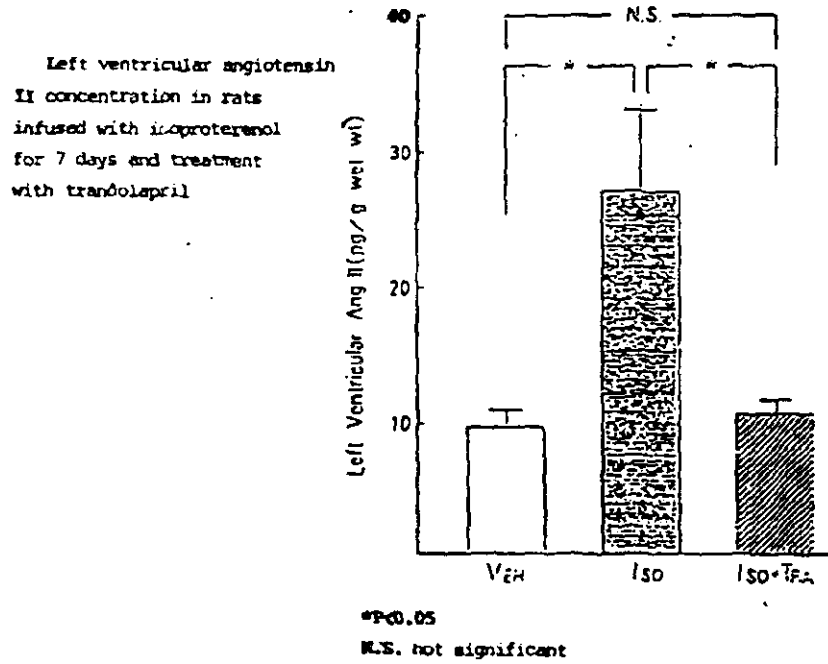
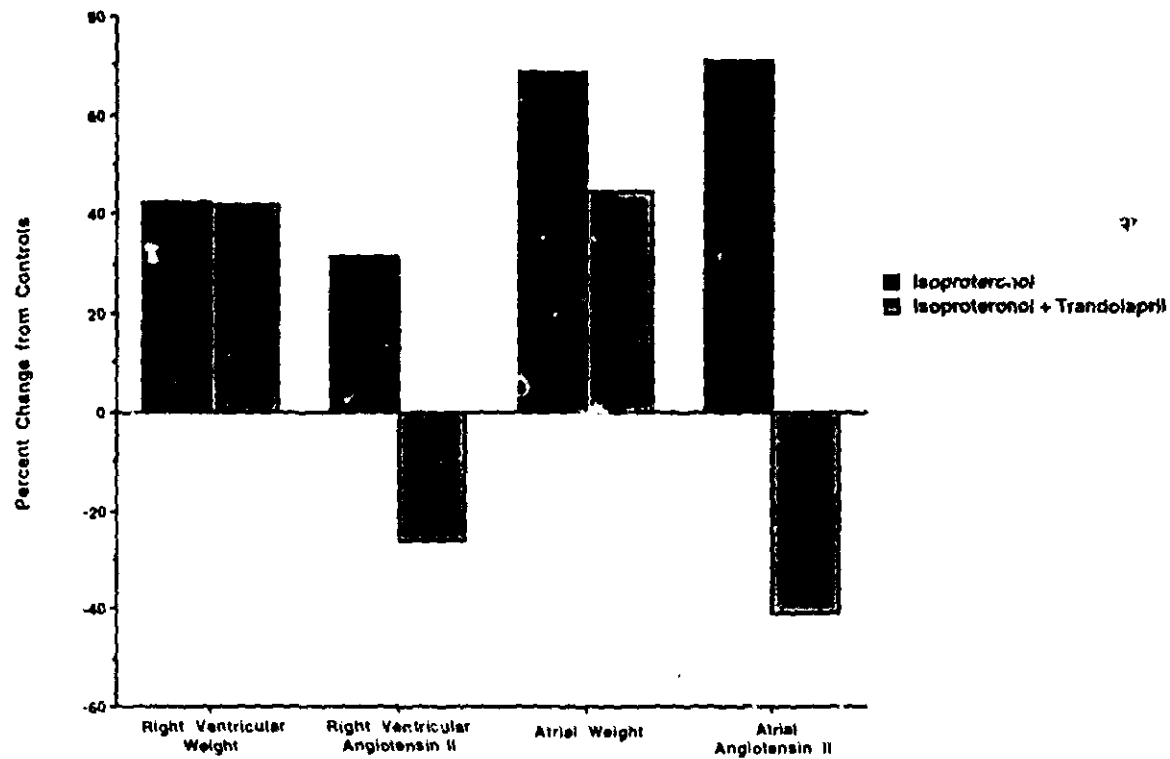


Figure 55

Lack of Correlation between Angiotensin II Concentration and Tissue Weight in Right Ventricles and Atria from Animals Treated with Isoproterenol with and without Concomitant Administration of Trandolapril



These results showed that stimulation of β -adrenergic receptors in the heart by isoproterenol produced cardiac hypertrophy (increased weight in atria, and right and left ventricles) which was associated with increased angiotensin II levels in these tissues. Concomitant administration of trandolapril reduced tissue angiotensin II levels, but reduced tissue weights in only the left ventricle (atria and right ventricular weights were unchanged). Although trandolapril had no effect on heart rates, systolic blood pressure was reduced by 19%, suggesting that the reduced cardiac hypertrophy was associated with both reduced left ventricular angiotensin II levels and reduced blood pressure. The lack of an association between reduced tissue angiotensin II levels in atria and right ventricles and lack of a reduction in their weight (in contrast to

the relationship of reduced left ventricular angiotensin II levels and reduced left ventricular weight) was left unresolved.

I.B.1.a.(8). Converting enzyme inhibitors induced regression of cardiac hypertrophy and reduced tissue angiotensin II in spontaneously hypertensive rats (Vol. 1.8 pp. 277-278 and Vol. 1.16 pp. 25-49):

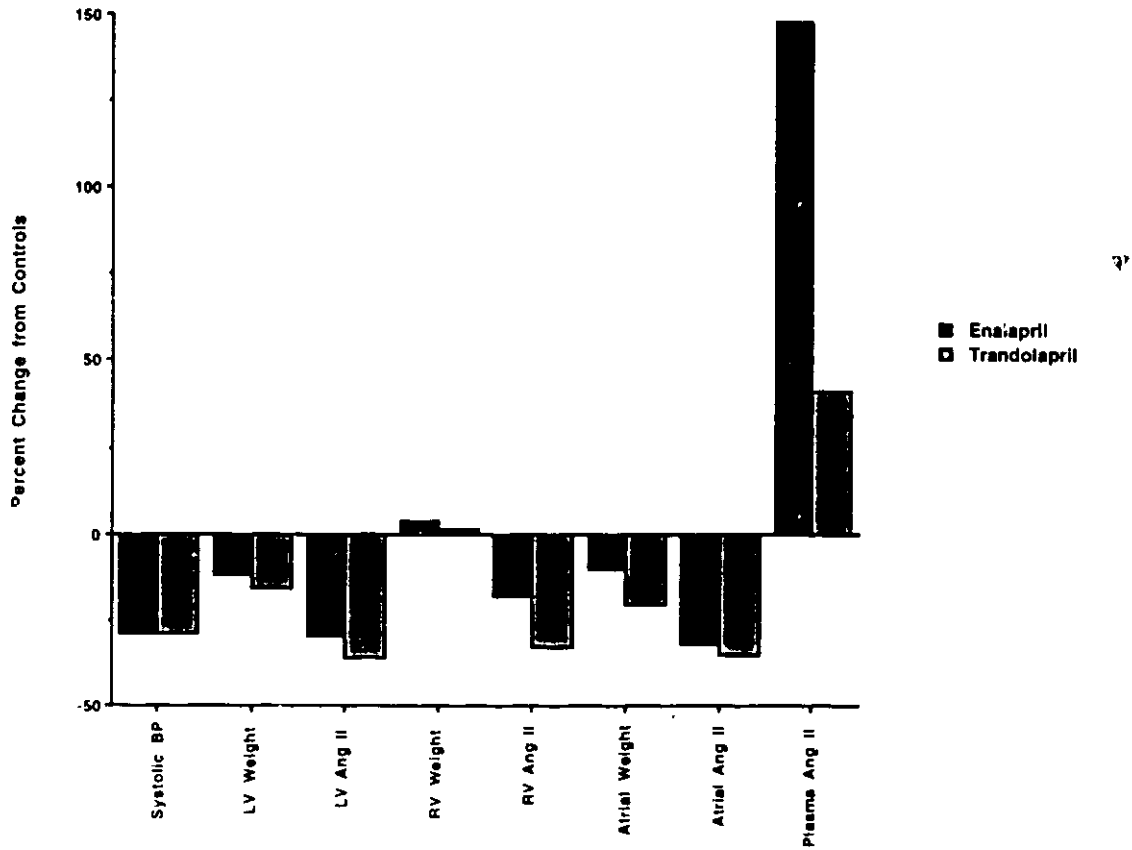
This study examined the effects of treatment for 4 weeks withtrandolapril or enalapril on cardiac hypertrophy in spontaneously hypertensive (SH) rats, and correlated these effects with tissue (cardiac) angiotensin II levels to determine whether ACE inhibition can reduce cardiac hypertrophy associated with hypertension.

SH rats were given eithertrandolapril (1.0 mg/kg/day) or enalapril (10.0 mg/kg/day) for 4 weeks in their drinking water. Controls received water only. After 4 weeks, rats were sacrificed, and the blood and heart (atria, left and right ventricles) were analyzed for angiotensin II levels.

Bothtrandolapril and enalapril reduced systolic blood pressure (-29%) after 4 weeks. Heart rates in all groups were unchanged. Both compounds reduced tissue angiotensin II levels (atria, right and left ventricles). These effects correlated with their corresponding tissue weights, except in the right ventricle where tissue weight was relatively unchanged. Both compounds increased plasma angiotensin II levels, although the increase bytrandolapril was not significant. These data are summarized in Figure 56.

Figure 56

Effects of Enalapril and Trandolapril Systolic Blood Pressure, Cardiac Weights and Angiotensin II Levels in the SH Rat



These results showed that left ventricular angiotensin II levels correlated better than circulating (plasma) angiotensin II levels with respect to left ventricular hypertrophy in this rat model of hypertension.

I.B.1.b. Rabbit

I.B.1.b.(1). Effect of trandolapril or trandolaprilat, respectively, on enzyme leakage of the ischemic reperfused rabbit heart preparation (Vol. 1.8 pp. 278-281 and Vol. 1.16 pp. 50-70):

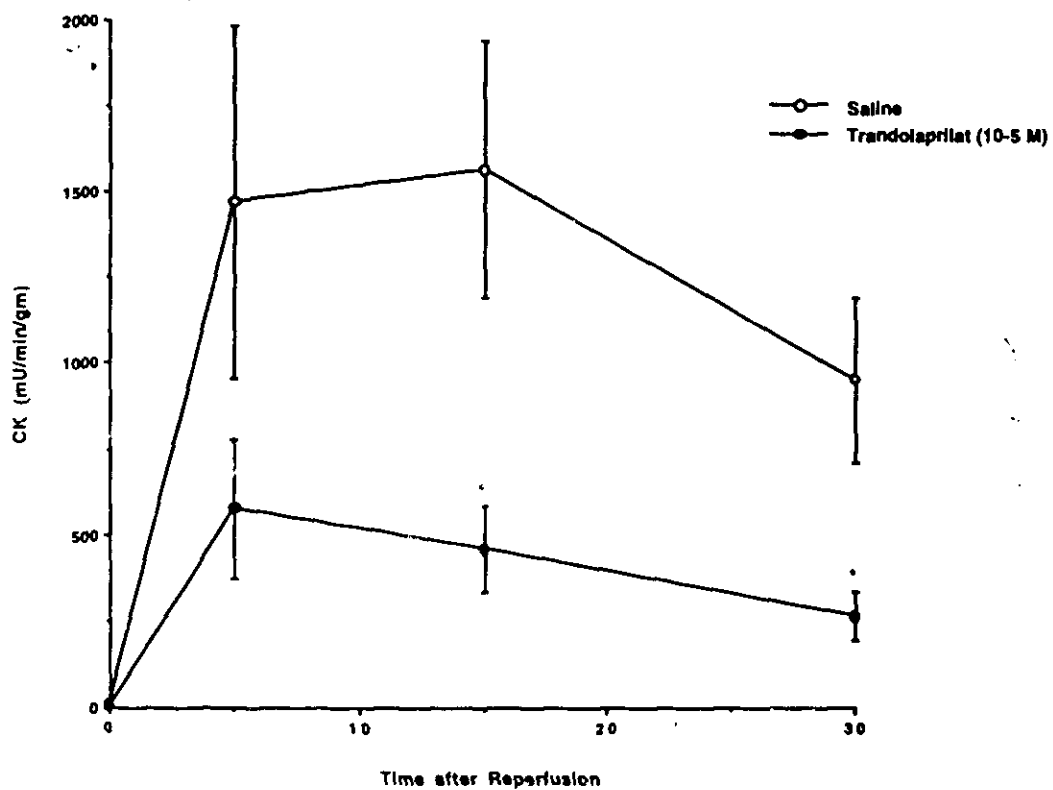
This study examined trandolapril and its active metabolite trandolaprilat for their ability to prevent leakage of CK and LDH after ischemia and reperfusion in isolated perfused rabbit hearts.

Isolated rabbit hearts were perfused with trandolapril (10^{-5} M) or trandolaprilat (10^{-5} and 10^{-6} M). Control hearts were perfused with saline. After 15 minutes of substance perfusion, ischemia was induced by reducing the perfusate flow to 2% of the initial flow for 60 min. The hearts were then reperfused for 30 min at the initial perfusate flow. At several times during the experiment, coronary perfusate samples were collected for analysis of CK and LDH levels by an enzymatic UV-spectrophotometric method.

Results showed that trandolaprilat at 10^{-5} M significantly reduced the leakage of CK (Figure 57) and LDH (Figure 58) caused by ischemia and reperfusion when compared to untreated control hearts. The reductions in enzyme leakage in hearts treated with trandolapril at 10^{-5} M or trandolaprilat at 10^{-6} M were not significant when compared to untreated control hearts. Coronary perfusion or myocardial contractile function was not improved when compared to untreated control hearts.

Figure 57

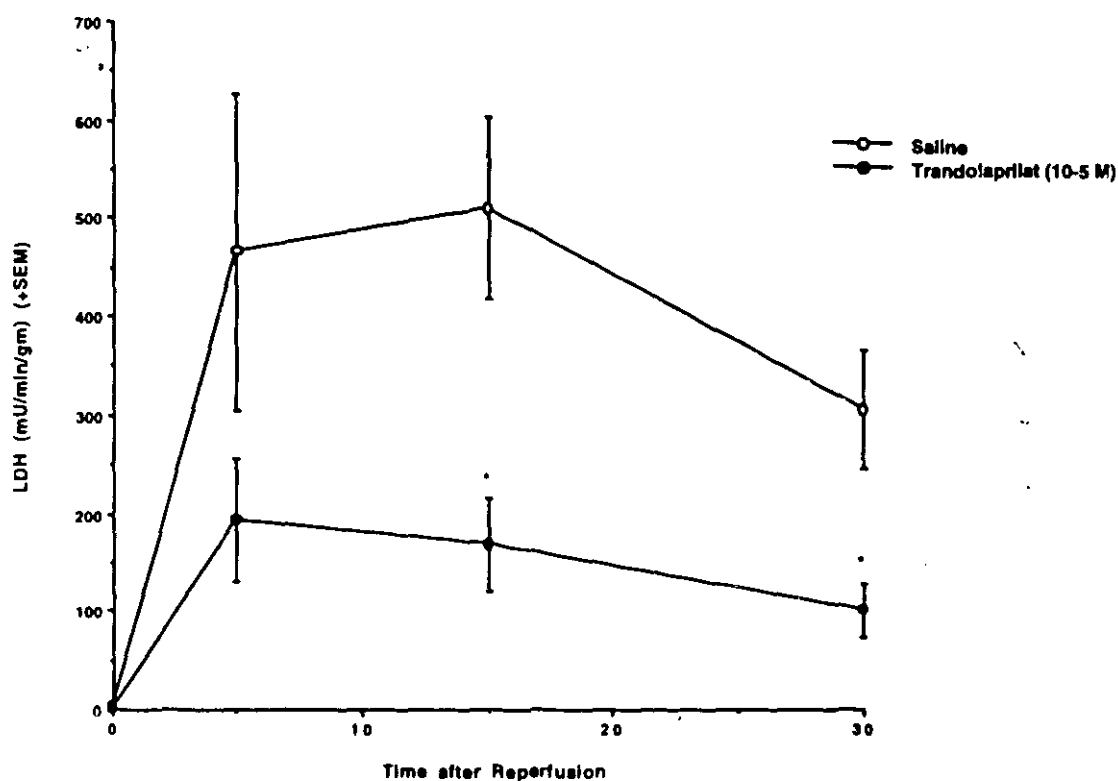
Effect of Trandolaprilat (10⁻⁵ M) on Leakage of CK from Isolated Perfused Rabbit Hearts following Ischemia and Reperfusion



(* = P < 0.05)

Figure 58

Effect of Trandolaprilat (10^{-5} M) on Leakage of LDH from Isolated Perfused Rabbit Hearts following Ischemia and Reperfusion



(* = P < 0.05)

These results showed that trandolaprilat was more effective than trandolapril in protecting the heart from ischemic damage in an isolated rabbit heart model.

I.B.1.b.(2). Protective effects of a converting enzyme inhibitor, trandolapril, on the induction of cardiac and vascular oncogenes by angiotensin I (Vol. 1.8 pp. 281-284 and Vol. 1.16 pp. 71-298):

Cardiac and vascular hypertrophy are the equivalent growth responses of the myocardium and vascular smooth muscle, respectively, carried out by terminally differentiated myocytes unable to undergo cell division. One of the earliest responses of myocytes to growth stimuli is the induction of a series of proto-oncogenes, such as c-

myc and c-fos. Further, c-myc induces the family of heat shock or stress proteins (HSP) that function to protect intracellular proteins from adverse or denaturing conditions.

Many recent studies have shown that myocardium and vascular smooth muscle possess receptors for angiotensin II, and that angiotensin I and II can induce proto-oncogene expression *in vivo*. Additionally, recent studies have shown that ACE inhibitors or angiotensin II receptor antagonists can apparently reverse or prevent the induction of cardiac and vascular hypertrophy. This study examined the effect oftrandolapril on induction of the proto-oncogenes, c-myc and c-fos, and heat shock protein 68 in left ventricle and aorta following injection of angiotensin I to rabbits.

Rabbits were chosen because they possess angiotensin II receptors in both heart and vessels. Rabbits were infused withtrandolapril at 0.5 mg/kg 15 min before injection with either angiotensin I (20 µg/kg/min for 60 min) or angiotensin II (7.5 µg/kg/min for 60 min). Controls received either nothing (non-infused), saline, angiotensin I alone, or angiotensin II alone. After treatment, rabbits were sacrificed and the hearts and aortas removed and frozen. The RNA was extracted and subjected to Northern analysis by hybridizing with probes specific for c-myc, c-fos, and HSP-68.

Hemodynamic data showed that treatment with angiotensin I and II temporarily (about 10 min) increased systolic carotid pressure. Pretreatment withtrandolapril prevented the angiotensin I-induced increase. Data on the effect oftrandolapril pretreatment on the angiotensin II-induced increase in systolic carotid pressure was not presented.

Northern blot analysis attempted to show that angiotensin I induced expression of c-fos and HSP-68 in the rabbit heart, and that this effect was prevented intrandolapril-pretreated animals (Figure 59; Sponsor's Figure 271). These data were marginally interpretable due to the poor quality of the photocopied Northern blots. Data from rabbit aorta was not interpretable again due to the poor quality of the photocopied Northern blots. It was claimed by the Sponsor thattrandolapril prevented the increase in expression of c-fos, c-myc, and HSP-68 in rabbit aorta by angiotensin I.

Figure 59

"Northern blot" - Total RNA in the rat or in the rabbit heart



Positive controls : liver CH : rat livers treated with cycloheximide - Heart HS : rat heart having undergone a heat shock.

Cont : control (group A) - Saline : controls (group B) - Angio I : group C - CEI + Saline : group E - CEI + Angio I : group F - Angio II : group D - CEI = trandolapril.

1 - 13 : numbers of the lanes - c- and r-fos : 2 forms of mRNA hybridizing with the c-fos probe - Lanes 1 and 2 : 25 µg of RNA. Lanes 3 to 13 : 50 µg of RNA.

18s : transcripts hybridizing with a specific synthetic probe of the form 18S of ribosomal RNA. This probe enables the quantity of RNA present on each lane to be checked.

It was concluded that angiotensin I and angiotensin II induced the expression of c-myc, c-fos, and HSP-68 in rabbit heart and aorta (the aorta data was not interpretable), and thattrandolapril at 0.5 mg/kg inhibited the effects of angiotensin I. According to the Sponsor, the data supported their hypothesis thattrandolapril might inhibit the development of cardiac hypertrophy and vascular hypertensive angiopathy resulting from arterial impedance, such as arterial hypertension and atherosclerosis. In this study, the data suggested an effect oftrandolapril on inhibiting the expression of genes associated with cellular growth (c-myc, c-fos, and HSP-68) in the heart and vessels, although the aorta data was of poor quality.

I.B.1.c. Dog

I.B.1.c.(1). Effects oftrandolapril on coronary blood distribution in the healthy and/or ischemic myocardium in dogs (Vol. 1,8 pp. 284-286 and Vol. 1,16 pp. 99-171):

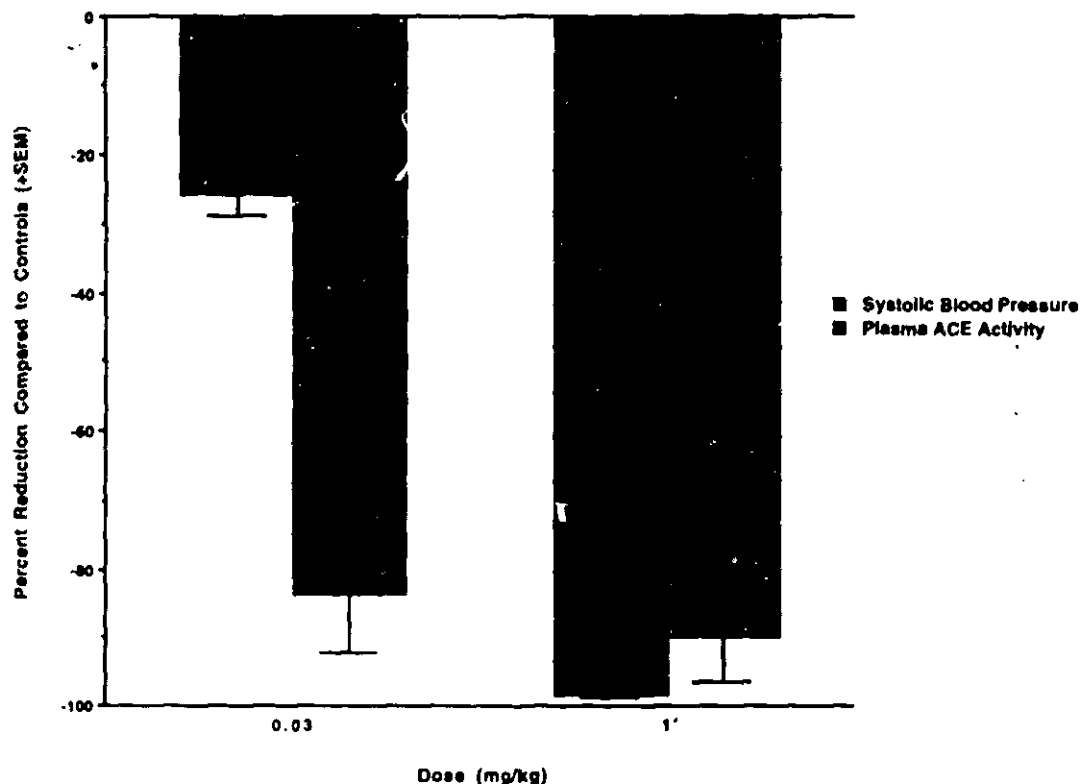
This study determined the effects oftrandolapril on hemodynamic parameters (left ventricular pressure, left ventricular telediastolic pressure (LVTDP) and dP/dt, systolic and diastolic blood pressures, heart rate, and cardiac output), plasma ACE activity, transmural distribution of regional myocardial flow (injection of radioactive microspheres), and intensity of electrical ischemia in healthy myocardium or myocardium made ischemic by coronary occlusion in anesthetized dogs.

The coronary (anterior interventricular) artery of dogs was occluded three successive times for 12 min each with a 45 min recovery period in between occlusions. Trandolapril (0.03 mg/kg) was injected 2 min before the second occlusion, and again (1.0 mg/kg) 2 min before the third occlusion. Radioactive microspheres were injected during all three occlusions to measure regional blood flows. In addition, a small dose of angiotensin I (300 ng/kg) was injected before and after the second and third occlusions to determine plasma ACE activity.

Effects oftrandolapril on blood pressure and plasma ACE activity are shown in Figure 60. Lower doses oftrandolapril (0.03 mg/kg) were more effective in reducing ACE activity than in reducing blood pressure.

Figure 60

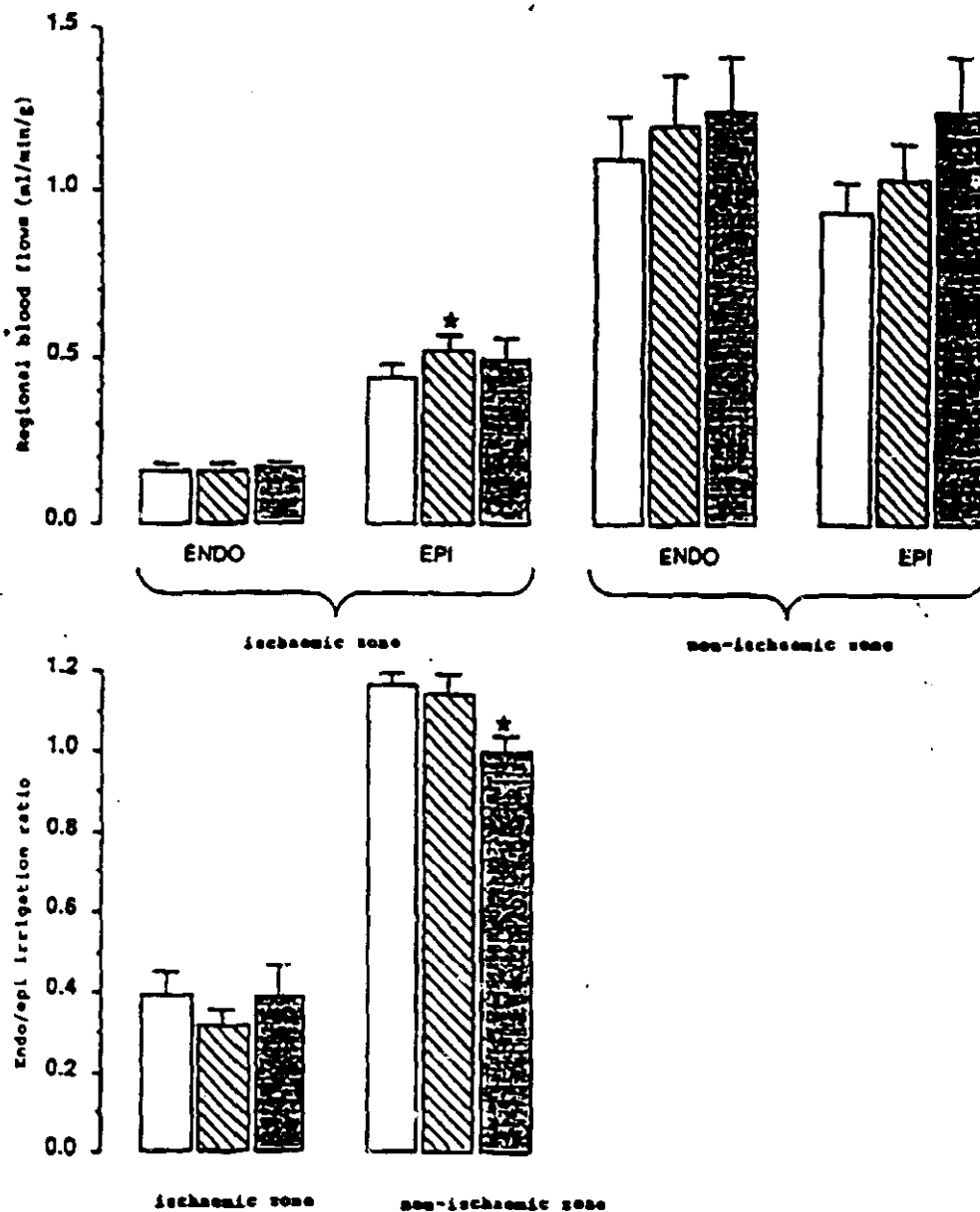
Effects of Trandolapril on Increase in Systolic Blood Pressure Induced by Angiotensin I and on Plasma ACE in Anesthetized Dogs



Increased left ventricular telediastolic pressure (evidence of induction of myocardial depression) was found after coronary occlusion (mean value of +35.2% increase when compared to before coronary occlusion). ST segment (mV) was increased +204% (mean). However, other hemodynamic parameters were minimally affected by coronary occlusion. Trandolapril (at 0.03 and 1.0 mg/kg) did not significantly affect these parameters.

Trandolapril slightly, but significantly ($P < 0.05$), increased blood flow from the endocardium to the epicardium in healthy (non-ischemic) tissue (Figure 61; Sponsor's Figure 275). Trandolapril (0.03 and 1.0 mg/kg) induced no other significant dose-dependent differences in regional blood flow (endocardium/epicardium ratio).

Figure 61



Effects of trandolapril on myocardial regional blood flows (endocardial: endo; epicardial: epi) and on the endocardium/epicardium (endo/epi) ratio in non-ischaemic and ischaemic myocardial zones during coronary occlusion (□), during coronary occlusion under 0.03 mg/kg trandolapril (▨) and during coronary occlusion under 1 mg/kg trandolapril (▩).

* Value significantly different from that observed during the control occlusion: p < 0.05.

These results showed that trandolapril at doses up to 1.0 mg/kg IV reduced ACE activity and the hypertensive effects of angiotensin I in anesthetized dogs. These effects were likely due to decreased peripheral resistance, since similar doses of trandolapril had no effect on cardiac output or heart rate. Regional blood flows (endocardium/epicardium ratio) were slightly increased in healthy myocardium, but not in ischemic tissue.

I.B.2. Renal Function

I.B.2.a. Dog

I.B.2.a.(1). Effects of intravenous (trandolapril) on renal function in the anesthetized dog (Vol. 1.8 pp. 287-289 and Vol. 1.16 pp. 172-235):

This study examined the effect of trandolapril (1 and 10 mg/kg) and enalapril (10 mg/kg) IV in dogs on renal function (urine volume, sodium excretion, chloride excretion, potassium excretion, reabsorption of free water, osmolar clearance, creatinine clearance, and p-aminohippuric acid clearance) for 90 min, and correlated these effects with any changes in heart rate and blood pressure.

When compared to baseline values, trandolapril at 10 mg/kg was shown to significantly ($P < 0.05$): increase urine volume (+168%), sodium excretion (+97%), chloride excretion (+145%), potassium excretion (+118%), and osmolar clearance (+65%). No significant changes were observed in measures of renal function (creatinine and p-aminohippuric acid clearance), heart rate or mean blood pressure.

These results showed that doses of trandolapril (10 mg/kg IV) which had marginal hemodynamic effects (heart rate and blood pressure) increased salt (Na^+ , K^+ , and Cl^-) excretion with little effect on renal function (clearance). Enalapril at 10 mg/kg had little effect on the parameters studied.

II. PHARMACOKINETICS:

II. A. ABSORPTION: The oral absorption of trandolapril and its conversion into its active metabolite trandolaprilat was studied in several species i.e. mice, rats, dogs and monkeys. Table 8 shows the maximal plasma concentration (C_{max}) and time to reach it (T_{max}) of trandolaprilat after a single oral administration of trandolapril. In mice, transformation of trandolapril into trandolaprilat was very rapid i.e. within 15 min after an oral administration and was essentially identical in either sex. However, the C_{max} was higher in the female mice compared to the male mice. In rats, complete de-esterification of trandolapril into the diacid metabolite, trandolaprilat, was rapid in the plasma reaching peak concentrations within 5 to 10 minutes (8.3 ± 2.6 min) following oral administration. In dogs, conversion of trandolapril to trandolaprilat was also rapid. however, T_{max} was prolonged as the dose increased indicating that the rate of appearance of trandolaprilat perhaps being retarded with increasing dose of

trandolapril. In cynomolgus female monkeys, transformation of trandolapril into trandolaprilat was independent of doses and the highest plasma concentrations were found at 2 to 3 hours after oral administration.

The absolute bioavailability was determined as the ratio of the area under the p.o. plasma concentration-time curve to the area under the i.v. plasma concentration-time curve. The bioavailability values of trandolaprilat were $37.29 \pm 7.92\%$, $42.99 \pm 19.76\%$ and $59.8 \pm 5.2\%$ for rats, dogs and monkeys, respectively.

II. B. DISTRIBUTION (including protein binding): Binding of trandolapril and trandolaprilat to plasma was determined in Beagle dogs and humans by incubating plasma *in vitro* at 37° C for four hours with various concentrations of [³H]-trandolapril and [³H]-trandolaprilat, respectively. Similarly, binding of only trandolaprilat to plasma was examined in Sprague-Dawley rats since trandolapril is very rapidly de-esterified to dicarboxylic acid, trandolaprilat, in the presence of rat plasma.

The mean percentages of trandolapril bound to dog and human plasma proteins were 81.4% and 83.2%, respectively and was constant over the concentration ranging from 0.1 to 10,000 ng/ml. In rat plasma, the degree of binding of trandolaprilat ranged from 59.8% (10,000 ng/ml) to 95.8% (0.1 ng/ml). In dog plasma the binding of trandolaprilat increased from 58.5% to 86% over the concentration range of 10,000 ng/ml to 0.1 ng/ml. Similarly, in human plasma the degree of binding of trandolaprilat rapidly increased with decreasing concentration of trandolaprilat, the values ranging from 65.1% (1000 ng/ml) to 94.2% (0.1 ng/ml). This negative concentration dependent binding of trandolaprilat in both dog and human plasma indicate saturation of the binding sites at low concentration.

The tissue distribution of orally administered [¹⁴C]-trandolapril (25 mg/kg) was examined in male and female mice. There was a rapid absorption with a peak tissue distribution in 2 hours. There was a marked affinity for the lung, attributable to the binding to angiotensin converting enzyme which is particularly prevalent in this tissue. Similarly, the radioactivity was found in the liver, external medulla of the kidneys, keratinized stomach wall, and the lumen of the digestive tract, particularly in its terminal portion. After 24 hours, little radioactivity was found in most tissues.

TABLE 3

Kinetic Parameters for Trandolaprilat (an Active Metabolite) after an Oral Administration of Trandolapril (Parent Compound)

<u>Species</u>	<u>Dose (mg/kg)</u>	<u>T_{max} (hr)</u>	<u>C_{max} (ug/ml)</u>
Mice (CD-1)			
<u>Male</u>	25	0.25	2.0
<u>Female</u>	25	0.25	4.57
Rat (SD)			
<u>Male</u>	1	0.14 ± 0.04	1.02 ± 0.35
Dog (Beagle)			
<u>Male</u>	1	0.7 ± 0.56	0.28 ± 0.15
	10	2.3 ± 0.9	2.63 ± 0.45
	50	1.7 ± 0.3	22.80 ± 3.40
	250	2.7 ± 0.7	172.00 ± 30.0
<u>Female</u>	10	1.3 ± 0.3	4.97 ± 0.44
	50	2.3 ± 0.9	24.90 ± 6.80
	250	4.0 ± 0.0	187.00 ± 14.0
Monkey (Cynomolgus)			
<u>Female</u>	1	2.3 ± 0.33	0.05 ± 0.01
	125	2.7 ± 0.33	23.17 ± 2.71

The distribution of radioactivity throughout the body of the albino and pigmented rats was studied post oral and intravenous administration of [¹⁴C]-trandolapril (1 mg/kg). The highest concentrations of radioactivity were observed in the gastrointestinal tract, liver, kidney, blood, skin, and lung after both oral and intravenous administration. No radioactivity was detected in the central nervous system. No difference in the distribution of the radioactivity was observed between the albino and the pigmented rats indicating that there was no preferential uptake or retention of radioactivity in the melanic tissues. Sprague-Dawley albino pregnant rats were divided into two groups. [¹⁴C]-trandolapril (1 mg/kg) was administered orally by gavage to one group on gestation day 14 and to the other group on gestation day 18. The highest concentrations occurred in the kidney, liver, lung, portions of the gastrointestinal tract, amniotic sac, placenta, and, to a lesser extent, blood. There was no evidence of placental transfer of radioactivity into the fetus at either the 14th or the 18th day of gestation. Radioactivity did not appear to cross the maternal blood-brain barrier.

Differences were observed in the pattern of distribution between rats dosed on either the 14th or the 18th day of gestation.

II. C. **METABOLISM:** The metabolic products of [¹⁴C]-trandolapril excreted in the urine and bile of rats and dogs were identified by HPLC and gas chromatography-mass spectroscopy. The major urinary and biliary metabolite of trandolapril in rats, following oral and intravenous dosing, was trandolaprilat (diacid metabolite) and accounted for 60-70% of the total radioactivity. There were several minor components present, one of which, dimethyl ester of trandolaprilat, accounted for 10-15% of the total radioactivity. No radioactive trandolapril was identified in urine and bile indicating that trandolapril was completely transformed into its metabolites in rats.

The major urinary metabolite of trandolapril in dog, post oral and intravenous administration, was trandolaprilat (21-44%). At least other two metabolites coded as B1 and B2 were identified. A small amount (3%) was detected as the parent compound.

II. D. **EXCRETION:** Excretion of radioactivity after the oral administration of [¹⁴C]-trandolapril (1 mg/kg) in biliary cannulated male animals (one dog and three rats) was studied. Following oral dosing, bile was collected for 24 hrs, and urine and feces were collected for 96 hrs from the dog. Bile, urine and feces were collected for 48 hrs from the rat.

The total recovery of radioactivity from the dog by 96 hrs after dosing was 94.1%. The percentage of dose recovered was: bile 24.07%, urine 18.86%, and feces 46.59%. The maximum rate of biliary elimination of radiolabel trandolapril occurred at 4.5 - 6 hrs after dosing. By 23-24 hrs after dosing the concentrations of radioactivity in the bile were negligible.

The total recovery of radioactivity from the rat by 48 hrs after dosing was 87%. The mean percentage of dose ($\pm$ SD) recovered was: bile $35.72 \pm 3.79\%$, urine $15.58 \pm 3.91\%$, and feces $35.90 \pm 10.82\%$. The majority of the radioactivity was excreted in the first 4 hrs.

III. TOXICOLOGY:

III. A. ACUTE TOXICITY:

Several acute toxicity studies were performed at _____ Scientific Division, _____ in accordance with GLP requirements. All animals were observed for 14 days post a single administration of Trandolapril. The number of animals per sex for each dose group was 5 for rodents and 3 for dogs. The strains of animals used were Swiss and NMRI mice, Sprague-Dawley rats, and Beagle dogs. Table 9 depicts the summary of the acute toxicity studies with LD₅₀.

TABLE 9

SUMMARY OF ACUTE TOXICITY WITH LD₅₀:

SPECIES	SEX	ROUTE	LD ₅₀ (mg/kg)
Mouse	M	Oral	4875
	F	Oral	3990
	M	I.P.	1010 - 1490
	F	I.P.	1220 - 1455
Rat	M	Oral	> 5000
	F	Oral	> 5000
	M	I.P.	1280 - 1530
	F	I.P.	1135 - 1765
Dog	M	Oral	> 1000
	F	Oral	> 1000

Dose proportional mortality was detected in both sexes after oral and I.P. administration in mice. Slight hypotonicity, piloerection, hunch back, locomotor difficulties or motor incoordination was noted only on day one. In rats, no mortality occurred after an oral administration, but dose proportional mortality was detected after I.P. dosing. Signs of hypothermia, marked hypotonicity, piloerection and epistaxis were observed. At necropsy, petechia and gross hemorrhage in the gastric atrium and the pancreas, thickened liver lobes and enlargement and thickening of the spleen and kidneys were seen. In dogs, mortality (50%) was observed only at 2000 mg/kg dose. Marked body weight loss with decreased food intake associated with hypotonicity, hypomotility, dehydration, weak pulse and respiratory difficulties were noted.

III. B. MULTI-DOSE

III. B. 1. Subchronic Toxicity

III. B. 1. a. 15 Day Oral Toxicology Study in the Rat:

Location of Data: Vol. # 18 of 418, dated September 28, 1994.

Testing Facility:

Study No./Dates: 84313/84314. Drug was administered from November 20 (M) or 21 (F), 1984 to December 4 (M) or 5 (F), 1984.

GLP Compliance: Yes

Animals: M/F Sprague-Dawley Rats, 6 weeks old.

Mode of Administration: Oral by gavage (Aqueous 0.5% methylcellulose suspension)

Dose Levels: 0, 20, 100 and 500 mg/kg/day.

No. of Animals: 5/sex/group

Observations/
Measurements: Clinical signs, mortality, moribundity and water intake daily; body weight twice a week; food consumption weekly; blood pressure 24 hrs after the 13th dose and 2 hrs after the 14th dose; hematology and clinical chemistry 23 hrs after the last dose; urinalysis between days 13 and 14; at necropsy organ were weighed and examined macroscopically and microscopically.

Drug-Associated Findings: There was no drug-induced mortality or clinical signs. In male rats dose-independent loss in body weight (12.8%, 10.5% and 8.6% at low-, mid- and high-doses, respectively) associated with decrease in food intake (25.6%, 18.5% and 21.6% at low-, mid- and high-doses, respectively) was detected. Plasma urea and creatinine were markedly elevated in both sexes indicating functional renal insufficiency in all the treated animals, independently of the dose, being more marked in the males than the females. Total Cholesterol, triglycerides, and total protein values (particularly globulins) were elevated in drug treated males. There was drug-induced increase in the frequency of renal lesions (presence of basophilic tubules in the cortex, tubular dilatation, nephritis).

III. B. 1. b. _____ 30 Day Oral Toxicology Study in Rats:

Location of Data: Vol.. # 18 & 19 of 418, dated September 28, 1994.

Testing Facility:

Study No./Dates: 85115 to 85120. Drug was administered from February 11 (M) or February 13 (F), 1985 to March 12 (M) or March 14 (F), 1985.

GLP Compliance: Yes

Animals: M/F Sprague-Dawely Rats, 6-7 weeks old.

Mode of Administration: Oral by gavage (Aqueous 0.5% methylcellulose suspension)

Dose Levels: 0, 4, 20 and 100 mg/kg/day

No. of Animals: 10/sex/group (Subdivided into two subgroups. one group received tap water as drinking water while second group received Ringer's solution diluted 1:3 as drinking water).

Observations/
Measurements: Clinical signs, mortality and moribundity daily; body weight and food and water consumption weekly; blood pressure at the end of the study; ophthalmoscopy before and at the end of the study; hematology, clinical chemistry and urinalysis at the end of the study; at necropsy organs were weighed and examined for gross and histopathology.

Drug-associated Findings: Mortality was low ; only 1 male in the 20 mg/kg/day treatment group receiving tap water died on day 31. There was drug-induced loss in body weight gains (19%, 26% and 21% at 4, 20 and 100 mg/kg/day dose, respectively) in males receiving tap-water to drink. However, this was not dose-dependent. Blood pressure determinations demonstrated that both the tap water and Ringer's saline group underwent a decrease in blood pressure which was generally dose-related. Dose-dependent significant decrease in angiotensin converting enzyme activity was detected at 20 and 100 mg/kg/day doses of all animals receiving both tap-water and Ringer's saline as drinking water. Numerous gastric ulcerations sites were seen at the 20 mg/kg/day dose in animals receiving tap-water to drink. Also, prenerotic lesions of the submaxillary salivary glands were seen in almost all animals receiving 20 and 100 mg/kg/day in the tap-water groups. Dose-dependent lesions of the renal tubules, more marked in the males than females, were detected in both groups of drinking water.

III. B. 1. c. 30 day Intravenous toxicology Study in the Rat:

Location of Data: Vol. # 20 of 418, dated September 28, 1994

Testing Facility:

Study No./Dates: 86225/86226. Drug was administered from June 24 (M) or July 1 (F), 1986 to July 23 - 24 (M) or July 30 - 31 (F), 1986.

GLP Compliance: Yes

Animals: M/F Sprague-Dawley Rats, 5 - 6 weeks old.

Mode of Administration: Intravenous

Dose Levels: 0, 1, 2.5 and 5 mg/kg/day

No. of Animals: 10/sex/group

Observations/
Measurements: Clinical signs, mortality, moribundity and water intake daily; body weight and food consumption weekly; blood pressure and ophthalmoscopy before and at the end of the study; hematology, clinical chemistry and urinalysis at the end of the study; at necropsy organs were weighed and examined macroscopically and microscopically.

Drug-Associated Findings: No drug-induced effect on mortality, blood chemistry, organ weights or histopathology was detected. Decrease in blood pressure and angiotensin converting enzymes were mainly reflecting the pharmacological activity of the active ingredient. The significant localized lesions in the caudal vein and the perivascular connective tissues (phlebitis, periphlebitis and thrombosis) were associated with the injection process. Intravenous administration of _____ to rats at doses of 1, 2.5 and 5 mg/kg/day was well tolerated with no drug-related toxic reactions.

III. B. 1. d. 15 Day Oral Toxicology Study in the Dog:

Location of Data: Vol. # 21 of 418, dated September 28, 1994

Testing Facility:

Study No./Dates: 84315/84316 . Drug was administered from December 5 (M) or 6 (F), 1984 to December 19 (M) or 20 (F), 1984.

GLP Compliance: Yes

Animals: M/F Beagle Dogs, 10 months old

Mode of administration: Oral by capsule

Dose Levels: 0, 10, 50 and 250 mg/kg/day

No. of animals: 2/sex/group

Observations/
Measurements: Clinical signs, mortality, moribundity and food intake daily; body weight twice a week; blood pressure on days 7 or 8 and on days 13 or 14; hematology, clinical chemistry and urinalysis at the end of the study; at necropsy organs were weighed and examined for gross and histopathology.

Drug-associated Findings: There was no drug-induced effect on mortality, clinical signs, blood pressure, ECG, organ weights or histopathology. There was a moderate loss in body weight gain in males at 250 mg/kg/day dose. Blood urea was slightly increased in 50 mg/kg/day dose group. A slight increase in serum alkaline phosphatase, urea and cholesterol levels with no concomitant histopathology of the liver and kidneys was observed at 250 mg/kg/day dose group. This reveals that 10 mg/kg/day is a no effect dose in dogs.

III. B. 1. e. 30 Day Oral Toxicity Study in Dogs:

Location of Data: Vol. # 21 of 418, dated September 28, 1994

Testing Facility:

Study No./Dates: 85121/85122. Drug was administered from May 21 (M) or 28 (F), 1985 to June 19 (M) or June 26 (F), 1985.

GLP Compliance: Yes

Animals: Beagle dogs, 8 months old.

Mode of Administration: Oral by capsule

Dose Levels: 0, 10, 50 and 250 mg/kg/day

No. of Animals: 3/sex/group

Observations/
Measurements: Clinical signs, mortality, moribundity and food consumption daily; body weight twice a week; blood pressure, ECG and ophthalmoscopy before and at the end of the study; hematology, clinical chemistry and urinalysis at the end of the study; at necropsy organs were weighed and examined macroscopically and microscopically.

Drug-associated Findings: Daily oral administration of 10, 50 or 250 mg/kg/day of to beagle dogs for 30 days did not induce mortality or abnormal clinical signs or any changes in body weight, food consumption, blood pressure, ECG waves and intervals, hematology, organ weights, macroscopic examinations, or microscopic examinations. There were moderate increases in males' alkaline phosphatase and plasma urea at the high dose (250 mg/kg/day); urea concentration was also elevated in the mid-dose (50 mg/kg/day) group's males. Serum cholesterol levels were slightly elevated in the females at the high dose. All treated animals demonstrated marked decreases in angiotensin converting enzyme levels. A few minor renal lesions such as basophilic tubules, dilated tubules and cells with pyknotic nuclei in the distal convoluted tubules were observed in the males of all treatment groups. Electrolyte supplementation with KCl and NaCl had little effect on results.

III. B. 1. f. 30 Day Intravenous Toxicology study in the Dog:

Location of Data: Vol. # 21 of 418, dated September 28, 1994

Testing Facility:

Study No./Dates: 86240/86241. Drug was administered from October 1 (M) or October 8 (F), 1986 to October 30 (M) or November 6(F), 1986.

GLP Compliance: Yes

Animals: Beagle dogs, 10 months old.

Mode of Administration : Intravenous

Dose Levels: 0, 0.25, 0.5 and 1.0 mg/kg/day

No. of Animals: 3/sex/group

Observations/
Measurements: Clinical signs, mortality, moribundity and food and water consumption daily; body weight weekly; blood pressure, ECG and ophthalmoscopy before and at the end of the study; hematology, clinical chemistry and urinalysis at the end of the study; at necropsy organs weights and were examined for gross and histopathology.

Drug-associated Findings: Intravenous administration of _____ to beagle dogs for 30 days at doses of 0.25, 0.5 or 1 mg/kg/day had no effect on mortality, clinical signs, body weight, food consumption, water intake, blood pressure, EKG, hematology or clinical chemistry. Drug-associated reduction in angiotensin converting enzyme was noted. Minimal tubular lesions and parietal and/or perivascular focal lesions of the cephalic veins were detected in most animals including controls.

III. B. 2. Chronic Toxicity:

III. B. 2. a. Oral Toxicity Study in Sprague-Dawley Rats for 26 weeks followed by a 6-weeks Recovery Period:

Location of Data: Vol. # 22 to 27 of 418, dated September 28, 1994.

Testing Facility:

Study No./Dates: 1202 TCR. Study was conducted in 1985.

GLP Compliance: Yes

Animals: M/F Sprague-Dawley Rats weighing 178 gm (M) and 149 gm (F).

Mode of Administration: Oral by gavage (Aqueous 0.5% methylcellulose suspension)

Dose Levels: 0, 0.25, 2.5 and 25 mg/kg/day

No. of Animals: 30/sex/dose/water-saline grouping (Water group received tap water and saline group received diluted Ringer's solution as drinking water) Ten of the 30 animals in each group were assigned to undergo 6 weeks of recovery after the 26-week dosing period.

Observations/Measurements: Clinical signs and mortality daily; body weight, food consumption and water intake weekly; ophthalmological examinations before treatment and in weeks 3, 12 and 24; hematology in weeks 4, 12/13 and 25/26; clinical chemistry and urinalysis in weeks 5, 14 and 26/27; at necropsy organs were weighed and examined macroscopically and microscopically. All these parameters were also examined at the end of the recovery period.

Drug-Associated Findings: During the 26 week (with 6 week recovery) rat oral study, the most frequently observed clinical sign was a marked soiling of the litter correlated with polyuria which appeared not to be dose related but possibly due to the pharmacological effect of the drug. The polyuria was most evident in males of both the drinking tap water and the saline groups. Slightly drug-related increased mortality was observed in the 2.5 and 25 mg/kg/day groups (Table 10). Animal deaths not considered to be drug related occurred during blood sampling anesthesia, without characteristic preceding clinical signs, or because of verified intubation errors. There was one death during the recovery period; a female (high-dose group) from the Ringer saline group was found dead (no prior clinical signs) on the last day of the recovery period. No sex relationship in mortality was noted so data

is combined for both sexes.

Table 10

Mortality in the 26 Week (6 Week Recovery) Rat Oral Study

<u>Treatment</u>	<u>Doses (mg/kg/day)</u>			
	<u>0</u>	<u>0.25</u>	<u>2.5</u>	<u>25</u>
Drinking Tap Water:				
Animals found dead	1/60	3/60	5/60	3/60
Animals euthenized	0/60	0/60	2/60	5/60
Probably drug related death	0/60	0/60	3/60	4/60
Drinking Ringer's Saline:				
Animals found dead	2/60	0/60	2/60	2/60
Animals euthenized	0/60	0/60	1/60	0/60
Probably drug related death	0/60	0/60	3/60	2/60

A slight (statistically significant) growth retardation was seen as early as the second dosing week in males drinking tap-water compared to control animals. The growth retardation was not dose-related (9%, 18%, 8% for the 0.25, 2.5, and 25 mg/kg/day groups, respectively, at the end of the treatment). A reduction in food consumption, sometimes statistically significant, was also observed in these animals of the tap-water group. There was no consistent significant, dose-related effects on growth or food consumption among the treated females drinking tap-water or the males and females drinking Ringer's solution.

A marked statistically significant decrease in angiotensin converting enzyme (ACE) activity was seen in all treated animals. Some increase in the levels of plasma urea (all doses) and creatinine (mid and high dose), which were marked in the males drinking tap water was seen; these changes were reversible. Kidney weights (both absolute and relative to body weight) increased in all treatment groups with all of the relative weights increasing significantly; after the recovery period, the differences were less marked. Some statistically significant decreases in absolute and relative (to body weight) heart weights were noted in high dose males (15% and 12%, respectively) drinking tap water and females (15% and 8%, respectively) drinking Ringer's saline. These changes tended to reverse during the recovery period. There was no correlation of histopathology with the heart weight changes, but the increase in kidney weights could be correlated with the incidence of microscopic observations of glomerulonephritis; the frequency of these lesions increased from the 2.5 mg/kg/day dose level and up. These lesions were also observed in the control group, and are characteristic of a spontaneous pathology in the rat, especially the male in which incidence increases with age. The sponsor considers that the majority of the modifications seen were due to the pharmacological activity of

III. B. 2. b. Toxicity Study in Rats by Oral route during 78 Weeks:

Location of Data: Vol. # 28 - 31 of 418, dated September 28, 1994

Testing Facility:

Study No./Dates: 2025 TCR. Drug was administered from May 29, 1986 to November 30, 1987 or December 12, 1987 (78 or 81 Weeks).

GLP Compliance: Yes

Animals: M/F Sprague/Dawley Rats, 6 Weeks Old

Mode of Administration: Oral gavage. (Aqueous 5% methylcellulose suspension)

Dose Levels: 0, 0.25, 1.5 and 9 mg/kg/day.

No. of Animals: 50/sex/group

Observations/
Measurements: Clinical signs and mortality daily; body weights and food consumption weekly during the first 13 weeks and then every two weeks; water intake in weeks 4, 13, 26, 52 and 78 for a period of 7 days; ophthalmoscopic examinations before treatment and in weeks 25, 51 and 78; hematology, clinical chemistry and urinalysis in weeks 13, 26, 52 and 78; at necropsy organs were weighed and examined macroscopically and microscopically

Drug-Associated Findings: No drug-induced clinical signs were observed except chromodacryorrhea, chromorhinorrhea, decrease or loss of activity, presence of abscess in urinogenital area in males, emaciation, piloerection, hypothermia, pallor of eyes and extremities and dyspnea which are generally detected in the aging rats. Mortality was higher in females than in males, but for each sex the distribution of mortality was normal and not related to the treatment (table 11).

Table 11
Mortality in the 78 Week Rat Oral Study

Sex	Males				Females			
	0	0.25	1.5	9	0	0.25	1.5	9
Doses mg/kg/day	0	0.25	1.5	9	0	0.25	1.5	9
Mortality	11/50	9/50	3/50	9/50	17/50	14/50	9/50	16/50
% of Mortality	22%	18%	6%	18%	34%	28%	18%	32%

The mortality was mostly associated with pituitary adenoma, primary hepatic tumors, mammary adenocarcinoma and tumors of the endometrium and stroma. There were no differences in the incidence of the total number of tumors or the number of tumor-bearing animals between treated and control groups. The rate of mortality and tumors and the factors contributing to them did not indicate any treatment or dose relationship. The number and percentage of animals with masses confirmed as tumors at microscopic examination are depicted in table 12.

Table 12
Number and Percentage of Tumors

Sex	Males				Females			
	0	0.25	1.5	9	0	0.25	1.5	9
Doses mg/kg/day	0	0.25	1.5	9	0	0.25	1.5	9
# of Animals	5/50	0/50	1/50	2/50	8/50	10/50	7/50	5/50
% of Tumors	10%	0%	2%	4%	16%	20%	14%	10%

The retardation in the body weight gain was somewhat dose-dependent in males (5.8%, 11.7% and 11% for the 0.25, 1.5 and 9 mg/kg/day groups, respectively, at the end of treatment) and females (11.7% and 14.4% for the 1.5 and 9 mg/kg/day dose groups, respectively, at the end of treatment). The growth retardation was seen as early as the second dosing week in the high dose (2.4%) males while in the mid (4.2%) and low (3.7%) dose males it was noted at the third and fourth dosing weeks, respectively. In female rats the retardation in body weight gain was in the first week of dosing for the mid-dose and in week 12 for the high-dose group. A reduction in food

consumption was associated with the retardation of the body weight gain and was sometimes statistically significant. At the end of treatment period food consumption decreased by 9%, 11.2% and 8% for the 0.25, 1.5 and 9 mg/kg/day dose groups, respectively in males and 8.6% and 12.4% for the 1.5 and 9 mg/kg/day dose groups, respectively in females.

The water intake in the high-dose males increased (about 27%) over the controls during the treatment period reaching to statistical significance in weeks 4 and 26. In the mid-dose males the water consumption increased (about 15%) over the controls and was only statistically significant in week 4. The water intake in the low-dose males was slightly increased in weeks 4 and 13 and slightly decreased in weeks 26, 52 and 78 but never attained statistical significance. This dose-related increase in the water consumption in the males could be drug-related effect. However, in females there was no consistent drug-related effect in the water consumption.

A slight changes in hematology parameters (mean hemoglobin, red blood cell count and packed cell volume), sometimes statistically significant, but not dose-dependent, were observed in both males and females. Therefore, it may not be of any toxicological significance at the examined doses up to 9 mg/kg/day for 78 weeks.

A slight decrease in serum sodium levels was observed in animals of either sex from the mid- and high-dose groups. A moderate increase in serum magnesium level was noted in males and females from the high-dose group in weeks 13, 26 and 52.

Slight to moderate increase in serum urea level with no change in creatinine level was observed in the high-dose group in weeks 26, 52 and 78. However, no drug-related pathological changes were detected in the kidney.

No drug-related statistical changes in organ weights or histopathology were noted. Therefore, the oral (gavage) administration of _____ to rats at dose levels of 0.25, 1.5 or 9 mg/kg/day for 78 weeks did not produce any histopathological evidence of toxicity or increase the incidence of total or malignant tumors in the treated animals compared to the controls.

III. B. 2. c. Oral Toxicity Study in Beagle Dogs for 26 Weeks Followed by a 6-Week Recovery Period:

Location of Data: Vol. # 32 to 41 of 418, dated September 28, 1995.

Testing Facility:

Study No./Dates: 1201 TCC. Drug was administered from February 28,

1986 to September 1-3, 1986. Recovery period began on August 31, 1986 and ended on October 13, 1986.

GLP Compliance: Yes

Animals: M/F Beagle Dogs -7.5 months of age.

Mode of Administration: Oral by Capsule

Dose Levels: 0, 2.5, 25, 250/125 and 125 mg/kg/day. Marked toxicity was detected at 250 mg/kg/day dose group on day 22. The dose was reduced to 125 mg/kg/day. All animals in this 250/125 mg/kg/day dose group were sacrificed on days 32 and 33. Subsequently second study was initiated with control and 125 mg/kg/day dose groups.

No. of Animals: 9/sex/group. One-hour after each daily feeding, 50 ml of an electrolyte solution (1% KCl plus 1% NaCl) was administered by intubation to 3 dogs of each sex from each dosing group. After 26 weeks of treatment, 3 animals of each sex from dosing group were chosen to continue evaluation for 6-week recovery; 1 of the 3 animals was chosen from the electrolyte supplement.

Observations/
Measurements: Clinical signs, mortality and food consumption daily; body weight weekly, water intake for 7 days in weeks 5, 12, 25 and 31; blood pressure, EKG and ophthalmological examination before treatment and in weeks 4, 13 and 26 (2-4 and 24 hours after treatment) and after the end of the recovery period; hematology and blood chemistry before and in weeks 4, 13 and 26 (18 hours after treatment) and at the end of the recovery period; urinalysis before and in weeks 4, 13 and 26 and at the end of the recovery period; at necropsy organs were weighed and examined for gross and histopathology.

Drug-Associated Findings: Treatment of beagle dogs with daily doses of 0, 2.5, 25, 250/125, or 125 mg/kg of _____ for 26 week resulted in hypotonia, vomiting, diarrhea, and dehydration, especially at the high dose levels (250/125 and 125 mg/kg/day). There were a few occurrences at the lower doses. Euthanasia was

apparently initiated if a dog had a recurrent clinical signs (i.e. vomiting or diarrhea). Dose-dependent mortality was detected at doses ≥ 25 mg/kg/day and no clear difference could be noted between the animals that were given the electrolyte solution or not. In the animals euthanized during the study, there were marked increases in fibrinogen levels, sedimentation rates, platelet counts, and activated partial thromboplastin times, hemoglobin levels, numbers of erythrocytes, and hematocrit increased or decreased depending on the clinical state of the animals. Administration of the electrolyte solution had no clear effect; all parameters returned to normal after 6 weeks of the recovery period. There were increases in urea and creatinine levels after about 4 weeks of treatment with 125 mg/kg/day and which were marked after 3 weeks of treatment with 250/125 mg/kg/day. Angiotensin converting enzymes activity was decreased by 2.5 mg/kg/day and inhibited completely by 25, 125, and 250/125 mg/kg/day of RU 44 570. The above itemized effects returned to control after the 6 week recovery period. A slight to moderate increases in several parameters such as alkaline phosphatase, 5'nucleotidase, ornithine carbonyltransferase, glutamate dehydrogenase and leucine aminopeptidase activities were noted at the mid- and high-dose groups in both the animals that were given the electrolyte solution or not. All these activities normalized on drug withdrawal. Urinary Na, K, Cl, Ca, and Mg outputs generally decreased at mid- and high-doses; after the 6 week recovery period urinalysis values were normal. Organ weights did not change due to There was some evidence of gastrointestinal irritation and pale kidneys following 125 and 250/125 mg/kg/day doses. These macroscopic findings correlated with microscopic evidence of gastrointestinal erosion and ulceration plus renal cortical tubular dilatation with epithelial collapse, but without necrosis. After the 6-week recovery period, only 2 animals that received the electrolyte solution at the 125 mg/kg/day dose had slight residual tubulonephrosis. There were no other histopathologic abnormalities.

III. B. 2. d. Toxicity Study in Beagle Dogs by Repeated Oral Administration for 52 Weeks.

Location of Data:	Vol. 42-45 of 418, dated September 28 1994
Testing Facility:	
Study No./Dates:	1875 TCC. Drug was administered from December 18, 1986 to December 17-22, 1987.
GLP Compliance:	Yes
Animals:	M/F Beagle Dogs, 6 months old
Mode of Administration:	Oral by Capsule

Dose Levels:	0, 0.25, 2.5 and 25 mg/kg/day
No. of Animals:	6/sex/group
Observations/ Measurements:	Clinical signs, food consumption and mortality daily; body weight weekly, water intake for 5 days in weeks 4, 12, 25 and 51; blood pressure, EKG and ophthalmoscopic examination before and in weeks 5, 13, 26 and 52 (2 - 4 hours post dosing); hematology, blood chemistry and urinalysis before and in weeks 5, 13, 26 and 52 of treatment; at necropsy organs were weighed and examined for gross and histopathology.

Drug-Associated Findings: Daily doses of 0, 0.25, 2.5 or 25 mg/kg administered for 52 weeks did not cause changes in body weight gain, food or water consumption in either sex. A slight dose-dependent increase in heart rate was observed in the treated males in weeks 13, 26 and 52 compared to controls at the corresponding time. However, significant difference was detected only in week 13. For the females, the heart rate values were essentially similar between control and treated animals at all times. In both sexes, the PQ, QRS and QT interval values were not affected by the treatment during the entire study. A slight dose-dependent decrease in systolic blood pressure values was observed only in males in weeks 13 and 52, but statistical significance was noted only in week 13. No drug-related changes were detected in hematology, ophthalmology or urinalysis. A significant decrease in both absolute and relative spleen weights was noted in males from the 0.25 mg/kg/day group and up. The absolute weights of kidneys and brain were significantly reduced in males from the 0.25 and 25 mg/kg/day dose groups, respectively. These changes in organ weights were not associated with histopathology and they were not dose-related. No death occurred during the study. Thus, over a period of 52 weeks, administered daily in doses as high as 25 mg/kg/day was well tolerated by the dog, and did not induce systemic toxicity.

III. C. CARCINOGENICITY:

III. C. I. Introduction

It is to be noted that, unless otherwise specified, the following conventions will be followed in this subdivision:

(Trandolapril), the ethyl ester prodrug of the angiotensin converting enzyme inhibitor, (Trandolaprilat), will be referred to as TRAN.

(Trandolaprilat) will be referred to as TRAN-AT.

ACEI refers to Angiotensin Converting Enzyme Inhibitor (s).

Sponsor, for whom experiments were conducted, and to whom the data were reported:

GLP COMPLIANCE: Yes.

STUDY DOCUMENTS ARCHIVED: YES, In CIT Archives for 5 years.

III. C.2. RU 44 570 (Trandolapril) Potential Tumorigenic Effects to Mice by Oral Administration (Gavage) 78 Weeks:

Location of Data: Vols. 45 through 48 of 418 dated Jun 16/89.

Study No./Dates 2036 TCS : 1/22/86 (FIRST DOSE) :
11/25/87

Animals: (Cri: CD-1 C-1CR BR) mice supplied by
 Mean weights ,
 28 g, males and 22 g females.

Mode of Administration: Orally, by gavage.

Dose Levels: The table showing dosing schedules and information is Table 13 reproduced below from Vol. 45, page 4 (using the uppermost page numbers.)

Table 13.

Groups	Dose levels (mg/kg/d)	Sex	Animals Number	Cages Number
T	0	Males	C02130 to C02189	1 to 60
A	1	Males	C02190 to C02249	61 to 120
B	5	Males	C02250 to C02309	121 to 180
C	25	Males	C02310 to C02369	181 to 240
T	0	Females	C02370 to C02429	241 to 300
A	1	Females	C02430 to C02489	301 to 360
B	5	Females	C02490 to C02549	361 to 420
C	25	Females	C02550 to C02609	421 to 480

Dose Rationale: Sponsor estimated the approximate maximum tolerated dose from the results of a 60 day study in mice of the same strain. Doses used were 0, 2, 10, and 50 mg/kg/d.

Vehicle: Vehicle used was a 0.5% suspension of methylcellulose in distilled water.

Test Substance Preparation: The test substance was diluted in the 0.5 % methylcellulose solution and homogenized using magnetic stirrers and ultrasonic mixers.

Test Substance Stability: Good, using sponsor's directions.

Animal Management:	Individually housed in polycarbonate cages, changed weekly, autoclavable. Animals were fed <i>ad lib.</i> with a pellet diet, and had free access to tap water.
Test Substance Administration:	Given daily using a plastic syringe with a metal dosing needle. Drug and control were administered at volumes of 10 ml/kg/d once daily at approximately the same time each day.
Clinical Examinations:	
Signs:	Once daily, at the same time.
Mass palpation:	After 6 mo., every 2 wk.
Mortality:	Exam 2/d. Sac. if debilitated or intox. Dead or if in extremis, sac., then do blood smear plus macroscopic examination.
Body weight:	At control, dosing day 1, once/week during 13 wks. of dosing, then @ 2 wk until the end of the study.
Food consumption:	Once/week (on a period of 8 days on wk. 1 and of 7 days until the end of the study) during the first 13 wks of treatment, on wk 18, then every 4 wk until the end of treatment. Calculated in g/animal/day, based on the amount given minus that remaining at the end of 7 days.
Water consumption:	Measured (on a period of 7 days) during treatment weeks 4, 13, 26, 52, and 78 in the first 10 males and 1st 10 females which survive from each group, except for control groups in wk 26. Measured as g/animal/day in terms of that given minus that remaining in bottles at the end of the experiment.

Laboratory Investigations:

Hematology:

In weeks 52 and 78, blood samples were taken by caudal vein incision from all the survivors and the moribund animals before sacrifice whenever possible. Blood smears were prepared and the differentials in white blood cells were counted. No other hematological parameters were evaluated.

Pathology:

Animals were sacrificed by the usual methods; a complete macroscopic exam was done before and after dissection.

The tissues and masses harvested from the animals dying during the study or sac. prematurely are shown below in Table 14, extracted from p. 7, Vol. 45.

Table 14.
Tissues Preserved-Mouse

aorta	spinal cord	testes and
caecum	cervical, thoracic	epididymides
brain including	and lumbar sections	thymus
medulla/pons,	skeletal muscle	thyroids and
cerebellar and	sciatic nerve	parathyroids
cerebral cortex	lymph nodes, cervical,	trachea
heart	thoracic and mesenteric	ureteres
colon	oesophagus	uterus, horns
duodenum	ovaries	and cervix
stomach	pancreas	vagina
femoral bone	skin	seminal vesicles
with articulation	lungs	urinary bladder
and bone marrow	prostate	eyes with optic nerves
liver	spleen	gall bladder
mammary glands	rectum	
parotid glands	kidneys	
mandibular glands	sternum with	
pituitary	bone marrow	
ileum	adrenals	
jejunum		

Table 15.
Microscopic Examinations-Mouse

All tissues required for microscopic examination were sectioned at approximately 4-5 microns and stained with hemalum-eosin.

Microscopical examinations were performed on:

- 1) all macroscopic lesions and tissues listed above:
 - . in all animals from the high dose level and control groups sacrificed at the end of the treatment period.
 - . in all animals that died or were sacrificed prematurely.
- 2) all macroscopical lesions from the intermediate and low dose level groups.

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Statistical Methods Used: The Kolmogorov-Smirnov Test was used to check the normalcy of the distribution. If abnormal, this test is performed after log transform of the values. If significant heterogeneity persists, analysis of variance is not performed and the Mann-Whitney test is used.

Bartlett's Test is used in the case of normal distribution of values according to the normal law of the analysis of variances. Dunnett's Test is used if no sig. heterogeneity of the variances can be found. If significant heterogeneity of the variances is found the Mann-Whitney Test is used.

The convention that $p < 0.05 = *$; $p < 0.01 = **$ is followed.

Results:

The palpable masses in mice (See Table 16) which were confirmed as tumors at microscopic examination were not dose-related events and we agree with sponsor's sense that the drug is devoid of significant effects on this parameter (from sponsor's table on page 12 of Vol. 45).

Table 16
Palpable Masses in Mice

Doses (mg/kg/d)	MALE				FEMALE			
	0	1	5	25	0	1	5	25
Number of animals	2/60	0/60	0/60	0/60	0/60	1/60	0/60	0/60
Percentage	3	0	0	0	0	2	0	0

Clinical Signs were monitored extensively. We disagree with sponsor's summary³⁷ that "the treatment did not induce any specific toxic clinical signs in the mouse". As can be seen in Tables 17 and 18 for male and female mice, respectively (from pp. 20 and 21 of Vol. 45), the incidences of clinical signs which appear to be

Table 17.
Summary of Principal Clinical Signs in Male Mice.

GROUPS DOSES (mg/kg/d)	T 0		A 1		B 5		C 25	
	n	%	n	%	n	%	n	%
<u>RESPIRATION</u>								
. Dyspnea	6	10	8	13	6	10	13	20
<u>APPEARANCE</u>								
. Pilorection	7	12	6	10	4	7	10	17
. Pallor of excrecites	5	8	3	5	6	10	2	3
. hunched posture	1	2	-	-	3	5	2	3
. swollen abdomen	3	5	6	10	-	-	1	2
. emaciation	4	7	2	3	1	2	2	3
. abnormalities at level of legs	5	8	-	-	2	3	4	7
. red and crusty ears	1	2	2	3	2	3	5	8
. cutaneous lesions	1	2	3	5	4	7	1	2
. ectopia testes	-	-	3	5	3	5	3	5
. swollen testes	-	-	4	7	3	5	-	-
. abnormalities of eyes (paller, opacity, exophthalmia)	6	10	6	10	3	5	6	10
<u>BEHAVIOR</u>								
. prostration	3	5	1	2	1	2	2	3
. hypothermia and/or tremors	1	2	5	8	6	10	7	12

n = Number of animals affected
% = Percentage of animals affected

Table 18.
Summary of Principal Clinical Signs in Female Mice.

GROUPS DOSES (mg/kg/d)	T 0		A 1		B 5		C 25	
	n	%	n	%	n	%	n	%
RESPIRATION								
. Dyspnea	10	17	12	20	10	17	11	18
APPEARANCE								
. Piloerection	8	12	7	12	9	15	11	18
. Pallor of extremities	8	11	6	10	3	5	10	17
. hunched posture	5	8	2	3	3	5	7	12
. swollen abdomen	8	13	6	10	5	8	4	7
. emaciation	5	8	5	8	4	7	?	3
. abnormalities at level of legs	2	3	3	5	1	2	1	2
. red and crusty ears	5	8	2	3	2	3	2	3
. cutaneous lesions	-	-	-	-	1	2	-	-
. abnormalities of eyes (pallor, opacity, exophthalmia)	4	7	6	10	6	10	1	2
BEHAVIOR								
. prostration	2	3	2	3	2	3	1	2
. hypothermia and/or tremors	5	8	9	15	8	13	14	23

n = Number of animals affected
% = Percentage of animals affected

significantly, and possibly dose-relatedly elevated, are hypothermia, and/or tremors in both sexes, and possibly piloerection and hunched posture in females only.

Mortality distribution in the mouse study is illustrated in Table 19, which is from the table on page 13 in Volume 45.

Table 19. Mortality Distribution in the Mouse Study.

Doses (mg/kg/d)	MALE				FEMALE			
	0	1	5	25	0	1	5	25
Animals/group	60	60	60	60	60	60	60	60
Killed prematurely	7	5	3	7	11	8	10	11
Found dead	0	3	6	4	6	6	2	4
Total	7	8	9	11	17	14	12	15
%	12	13	15	18	28	23	20	25

There were no dose-related trends and no marked differences in mortality between the treated and the control groups. The contributing factors to mortality or premature sacrifice were comparable among the control and treated groups. Amyloidosis and malignant lymphoma were the usual contributory factors. Additionally, endometrial sarcoma, with metastases in some organs was a contributory factor for the death of some of the females of the control, low, and intermediate dose groups. Sponsor does not comment on the incidence of endometrial sarcoma in the high dose group. However, this sarcoma was not listed in Factors Contributing to Death for the high dose group (pp. 56-57, Vol. 45).

In addition, misdosing was a cause of death in some animals from all groups; 16 animals died after misdosing. Seventeen animals were replaced in the first week of the study by spare animals and one animal evidenced cutaneous lesions. The replacement animals did not show any chronological time lag.

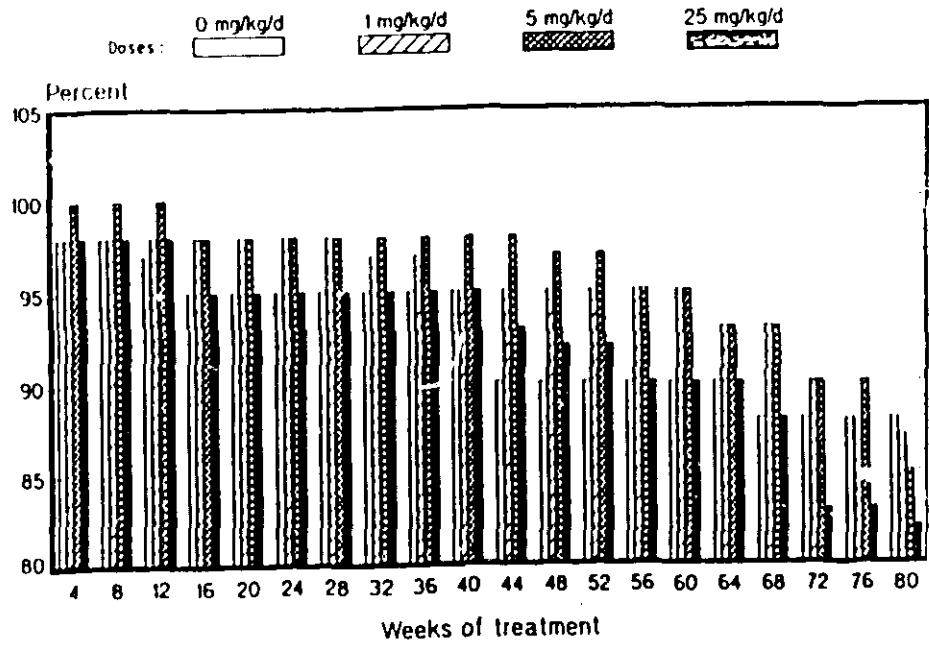
The percentages of surviving animals were evaluated every 4 weeks, and the collected data were presented by sex as the histograms shown in Figure 62. Sponsor concluded that the survival rates of the control groups were very close to the survival rates of the treated groups. Examination of the data supports sponsor's conclusion.

Body weight changes were monitored for all dose groups of both sexes for the duration of the experiment; weekly for 14 weeks and biweekly to the 78th week. The male animals receiving drug showed an approximately dose-related decrease in body weight gains when compared with the control group's gains. Figure 63A (from Vol. 45) demonstrates this response by males. When sponsor calculated the significance of the differences between the control and the high dose (25 mg/kg/day) treatment groups, all of the time intervals were significantly different; i.e., all of the time intervals from 16 weeks to the end of the study were significantly different at the 0.01 level. A plateau level of approximately 8% depression of body weight gain was maintained from weeks 54 to 78. A differential of this magnitude would seem to justify the choice of the high dose in males.

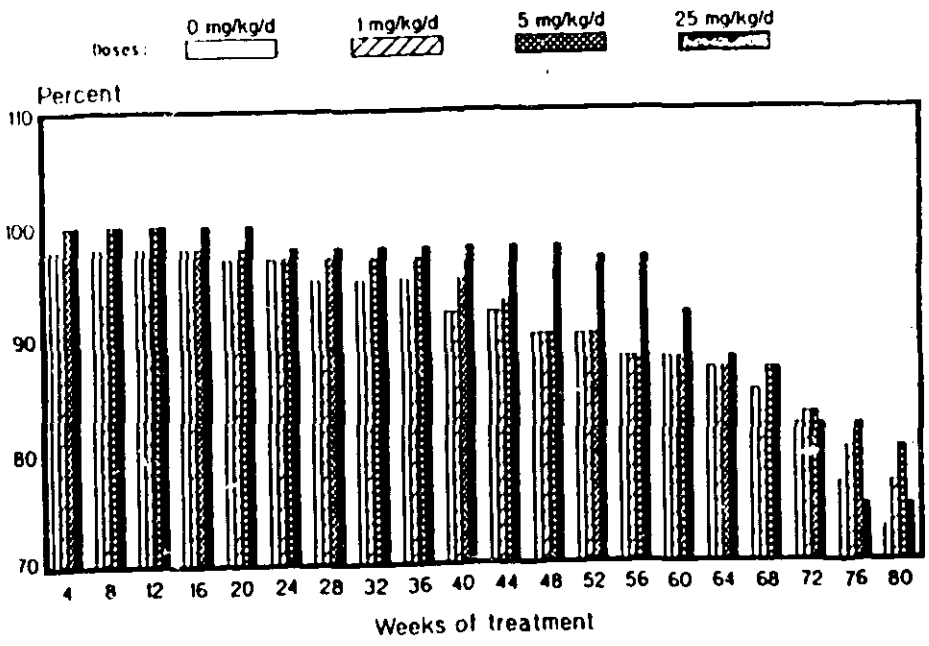
Figure 63 B demonstrates that there was no consistent difference between the body weight changes of the treated female groups when compared with that of the female control group.

Figure 62.
Mouse Survival Rates With and Without Trandolapril Treatment

SURVIVAL RATE - MALE



SURVIVAL RATE - FEMALE



BODY WEIGHT CHANGES IN MICE TREATED WITH TRANDOLAPRIL
 FIGURE 63 A: MALES

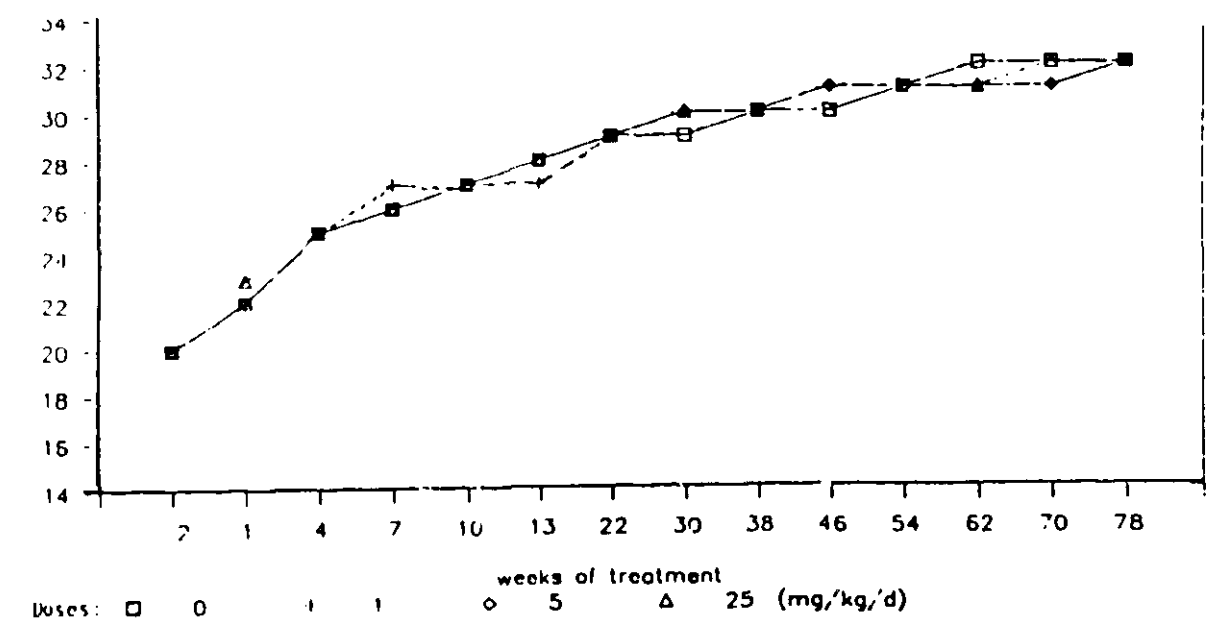
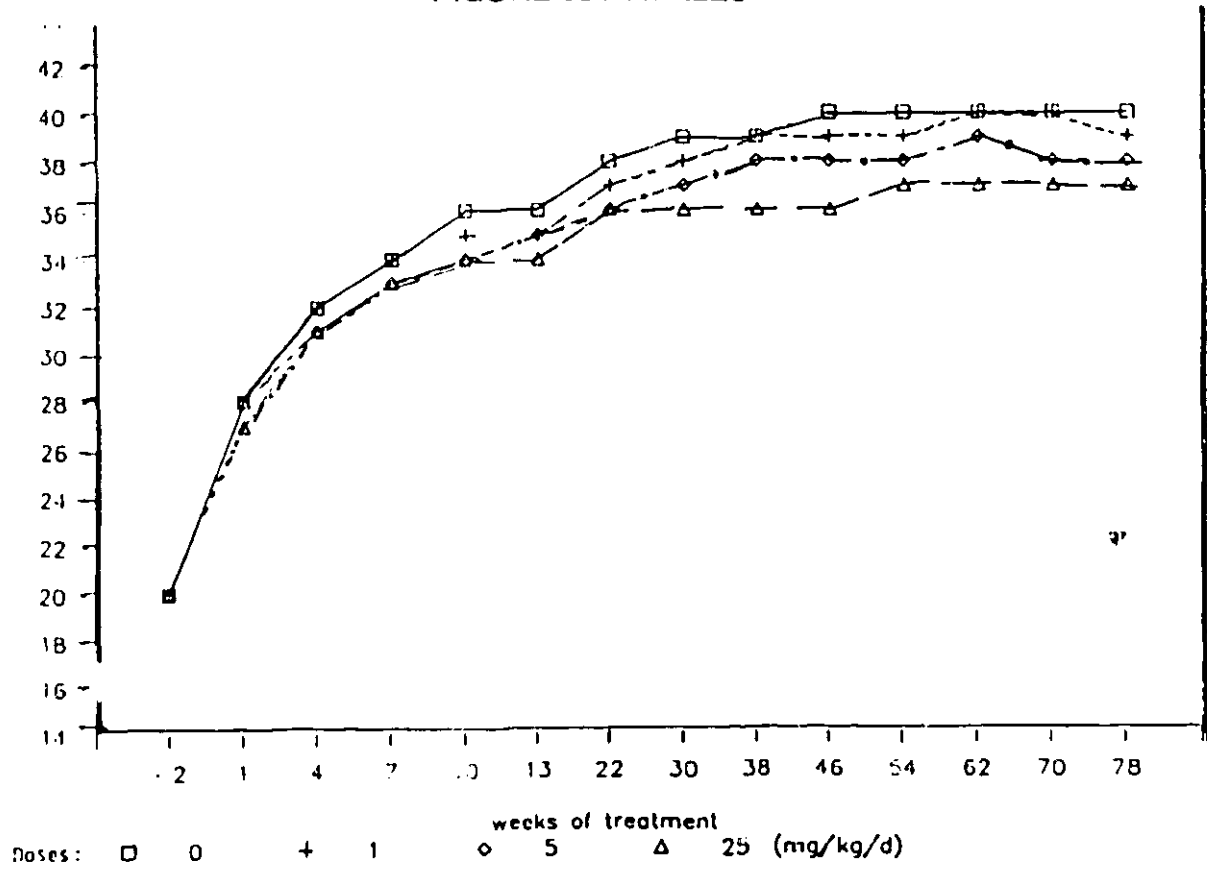


FIGURE 63 B: FEMALES

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Mean food consumption was examined in both sexes. The changes in consumption were quite small in both sexes. However, there seemed to be a trend toward greater consumption by the mid and high doses of both sexes during the first four weeks of the study. This was followed by decreased consumption by both sexes until the end of the study. Within the above framework, there were several statistically significant changes.

Mean water consumption was also studied, although the study was quite cursory. Males appeared to show greater increases in consumption by the mid and high doses than did the females. However, few of the differences reached the level of significance.

Laboratory Investigations:

Hematology: There were minimal and/or transient changes in neutrophils, monocytes, and lymphocytes in males, plus changes in the percentage of eosinophils. These changes were not considered to be of any toxicological significance. Anomalies encountered were not treatment related since their incidences were comparable amongst all groups.

Pathology:

A. Summary Tables of General Macroscopic Pathological Observations in Mouse Studies:

Tabular Units: Incidence of Necropsy Findings by Organ/Dose Group/Sex.

Dose Groups:

T = Control, A = 1 mg/kg/day, B = 5 mg/kg/day, C = 25 mg/kg/day

Sex: M = Male, F = Female

Mouse Pathology References: Vol. 45, pp. 28-30

Tables: Appendix I, pp. 87-94.

CONCLUSIONS: The incidence of pale kidneys among females of the high and mid-dose groups was increased compared with controls. Sponsor does not specify if the differences were statistically significant, but it appears likely. This agrees with microscopic findings which will be specified in the next section.

Changes in other organs were reported as common findings in old mice, including all dose groups and controls, therefore, the findings were judged to be of no toxicological significance by sponsor. This appears to be a reasonable conclusion.

It should be noted that the summary tables of general macroscopic, non-neoplastic, and neoplastic data have been separated out and explanatory notes have been added. This was done by the reviewer to enhance the utility of the data by improving the flow of information from the tables.

Table 20. a. Summary Table of Macroscopic Pathology

ORGAN/FINDING	DOSE GROUP:		T		A		B		C	
	SEX:	NO. ANIMALS:	M	F	M	F	M	F	M	F
			60	60	60	60	60	60	60	60
GENERAL OBSERVATIONS										
- Sacrificed			7	11	5	8	3	10	6	11
AORTA										
HEART										
CECUM										
- DISTENDED WITH GAS									1	1
- THICKENED			1							
- GREYISH COLOR					1					
- CONTENTS							1			1
- DEPOSIT			2		6	1	2	4	1	1
- FOCUS									1	
- FOCI					1	2	5	2	6	2
COLON										
- DISTENDED WITH GAS										1
- CONTENTS							1			
RECTUM										
BRAIN										
- ADHESION										1
OVARIES										
- HEMORRHAGIC CYST				9		8		6		5
- SEROUS CYST				33		32		29		35
- ENLARGED				5				3		3
- WHITISH COLOR				1		1		5		1
- BLACKISH COLOR				1						1
- REDDISH COLOR				3		1		2		1
- FIRM						1				
- FOCUS						2				
- FOCI				1				1		1
- CYST				38		39		32		36
DUODENUM										
- DISTENDED WITH GAS								1		1 1
- DILATATION								1		
- THINNED								2		
- REDDISH COLOR								1		
- CONTENTS				1		2	1			1
JEJUNUM										
- DISTENDED WITH GAS								1		1 1
- DILATATION								1		
- THINNED								1		
- REDDISH COLOR								1		
- CONTENTS						2	1			1
- NODULE				1						
ILEUM										
- DISTENDED WITH GAS								1		1 1
- DILATATION								1		
- THICKENED				1					1	
- ADHESION								1		
- CONTENTS				1	1		1	1		
STOMACH										
- DILATATION								1		
- THINNED								1		
- THICKENED				3	3	1		2		1
- YELLOWISH COLOR										1
- REDDISH COLOR				1	1			1		1
- CONTENTS				1		1		3		1 1
- DEPOSIT										1
- ULCERATION										
MESENT. LYMPH NODE										
- ENLARGED				3	4	3	4	2	6	7
- REDDISH COLOR				1	3		6	2		6 5
- GREENISH COLOR										1
- EDEMA										
FEMORAL BONE (-B.M)										

Table 20. b. Summary Table of Macroscopic Pathology.

ORGAN/FINDING	DOSE GROUP:		T		A		B		C	
	SEX:	NO. ANIMALS:	M	F	M	F	M	F	M	F
			60	60	60	60	60	60	60	60
LIVER										
- ACCENTUATED LOBULAR PATTERN					1					
- CONGESTION							1			
- ENLARGED			1	3		2		2		2
- REDUCED IN SIZE			1		2					
- IRREGULAR SURFACE				1		1				1
- THINNED								1		
- PALENESS			1	2	3	3	1	3	1	4
- WHITISH COLOR					1					
- BROWNISH COLOR										1
- REDDISH COLOR					1					
- FOCUS			1				1			
- FOCI				2	3	1				6
- CYST			1				1			
- MASS			5		9		4		3	
- NODULE			6	1	5	2	3	2	1	
- AREA			1							
PAROTID GLANDS										
- EDEMA					1					
MANDIBULAR GLANDS										
- REDUCED IN SIZE				1		3	1			1
- EDEMA						1				
CERVICAL LYMPH NODE										
- ENLARGED			1	5	3		2	1	3	
- REDDISH COLOR				1						
- NOT FOUND				1						
PITUITARY GLAND										
- REDDISH COLOR										1
STERNUM (- B.M.)										
SKELETAL MUSCLE										
- MASS				1						
SCIATIC NERVE										

Table 20. b. (Continued) Summary Table of Macroscopic Pathology.

SKIN										
- ALOPECIA				1						
- SORE		1								
- NODULE				1						
- ULCERATION								1		
- AREA				1		1				
MAMMARY GLAND										
URINARY BLADDER										
- DILATATION		1				2			2	
- THICKENED		1								
- CONTENTS		4								
SPINAL CORD										
ESOPHAGUS										
- DILATATION								1		
- CONTENTS								1		
- NODULE			1					1		
- PERFORATION		3	2			2		1		2
ADRENAL GLANDS										
- REDUCED IN SIZE		1		1				1		
- WHITISH COLOR				1						1
- REDDISH COLOR					1					
- FOCI										1
- CYST		1						1		
THYROID GLAND										
- ENLARGED		1		1						1
- WHITISH COLOR										1
TRACHEA										
PANCREAS										
- REDDISH COLOR		1		1		1				
- NODULE						1				
- EDEMA		1	3	1	2	2	4	1	3	

Table 20. d. Summary Table of Macroscopic Pathology

ORGAN/FINDING	DOSE GROUP:		A		B		C		
	SEX:	T	M	F	M	F	M	F	
	NO. ANIMALS:	60	60	60	60	60	60	60	
UTERUS									
- ENLARGED			16		23		13		15
- DILATATION			5		2		2		4
- IRREGULAR SURFACE			1		1		2		5
- THICKENED									1
- FIRM							1		
- CONTENTS			3						2
- FOCUS									1
- CYST			14		17		19		21
- MASS			7		5		5		2
- NODULE			3		1		3		7
VAGINA									
- NODULE					1				
SEMINAL VESICLES									
- ENLARGED			21		20		15		17
- REDUCED IN SIZE					2		1		2
- WHITISH COLOR			1		1				
- BROWNISH COLOR			2						1
- REDDISH COLOR			2				2		1
- GREENISH COLOR			1				1		
EYES									
- CLOUDY									1
- WHITISH COLOR					1		1	1	
- MASS					1				
OPTIC NERVE									
GALL BLADDER									
- DILATATION					3				1
- DEPOSIT									1
SUBCUTANEOUS TISSUE									
- DEPOSIT					1				
- MASS			5	1	1	1			
- NODULE							1		
- EDEMA			2		2	2	3		
LYMPH NODES									
- ENLARGED			5	7	2	4	3	4	2
- REDUCED IN SIZE							1		
- REDDISH COLOR							1	1	1
- GREENISH COLOR									1
OTHER ORGANS/TISSUES									
- ENLARGED						2			2
- THICKENED			1			1			1
- BLACKISH COLOR					1		2		3
- REDDISH COLOR						1			2
- ADHESION									1
- CONTENTS					1				1
- FOCI				1			1		1
- MASS			1				1		1
- NODULE				1					1
- EDEMA						1			

END OF REPORT SECTION

B. Summary Tables of Non-Neoplastic Findings in Mouse Studies.

(Tabular Units: Incidence of Animals with Non-Neoplastic Lesions by Organ/Dose Group/Sex).

Dose Groups: T = Control, A = 1 mg/kg/day, B = 5 mg/kg/day, C = 25 mg/kg/day.

Sex: M = Male, F = Female.

Mouse Pathology References: Vol. 45, pp. 28-30--Tables: Appendix I, pp. 95-101.

CONCLUSIONS: Kidneys showed elevated incidence of focal nephropathy among the female mice of the mid (10/24 [42 %]) and high dose (18/60 [30 %]) groups compared with the mean control group's incidence (5/60 [8 %]). It has been sponsor's experience that this is within the range of spontaneous incidence (50-60 %) in old mice; however, sponsor feels "that the relationship of the increased incidence of nephropathy to the treatment with the test substance cannot be ruled out". Sponsor feels that "the severity and morphological character of the other changes encountered in the kidneys, are comparable in all groups including the control group". Since these changes are commonly recorded findings in mice, they are considered to be of no toxicological significance.

Liver terminology and criteria for morphological diagnosis of the proliferative hepatic lesions were as reported by Maronpot, *et al.* (Tox. Path. 14:263-273, 1986) The diagnostic terms used were foci of cellular alteration and nodular hyperplasia; these non-neoplastic alterations were found among mice of all groups without indication of a dose-related relationship in incidence or morphological character of the hepatic cells. Other liver changes were comparable among all groups including the controls.

Other organs and tissues showed changes which are commonly recorded senility changes in mice, and are therefore not considered to be of toxicological significance.

Summary tables and discussions of neoplastic findings in the mouse studies begin on page 75. Codes for animal status at necropsy are found on page 84.

Table 21. a. Summary of Incidence of Non-Neoplastic Lesions

ORGAN/FINDING	DOSE GROUP: SEX: NO. ANIMALS:	T		A		B		C	
		M	F	M	F	M	F	M	F
AORTA	NO. EXAM.: 60	60	60	8	14	9	12	58	60
- CARTILAGENOUS METAP.									1
HEART	NO. EXAM.: 60	60	60	8	14	9	12	60	60
- AMYLOIDOSIS.		1	2	1		3		3	3
- MYOCARDIAL DEG. FIBR.		1	1					1	2
- CHRONIC PERICARDITIS								1	
- PERIVASCULITIS, MYOC.									1
- MYOCARDITIS.			1						
CECUM	NO. EXAM.: 60	60	60	16	15	14	18	58	59
- AMYLOIDOSIS.		2	1	7	1	5	4	5	5
- PSEUDOMELANOSIS.			1	7	2	1	6	4	
COLON	NO. EXAM.: 60	60	60	8	13	9	12	60	60
- AMYLOIDOSIS.			1					1	1
RECTUM	NO. EXAM.: 60	60	59	8	13	9	12	60	60
- SQUAMOUS METAPLASIA.			1						
BRAIN	NO. EXAM.: 60	60	60	8	14	9	12	60	60
- VACUOLAT. WHIT. MATTER		4	18		1		3	3	11
OVARIES	NO. EXAM.: 60	60	60	47		44		60	60
- PARAOVARIAN CYST.		36		37		27		35	
- FOLLICULAR CYSTS.		25		28		28		38	
- AMYLOIDOSIS.		7		3		10		12	
- NO CORPORA LUTEA.		8		9		6		14	
- FOLLICULAR ATRESIA.		17		10		13		21	
- CHRONIC VASCULITIS.		1							
- NO GROWING FOLLICLES		1							
- CEROID LADEN MACROP.									1
DUODENUM	NO. EXAM.: 60	60	60	8	15	6	12	59	60
- AMYLOIDOSIS.		7	11	3	6	1	1	11	15
- CHRONIC INFLAMMATION									1
JEJUNUM	NO. EXAM.: 60	60	60	8	15	6	12	60	60
- AMYLOIDOSIS.		7	10	3	2	2	1	9	12
ILEUM	NO. EXAM.: 60	60	59	8	14	8	12	57	59
- AMYLOIDOSIS.		9	13	3	4	4	1	19	18
STOMACH	NO. EXAM.: 60	60	60	11	15	9	14	59	60
- EPITH. CEL. HYPERPLAS.		2		2		1			
- AMYLOIDOSIS.		1				1			1
- GLANDULAR DILATATION				1					
MESENT. LYMPH NODE	NO. EXAM.: 56	54	54	11	17	6	18	57	56
- REACTIV LYMPH NODE.		3		1		1		1	
- CEROID LADEN MACROP.		19	10	3		1		10	16
- REACTIVE HISTIOCYTO.		25	26	8	5	3	6	23	21
- ERYTHROPHAGOCYTOSIS.		4	2	1		1	1	9	3
- CONGESTION.		4	5	6		2	3	6	8
- AMYLOIDOSIS.		7	8	3	4	3	3	10	13
- DILATED LYMPH SINUS.		3				1		3	3
- LYMPHOID CELL HYPER.				1					
FEMORAL BONE(-B.M)	NO. EXAM.: 60	60	58	7	14	9	12	59	59
- HYPERP. OF MYELO. ELE.				3	1	1	5		
LIVER	NO. EXAM.: 60	60	60	23	16	16	15	60	60
- STEATOSIS.		9	2	1		2		7	
- TELANGIECTASIS.		1							1
- MONONUCLEAR CEL. ACC.		19	21	3	1	3	2	25	23
- NODULAR HYPERPLASIA.		7		5		3	1	2	
- HEPATOCELLULAR CYSTS		1							
- PERICHOLANGITIS.		3							
- FOC. CHR. HEP. CEL. NEC.		2	6		1		1	4	6
- AMYLOIDOSIS, POR. TR.		1	4	2	1	3	2	2	5
- HEPATIC LEUCOCYTOSIS		2							
- ALTER. CEL. FOC., ACID.		1	1	1			1		
- KUPF. CEL. LOAD. PIGME.									1
- PFLIOSIS.									1
- CONGESTION.			1						
- ALTER. CEL. FOCI, VACU.						2			
- EXTRAMEDUL. HAEMATOP.					1				
- LARGE CYST WITH FIB.					1				

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Table 21. b. Summary of Incidence of Non-Neoplastic Lesions

ORGAN/FINDING	DOSE GROUP: SEX: NO. ANIMALS:	T		A		B		C	
		M	F	M	F	M	F	M	F
PAROTID GLANDS	NO. EXAM.:	60	60	8	14	9	12	60	60
- INTERS. MONO. CEL. AGG.		6	6		2			8	2
- AMYLOIDOSIS.		1	10	2	1	4	1	8	12
MANDIBULAR GLANDS	NO. EXAM.:	60	59	8	17	10	12	60	60
- INTERS. MONO. CEL. AGG.		21	4	2	3		1	16	10
CERVICAL LYMPH NODE	NO. EXAM.:	56	57	11	10	8	11	53	56
- REACTIVE HISTIOCYTO.		13	3		7		1	8	5
- CEROID LADEN MACROP.		25	23		1		3	28	18
- REACTIVE LYMPH NODE.		1	1		1				
- PLASMOCYTOSIS.		1			1				
- CONGESTION.			1		1				
- ERYTHROPHAGOCYTOSIS.			1						
PITUITARY GLAND	NO. EXAM.:	60	60	8	16	8	12	60	59
- DEVELOPMENTAL CYST.			1						
- SINUSOIDAL ECTASIA.									1
STERNUM (+ B.M.)	NO. EXAM.:	60	60	18	16	18	14	60	60
- OSTEOSCLEROSIS.			6						1
- DEFORMATION.			3	7	10	2	9	2	10
- HYPERPL. MYELOID ELE.					1	1		4	
SKELTAL MUSCLE	NO. EXAM.:	60	60	8	16	9	12	60	60
- LYMPHOCTIC MYOSITIS			1						
- MYODEGENER. AND FIBR.			1						
SKIN	NO. EXAM.:	59	59	11	16	9	12	60	59
- SUPPURATIVE CELLULI.					3		1	1	
- EPIDERMAL CYST.					1				
URINARY BLADDER	NO. EXAM.:	60	57	8	14	9	12	60	59
- ACUTE CYSTITIS.			1		1				
- FOLLICULAR CYSTITIS.				12	1	1	1	3	1
ESOPHAGUS	NO. EXAM.:	60	60	8	14	9	12	60	60
- NECROTISING INFLAMM.			2	2		1	1		2
- AMORPH. SUBS. IN LUMEN.				1					
- ABSCESS.							1		
ADRENAL GLANDS	NO. EXAM.:	58	60	10	16	10	12	60	59
- AMYLOIDOSIS.			6	9	4	1	5	1	11
- CEROID DEGENERATION.			2	9		1	1	1	7
- CYSTDEGENERATION.			1						
- FOCI CORT. CEL. ALTER.									2
- GLOMERUL. CEL. HYPERP.				11		1		2	
- PELIOSIS.				3		2		4	
- PARACORTICAL CYST.							1		
THYROID GLAND	NO. EXAM.:	59	60	9	13	8	12	59	60
- CYSTIC FOLLICLES.			8	8		1	2	3	6
- AMYLOIDOSIS.			1	9	1	1	2	1	6
- NODULAR HYPERPLASIA.					1				
TRACHEA	NO. EXAM.:	60	60	8	16	9	12	60	60
- NECROTISING INFLAMM.			1						
PANCREAS	NO. EXAM.:	60	60	10	15	10	14	60	60
- LIPOMATOSIS.			4						3
- AMYLOIDOSIS.			1			1			
- OEDEMA.			1	3	1	1	2	4	1
- CHRONIC PANCREATITIS			1						
- INTERST. MONO. CEL. AG.									1
- INTERST. FIBROPLASIA.									
- CONGESTION.					1	1			
SPLEEN	NO. EXAM.:	60	60	11	21	11	16	60	60
- EXTRAMEDUL. HAEMATOP.			2	2	1	2		2	1
- MACROPH. LOAD. HAEMOS.			1						
- LYMPHORET. CEL. HYPER.			5	4	5	7	2	3	1
- CONGESTION.			1			2			3
- AMYLOIDOSIS.			4	3	2	4	2	3	5
- AGGREG. FOAM. MACROPH.					1				
THYMUS	NO. EXAM.:	51	53	4	13	5	11	44	52
- INVOLUTION.			50	50	4	11	4	8	43

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Table 21. c. Summary of Incidence of Non-Neoplastic Lesions

ORGAN/FINDING	DOSE GROUP: SEX: NO. ANIMALS:	T		A		B		C	
		M	F	M	F	M	F	M	F
		60	60	60	60	60	60	60	60
THORACIC LYMPH NODE	NO. EXAM.:	51	53	3	10	3	0	47	49
- CEROID LADEN MACRO.		9	4	1			1	2	5
- REACTIVE LYMPH NODE.		2	5		1		1	1	3
- REACTIVE HISTIOCYTO.		11	18		1		1	11	3
- LYMPHOID CELL HYPER.		1	1		2				
- ERYTHROPHAGOCYTOSIS.								2	1
- CONGESTION.									1
LUNGS	NO. EXAM.:	60	60	14	17	13	16	60	60
- ALVEOLAR HAEMORRHAG.		12	10	3	1	1	3	9	6
- CHRON. INTERST. PNEUM.		7	10	3	4	1	5	9	12
- ADENOMATOUS HYPERPL.		3	1					2	1
- ALVEOLAR OEDEMA.						1	1	2	
- AMYLOIDOSIS.			1						
- GRANULOMA.									1
- CONGESTION.					2				
- SUPPURATIVE PNEUMON.							1		
- FOAMY ALVEOL. MACROP.				1					
KIDNEYS	NO. EXAM.:	60	60	18	22	14	24	60	60
- CORTI. AREA. TUBU. BAS.		17	6	2	1	3	5	25	14
- INTERS. MONON. CEL. AG.		37	17	2	4	5	9	38	19
- PELVIC DILATATION.		2					1	3	
- AMYLOIDOSIS, GLOMER.		6	1	7	5	5	9	11	16
- RENAL CYST.		2	1	5		3	1	6	3
- AMYLOIDOSIS, INTERS.		1	6		3			1	3
- FOCAL NEPHROPATHY.		3	5	7	5	2	10	5	10
- TUBULAR CYST DILATA.		2							
- TUB. EPITH. CEL. HYPER.			1	1					2
- ATROPH. CONTRACT. KID.							1		1
- PERIGLOMERULAR FIBR.									1
- TUBULAR LIPOFUSCINO.					1				2
- SUPPURATIVE NEPHRIT.				1					
TESTES	NO. EXAM.:	60		14		16		60	
- AMYLOIDOSIS.		3		5		3		7	
- DEGENER. SEMI. TUBULES		6		5		7		7	
- ARREST SPERMATOGENE.		6		5		7		7	
- GRANULOMAT. ORCHITIS.		1							
- INTERSTIT. CEL. HYPER.				2		4		3	
- CYST DEGENERATION.						1			
EPIDIDYMISS	NO. EXAM.:	60		10		10		60	
- OLIGOSERMIA.		6		1		3		7	
- SPERMATIC GRANULOMA.				1					
PROSTATE	NO. EXAM.:	60		9		10		59	
- EPITHELIAL CEL. ATRO.		14		1		1		5	
- CHRONIC PROSTATITIS.		1							
- ACUTE PROSTATITIS.		1							
- SUPPURATIVE PROSTAT.				1					
UTERUS	NO. EXAM.:		60		46		40		60
- ENDOMET. CYST. HYPERP.			46		38		34		50
- ENDOMET. EPITH. ATROP.			2		2		2		4
VAGINA	NO. EXAM.:		58		18		12		59
- HYPER. BASAL CEL. LAY.			41		13		6		48
- HYPERKERATINIZATION.			33		10		6		31
- MUCIFIC. OF VAG. EPIT.			5		1				2
- EPITHELIAL CEL. ATRO.			4		2		4		6
SEMINAL VESICLES	NO. EXAM.:		60		26		25		60
- INCREASE SECRET. ACT.			18		20		16		16
- SEMINAL VESICULITIS.			1		1				
EYES	NO. EXAM.:	60	60	10	14	10	12	60	60
- CORNEAL OEDEMA.									1
- FOCAL KERATITIS.							1		
- CHRON. GRANULOM. LESI.					1				

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C. Summary Tables of Non-Neoplastic Findings in Mouse Studies

(Tabular Units: Incidence of Animals with Non-Neoplastic Lesions by Organ/Dose Group/Sex, as Percent of Total Animals Examined.)

Dose Groups: T = Control, A = 1 mg/kg/day, B = 5 mg/kg/day, C = 25 mg/kg/day.

Sex: M = Male, F = Female.

Mouse Pathology References: Vol. 45, pp. 28-30. Tables: Appendix I, pp. 102-108.

Kidneys: There was increased incidence of focal nephropathy among the female mice of the mid- and high- dose groups

Table 22. a. Incidence of Non-Neoplastic Lesions (As % of Total)

DOSE GROUP: SEX: NO. ANIMALS:	T		A		B		C	
	M	F	M	F	M	F	M	F
AORTA	60	60	60	60	60	60	60	60
- CARTILAGINOUS METAP.								1.7
HEART	60	60	60	60	60	60	60	60
- ARTERIOSCLEROSIS	1.7	3.3	n(10)		n(10)		5.0	5.0
- MYOCARDIAL DEG. FIBR.	1.7	1.7					1.7	3.3
- CHRONIC PERICARDITIS							1.7	
- PERIVASCULITIS, MYOC.		3.3						1.7
- MYOCARDITIS		1.7						
CECUM	60	60	60	60	60	60	60	60
- ARTERIOSCLEROSIS	1.3	1.7	63.0	6.7	25.7	22.3	0.1	0.5
- PSEUDOPOLYPOSIS		5.7	43.0	13.3	7.1	33.3	6.9	
COLON	60	60	60	60	60	60	60	60
- ARTERIOSCLEROSIS	1.7						1.7	1.7
RECTUM	60	60	60	60	60	60	60	60
- SQUAMOUS METAPLASIA	1.7							
BRAIN	60	60	60	60	60	60	60	60
- VACUOLAT. WHIT. MATTER	6.7	30.0		7.1	25.0		5.0	10.3
OVARIES	60	60	60	60	60	60	60	60
- PARAOVARIAN CYST	60.0		78.7		81.6		58.3	
- FOLLICULAR CYSTS	41.7		55.6		62.6		63.3	
- ARTERIOSCLEROSIS	11.7		6.6		22.7		20.0	
- NO CORPORA LUTEA	13.3		19.2		12.6		23.3	
- FOLLICULAR ATRESIA	20.3		21.3		29.6		33.0	
- CHRONIC VASCULITIS	1.7							
- NO GROWING FOLLICLES	1.7							
- CYSTIC LADEN MACROP.								1.7
DUODENUM	60	60	60	60	60	60	60	60
- ARTERIOSCLEROSIS	11.7	16.3	n(10)	26.7	n(10)	0.3	10.6	15.0
- CHRONIC INFLAMMATION							1.7	
JEJUNUM	60	60	60	60	60	60	60	60
- ARTERIOSCLEROSIS	12.7	16.7	n(10)	13.3	n(10)	0.3	15.0	20.0
ILEUM	60	60	60	60	60	60	60	60
- ARTERIOSCLEROSIS	15.0	22.0	n(10)	28.6	n(10)	0.3	33.3	38.3
STOMACH	60	60	60	60	60	60	60	60
- EPITH. CEL. HYPERPLAS.		3.3	18.2		7.1			
- ARTERIOSCLEROSIS		1.7			n(10)			1.7
- GLANDULAR DILATATION				6.7				
MESENT. LYMPH NODE	60	60	60	60	60	60	60	60
- REACTIVE LYMPH NODE	5.4		5.9		5.6		1.8	
- CYSTIC LADEN MACROP.	33.9	10.3	17.7		n(10)	14.7	17.5	28.6
- REACTIVE HISTIOCYTOSIS	44.6	68.2	72.7	29.4	n(10)	33.3	60.4	37.5
- ERYTHROPHAGOCYTOSIS	7.1	3.7	5.9		n(10)	5.6	15.0	3.4
- CONGESTION	7.1	9.3	35.3		n(10)	14.7	10.5	14.3
- ARTERIOSCLEROSIS	12.5	14.0	27.3	23.5	n(10)	16.7	17.5	23.2
- DILATED LYMPH SINUS	5.4					5.6	5.3	5.4
- LYMPHOID CELL HYPER.			5.9					
FEMORAL BONE (L.F.M.)	60	60	60	60	60	60	60	60
- HYPERP. OF MYELO. ELEM.			n(10)	7.1	n(10)	41.7		
LIVER	60	60	60	60	60	60	60	60
- STEATOSIS	15.0	3.3	4.4		13.3		11.7	
- TELANGIECTASIS	1.7						1.7	
- MONONUCLEAR CEL. ACC.	31.7	35.0	13.0	6.3	18.8	13.3	41.7	38.3
- NODULAR HYPERPLASIA	11.7		21.7		18.8	6.7	3.3	
- HEPATOCELLULAR CYSTS	1.7							
- PERICHOLANGITIS	5.0				1			
- FOC. CHR. HEP. CEL. NEC.	3.3	10.0		6.3		4.7	6.7	10.0
- ARTERIOSCLEROSIS, PORT.	1.7	6.7	0.7	6.3	18.8	13.3	3.3	0.3
- HEPATIC LEUCOCYTOSIS	3.3							
- ALTER. CEL. FOC. ACID.	1.7	1.7	4.4			4.7		
- KUPF. CEL. LOAD. PIGME.								1.7
- PELIOSIS								1.7
- CONGESTION		1.7						
- ALTER. CEL. FOCI. VACU.					12.5			
- EXTRAMEDULL. HEMATOPO.				6.3				
- LARGE CYST WITH FIB.				6.3				

Table 22. b. Incidence of Non-Neoplastic Lesions (As % of Total)

DOSE GROUP: SEX: NO. ANIMALS:	T		A		B		C	
	M	F	M	F	M	F	M	F
	60	60	60	60	60	60	60	60
PAROTID GLANDS	60	60	8	14	9	12	60	60
- INTERS. MONO. CEL. AGG.	10.0	6.7		14.3			13.3	3.3
- AMYLOIDOSIS.	1.7	16.7	n<10	7.1	n<10	8.3	13.3	20.0
MANDIBULAR GLANDS	60	59	8	17	10	12	60	60
- INTERS. MONO. CEL. AGG.	35.0	6.8	n<10	17.7		8.3	26.7	16.7
CERVICAL LYMPH NODE	54	57	11	10	8	11	53	56
- REACTIVE HISTIOCYTO.	23.2	5.3	63.6			9.1	15.1	8.9
- CEROID LADEN MACROP.	44.6	40.4		10.0		27.3	52.8	32.1
- REACTIVE LYMPH NODE.	1.8	1.8	9.1					
- PLASMOCYTOSIS.	1.8		9.1					
- CONGESTION.		1.8		10.0				
- ERYTHROPHAGOCYTOSIS.		1.8						
PITUITARY GLAND	60	60	8	14	8	12	60	59
- DEVELOPMENTAL CYST.	1.7							
- SIMUSOIDAL ECTASIA.								1.7
STERNUM (+ B.M.)	60	60	10	16	18	16	60	60
- OSTEOSCLEROSIS.	10.0							1.7
- DEFORMATION.	5.0	11.7	55.6	12.5	50.0	14.3	14.7	0.2
- HYPERPL. MYELOID ELE.			5.6	6.3		28.6		
SKELETAL MUSCLE	60	60	8	14	9	12	60	60
- LYMPHOCYTIC MYOSITIS	1.7							
- MYODEGENER. AND FIBR.	1.7							
SKIN	59	59	11	16	9	12	60	59
- SUPPURATIVE CELLULI.			27.3		n<10	0.3		
- EPIDERMAL CYST.			9.1					
URINARY BLADDER	60	57	8	14	9	12	60	59
- ACUTE CYSTITIS.	1.7		n<10					
- FOLLICULAR CYSTITIS.		21.1	n<10	7.1	n<10	25.0	1.7	25.4
ESOPHAGUS	60	60	8	14	9	12	60	60
- NECROTISING INFLAMM.	3.3	3.3		7.1		0.3	3.3	
- AMORPH. SUBS. IN LUME.		1.7						
- ABSCESS.					n<10			
ADRENAL GLANDS	58	60	10	14	10	12	60	59
- AMYLOIDOSIS.	10.3	15.0	40.0	7.1	50.0	8.3	18.3	20.3
- CEROID DEGENERATION.	3.3	15.0		7.1	10.0	8.3	11.7	25.4
- CYSTDEGENERATION.	1.7							
- FOCY CORT. CEL. ALTER.							3.3	
- GLOMERUL. CEL. HYPERP.		18.3		7.1		16.7		23.7
- PELIOSIS.		5.0		16.3		33.3		8.5
- PARACORTICAL CYST.					10.0			
THYROID GLAND	59	60	9	13	8	12	59	60
- CYSTIC FOLLICLES.	13.6	13.3		7.7	n<10	25.0	10.2	20.0
- AMYLOIDOSIS.	1.7	15.0	n<10	7.7	n<10	8.3	10.2	11.7
- NODULAR HYPERPLASIA.			n<10					
TRACHEA	60	60	8	14	9	12	60	60
- NECROTISING INFLAMM.	1.7							
PANCREAS	60	60	10	15	10	16	60	60
- LIPOMATOSIS.	6.7						5.0	
- AMYLOIDOSIS.	1.7			6.7				
- OEDEMA.	1.7	5.0	10.0	6.7	20.0	28.6	1.7	5.0
- CHRONIC PANCREATITIS	1.7							
- INTERST. MONO. CEL. AG.							1.7	
- INTERST. FIBROPLASIA.								1.7
- CONGESTION.			10.0	6.7				
SPLEEN	60	60	11	21	11	16	60	60
- EXTRAMEDUL. HAEMATOP.	3.3	3.3	27.3	9.5			3.3	1.7
- MACROPH. LOAD. HAEMOS.	1.7							
- LYMPHORET. CEL. HYPER.	8.3	6.7	45.5	33.3	18.2	18.8	1.7	5.0
- CONGESTION.	1.7			9.5				5.0
- AMYLOIDOSIS.	6.7	5.0	18.2	19.1	18.2	18.8	8.3	6.7
- AGGREG. FOAM. MACROPH.			9.1					
THYRUS	51	33	4	13	5	11	44	52
- INVOLUTION.	98.0	44.3	n<10	86.6	n<10	72.7	97.7	92.3

Table 22. c. Incidence of Non-Neoplastic Lesions (As % of Total)

DOSE GROUP: SEX: NO. ANIMALS:	T		A		B		C	
	M	F	M	F	M	F	M	F
	60	60	60	60	60	60	60	60
THORACIC LYMPH NODE	51	53	3	10	3	8	47	49
- GEROID LADEN MACRO.	17.7	7.6	n<10			n<10	4.3	10.2
- REACTIVE LYMPH NODE.	3.9	9.4		10.0		n<10	2.1	6.1
- REACTIVE HISTIOCYTO.	21.6	34.0		10.0		n<10	23.4	6.1
- LYMPHOID CELL HYPER.	2.0	1.9		20.0				
- ERYTHROPHAGOCYTOSIS.							4.3	2.0
- CONGESTION.								2.0
LUNGS	60	60	14	17	13	16	60	60
- ALVEOLAR HAEMORRHAG.	20.0	16.7	21.4	5.9	7.7	18.8	15.0	10.0
- CHRON. INTERST. PNEUM.	11.7	16.7	21.4	23.5	7.7	31.3	15.0	20.0
- ADENOMATOUS HYPERPL.	5.0	1.7					3.3	1.7
- ALVEOLAR OEDEMA.					7.7	6.3	3.3	
- AMYLOIDOSIS.		1.7						
- GRANULOMA.								1.7
- CONGESTION.				11.8		6.3		
- SUPPURATIVE PNEUMON.						6.3		
- FOAMY ALVEOL. MACROP.				7.1				
KIDNEYS	60	60	18	22	14	24	60	60
- CORTI. AREA. TUBU. BAS.	28.3	10.0	11.1	4.6	21.4	20.8	41.7	23.3
- INTERS. MONON. CEL. AG.	61.7	28.3	11.1	18.2	35.7	37.5	63.3	31.7
- PELVIC DILATATION.	3.3					4.2	5.0	
- AMYLOIDOSIS, GLOMER.	10.0	16.7	38.9	22.7	35.7	37.5	18.3	26.7
- RENAL CYST.	3.3	1.7	27.8		21.4	4.2	10.0	5.0
- AMYLOIDOSIS, INTERS.	1.7	10.0		13.6			1.7	5.0
- FOCAL NEPHROPATHY.	5.0	8.3	38.9	22.7	14.3	41.7	8.3	30.0
- TUBULAR CYST DILATA.	3.3							
- TUB. EPITH. CEL. HYPER.		1.7	5.6					3.3
- ATROPH. CONTRACT. KID.						4.2		1.7
- PERIGLOMERULAR FIBR.								1.7
- TUBULAR LIPOFUSCINO.				4.6				3.3
- SUPPURATIVE NEPHRIT.			5.6					
TESTES	60		14		16		60	
- AMYLOIDOSIS.	5.0		35.7		18.8		11.7	
- DEGENER. SEMI. TUBULES	10.0		35.7		43.8		11.7	
- ARREST SPERMATOGENE.	10.0		35.7		43.8		11.7	
- GRANULOMAT. ORCHITIS.	1.7							
- INTERSTIT. CEL. HYPER.			14.3		25.0		5.0	
- CYST DEGENERATION.					6.3			
EPIDIDYMHIS	60		10		10		60	
- OLIGOSERMIA.	10.0		10.0		30.0		11.7	
- SPERMATIC GRANULOMA.			10.0					
PROSTATE	60		9		10		59	
- EPITHELIAL CEL. ATRO.	23.3		n<10		10.0		8.5	
- CHRONIC PROSTATITIS.	1.7							
- ACUTE PROSTATITIS.	1.7							
- SUPPURATIVE PROSTAT.			n<10					
UTERUS	60	60	46	40	40	60	60	60
- ENDOMET. CYST. HYPERP.	76.7		82.6		85.0		81.3	
- ENDOMET. EPITH. ATROP.	3.3		4.4		5.0		6.7	
VAGINA	58	58	18	12	12	59	59	59
- HYPER. BASAL CEL. LAY.	70.7		72.2		50.0		81.4	
- HYPERKERATINIZATION.	56.9		55.6		50.0		52.5	
- MUCIFIC. OF VAG. EPIT.	8.6		5.6				3.4	
- EPITHELIAL CEL. ATRO.	6.9		11.1		33.3		10.2	
SEMINAL VESICLES	60	60	26	25	25	60	60	60
- INCREASE SECRET. ACT.	30.0		76.9		64.0		26.7	
- SEMINAL VESICULITIS.	1.7		3.9					
EYES	60	60	10	14	10	12	60	60
- CORNEAL OEDEMA.							1.7	
- FOCAL KERATITIS.					10.0			
- CHRON. GRANULOH. LESI.			10.0					

Table 22. d. Incidence of Non-Neoplastic Lesions (As % of Total)

DOSE GROUP:	T		A		B		C	
SEX:	M	F	M	F	M	F	M	F
NO. ANIMALS:	60	60	60	60	60	60	60	60
SUBCUTANEOUS TISSUE	7	1	1	4	2	4	.1	
- SUPPURATIVE CELLULI.	n<10		n<10	n<10				
- SUBCUTANEOUS ABSCESS	n<10							
- CYST.					n<10		n<10	
LYMPH NODES	5	7	2	4	3	5	2	3
- LYMPHOID CELL HYPER.	n<10	n<10	n<10		n<10	n<10		
- REACTIVE LYMPH NODE.	n<10	n<10		n<10				
- REACTIVE HISTIOCYTO.	n<10	n<10	n<10	n<10		n<10		n<10
- AMYLOIDOSIS.							n<10	
- CEROID LADEN MACROP.				n<10		n<10	n<10	
- CONGESTION.								n<10
- ERYTHROPHAGOCYTOSIS.						n<10		
OTHER ORGANS/TISSUES	3	2	1	3	5	2	9	4
- ACUTE URETHRITIS.	n<10							
- EPIT. CEL. HYPE. H. GLA.							n<10	
- CYST. DILAT., PREP. GL.					n<10		n<10	
- VASCULAR ECTAS., EAR.							n<10	
- CHROMODACRYORRHEA.				n<10	n<10	n<10		n<10
- FOCAL CHRONIC PERIT.								n<10

END OF REPORT SECTION

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D. Summary of the Incidence of Neoplastic Findings in Mouse Studies.

(Tabular Units, First Page, Number of Animals with Neoplastic Lesions by Organ/Dose Group/Sex, for 78 Weeks, as Previously Defined).

(Tabular Units, Next Two Pages, by Number of Animals with Neoplastic Lesions as Above, but Grouped by Findings Per 200-Day Intervals).

Dose Groups: T= Control, A= 1 mg/kg/day, B=5 mg/kg/day, C=25 mg/kg/day.

Sex: M = Male, F = Female

Mouse Pathology References: Vol. 45, pp. 28-30; Tables: Appendix I, pp. 109-114

Liver terminology and criteria were as reported by Moronpot, et al. (Tox. Path.14:263-273, 1986). The diagnostic terms used were hepatocellular adenoma and hepatocellular carcinoma. The incidence and morphological types of hepatic tumors reported by sponsor in all groups were similar to those seen in old mice, without any indication of treatment or dose relationship.

Lungs were free of treatment related effects in the distribution of tumors.

Lymphoreticular tissue: Lymphomas were found primarily in the liver, spleen nodes, and also in the heart, thymus, lungs, salivary glands and kidneys of some animals. The occurrences of these tumors were found in both controls and treated animals and were not affected by drug treatment.

Other neoplastic lesions: The usually reported incidences in the female genital organs and the skin occurred commonly without drug treatment related variations.

Table 23. b. Neoplastic Findings Per 200 Day Intervals
Days 1-200

ORGAN/FINDING	DOSE GROUP:									
	SEX		T		A		B		C	
	M	F	M	F	M	F	M	F	M	F
	NO. ANIMALS:		3	3	1	5	1	2	3	1
HEMOLYMPHORET. SYS.	NO. EXAM.:			1		1				
- MALIGNANT LYMPH.P.D.				1		1				
THYMUS	NO. EXAM.:		3	3	1	5	1	2	2	1
- THYMOHA.										1
SUBCUTANEOUS TISSUE	NO. EXAM.:		3	1				1	1	
- HISTIOCYTOMA.				1				1		

Table 23. c. Neoplastic Findings Per 200 Day Intervals
Days 201-400

ORGAN/FINDING	DOSE GROUP:									
	SEX		T		A		B		C	
	M	F	M	F	M	F	M	F	M	F
	NO. ANIMALS:		3	4	2	2	2	5	3	2
HEMOLYMPHORET. SYS.	NO. EXAM.:		1					2		
- MALIGNANT LYMPH.P.D.			1					1		
- MYELOID LEUKEMIA.								1		
OVARIES	NO. EXAM.:			4		2		5		2
- GRANULOSA CELL TUMO.				1						
SKELETAL MUSCLE	NO. EXAM.:		3	4	2	2	2	5	3	2
- HISTIOCYTOMA.				1						
LUNGS	NO. EXAM.:		3	4	2	2	2	5	3	2
- BRONCHI./ALVEO.ADEN.				1						
UTERUS	NO. EXAM.:			4		2		5		2
- ENDOMETR. STRO. POLYP.				1						
- ENDOMETRIAL SARCOMA.								1		
SUBCUTANEOUS TISSUE	NO. EXAM.:		1			2				
- HISTIOCYTOMA.						1				
- FIBROHISTIOCYTOMA.			1							
OTHER ORGANS/TISSUES	NO. EXAM.:			1		1		1		3
- HAEMANGIOMA.								1		

Table 23. d. Neoplastic Findings Per 200 Day Intervals
Days 401-563

ORGAN/FINDING	DOSE GROUP: SEX : NO. ANIMALS:	T		A		B		C	
		M	F	M	F	M	F	M	F
		1	10	5	7	6	5	5	12
HEMOLYMPHRET. SYS.	NO. EXAM.:		2			1	1		
- MALIGNANT LYMPH. P.D.						1	1		
- MALIGNANT LYMPH. H.T.			2						
OVARIES	NO. EXAM.:		10		6		5		12
- PAPILLARY CYSTADENO.			1						
LIVER	NO. EXAM.:	1	10	5	7	6	5	5	12
- HEPATOCELLULAR ADEN.				2				2	
- ENDOMETR. SARCO., MET.			2		2		1		
URINARY BLADDER	NO. EXAM.:	1	10	5	7	6	5	5	12
- ENDOMETR. SARCO., META.					1				
PANCREAS	NO. EXAM.:	1	10	5	7	6	5	5	12
- ENDOMETR. SARCO., META.					1				
THYMUS	NO. EXAM.:	1	10	1	7	4	5	3	12
- THYMOMA.					1				
LUNGS	NO. EXAM.:	1	10	5	7	6	5	5	12
- BRONCHI./ALVEO. ADEN.			2						
KIDNEYS	NO. EXAM.:	1	10	5	7	6	5	5	12
- ENDOMETR. SARCO., META.							1		
UTERUS	NO. EXAM.:		10		7		5		12
- LEIOMYOSARCOMA.							1		
- ENDOMETRIAL SARCOMA.			3		2		1		
- HAEMANGIOMA.									1
VAGINA	NO. EXAM.:		10		7		5		12
- HAEMANGIOPERICYTOMA.									1
SUBCUTANEOUS TISSUE	NO. EXAM.:	1		1	2	2	2		
- FIBROHISTIOCYTOMA.			1						
LYMPH NODES	NO. EXAM.:		2		1	1	1	1	1
- ENDOMETR. SARCO., META.					1				
OTHER ORGANS/TISSUES	NO. EXAM.:					2		1	1
- LIPOMA, ADIPOSE TIS.								1	

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E. Summary Tables of Neoplastic Findings in Mouse Studies

(Tabular Units: Incidence of Animals with Neoplastic Lesions by Organ / Dose Group /Sex, as Percent of Total Animals Examined)

Dose Groups:

T= Control, A = 1 mg/kg/day, B= 5 mg/kg/day, C = 25 mg/kg/day

Sex: M = Male, F = Female

Mouse Pathology References

Vol. = 45, pp. 28-30

Tables on pages 115-116

F. Summary of the Incidences of Neoplastic Findings in Mouse Studies Grouped Per 200-Day Intervals

Tabular Units: Incidence of Animals with Neoplastic Lesions by Organ / Dose Group/ Sex, as Percent of Total Animals Examined)

Dose Groups:

T=Control, A=1 mg/kg/day, B=5 mg/kg/day, C=25 mg/day

Sex: M=Male, F=Female

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Mouse Pathology References

Vol. = 45, pp. 28-30

Tables on pages 117-120

Table 25. a. Neoplastic Findings Per 200 Day Intervals
Days 1-200 (as Percent of Total Animals Examined)

DAYS : 1- 200									
NEOPLASTIC FINDINGS PER 200 DAYS INTERVALS.									
DOSE GROUP:	T		A		B		C		
SEX :	M	F	M	F	M	F	M	F	
NO. ANIMALS:	3	3	1	5	1	2	3	1	
HEMOLYMPHORET. SYS.		1		1					
- MALIGNANT LYMPH.P.D.		n<10		n<10					
THYMUS	3	3	1	5	1	2	2	1	
- THYMOOMA.								n<10	
SUBCUTANEOUS TISSUE	3	1				1	1		
- HISTIOCYTOMA.		n<10				n<10			

Table 25. b. Neoplastic Findings Per 200 Day Intervals
Days 201-400 (as Percent of Total Animals Examined)

DAYS : 201- 400									
NEOPLASTIC FINDINGS PER 200 DAYS INTERVALS.									
DOSE GROUP:	T		A		B		C		
SEX :	M	F	M	F	M	F	M	F	
NO. ANIMALS:	3	4	2	2	2	5	3	2	
HEMOLYMPHORET. SYS.	1					2			
- MALIGNANT LYMPH.P.D.	n<10					n<10			
- MYELOID LEUKEMIA.						n<10			
OVARIES		4		2		5		1	
- GRANULOSA CELL TUMO.		n<10							
SKELETAL MUSCLE	3	4	2	2	2	5	3	2	
- HISTIOCYTOMA.		n<10							
LUNGS	3	4	2	2	2	5	3	2	
- BRONCHI./ALVEO.ADEN.	n<10								
UTERUS		4		2		5		2	
- ENDOMETR.STRO.POLYP.		n<10							
- ENDOMETRIAL SARCOMA.						n<10			
SUBCUTANEOUS TISSUE	1			2					
- HISTIOCYTOMA.				n<10					
- FIBROHISTIOCYTOMA.	n<10								
OTHER ORGANS/TISSUES		1		1	1		3		
- HAEMANGIOMA.					n<10				

Table 25. c. Neoplastic Findings Per 200 Day Intervals
Days 401-563 (as Percent of Total Animals Examined)

DAYS 401- 563
 NEOPLASTIC FINDINGS PER 200 DAYS INTERVALS.

DOSE GROUP:	T		A		B		C	
SEX :	M	F	M	F	M	F	M	F
NO. ANIMALS:	1	10	5	7	6	5	5	12
HEMOLYMPHORET. SYS.		2			1	1		
- MALIGNANT LYMPH. P.D.					n<10	n<10		
- MALIGNANT LYMPH. H.T.		n<10						
OVARIES		10		6		5		12
- PAPILLARY CYSTADENO.		10.0						
LIVER	1	10	5	7	6	5	5	12
- HEPATOCELLULAR ADEN.			n<10				n<10	
- ENDOMETR. SARCO., MET.		20.0		n<10		n<10		
URINARY BLADDER	1	10	5	7	6	5	5	12
- ENDOMETR. SARCO., META.				n<10				
PANCREAS	1	10	5	7	6	5	5	12
- ENDOMETR. SARCO., META.				n<10				
THYMUS	1	10	1	7	4	5	3	12
- THYMOOMA.				n<10				
LUNGS	1	10	5	7	6	5	5	12
- BRONCHI./ALVEO. ADEN.		20.0						
KIDNEYS	1	10	5	7	6	5	5	12
- ENDOMETR. SARCO., META.						n<10		
UTERUS		10		7		5		12
- LEIOMYOSARCOMA.						n<10		
- ENDOMETRIAL SARCOMA.		30.0		n<10		n<10		
- HAEMANGIOMA.								8.3
VAGINA		10		7		5		12
- HAEMANGIOPERICYTOMA.								8.3
SUBCUTANEOUS TISSUE	1		1	2	2	2		
- FIBROHISTIOCYTOMA.	n<10							
LYMPH NODES		2		1	1	1	1	1
- ENDOMETR. SARCO., META.				n<10				

DAYS 401- 563
 NEOPLASTIC FINDINGS PER 200 DAYS INTERVALS.

DOSE GROUP:	T		A		B		C	
SEX :	M	F	M	F	M	F	M	F
NO. ANIMALS:	1	10	5	7	6	5	5	12
OTHER ORGANS/TISSUES					2		1	1
- LIPOMA, ADIPOSE TIS.							n<10	

END OF REPORT SECTION

G. Summary Tables From Mouse Pathology Reports Evaluating Status of Neoplastic Lesions (Page 75 to 87)

Dose Groups:

T = Controls, A = 1 mg/kg/day, B = 5 mg/kg/day, C = 25 mg/kg/day.

Sex: M = Male, F = Female.

Mouse Pathology References: Vol. 45, pp. 121-124

Tables: Appendix I, pp. 121-124.

CODES FOR ANIMALS' STATUS AT NECROPSY

K0 = Terminal Sacrifice:

Incl. = Indicates inclusion of all sacrificed and moribund animals:

+ Includes all animals which are moribund or which have died.

CONCLUSIONS: Sponsor concludes (Neoplastic Findings, Page 16, Volume 45) that, "The number of animals with neoplasms (Tables 26 a and 27 a), the number of animals with more than one primary neoplasm (Tables 26 b and 27 b), and the number of benign and malignant tumors (26 e and 27 e) was comparable among all groups, including the control group." [The information within the parentheses was added by the reviewer.] Sponsor's data were slightly modified to illustrate the above in the table which follows as Table 26, Evaluation of Neoplastic Lesions. Examination of the data indicated that the different doses and methods of evaluation quite uniformly demonstrated the absence of neoplastic tissue occurrence.

In addition, sponsor found that the incidental tumor analysis, as assessed from the incidental neoplastic lesions observed at 200 day intervals in the animals found dead or sacrificed prematurely before the end of the study (see table of neoplastic findings in Appendix I), showed that the treatment with the test substance did not induce decreases in the latency of any of the neoplastic lesions in the treated animals.

Kidneys displayed an histiosarcoma in one control male mouse. The treated animals showed none.

Liver, as noted above, was described by the Maronpot criteria. The diagnostic terms used were hepatocellular adenoma and hepatocellular carcinoma. Sponsor reported that all of the tumors observed were similar to those seen in old mice and were without evidence of treatment or dose related effects.

There were no treatment-related effects on lung tumor distribution.

Table 26. Evaluation of the Number and Nature of Neoplastic Lesions at Necropsy Following Terminal Sacrifice (K0) in the Mouse Carcinogenicity Study (Volume 45, pp. 121-122)

a. Number of Animals with Neoplasms

DOSE GR:	T		A		B		C		TOTAL	
SEX :	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	53	43	39	43	37	43	49	45	173	174
NO. AFF.:	15	8	12	8	7	7	4	4	38	27
% :	28.3	18.6	30.8	18.6	18.9	16.3	8.2	8.9	21.4	15.5

b. Number of Animals with More than One Primary Neoplasm

DOSE GR:	T		A		B		C		TOTAL	
SEX :	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	53	43	39	43	37	43	49	45	178	174
NO. AFF.:	2	0	0	0	0	1	0	1	2	2
% :	3.8	0.0	0.0	0.0	0.0	2.3	0.0	2.2	1.1	1.2

c. Number of Animals with Metastases

DOSE GR:	T		A		B		C		TOTAL	
SEX :	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	53	43	39	43	37	43	49	45	178	174
NO. AFF.:	1	0	0	0	0	0	0	0	1	0
% :	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0

d. Number of Primary Neoplasms/Group/Sex

DOSE GR:	T		A		B		C		TOTAL	
SEX :	M	F	M	F	M	F	M	F	M	F
PRIM. T.:	17	8	12	8	7	8	4	5	40	29

e. Number of Benign, Unclassified, Malignant Neoplasms/Group/Sex

DOSE GR:	T		A		B		C		TOTAL	
SEX :	M	F	M	F	M	F	M	F	M	F
BENIGN :	15	6	12	6	7	8	3	2	37	22
UNCLASS.:	0	0	0	0	0	0	0	0	0	0
MALIGN.:	2	2	0	2	0	0	1	3	3	7

Table 27. Evaluation of the Number and Nature of Neoplastic Lesions at Necropsy Following Inclusion of all Sacrificed, Dead, and Moribund Animals (KO, Incl., and +) as Shown on pp. 123-124 of Vol. 45.

a. Number of Animals with Neoplasms

DOSE GR:	T		A		B		C		TOTAL	
	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	60	60	47	57	46	55	60	60	213	232
NO. AFF.:	18	19	14	13	9	13	7	7	48	52
%	30.0	31.7	29.8	22.8	19.6	23.6	11.7	11.7	22.5	22.4

b. Number of Animals with Greater than One Primary Neoplasm

DOSE GR:	T		A		B		C		TOTAL	
	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	60	60	47	57	46	55	60	60	213	232
NO. AFF.:	3	2	0	0	0	2	0	1	3	5
%	5.0	3.3	0.0	0.0	0.0	3.6	0.0	1.7	1.4	2.2

c. Number of Animals with Metastases

DOSE GR:	T		A		B		C		TOTAL	
	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	60	60	47	57	46	55	60	60	213	232
NO. AFF.:	1	2	0	2	0	1	0	0	1	5
%	1.7	3.3	0.0	3.5	0.0	1.8	0.0	0.0	0.5	2.2

d. Number of Primary Neoplasms/Group/Sex

DOSE GR:	T		A		B		C		TOTAL	
	M	F	M	F	M	F	M	F	M	F
PRIM. T.:	21	21	14	13	9	15	7	8	51	57

e. Number of Benign, Unclassified, Malignant Neoplasms /Group/Sex

DOSE GR:	T		A		B		C		TOTAL	
	M	F	M	F	M	F	M	F	M	F
BENIGN:	18	13	14	8	8	9	6	5	46	35
UNCLASS:	0	0	0	0	0	0	0	0	0	0
MALIGN.:	3	8	0	5	1	6	1	3	5	22

Lymphoreticular tissue, as lymphomas, involved primarily the liver, spleen and lymph nodes, and in some animals also the heart, thymus, lungs, salivary glands, and kidneys. These tumors occurred in both control and treated groups without modification of occurrence related to treatment.

Other neoplastic lesions: Other neoplastic lesions reported by sponsor included occurrence in female genital organs and skin. These lesions were the same as those commonly occurring in mice, which were without significant treatment related variation.

III. C.3 (Trandolapril) Potential Tumorigenic Effects in Long Term Administration (Gavage) to Rats for 104 Weeks:

Location of Data: Vols. 49 through 54 of 418 dated June 19, 1989.

Study No./Dates: 2026 TCR : 1/22/86 (FIRST DOSE) : 1/29/88 (END of NECROPSY).

Animals: Sprague Dawley CrI CD (SD) BR rats, supplied by Charles River . Mean initial body weights, males 208 g at 7 wk of age, and females 148 g at 6 wk of age.

Mode of Administration: Orally, by gavage.

Dose Levels: Table 27, showing dosing schedules and information is reproduced below from Vol. 49, p. 4 (using the uppermost of the of the paired numbers in the upper right hand corner.

Table 27.

Groups	Dose levels (mg/kg/d)	Sex	Animals Number	Cages Number
T	0	Males	C00018 to C00077	1 to 30
A	0.25	Males	C00078 to C00137	31 to 60
B	2	Males	C00138 to C00197	61 to 90
C	8	Males	C00198 to C00257	91 to 120

T	0	Females	C00258 to C00317	121 to 150
A	0.25	Females	C00318 to C00377	151 to 180
B	2	Females	C00378 to C00437	181 to 210
C	8	Females	C00438 to C00497	211 to 240

*Note the comment on dosing at the beginning of the results section.

Vehicle: The vehicle used was a 0.5% suspension of methyl cellulose in distilled water.

Test Substance Preparation: The test substance was diluted in the 0.5% methylcellulose solution and homogenized with magnetic stirrers and ultrasonic mixers.

Test Substance Stability: Good, using sponsor's directions.

Animal Management: Housed 2 animals per polycarbonate cages, changed weekly, autoclavable. Animals were fed *ad lib.* with a pellet diet, and had free access to tap water.

Test Substance Administration: The test substances were administered by gavage using syringes with metal dosing needles. Each animal received 2.5 ml/kg/d, with the amount adjusted to the most recent body weight. Doses were administered at the same time (AM) each day for 104-106 wk.

Clinical Examinations:

Signs: Once daily, at approx. the same time.

Mass palpation: After 5 mo. every 2 wk.

Mortality: Twice/Day, every day. Sac. if debilitated or intox. Dead or in extremis killing, do blood smear and macroscopic exam.

Bodyweight: At control, dosing day 1, once/week during 13 wks. of dosing, then @ 2 wk until the end of the study.

Food consumption: Once/week (on a period of 8 days on wk. 1 and of 7 days until the end of the study) during the first 13 wks of treatment, on wk 18, then every 4 wk until the end of treatment. Calculated in g/animal/day, based on the amount given minus that remaining at end of 7 days.

Water consumption: Measured (on a period of 7 days) during treatment weeks 4, 13, 26, 52, and 78 in the 1st 10 males and 10 females which survive from each group, except for control groups in 26th wk. Measured as ml/animal /day in terms of that given minus that remaining in the bottle at the end of the experiment.

Laboratory Investigations:
Hematology:

In weeks 52 and 78, blood samples were taken by caudal vein incision from all of the survivors and the moribund animals (before sacrifice), when possible. Blood smears were prepared and a differential white cell count was done. No other hematological parameters were evaluated.

Pathology:

Animals were sacrificed by the usual methods. A complete macroscopic examination was done before and after dissection was performed

The tissues and masses harvested from the animals were treated as shown below in Table 28 (sponsor's p. 8, Vol. 49).

Table 28.
Tissues Preserved-Rat

aorta	spinal cord	testes and epididymides
caecum	cervical, thoracic and lumbar sections	thymus
brain including medulla/pons, cerebellar and cerebral cortex	skeletal muscle	thyroids and parathyroids
heart	sciatic nerve	trachea
colon	lymph nodes, cervical, thoracic and mesenteric	uterus, horns and cervix
duodenum	oesophagus	vagina
stomach	ovaries	seminal vesicles
femoral bone and articulation with bone marrow	pancreas	urinary bladder
liver	skin	eyes with optic nerves
mammary glands (with skin)	lungs	masses
parotid glands	prostate	
mandibular glands	spleen	
pituitary	rectum	
ileum	kidneys	
jejunum	sternum with bone marrow	
	adrenals	

**Table 29
Microscopic Examination-Rat**

All tissues in paraplast blocks required for microscopical examination were sectioned at approximately 4 microns in thickness and stained with hemalum-eosine.

Microscopical examinations were performed on:

- 1) all gross abnormalities and tissues listed in all animals of the high dose level and control groups.
- 2) all gross abnormalities and tissues listed in all animals that died or were sacrificed prematurely.
- 3) all tissues showing macroscopical abnormalities from the intermediate and low dose level groups.

Statistical Methods Used: As described for the mouse studies in the above sections.

Results:

It is important to note that sponsor requested the Study Director to reduce the high dose (16 mg/kg/day) at the 17th week to 8 mg/kg/day until the end of the study. The dose modification was at "the request of the sponsor following biochemical and histological results obtained in an anterior (circa, preceding) study" (see page 4, Vol. 49, when data became available).

The palpable masses in rats which were confirmed as tumors at microscopic examination, are shown below in Table 30 (sponser's Table on p. 12, Vol. 49). Reviewer is in accord with sponsor in that "there is no treatment or dose-relatedness either in morphological type, size, or multiplicity."

**Table 30
Palpable Masses in Rats**

Sex	Males				Females			
	0	0.25	2	8	0	0.25	2	8
Doses (mg/kg/d)								
Number of animals	9	6	11	8	31	36	27	26
Percentage	15	10	18	13	52	60	45	43

The mortality distribution in this study is shown in Table 31 below (from sponsor's p. 13, Vol. 49)

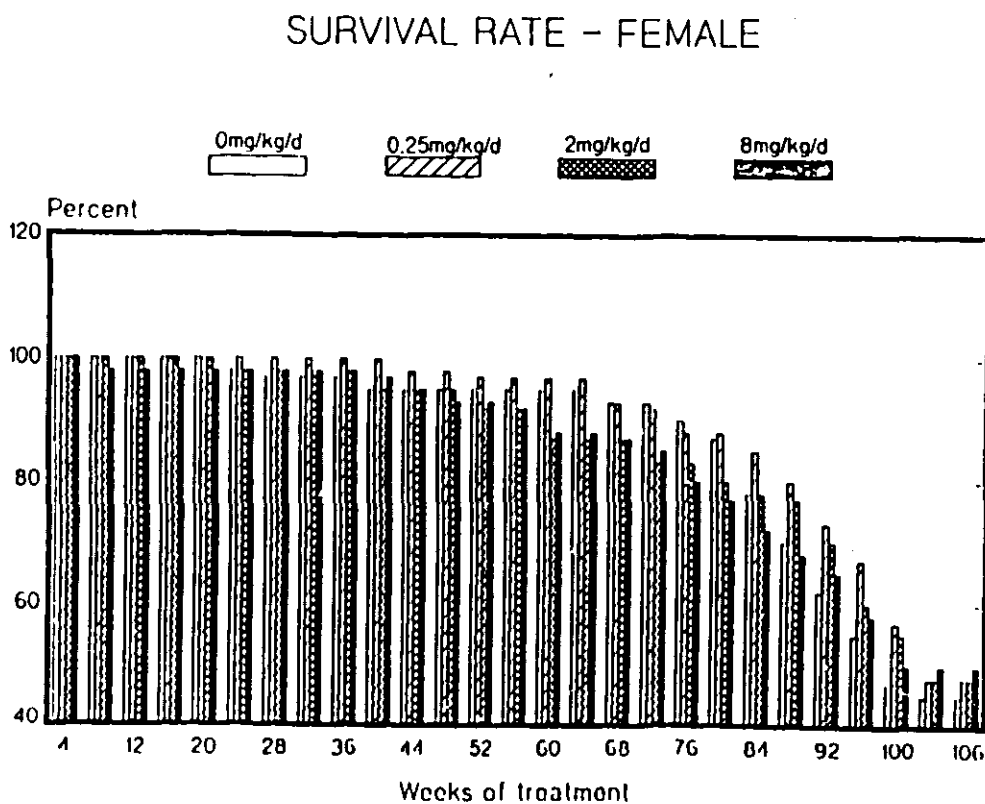
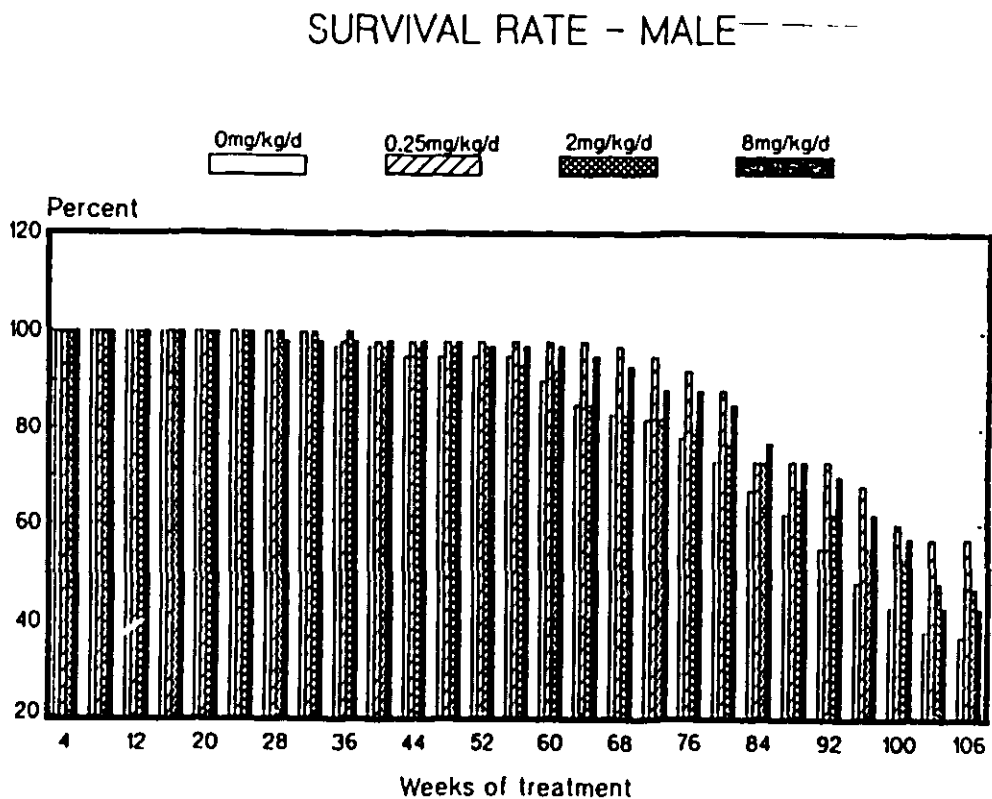
Table 31
Mortality Distribution in Rats Treated with TRAN

Sex	Males				Females			
	0	0.25	2	8	0	0.25	2	8
Doses (mg/kg/d)								
Animals/Group	60	60	60	60	60	60	60	60
Killed prematurely	26	18	25	29	29	24	25	26
Found dead	12	8	7	5	4	7	6	4
Total	38	26	32	34	33	31	31	30
2	63	43	53	57	55	52	52	50

There were similar rates of mortality in the treated and control groups, as can be seen in Table 31. Figure 64 also shows that the survival rate in male rats is quite uniformly higher in those animals treated with the higher doses, while in the female rats the survival rate seems to be higher in those animals from the lower dose groups. However, not too much can be made of this since no supporting statistical evaluation was carried out.

Sponsor reports that a major factor contributing to the death and morbidity of treated and control animal groups was the occurrence of a pituitary tumor. Additionally, sponsor reports that there was comparable distribution of factors contributing to death or morbidity among animals of all groups and there was "no disturbance as a result of treatment".

Figure 64.
Rat Survival Rates With and Without Trandolapril Treatment



Body weight gains were retarded to some extent in both sex-groups and by most of the utilized doses (see Figures 65 A and 65 B, from sponsor's Figures on pp. 74 and 75 in Vol. 49). The low dose group (0.25 mg/kg/day) males showed a very slight retardation of body weight gain from week 3 to the end of the study. The difference was approximately 7% less than mean control. During the interval of 3 to 66 weeks, the difference between the mean controls and the dosed animals (0.25 mg/kg/day) was statistically significant.

In the mid and high dose male groups, the body weight gain was slightly less than the mean control from week 2 to the end of the treatment. The mid-dose group was significantly lower than control for week 2 to week 90 and for week 2 to week 92 for the high dose group. For the above described dose groups, the differences were statistically significant and averaged approximately 12%. From these dates to the end of the treatment, the differences were occasionally statistically significant relative to control. The mean values were approximately 5% and 7 %, respectively, for the mid and high dose groups.

The low dose females showed a slight retardation of body weight gain from week 4 to week 66. The average mean difference from control was 6% and was irregularly statistically significant. From week 68 to the end of dosing, the body weights of the low dose group oscillated around control without achieving statistical significance. The mid and high doses apparently caused slight decreases in body weight gain from weeks 2 and 3 to the end of the study, respectively. These changes were about 7% and statistically significant until week 86 for the low dose group and until week 78 for the high dose group.

Food consumption by the treated rats was slightly lower than by the mean controls. This difference was similar in males and females and was about 7%. This change was generally significant for the first 13 weeks of treatment and after that time only occasionally so. Efficiency of food utilization (the mean amount of food eaten per unit of bodyweight gain) during the growth period (first 13 weeks of treatment) did not differ between the control and the treated animals.

Water consumption--males: Water consumption by males of the low dose groups did not vary significantly from control. The mid- and high-dose animals showed increases in consumption during weeks 4 and 14 which were not dose-related. These statistically significant differences were about 55% and 40% in weeks 4 and 14, respectively, compared with control. Sponsor did not report any other significant differences.

Water consumption--
females: Sponsor did not report any constant pattern of change.

RAT BODYWEIGHT CHANGES (GRAMS) WITH TRANDOLAPRIL
 FIGURE 65A: MALES

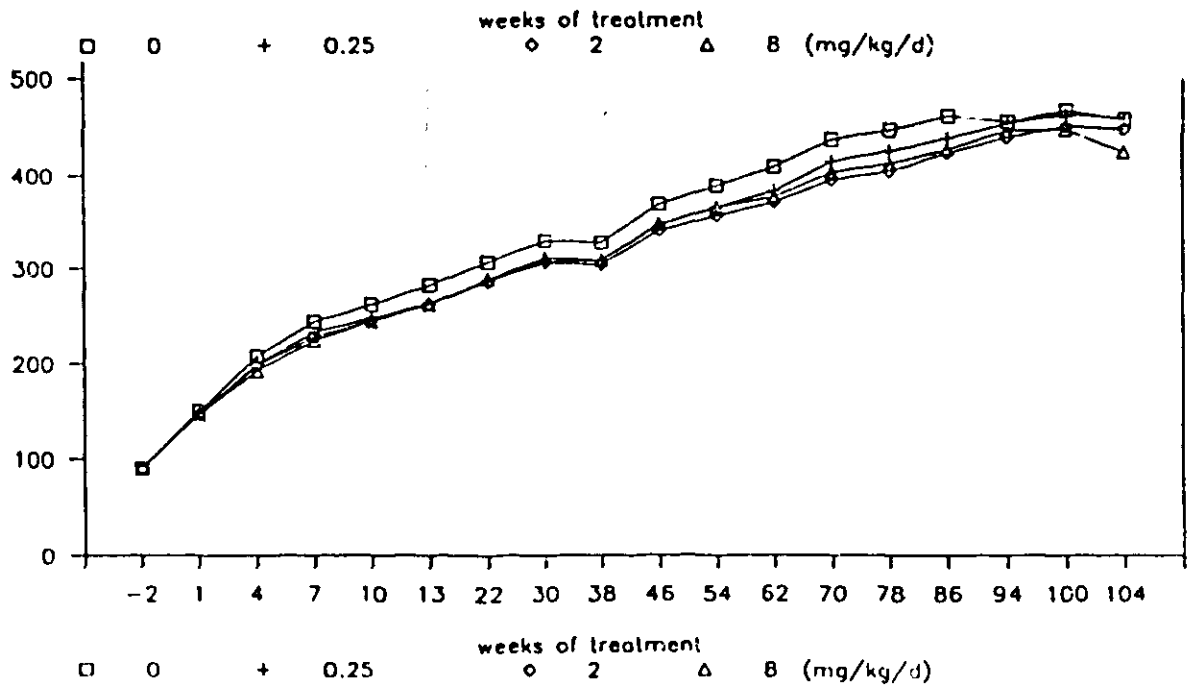
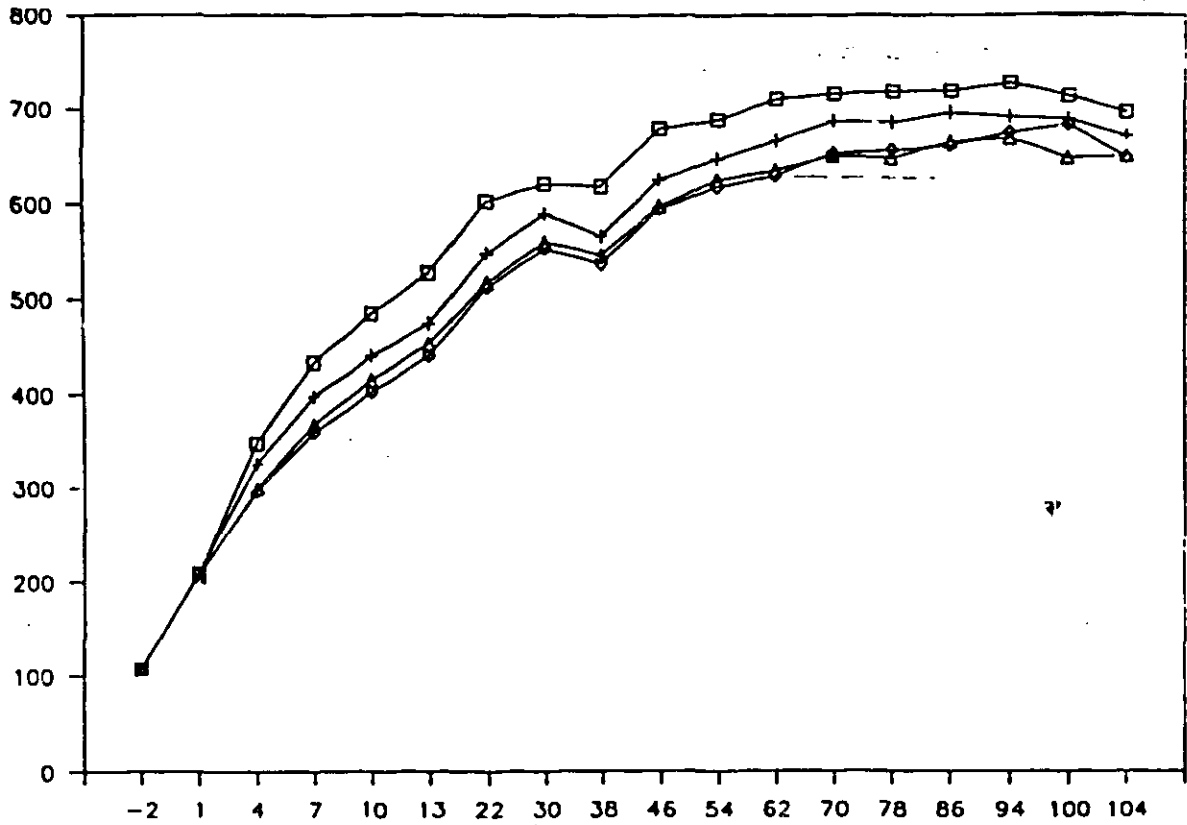


FIGURE 65 B: FEMALES

Clinical Signs were noted at similar frequencies in the control and treated groups and were attributed to spontaneous occurrences in aged rats. Scalded litter was observed in the male mid and high dose treated groups which was considered to be treatment-related since increasing the frequency of litter changes corrected the problem.

Laboratory Investigations:

Hematology: Sponsor reported slight changes in blood parameters lasting for a few weeks were usually seen. Although some of these changes sometimes achieved statistical significance, the changes were slight and usually within the normal limits of variation seen in aged rats. Prematurely sacrificed animals did not show any drug related changes.

In summary, the above specified changes were not considered to be toxicologically significant.

Pathology:

A. Summary Tables of General Macroscopic Pathological Observations in Rat Studies (p. 16)

Tabular Units: Incidence of Necropsy Findings by Organ/Dose Group/Sex

Dose Groups:

T = Control. A = 0.25 mg/kg/day. B = 2 mg/kg/day. C = 8 mg/kg/day

Sex: M= Male, F=Female

Rat Pathology References: Vol. 49, p. 20

Tables: Appendix I, pp. 125-135

Table 32. a. Summary Table of Macroscopic Pathology

ORGAN/FINDING	DOSE GROUP:		T		A		B		C	
	SEX:		M	F	M	F	M	F	M	F
	NO. ANIMALS:		60	60	60	60	60	60	60	60
GENERAL OBSERVATIONS										
- Died			12	4	8	6	7	6	5	4
- Sacrificed			22	29	16	25	25	25	27	26
- MASS								1		
.....										
AORTE										
- ENLARGED										1
.....										
HEART										
- ENLARGED							1			
- THICKENED			1					1		
- PALENESS				1						
- WHITISH COLOR								1		
- ADHESION				1						
- FOCI				1						
- MASS						1				1
- NODULE			1		1					
.....										
CECUM										
- DISTENDED WITH GAS			2				1	1		
- DISTENDED WITH FECES					1					
- DILATATION			1				2			
- CONGESTION			1							2
- THICKENED			1		1		2	1		
- YELLOWISH COLOR			1							
- BLACKISH COLOR						1				
- REDDISH COLOR							2			2
- GREENISH COLOR					1					
- CONTENTS			1	1	1		4	1		
- FOCUS							2			
- FOCI							1			
- ORELEM										1
- AREA							1			
.....										
COLON										
- DISTENDED WITH GAS							1			
- DISTENDED WITH FECES					1					
- DILATATION					1		2			
- IRREGULAR SURFACE							1			
- CONGESTION							1			
- WHITISH COLOR										1
- CONTENTS							3			
- NODULE							1			
.....										
RECTUM										
- DISTENDED WITH FECES					1					
- DILATATION					1		2			
.....										
BRAIN										
- FOCUS						1				
- AREA										1
.....										
DUODENUM										
- DISTENDED WITH GAS			1				1	1		
- DILATATION							1			
- CONTENTS					1		2	2		1
- NODULE										1
.....										
ILEUM										
- DISTENDED WITH GAS			2		1		1	2		1
- ENLARGED					1		1			
- DILATATION			1				1			
- CONTENTS				1	1		2	1		4
- NODULE							1			
.....										
JEJUNUM										
- DISTENDED WITH GAS			1		1		1	1		
- ENLARGED					1					
- DILATATION										
- CONTENTS				1	1		4	2		4
- MASS										
- NODULE							1			1
.....										

Table 32. b. Summary Table of Macroscopic Pathology

ORGAN/FINDING	DOSE GROUP:		T		A		B		C	
	SEX:		M	F	M	F	M	F	M	F
	NO. ANIMALS:		60	60	60	60	60	60	60	60
STOMACH										
- DISTENDED WITH GAS			1							
- DILATATION					1		2	1		
- IRREGULAR SURFACE							2		1	
- THICKENED			3	1	4		3	1	3	2
- REDDISH COLOR					1		1	1		
- GREENISH COLOR					1					
- ADHESION			1							
- CONTENTS					1		1	1	1	
- DEPOSIT					1					1
- FOCUS									1	
- FOCI			2	2	1	1	5	1	2	3
- NODULE										1
- ULCERATION			2	2	2	1	5	1	1	1
- AREA			1	1	1	1			1	
.....										
MESEN. LYMPH NODE										
- ENLARGED					1	1			1	
- BLACKISH COLOR						1				
- REDDISH COLOR					1	2			4	1
.....										
FEMORAL BONE (R.H.)										
- ENLARGED			1							
- GREENISH COLOR			1							
.....										
LIVER										
- ACCENTUATED LOBULAR PATTERN								2		
- NODULE										1
- ENLARGED			5	2	4	3	3	3	1	1
- REDUCED IN SIZE				1						
- IRREGULAR SURFACE			1		1	1	1		1	
- FATTINESS			3	6	3	6	1	3	2	2
- IRREGULAR COLOR			1	1	1	3	3	4	2	
- YELLOWISH COLOR				1						1
- GREENISH COLOR			1		1					
- FRIABLE				1						
- FOCUS			4	8	3	5	1	5	5	6
- FOCI			1	2	1	2	5	3	5	1
- HEMORRHAGE			1							
- CYST					2			2	1	1
- MASS			2		5	2	1		1	
- NODULE					3	2		2	1	
- AREA				1				2	2	
.....										
PAROTID GLANDS										
.....										
MANDIBULAR GLANDS										
- ENLARGED			1		1					
- GREENISH COLOR			1		1					
- FIRM			1							
- OEDEMA								1		
.....										
CERVICAL LYMPH NODE										
- ENLARGED			3	1	3	1	3			2
- YELLOWISH COLOR					1					
- REDDISH COLOR			1	3	1	2	1	2	1	2
- GREENISH COLOR			1		1		2		1	
.....										

Table 32. d. Summary Table of Macroscopic Pathology

ORGAN/FINDING	DOSE GROUP:		T		A		B		C	
	SEX:	NO. ANIMALS:	M	F	M	F	M	F	M	F
OVARIES										
- SEROUS CYST				8		12		7		7
- CYST				8		12		9		7
.....										
URINARY BLADDER										
- ENLARGED				1						
- DILATATION				2		2		1		2
- THICKENED								1		
- REDDISH COLOR				1						
- CONTENTS				3	1					2
.....										
PANCREAS										
- REDUCED IN SIZE					1		2	1		
- WHITISH COLOR						1				1
- GREENISH COLOR						1				
- FOCI							1			
- NODULE			1	3	5	1	1	1	1	1
- OEDEMA										1
.....										
SPLEEN										
- ENLARGED			1	2	2	1	4	1		2
- PALENESS			1				1			
- IRREGULAR COLOR			1							
- ADHESION			1		1					
- CLOT					1					
- FOCUS					2					
- CYST										1
- MASS						1				
- NODULE					1			1		
- AREA			1		1		1			
.....										
THYMUS										
- ENLARGED				1	1	1				
- REDUCED IN SIZE						1		2		1
- GREENISH COLOR					1				1	
- NOT FOUND			4					1		
- CYST			1							
- OEDEMA										F
.....										
THORACIC LYMPH NODE										
- ENLARGED			4	1	3	3	3	1	1	1
- YELLOWISH COLOR					1					
- REDDISH COLOR				2	1	2	1		2	1
- GREENISH COLOR			1		1		3		1	
- ADHESION						1				
- NOT FOUND					1					
.....										
LUNGS										
- NOT COLLAPSED			6		1	1	2			1
- DILATATION					1					1
- IRREGULAR SURFACE			2	1						
- IRREGULAR COLOR			1							1
- YELLOWISH COLOR					1					
- GREYISH COLOR					1					
- REDDISH COLOR			4	1	2	2	2	3	5	
- FOCI				1	2	4	2	2	1	3
- MASS					1					
- NODULE			2	1	1			1	2	1
.....										
PROSTATE										
- ENLARGED			1		2		3			2
- REDUCED IN SIZE			3		1		1			
- IRREGULAR SURFACE							1			
- BROWNISH COLOR			3		2		2			5
- REDDISH COLOR			2		1					1
- GREENISH COLOR			2				2		2	
- ADHESION					1					
- FIRM							1			
- CONTENTS			1		1					
- OEDEMA										1
- AREA								1		
.....										

Table 32. e. Summary Table of Macroscopic Pathology

ORGAN/FINDING	DOSE GROUP:		A		B		C	
	M	F	M	F	M	F	M	F
NO. ANIMALS:	60	60	60	60	60	60	60	60
KIDNEYS								
- PELVIC DILATATION	6	3	2	1	3	4	2	3
- ENLARGED	6		4	1	6		4	
- REDUCED IN SIZE				2				
- DILATATION	4	3	2	2	3	4	3	3
- IRREGULAR SURFACE	10	4	4	1	11	1	14	7
- PALENESS	4	5	9	3	5	3	8	4
- IRREGULAR COLOR	1				1		2	
- BROWNISH COLOR	1		1		2		2	
- YELLOWISH COLOR	1	1						
- GREYISH COLOR				1				
- CONTENTS		2		1				
- DEPOSIT								1
- FOCUS								1
- FOCI					3		2	
- CYST	10		10	4	13	7	14	10
- NODULE				1	1		2	
- DISCHARGE					1			
.....								
URETERS								
- DILATATION	1	2	2	1		2	2	1
- NOT FOUND		1						
- CONTENTS	1	1		1			1	
.....								
TESTES								
- ENLARGED	2		1		3		3	
- REDUCED IN SIZE	7		10		5		7	
- IRREGULAR COLOR			4		3		2	
- WHITISH COLOR	2				1		1	
- PURPLISH COLOR	3		1		1		6	
- FIRM	1							
- SOFT	10		3		3		3	
- MASS								
- NODULE			1		2			
- TRANSLUCENT			1		1			
.....								
EPIDIDYMIDES								
- ENLARGED							1	
- REDUCED IN SIZE	7		6		3		4	
- DILATATION	1							
- IRREGULAR SURFACE			1					
- FIRM	1							
- NOT FOUND					1			
- FOCUS							1	
.....								
UTERUS								
- HYDROMETRA				1		1		
- ENLARGED				1		3		2
- DILATATION		5		2		2		2
- BLACKISH COLOR						1		
- CONTENTS		4		2		1		2
- CYST		2		3		4		2
- NODULE				1		2		2
.....								
VAGINA								
- DILATATION		1		1				
- CONTENTS		2		2		1		2
- CYST				1				
- MASS								1
- NODULE						1		
- DISCHARGE						1		
.....								
SEMINAL VESICLES								
- ENLARGED	1		4		6		2	
- REDUCED IN SIZE	1		9		4		10	
- REDDISH COLOR	1							
- GREENISH COLOR			3					
- CONTENTS	1							1
- MASS			1					
- TRANSLUCENT			1		1			
.....								
EYES								
- CLOUD						3		
- PERFORATION				2				
- WHITISH COLOR	1		2		1		1	1

Table 32. f. Summary Table of Macroscopic Pathology

ORGAN/FINDING	DOSE GROUP:		T		A		B		C	
	SEX:	NO. ANIMALS:	M	F	M	F	M	F	M	F
OPTIC NERVE		1								
.....										
LYMPH NODES		1								
- ENLARGED			8	3	10	3	10	1	9	5
- WHITISH COLOR										1
- YELLOWISH COLOR					1					
- GREYISH COLOR				1						
- REDDISH COLOR			4		2	4	5	1	7	2
- GREENISH COLOR			1		1		4		1	
- FIRM										1
- CYST					3	1	2		6	
- TRANSLUCENT									1	
.....										
OTHER ORGANS/TISSUES		1								
- ENLARGED			2		4	1	2		1	
- CONGESTION									1	
- THICKENED					2		1	3	1	5
- WHITISH COLOR			1						1	
- YELLOWISH COLOR						1				
- BLACKISH COLOR							1			
- REDDISH COLOR									1	
- ADHESION					1		1			
- FIRM			1							
- CONTENTS					1	1				
- FOCI				1	1	1				
- HEMATOMA					1		1			
- CYST							1		1	
- MASS				1	3	2	1		1	
- NODULE			1		4	3	2		4	
- OEDEMA			1							
- PERFORATION					1					
- AREA			1							
.....										
SUBCUTANEOUS TISSUE		1								
- MASS			9	25	6	32	11	28	9	25
- NODULE			2	1		1				
- OEDEMA								2		

END OF REPORT SECTION

B. Summary Tables of Non-Neoplastic Findings in Rat Studies.

(Tabular Units: Incidence of Animals with Non-Neoplastic Lesions by Organ/Dose Group/Sex).

Dose Groups: T=Control, A=0.25 mg/kg/day, B=2 mg/kg/day, C=8 mg/kg/day

Sex: M=Male, F=Female.

Rat Pathology References: Vol. 49, p 20: Appendix I, pp. 136-143.

Status at Necropsy: K0=Terminal Sacrifice, Incl.=includes all (sacrificed and moribund), + = moribund or died.

CONCLUSIONS : Sponsor concludes that the changes seen in the **kidneys** are commonly found in old rats, and their appearance did not indicate treatment or dose-related effects on morphology or incidence .

Sponsor used the **liver** terminology and criteria of Moronpot, *et al.* (Toxicology Pathology 14:263-273) for the morphologic diagnosis of the proliferative hepatic lesions. The diagnostic terms used were "foci of cellular alteration and nodular hyperplasia", and these findings were seen among rats of all groups with no indication of treatment or dose relationship either in the incidence or in the morphological character of the hepatic cells. The incidence, severity, and the morphological character of the other changes encountered in the liver were comparable among all groups and were similar to those recorded spontaneously in the old rats.

Other organs and tissues: The changes found in the other organs and tissues were commonly seen senility changes in the laboratory rats, and, consequently, are not regarded as being of any toxicological significance.

Table 33. a. Summary of Incidence of Non-Neoplastic Lesions

ORGAN/FINDING	DOSE GROUP: SEX: NO. ANIMALS:	T		A		B		C	
		M	F	M	F	M	F	M	F
AORTA	NO. EXAM.:	60	60	26	31	31	31	60	60
- CARTILAGENOUS METAP.		1						2	3
- BASOPH. DEG. TUM. MED.		2		1					
HEART	NO. EXAM.:	60	60	26	31	32	32	60	60
- MYOCARD. DEGEN. FIBRO.		32	24	9	1	9	2	22	15
- CARTILAG. META. ATRI.		9	4		1	2	1	4	3
- ENDOCARDIOSIS.		1							
- PERICARDITIS.		1	1				1		
- ENDOCARDIAL DISEASE.		1							
- FIBROMYX. CHAM. CAR. V.		3	8			4		5	1
- ENDOCARDITIS.		1							
- MYOCARDITIS.			1				1		
CECUM	NO. EXAM.:	53	59	24	27	30	29	59	60
- ACUTE TYPHLITIS.				1		1	1	2	
- VASCULAR ECTASIA.								2	
- SUBMUCOSAL OEDEMA.						1		1	
RECTUM	NO. EXAM.:	52	60	24	27	29	28	60	59
- SQUAMOUS METAPLASIA.		5	4				1	5	6
- PROLAPSE.									
ERAIN	NO. EXAM.:	60	60	26	31	32	31	60	60
- VACUOL. OF WHITE MAT.		5	7	1				2	6
STOMACH	NO. EXAM.:	57	60	27	31	32	31	59	60
- ULCERATION.		3		1	1	6		3	2
- ABSCESS.		1							
- SUBMUCOSAL OEDEMA.		4	2	3		4	1	4	3
- SUBMUC. INFL. CEL. AGG.		3	2	3		4	1	4	4
- FOCAL GASTRITIS.						1		1	
- VASCULAR ECTASIA.			1						
- PSEUDOMELANOSIS.				2					1
- SEROSAL CYST.									1
- EPITHEL. CEL. HYPERPL.				1	1				
MESENT. LYMPH NODE	NO. EXAM.:	57	60	26	32	32	31	60	60
- CEROID LADEN MACROP.		21	38	11	18	6	10	28	26
- REACTIVE HISTIOCYTO.		37	44	10	21	13	19	32	39
- PLASMOCYTOSIS.		1							
- FIBRINOID NECROSIS.		1							
- CONGESTION.		1	1	1	2	2	2	4	1
- ERYTHROPHAGOCYTOSIS.		1		2	2			1	1
- DILATED LYMPH SINUS.		2	1	1					
- REACTIVE LYMPH NODE.		2						2	
- LYMPHOID CEL. HYPERP.		1							1
FEMORAL BONE(=B.M)	NO. EXAM.:	60	60	25	31	32	30	60	59
- MEGAKARYOCYTOSIS.		4						3	
- RETICUL. CEL. HYPERPL.		2	1						1
LIVER	NO. EXAM.:	60	60	41	39	52	40	60	60
- STEATOSIS.		16	13	10	6	6	6	9	3
- PERICHOLANGITIS.		50	31	15	7	15	12	39	12
- ALTER. CEL. FOC. VACU.		6	17	6	9	10	8	5	19
- FOC. SINUSOIDAL ECTA.		9	9	7	5	5	6	9	4
- HEPATOCELLULAR CYSTS		16	3	6	1	4	2	15	2
- BILIARY CYSTS.		6	2				2	1	
- ALTER. CEL. FOC. ACID.		13	25	5	10	6	11	5	17
- FOC. CHR. HEP. CEL. NEC.		2	1		2	1		1	1
- ALTER. CEL. FOC. MIXED		3		1	2		3		
- NODULAR HYPERPLASIA.		4	6	5	5	3	4	4	3
- BIL. DUCTUL. HYP. FIRO.		6	7	4	3	8	1	10	5
- CHRONIC VEN. CONGEST.									1
- AREA OF NECR. FIBROS.									1
PANCREAS GLANDS	NO. EXAM.:	60	60	26	31	32	31	60	60
- CYTOMEGALY.		7	1	2		1		2	
- LIPOMATOSIS.		1							
- DILATED ACINI.		2							
- INTERS. MONO. CEL. AGG.		2		1			2		2
- CHRONIC SIALOADENIT.		1							2

Table 33. b. Summary of Incidence of Non-Neoplastic Lesions

ORGAN/FINDING	DOSE GROUP: SEX: NO. ANIMALS:	T		A		B		C	
		M	F	M	F	M	F	M	F
MANDIBULAR GLANDS	NO. EXAM.:	60	60	26	31	32	31	60	60
- SIALOADENITIS.		1				2			
- CYTOMEGALY.		2						3	
- INTERST. MONO. CEL. AG.								1	1
CERVICAL LYMPH NODE	NO. EXAM.:	59	60	26	31	32	31	60	60
- REACTIVE HISTIOCYTO.		17	17	7	11	7	5	16	22
- CEROID LADEN MACROP.		6	16	1	1	1	2	5	7
- CONGESTION.		8	7	2	2	2	4	3	1
- PLASMOCYTOSIS.		9	8	2	1	4	4	6	2
- FOCAL FIBROSIS.		1							
- LYMPHOID CELL HYPER.		1	1	1					5
- REACTIVE LYMPH NODE.		2						2	2
- ERYTHROPHAGOCYTOSIS.			3	1	1		1	4	1
- DILATED LYMPH SINUS.						1			
PITUITARY GLAND	NO. EXAM.:	60	60	33	40	43	46	60	60
- DEVELOPMENTAL CYST.		2		1				2	
STERNUM (- B.M.)	NO. EXAM.:	60	60	26	31	32	31	60	60
- MEGAKARYOCYTOSIS.		10	1					14	
- PLASMOCYTOSIS.		1							
- RETICUL. CEL. HYPERPL.		1	2					4	
SKELETAL MUSCLE	NO. EXAM.:	60	60	29	31	32	31	60	60
- HAEMATOMA.				1					
- LYMPHOCYTIC MYOSITIS							2		
SKIN	NO. EXAM.:	57	60	37	34	39	34	59	59
- EPIDERMITIS.		5	2	5		1	1	4	1
- ACUTE CELLULITIS.		2		2				1	
- ABSCESS.		1				1			
- SUPPURATIVE CELLULI.		4	1			1		3	
- SUBACUTE CELLULITIS.		1	1	3					
- EPIDERMAL CYST.		1					1	2	
MAMMARY GLAND	NO. EXAM.:	57	60	26	46	52	43	60	59
- GYNECOMASTIA.		2		1		3		5	
- GALACTOCELE.		1	1		1				1
- DILATED DUCTS.			2						
ESOPHAGUS	NO. EXAM.:	59	60	26	31	32	31	60	60
- NECROTISING INFLAMM.		1		1		2		1	
- SUBMUCOSAL HAEMORRH.				1		2			
- PARAESOPHAGEAL ABSCE.						1			
ADRENAL GLANDS	NO. EXAM.:	59	60	30	35	35	37	60	60
- CERCOID DEGENERATION.		37	36	9	15	6	10	27	26
- SINUSOIDAL ECTASIA.		18	1	5	3	3	1	10	
- FOCUS OF CEL. ALTERAT.		5	3	1	1	2	2	5	4
- FOCI OF CEL. ALTERAT.		10	6	0	2	4	2	17	15
- MEDULLARY HYPERPLAS.		9	6	1	1	4	2	9	8
- CORTIC. CYST DEGENER.		3	42		20	1	25	2	35
- MODULAR HYPERPLASIA.		7	7		2		2	3	11
- HAEMATOCYST.			25		2	2	2	2	1
- PIELOSIS.			39		21		19		41
- FOCAL CORTICAL NECR.			1			1			
- CORTICAL ATROPHY.					1		2		
THYROID GLAND	NO. EXAM.:	60	59	31	31	32	31	60	60
- FOLLICULAR CYSTS.		5				3		1	
- PARAFOLL. CEL. HYPERP.		4	2	1	2		1	2	2
- NODULAR HYPERPLASIA.		3	4					2	
- FOLLIC. CEL. HYPERPLA.		2						1	
- GIANT FOLLICLES.		2						3	
- ULTIMERANCHIAL CYST			3				1	2	
OVARIES	NO. EXAM.:		60		36		36		60
- FOLLICULAR ATRESIA.			55		28		32		57
- FEW CORPORA LUTEA.			9		1		3		12
- NO CORPORA LUTEA.			31		23		17		34
- DEGENERATED CORP. LU.			1						
- FOLLICULAR CYSTS.			19		5		6		20
- PARAOVARIAN CYST.			7		12		6		3
- LUTEAL CYST.			1		2		2		2
URINARY BLADDER	NO. EXAM.:	58	58	25	32	31	29	60	60
- ACUTE CYSTITIS		2	1						

Table 33. d. Summary of Incidence of Non-Neoplastic Lesions

ORGAN/FINDING	DOSE GROUP: SEX: NO. ANIMALS:	T		A		B		C	
		M	F	M	F	M	F	M	F
		60	60	60	60	60	60	60	60
URETERS	NO. EXAM.:	60	60	26	32	32	31	59	60
- ACUTE URETERITIS.		1			1				
- EPITHEL. CEL. HYPERPL.					1				
TESTES	NO. EXAM.:	60		39		41		60	
- INTERSTITIAL OEDEMA.		2		4		4		4	
- DEGENER. SEMIN. TUBUL.		11		13		10		13	
- INHIBITION OF SPERM.		11		13		9		14	
- POLYARTERITIS NODOSA		4		3		3		6	
- INTERSTIT. CEL. HYPER.		1		6		2		5	
- CYST DEGENERATION.		2							
EPIDIDYMIDES	NO. EXAM.:	60		32		36		60	
- OLIGOSPERMIA.		11		10		8		12	
- EPITHEL. CEL. ATROPHY.		5		3				2	
- SPERMATIC GRANULOMA.		1		1				1	
UTERUS	NO. EXAM.:	60		35		38		59	
- CONDENSAT. ENDOM. STR.		52		29		30		46	
- DECR. NUM. ENDOM. GLAN.		52		29		30		46	
- ENDOM. EPITH. CEL. ATR.		10		4				5	
- PARAUTERINE CYST.		1				1			
- ENDOMETRITIS.		1				2			
- HYDROMETRA.		8		4		4		6	
- INCREASE VASCULARITY		6		1		4		5	
- EPITH. CEL. HYPERTROP.		4				5		8	
- EPITH. CEL. HYPERPLAC.		3						2	
- ENDOMETR. CYST. HYPER.				1				1	
- INFLAMMATORY POLYP.						1		1	
VAGINA	NO. EXAM.:	59		32		29		58	
- MUCIFICAT. VAG. EPITH.		18		9		9		25	
- EPITHELIAL CELL ATR.		29		16		14		17	
- HYPERPLAS. BAS. CEL. L.		11		6		5		16	
- HYPERKERATOSIS.		5		1		4		6	
- INFLAMMATORY POLYP.		1				1		1	
- LUMEN CONTAINS LEUC.								2	
- CYST.						1			
- VAGINITIS.						1			
SEMINAL VESICLES	NO. EXAM.:	60		32		35		60	
- DECREASE SECRET. ACT.		5		7		3		9	
- EPITHEL. CEL. ATROPHY.		1		1				2	
- SEMINAL VESICULITIS.		1						1	
- INCREASE SECRET. ACT.				5		7		2	
EYES	NO. EXAM.:	60	60	26	31	36	31	60	60
- CHRON. DACYOGADENITIS.			12		7	1	6		8
- SQUAM. MET., HARD. GLA.									1
- ACUTE KERATITIS.						1			
LYMPH NODES	NO. EXAM.:	8	3	13	4	13	2	17	5
- CONGESTION.		3		1	2	4	2	5	2
- REACTIVE HISTIOCYTO.		3	2	1		4			1
- DILATED LYMPH SINUS.		1		7	1	5		11	
- REACTIVE LYMPH NODE.		2				1			1
- PLASMOCYTOSIS.		1				1		1	
- CEROID LADEN MACROP.		1		1		1		3	
- LYMPHOID CEL. HYPERF.			1	2	1	1		1	2
- ERYTHROPHAGOCYTOSIS.				1				4	1
OTHER ORGANS/TISSUES	NO. EXAM.:	5	3	14	6	10	3	9	5
- CYST DEGEN., PARTHYR.		1							
- EPIDERMAL CYST, EAR.									1
- POLYARTER. NOD. MESE.									1
- ACUTE URETERITIS.									1
- CHRON. INFL., LACR. GL.									1
- CHRON. INFLAM., CRAN.				1		1			
- CHROMODACRYORRHEA.				1					
- HAEMORRHAGES, CRANI.						1			
SUBCUTANEOUS TISSUE	NO. EXAM.:	14	28	9	33	13	31	11	25
- ABSCESS.			2	3		1			2
- EPIDERMAL CYST.							1		
- SUBCUTANEOUS OEDEMA							1		

C. Summary Tables of Non-Neoplastic Findings in Rat Studies

(Tabular Units: Incidence of Animals with Non-Neoplastic Lesions by Organ/ Dose Group/ Sex, as Percent of Total Animals Examined).

Dose Groups: T = Control, A = 0.025 mg/kg/day, B = 2 mg/kg/day, C = 8 mg/kg/day.

Sex: M=Male, F=Female.

Rat Pathology References: Vol. 49, p. 20; Appendix I, pp. 144-151.

Status at Necropsy: K0=Terminal Sacrifice, Incl.= includes all (sacrificed and moribund), + = moribund or died.

2'

Table 34. a. Summary of Incidence of Non-Neoplastic Lesions

DOSE GROUP: SEX: NO. ANIMALS:	T		A		B		C	
	M	F	M	F	M	F	M	F
AORTA	60	60	26	31	32	31	60	60
- CARTILAGENOUS METAP.	1.7							
- BASOPH. DEG., TUM. MED.	3.3		3.3				3.3	5.0
HEART	60	60	26	31	32	32	60	60
- MYOCARD. DEGEN. FIBRO.	53.3	40.0	34.6	3.2	28.1	6.3	36.7	25.0
- CARTILAG. META. ATRI.	15.0	6.7		3.2	6.3	3.1	6.7	5.0
- ENDOCARDIOSIS.	1.7							
- PERICARDITIS.	1.7	1.7				3.1		
- ENDOCARDIAL DISEASE.	1.7							
- FIBROCYT. CHAN. CAR. V.	5.0	13.3			12.5		8.3	1.7
- ENDOCARDITIS.	1.7							
- MYOCARDITIS.		1.7				3.1		
CECUM	53	59	24	27	30	29	59	60
- ACUTE TYPHLITIS.			4.2		3.3	3.5	3.4	
- VASCULAR ECTASIA.							3.4	
- SUBMUCOSAL OEDEMA.					3.3		1.7	
RECTUM	52	60	24	27	29	28	60	59
- SQUAMOUS METAPLASIA.	9.6	6.7				3.6	8.3	10.2
- PROLAPSE.					3.5			
BRAIN	60	60	26	31	32	31	60	60
- VACUOL. OF WHITE MAT.	5.0	11.7	3.9				3.3	10.0
STOMACH	57	60	27	31	32	31	59	60
- ULCERATION.	5.3		3.7	3.2	10.0		5.1	3.3
- ABSCESS.	1.8							
- SUBMUCOSAL OEDEMA.	7.0	3.3	11.1		12.5	3.2	6.2	5.0
- SUBMUC. INFL. CEL. AGG.	5.3	3.3	11.1		12.5	3.2	6.2	6.7
- FOCAL GASTRITIS.					3.1		1.7	
- VASCULAR ECTASIA.		1.7						
- PSEUDOMELANOSIS.			7.4					1.7
- SEROUS CYST.								2.7
- EPITHEL. CEL. HYPERPL.			3.7	3.2				
MESENT. LYMPH NODE	57	60	24	31	32	31	60	60
- CEROID LADEN MACROP.	36.8	63.3	42.3	55.3	18.8	32.3	46.7	43.3
- REACTIVE HISTIOCYTO.	64.9	73.3	38.5	65.6	40.6	61.3	53.3	65.0
- PLASMOCYTOSIS.	1.8							
- FIBRINOID NECROSIS.	1.0							
- CONGESTION.	1.8	1.7	3.9	4.3	6.3	6.5	6.7	1.7
- ERYTHROPHAGOCYTOSIS.	1.8		7.7	6.3			1.7	1.7
- DILATED LYMPH SINUS.	3.3	1.7	3.9					
- REACTIVE LYMPH NODE.	3.5						3.3	
- LYMPHOID CEL. HYPERP.	1.8							1.7
FEMORAL BONE (-B.M.)	60	60	25	31	32	30	60	59
- MEGALARYOCYTOSIS.	6.7						5.0	
- RETICUL. CEL. HYPERPL.	3.3	1.7						1.7
LIVER	60	60	41	39	39	40	60	60
- STEATOSIS.	26.7	21.7	24.4	15.4	20.5	15.0	15.0	5.0
- PERICHOANGITIS.	83.3	51.7	36.6	18.0	38.5	30.0	65.0	20.0
- ALTER. CEL. FOC., VACU.	13.3	28.3	14.6	23.1	25.6	20.0	8.3	31.7
- FOC. SINUSOIDAL ECTA.	15.0	15.0	17.1	12.8	12.8	15.0	15.0	6.7
- HEPATOCELLULAR CYSTS	26.7	5.0	14.6	2.6	10.3	5.0	25.0	3.3
- BILIARY CYSTS.	10.0	3.3				5.0	1.7	
- ALTER. CEL. FOC., ACID.	21.7	41.7	12.2	25.6	15.4	27.5	8.3	28.3
- FOC. CHR. HEP. CEL. NEC.	5.0	1.7		5.1	2.6		1.7	1.7
- ALTER. CEL. FOC., MIXED	5.0		2.4	5.1		7.5		
- NODULAR HYPERPLASIA.	6.7	10.0	12.2	12.8	7.7	10.0	6.7	5.0
- BIL. DUCTUL. HYP. FIRO.	23.3	11.7	9.8	7.7	20.5	2.5	15.7	5.0
- CHRONIC VEN. CONGEST.							1.7	
- AREA OF NECR. FIBROS.							1.7	
PAROTID GLANDS	60	60	26	31	32	31	60	60
- CYTOMEGALY.	11.7	1.7	7.7		3.1		3.3	
- LIPOMATOSIS.	1.7							
- DILATED ACINI.	3.3							
- INTER. MONO. CEL. AGG.	3.3		3.5			6.5		3.3
- CHRONIC SIALADENIT.	1.7						3.3	

Table 34. b. Summary of Incidence of Non-Neoplastic Lesions

DOSE GROUP: SEX: NO. ANIMALS:	T		A		B		C	
	M	F	M	F	M	F	M	F
	60	60	60	60	60	60	60	60
MANDIBULAR GLANDS	60	60	26	31	32	31	60	60
- SIALOADENITIS	1.7					6.5		
- CYTOMEGALY	3.3						5.0	
- INTERST. MONO. CEL. AG.							1.7	1.7
CERVICAL LYMPH NODE	59	60	26	31	32	31	60	60
- REACTIVE HISTIOCYTO.	26.6	28.3	26.9	35.5	21.9	16.1	26.7	38.3
- CEROID LADEN MACROP.	13.6	23.3	3.9	3.2	3.1	6.5	8.3	11.7
- CONGESTION	13.6	11.7	7.7	6.5	6.3	12.9	5.0	1.7
- PLASMOCTOSIS	15.3	13.3	7.7	3.2	12.5	12.9	10.0	3.3
- FOCAL FIBROSIS	1.7							
- LYMPHOID CELL HYPERP.	1.7	1.7	3.5					8.3
- REACTIVE LYMPH NODE	3.4						3.3	3.3
- ERYTHROPHAGOCYTOSIS		3.0	3.9	3.2		3.2	6.7	1.7
- DILATED LYMPH SINUS					3.1			
PITUITARY GLAND	60	60	33	40	43	46	60	60
- DEVELOPMENTAL CYST	3.3		3.0				3.3	
STERNUM (= B.M.)	60	60	26	31	32	31	60	60
- MEGAKARYOCYTOSIS	16.7	1.7					23.3	
- PLASMOCTOSIS	1.7							
- RETICUL. CEL. HYPERPL.	1.7	3.3					6.7	
SKELETAL MUSCLE	60	60	29	31	32	31	60	60
- NEMATOMA			3.5					
- LYMPHOCTIC MYOSITIS						3.2		
SKIN	59	60	37	34	39	34	59	59
- EPIDERMITIS	8.5	3.3	13.5		2.6	2.9	6.3	1.7
- ACUTE CELLULITIS	3.4		5.4				1.7	
- ABSCESS	1.7				2.6			
- SUPPURATIVE CELLUL.	6.8	1.7			2.6		5.1	
- SUBACUTE CELLULITIS	1.7	1.7	8.1					
- EPIDERMAL CYST	1.7					2.9	1.7	
MAMMARY GLAND	59	60	26	44	32	43	60	59
- GYNECOMASTIA	3.4		3.9		9.4		2.3	
- GALACTOCYCLE	1.7	1.7		2.2				1.7
- DILATED DUCTS		3.3						
ESOPHAGUS	59	60	26	31	32	31	60	60
- NECROTISING INFLAMM.	1.7		3.9			6.5	1.7	
- SUBMUCOSAL HAEMORRH.			3.9			3.2		
- PARAESOPHAGEAL ABS.						3.2		
ADRENAL GLANDS	59	60	30	35	37	37	60	60
- CEROID DEGENERATION	62.7	60.0	30.0	42.9	18.2	27.0	45.0	43.3
- SIMUSOIDAL ECTASIA	30.5	1.7	16.7		9.1	2.7	16.7	
- FOCUS OF CEL. ALTERAT.	8.5	5.0	3.3	2.9	6.1	5.4	8.3	6.7
- FOCI OF CEL. ALTERAT.	17.0	10.0	26.7	5.7	12.1	5.4	28.3	25.0
- MEDULLARY HYPERPLAS.	15.3	10.0		2.9	12.1	5.4	15.0	13.3
- CORTIC. CYST DEGENER.	5.1	70.0		57.1	3.0	70.3	3.3	58.3
- MODULAR HYPERPLASIA	11.9	11.7		5.7		5.4	5.0	18.3
- NEMATOCYST		61.7		5.7	6.1	5.4	1.7	1.7
- PELIOSIS		65.0		60.0		51.4		68.3
- FOCAL CORTICAL NECR.		1.7			3.0			
- CORTICAL ATROPHY				2.9		5.4		
THYROID GLAND	60	59	31	31	32	31	60	60
- FOLLICULAR CYSTS	8.3				9.4		1.7	
- PARAFOL. CEL. HYPERP.	6.7	3.4	3.2	6.5		3.2	3.3	3.3
- MODULAR HYPERPLASIA	5.0	6.8					3.3	
- FOLLIC. CEL. HYPERPLA.	3.3						1.7	
- GIANT FOLLICLES	3.3						5.0	
- ULTIMOBRANCHIAL CYST		5.1				3.2	3.3	
OVARIES		60		38		38		60
- FOLLICULAR ATRESIA		91.7		73.7		100.0		95.0
- FEW CORPORA LUTEA		15.0		2.6		7.9		20.0
- NO CORPORA LUTEA		51.7		60.5		66.7		36.7
- DEGENERATED CORP. LU.		1.7						
- FOLLICULAR CYSTS		31.7		13.2		10.5		31.3
- PARAOVARIAN CYST		11.7		31.6		21.1		5.0
- LUTEAL CYST		1.7		5.3		5.3		5.0
URINARY BLADDER	58	59	25	32	32	29	60	60
- ACUTE CISTITIS	3.5	1.7						
- NYAL. PROT. MAT. IN LU.	5.2						8.3	
- THINNING EPIT. CELL. LI.							1.7	
- EPITHEL. CEL. HYPERPL.				3.1				

Table 34. d. Summary of Incidence of Non-Neoplastic Lesions

DOSE GROUP: SEX: NO. ANIMALS:	T		A		B		C	
	M	F	M	F	M	F	M	F
	60	60	60	60	60	60	60	60
URETERS	60	60	26	32	32	31	59	60
- ACUTE URETERITIS.	1.7			3.1				
- EPITHEL. CEL. HYPERPL.				3.1				
TESTES	60		39		41		60	
- INTERSTITIAL OEDEMA.	3.3		10.3		9.8		6.7	
- DEGENER. SEMIN. TUBUL.	18.3		33.3		24.4		21.7	
- INHIBITION OF SPERM.	18.3		33.3		22.0		23.3	
- POLYARTERITIS NODOSA	6.7		7.7		7.3		10.0	
- INTERSTIT. CEL. HYPER.	1.7		15.4		6.9		6.3	
- CYST DEGENERATION.	1.7							
EPIDIDYMIDES	60		32		36		60	
- OLIGOSPERMIA.	18.3		31.3		22.2		20.0	
- EPITHEL. CEL. ATROPHY.	8.3		9.4				3.3	
- SPERMATIC GRANULOMA.	1.7		3.1				1.7	
UTERUS		60		35		38		59
- CONDENSAT. ENDOM. STR.		86.7		82.9		79.0		78.0
- DECR. MUM. ENDOM. GLAN.		86.7		82.9		79.0		78.0
- ENDOM. EPITH. CEL. ATR.		16.7		11.4				8.5
- FOLICULAR CYST.		1.7				2.6		
- ENDOMETRITIS.		1.7				5.3		1.7
- HYDROMETRA.		13.3		11.4		10.5		13.6
- INCREASE VASCULARITY		10.0		2.9		10.5		6.5
- EPITH. CEL. HYPERTROP.		10.0				13.2		13.4
- EPITH. CEL. HYPERPLAS.		5.0						3.4
- ENDOMETR. CYST. HYPER.				2.9				1.7
- INFLAMMATORY POLYP.						2.6		1.7
VAGINA		59		32		29		58
- MUCIFICAT. VAG. EPITH.		30.5		28.1		31.0		43.1
- EPITHELIAL CELL ATR.		49.2		50.0		68.3		29.3
- HYPERPLAS. EAS. CEL. L.		18.6		18.8		17.2		27.6
- HYPERKERATOSIS.		8.5		3.1		13.8		10.3
- INFLAMMATORY POLYP.		1.7				3.5		1.7
- LUMEN CONTAINS LEUC.								1.7
- CYST.				3.1				
- VAGINITIS.						3.5		
SEMINAL VESICLES	60		32		35		60	
- DECREASE SECRET. ACT.	8.3		21.9		8.6		15.0	
- EPITHEL. CEL. ATROPHY.	1.7		3.1				3.3	
- SEMINAL VESICULITIS.	1.7						1.7	
- INCREASE SECRET. ACT.			15.6		20.0		3.3	
EYES	60	60	26	21	36	31	60	60
- CHRON. DACYOADENITIS.		20.0		22.6		2.8	19.4	13.3
- SQUAM. MET. HARD. GLA.								1.7
- ACUTE KERATITIS.					2.8			
LYMPH NODES	8	3	13	4	13	2	17	5
- CONGESTION.	n<10		7.7	n<10	30.8	n<10	29.4	n<10
- REACTIVE HISTIOCYTO.	n<10	n<10	7.7		30.8			n<10
- DILATED LYMPH SINUS.	n<10		51.9	n<10	38.5		64.7	
- REACTIVE LYMPH NODE.	n<10				7.7			n<10
- PLASMOCYTOSIS.	n<10				7.7		5.9	
- CEROID LADEN MACROP.	n<10		7.7		7.7		17.7	
- LYMPHOID CEL. HYPERP.		n<10	15.4	n<10	7.7		5.9	n<10
- ERYTHROPHAGOCYTOSIS.			7.7				23.5	n<10
OTHER ORGANS/TISSUES	5	3	14	6	10	3	9	5
- CYST DEGEN. PARTHYR.	n<10							
- EPIDERMAL CYST, EAR.							n<10	
- POLYARTER. NOD. MESE.							n<10	
- ACUTE URETERITIS.							n<10	
- CHRON. INFL. LACR. GL.							n<10	
- CHRON. INFLAM. CRAN.			7.1		10.0			
- CHROMODACRYOPHEA.			7.1					
- HAEMORRHAGES, CRANI.					10.0			
SUBCUTANEOUS TISSUE	14	23	9	33	13	31	11	26
- ABSCESS.		7.1	n<10		15.4		12.2	
- EPIDERMAL CYST						3.2		
- SUBCUTANEOUS OEDEMA.						3.2		

END OF REPORT SECTION

D. Summary of the Incidence of Neoplastic Findings in Rat Studies.

(Tabular Units, Pages 1 and 2, Number of Animals with Neoplastic Lesions by Organ/Dose Group/Sex for 104 Weeks, as Previously Defined).

(The Six Tabular Units Remaining Were Calculated as the Number of Animals with Neoplastic Lesions/Organ/Dose Group/Sex, for 104 Weeks, But Grouped by Findings Per 200-Day Intervals).

Dose Groups: T=Control, A=0.025 mg/kg/day, B=2 mg/kg/kg/day, C=8 mg/kg/day.

Rat Pathology References: Vol. 49, p. 20: Appendix 1, pp.152-159.

Status at Necropsy: K0=Terminal Sacrifice, Incl.=includes all (sacrificed and moribund), + = moribund or died.

CONCLUSIONS: Sponsor reports that the number of animals with neoplasms, the number of animals with more than one primary neoplasm, and the number of benign and malignant tumors were comparable among all groups, including the control group.

Additionally, incidental tumor analysis was considered as showing that the drug (TRAN) did not produce a decrease in the latency of tumor appearance in the treated animals when compared with controls. This was assessed from the evaluation of the incidental neoplastic lesions observed at each 200 day interval in the animals that died or were sacrificed prematurely before the end of the study (see Table 35).

The incidence and morphological types of hepatic tumors reported by sponsor (hepatocellular adenoma and carcinoma) were similar to those reported for the control values of the rat strain used and showed no indication of treatment or dose relationship.

Kidney tubular cell adenoma (found in 1/60 high dose males, or 1.7%) and **nephroblastomas** (found in 1/46 males, or in 2.2%) were compared with their respective historical controls (2% and 3%) and were found not to be statistically significant. Sponsor concluded that there were no treatment-related effects on kidney or lung tumor distribution.

Lymphoreticular tissue, lymphomas and myeloid leukemias, were found involving many tissue groups, control as well as treated. Sponsor did not consider these effects to be significant.

Table 35. a. Summary of the Incidence of Neoplastic Findings

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX STATUS AT NECROPSY: KO, INCL. *

ORGAN/FINDING	DOSE GROUP: SEX NO. ANIMALS:	T		A		B		C	
		M	F	M	F	M	F	M	F
HEMOLYMPHORET. SYS.	NO. EXAM.:	1		3	1	4	2	2	1
- MYELOID LEUKEMIA.		1		1		1		1	
- MALIGN. LYMPH., MET. T.				1		1		1	1
- MALIGN. LYMPH., LYM-B.				1	1	1			
- MALIGN. LYMPH., IMMUN.						2			
- MALIGN. LYMPH., LYM-C.							1		
HEART	NO. EXAM.:	60	60	26	31	32	32	60	60
- HAEMANGIOENDOTHELIO.					1		1	1	
BRAIN	NO. EXAM.:	60	60	26	31	32	31	60	60
- EPENDYMOA.			1				1		1
- ASTROCYTOMA.									1
DUODENUM	NO. EXAM.:	51	60	23	28	25	28	56	60
- LEIOMYOMA.									1
LIVER	NO. EXAM.:	60	60	41	39	39	40	60	60
- HEPATOCELLULAR ADEN.		2		5	3	1		1	
- DUCTUL. ADENOC. META.					1				
- HEPATOCELLULAR CARC.							1		
PITUITARY GLAND	NO. EXAM.:	60	60	33	40	42	46	60	60
- ACIDO. CHROMO. CEL. AD.		18	31	14	27	24	24	22	27
- CHROMOPHOBE CEL. ADE.		13	8	6	2	3	4	10	15
- ACIDOPHIL CELL ADEN.		15	15	6	6	3	11	10	13
- ADEN. OF PARS INTERM.		1							
SKELETAL MUSCLE	NO. EXAM.:	60	60	29	31	32	31	60	60
- RHABDOMYOMA.					1				
- LIPOSARCOMA.					1				
SKIN	NO. EXAM.:	59	60	37	34	39	34	59	59
- PILOMATRICOMA.		2		2		3		3	
- FIBROUS HISTIOCYTOMA		2		3	1	2	1	2	1
- LIPOMA.		2		2		1		1	
- FIBROMA.		1	1	3		5		2	
- TRICHOFOLLICULOMA.		3				1		2	
- BASAL CELL TUMOUR.		1	1	1	1	1		1	
- HISTIOCYTOMA.		1	1				1		1
- FIBROSARCOMA.								1	1
- MALIGN. HISTIOCYTOMA.									1
- FIBROLOPOMA.					1				
MAMMARY GLAND	NO. EXAM.:	59	60	26	46	32	43	60	59
- FIBROADENOMA.			14		19		20	1	13
- ADENOMA.			6		8		1		6
- CYSTADENOMA.			7		6		6		2
- ADENOFIBROMA.			1		1		3		4
- ADENOCARCINOMA.			2		1				
- DUCTULAR ADENOCARCI.					2				
ADRENAL GLANDS	NO. EXAM.:	59	60	30	35	31	37	60	60
- PHEOCHROMOCYTOMA.			8		2		3	1	6
THYROID GLAND	NO. EXAM.:	60	59	31	31	32	31	60	60
- PARAFOLL. CEL. ADENOMA			1		1		1		1
- FOLLIC. CEL. ADENOMA.			4		3				2
- FOLLIC. CEL. CARCINOMA			1						
PANCREAS	NO. EXAM.:	59	60	28	33	23	31	60	60
- ACINAR CELL ADENOMA.			1		4		1		3
- ISLET CELL ADENOMA.			2	2					
SPLEEN	NO. EXAM.:	59	60	28	31	32	31	60	60
- CAVERNOUS HAEMANGIO.					1				
LUNGS	NO. EXAM.:	60	60	30	32	31	31	60	60
- MAMM. ADENOCARC. MET.			1						
- BRONCHIO-ALVE. CARCI.					1				
- BRONCHIO-ALVE. CARCI.					1				

Table 35. b. Summary of Incidence of Neoplastic Findings

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX STATUS AT NECROPSY: KO, INCL. +									
ORGAN/FINDING	DOSE GROUP: SEX NO. ANIMALS:	T		A		B		C	
		M	F	M	F	M	F	M	F
KIDNEYS	NO. EXAM.:	58	60	39	36	46	36	60	60
- TUBULAR CELL ADENOMA								1	
- NEPHROBLASTOMA						1			
TESTES	NO. EXAM.:	60		39		41		60	
- INTERSTIT. CEL. TUMOUR		2		3		5		4	
UTERUS	NO. EXAM.:		60		35		38		59
- ENDOMETR. STROM. POLYP					1				2
VAGINA	NO. EXAM.:		59		32		29		58
- HAEMANGIOMA									1
SEMINAL VESICLES	NO. EXAM.:	60		32		35		60	
- BRON. ALVEO. CARC., ME.				1					
LYMPH NODES	NO. EXAM.:	8	3	13	4	13	2	17	5
- MALIG. HISTIOCYT., ME.									1
- BRON. ALVEO. CARC., ME.				1					
OTHER ORGANS/TISSUES	NO. EXAM.:	5	3	14	6	10	3	9	5
- LIPOMA, ADIPOSE TIS.				2	2	3		1	
- MESOTHELIOMA								1	
- HAEMANGIOMA, MESENT.			1						
- HAEMAGIOPERIC., MES.					1				
- PAPILOMA, TONGUE				1					

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Table 35. c. Neoplastic Findings Per 200 Day Intervals
Days 201-400

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX									
STATUS AT NECROPSY: + ONLY									
DAYS : 201- 400									
NEOPLASTIC FINDINGS PER 200 DAYS INTERVALS									
ORGAN/FINDING	DOSE GROUP:	T		A		B		C	
		SEX	M	F	M	F	M	F	M
	NO. ANIMALS:	3	1		2	5	5	1	4
HEMOLYMPHORET. SYS.	NO. EXAM.:				1	2			
- MALIGN. LYMPH., MET. T.						1			
- MALIGN. LYMPH., LYM-B.					1	1			
PITUITARY GLAND	NO. EXAM.:	3	1		2	5	5	1	4
- ACIDO. CHROMO. CEL. AD.							1		1
- ACIDOPHIL CELL ADEN.							1		
SKIN	NO. EXAM.:	3	1		2	5	5	1	4
- HISTIOCYTOMA.		1							
- FIBROSARCOMA.									1
OTHER ORGANS/TISSUES	NO. EXAM.:					2	1		
- LIPOMA, ADIPOSE TIS.						1			

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Table 35. e. Days 601-738

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX STATUS AT NECROPSY: + ONLY DAYS : 601- 738 NEOPLASTIC FINDINGS PER 200 DAYS INTERVALS									
ORGAN/FINDING	DOSE GROUP: SEX NO. ANIMALS:	T		A		B		C	
		M	F	M	F	M	F	M	F
HEMOLYMPHORET. SYS.	NO. EXAM.:	17	18	10	21	15	18	18	11
- MYELOID LEUCEMIA.						2	2	1	
- MALIGN. LYMPH. IMMUN.							1	1	
- MALIGN. LYMPH. LYM-C.						2			
							1		
HEART	NO. EXAM.:	17	18	10	21	15	18	18	11
- HAEMANGIOENDOTHELIO.								1	
BRAIN	NO. EXAM.:	17	18	10	21	15	18	18	11
- EPENDYMOA.			1						
DUODENUM	NO. EXAM.:	14	18	8	19	12	16	17	11
- LEIOMYOMA.								1	
LIVER	NO. EXAM.:	17	18	10	21	15	18	18	11
- HEPATOCELLULAR ADEN.								1	
- DUCTUL. ADENOC. META.					1				1
- HEPATOCELLULAR CARC.							1		
PITUITARY GLAND	NO. EXAM.:	17	18	9	21	15	16	18	11
- ACIDO. CHROMO. CEL. AD.		8	17	2	17	7	11	6	6
- CHROMOPHOBE CEL. ADE.		5		4	2	3	3	3	2
- ACIDOPHIL CELL ADEN.		3	1	1	1	2			1
SKIN	NO. EXAM.:	17	18	10	21	15	18	17	11
- PILOMATRICOMA.						1		2	
- FIBROUS HISTIOCYTOMA					1	1	1	2	1
- LIPOMA.		1							
- FIBROMA.						1			
- TRICHOFOLLICULOMA.		2				1		1	
- BASAL CELL TUMOUR.		1	1						
- FIBROSARCOMA.								1	
MAMMARY GLAND	NO. EXAM.:	17	18	10	21	15	18	18	11
- FIBROADENOMA.			3		7		10	2	3
- ADENOMA.			3		3		1		1
- CYSTADENOMA.			2		4		3		
- ADENOFIBROMA.					1				2
- LUCTULAR ADENOCARCI.					2				
ADRENAL GLANDS	NO. EXAM.:	17	18	10	21	14	18	18	11
- PHEOCHROMOCYTOMA.		2			2	2	1	4	
THYROID GLAND	NO. EXAM.:	17	18	10	21	15	18	18	11
- PARAFOLL. CEL. ADENOMA							1		
- FOLLIC. CEL. ADENOMA.		2							1
- FOLLIC. CEL. CARCINOMA		1							
PANCREAS	NO. EXAM.:	17	18	10	20	15	18	18	11
- ACINAR CELL ADENOMA.				1					
- ISLET CELL ADENOMA.		1	1						
LUNGS	NO. EXAM.:	17	18	10	21	15	18	18	11
- FIBROUS HISTIOCYTOMA				1					
TESTES	NO. EXAM.:	17		10		15		18	
- INTERSTIT. CEL. TUMOUR						1		1	
UTERUS	NO. EXAM.:		18		21		18		11
- ENDOMETR. STROM. POLYP									1
OTHER ORGANS/TISSUES	NO. EXAM.:			2	2	3	1	3	2
- LIPOMA, ADIPOSE TIS.					1				
- MESOTHELIOMA.								1	
- HAEMANGIOPERIC. MES.					1				

E. Summary of the Incidence of Neoplastic Findings in Rat Studies.

(Tabular Units, Pages 1 and 2, Number of Animals with Neoplastic Lesions by Organ/Dose Group/Sex, as Percent of Total Animals Examined for 104 Weeks, as Previously Defined)

(The Six Tabular Units Remaining were Calculated as the Number of Animals with Neoplastic Lesions/Organ/Dose Group/Sex, as Percent of Total Animals Examined for 104 Weeks, But Grouped by Findings Per 200-Day Intervals)

Dose Groups: T=Control, A=0.025 mg/kg/day, B=2 mg/kg/kg/day, C=8 mg/kg/day.

Rat Pathology References: Vol. 49, p. 20: Appendix 1, pp.160-167.

Status at Necropsy: K0=Terminal Sacrifice, Incl.=includes all (sacrificed and moribund), + = moribund or died.

Parafollicular cell adenoma of the **thyroid glands** was found in one male rat each from the control group, the low and high dose groups, and in a female rat from the intermediate dose group. **Follicular cell adenomas** occurred in 4/60 control male rats (6.7%), in 3/31 male rats (9.7%) from the low dose group, and in 2/60 females from the high dose group. **Follicular cell carcinomas** were found in 1/60 (1.7%) of the male rats from the control group.

Thyroid tumors were not treatment-related in their effects, and they were within the normal range for old rats in all dosing groups.

The incidence and morphological types of **pituitary gland tumors** found in this experiment were similar to the old rats and there were no differences between treated and control groups.

Mammary gland tumors did not differ significantly between control and treated groups nor were there patterns of treatment or dose relationship.

Sponsor concluded that the drug had neither carcinogenic potential nor increased incidence of spontaneously occurring tumors.

Table 36. a. Summary of the Incidence of Neoplastic Findings (as % of Total)

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX IN % STATUS AT NECROPSY: KO, INCL. +

DOSE GROUP: SEX NO. ANIMALS:	I		A		B		C	
	M 60	F 60	M 60	F 60	M 60	F 60	M 60	F 60
HEMOLYMPHOCYT. SYS.	1		3	1	4	2	2	1
- MYELOID LEUKEMIA.	n<10		n<10			n<10	n<10	
- MALIGN. LYMPH., MET. T.			n<10		n<10		n<10	n<10
- MALIGN. LYMPH., LYM-B.			n<10	n<10	n<10			
- MALIGN. LYMPH., IMMUN.					n<10			
- MALIGN. LYMPH., LYM-C.						n<10		
HEART	60	60	26	31	32	32	60	60
- HAEMANGIOENDOTHELIO.				3.2		3.1	1.7	
BRAIN	60	60	26	31	32	31	60	60
- EPENDYMOMA.		1.7				3.2		1.7
- ASTROCYTOMA.								1.7
DUODENUM	51	60	23	28	28	28	58	60
- LEIOMYOMA.							1.7	
LIVER	60	60	41	39	39	40	60	60
- HEPATOCELLULAR ADEN.	3.2		12.2	7.7	2.6		1.7	
- DUCTUL. ADENOC., META.				2.6				
- HEPATOCELLULAR CARC.						2.5		
PITUITARY GLAND	60	60	33	40	43	46	60	60
- ACIDO. CHROMO. CEL. AD.	30.0	51.7	42.4	67.5	55.8	52.2	36.7	45.0
- CHROMOPHOBE CEL. ADE.	21.7	13.3	18.2	5.0	7.0	8.7	16.7	25.0
- ACIDOPHIL CELL ADEN.	25.0	25.0	18.2	15.0	7.0	23.9	16.7	21.7
- ADEN. OF PARS INTERM.	1.7							
SKELETAL MUSCLE	60	60	29	31	32	31	60	60
- RHABDOMYOMA.			3.5					
- LIPOSARCOMA.			3.5					
SKIN	59	60	37	34	39	34	59	59
- PILOMATRICOMA.	3.4		5.4		7.7		5.1	
- FIBROUS HISTIOCYTOMA	3.4		8.1	2.9	5.2	2.9	3.4	1.7
- LIPOMA.	3.4			5.9		2.9	1.7	
- FIBROMA.	1.7	1.7	8.1		12.8		3.4	
- TRICHOFOOLLICULOMA.	5.1				2.6		1.7	
- BASAL CELL TUMOUR.	1.7	1.7	2.7	2.9	2.6		1.7	
- HISTIOCYTOMA.	1.7	1.7				2.9		1.7
- FIBROSARCOMA.							1.7	1.7
- MALIGN. HISTIOCYTOMA.								1.7
- FIBROLOPOMA.			2.7					
MAMMARY GLAND	59	60	26	46	32	43	60	59
- FIBROADENOMA.		23.3		41.3		46.5	1.7	22.0
- ADENOMA.		10.0		17.4		2.3		10.2
- CYSTADENOMA.		11.7		13.0		14.0		3.4
- ADENOFIBROMA.		1.7		2.2		7.0		6.8
- ADENOCARCINOMA.		3.3		2.2				
- DUCTULAR ADENOCARCI.				4.4				
ADRENAL GLANDS	59	60	10	35	33	37	60	60
- PHEOCHROMOCYTOMA.	13.6		6.7	5.7	9.1	2.7	10.0	8.3
THYROID GLAND	60	59	31	31	32	31	60	60
- PARAFOLL. CEL. ADENOMA	1.7		3.2			3.2	1.7	
- FOLLIC. CEL. ADENOMA.	6.7		9.7					3.3
- FOLLIC. CEL. CARCINOMA	1.7							
PANCREAS	59	60	29	33	33	31	60	60
- ACINAR CELL ADENOMA.	1.7		14.3		3.0		5.0	1.7
- ISLET CELL ADENOMA.	3.4	3.3						
SPLEEN	59	60	28	31	32	31	60	60
- CAVERNOUS HAEMANGIO.			3.6					
LUNGS	60	60	30	32	33	34	60	60
- MAMM. ADENOCARC. (MET.)		1.7						
- BRONCHIO-ALVE. CARCI.			3.3					
- FIBROUS HISTIOCYTOMA			3.3					

Table 36. b. Summary of the Incidence of Neoplastic Findings (as % of Total)

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX IN % STATUS AT NECROPSY: KO, INCL. *

DOSE GROUP: SEX NO. ANIMALS:	T		A		B		C	
	M	F	M	F	M	F	M	F
KIDNEYS	58	60	39	36	46	36	60	60
- TUBULAR CELL ADENOMA							1.7	
- NEPHROBLASTOMA					2.2			
TESTES	60		39		41		60	
- INTERSTIT. CEL. TUMOUR	3.3		7.7		12.2		6.7	
UTERUS		60		35		38		59
- ENDOMETR. STROM. POLYP				2.9				3.4
VAGINA		59		32		29		58
- HAEMANGIOMA								1.7
SEMINAL VESICLES	60		32		35		60	
- BRON. ALVEO. CARC., ME.			3.1					
LYMPH NODES	8	3	13	4	13	2	17	5
- MALIG. HISTIOCYT., ME.								n<10
- BRON. ALVEO. CARC., ME.			7.7					
OTHER ORGANS/TISSUES	5	3	14	6	10	3	9	5
- LIPOMA, ADIPOSE TIS.			14.3	n<10	30.0		n<10	
- MESOTHELIOMA							n<10	
- HAEMANGIOMA, MESENT.		n<10						
- HAEMANGIOPERIC., MES.				n<10				
- PAPILLOMA, TONGUE				7.1				

Table 36 c. Neoplastic Findings (as % of Total) Per 200 Day Intervals
Days 201-400

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX IN 3										
STATUS AT NECROPSY: + ONLY										
DAYS 1 201- 400										
NEOPLASTIC FINDINGS PER 200 DAYS INTERVALS										
DOSE GROUP:	T		A		E		C			
SEX :	M	F	M	F	M	F	M	F	M	F
NO. ANIMALS:	3	1		2	5	5	1	4		
HEMOLYMPHORET. SYS.										
- MALIGN. LYMPH. NET. T.				1		2				
- MALIGN. LYMPH. LYM-B.				n<10		n<10				
.....										
PITUITARY GLAND										
- ACIDO. CHROMO. CEL. AD.	3	1		2	5	5	1	4		
- ACIDOPHIL CELL ADEN.						n<10		n<10		
						n<10				
.....										
SKIN										
- HISTIOCYTOMA.	3	1		2	5	5	1	4		
- FIBROSARCOMA.	n<10							n<10		
.....										
OTHER ORGANS/TISSUES										
- LIPOMA, ADIPOSE TIS.					2	1				
					n<10					
.....										

Table 36. d. Days 401-600

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX IN A STATUS AT NECROPSY: + ONLY									
DAYS : 401- 600									
NEOPLASTIC FINDINGS PER 200 DAYS INTERVALS									
DOSE GROUP:	T		A		B		C		
SEX :	M	F	M	F	M	F	M	F	
NO. ANIMALS:	18	12	15	8	12	7	14	14	
HEMOLYMPHORET. SYS.	1		3				1		
- MYELOID LEUKEMIA.	n<10		n<10						
- MALIGN. LYMPH. .NET. T.			n<10				n<10		
- MALIGN. LYMPH. .LYM-B.			n<10						
HEART	18	12	15	8	12	7	14	14	
- HAEMANGIOENDOTHELIC.				n<10					
BRAIN	18	12	15	8	12	7	14	14	
- EPENDYMOMA.						n<10			7.1
- ASTROCYTOMA.									7.1
LIVER	18	12	15	8	12	7	14	14	
- HEPATOCELLULAR ADEM.	5.6								
PITUITARY GLAND	18	12	15	8	12	7	14	14	
- ACIDO. CHROMO. CEL. AD.	33.3	58.3	46.7	n<10	75.0	n<10	78.6	71.4	
- CHROMOPHOBE CEL. ALE.	16.7		6.7				14.3	14.3	
- ACIDOPHIL CELL ADEM.	22.2	8.3	13.3				7.1	7.1	
SKIN	18	12	15	8	12	7	14	13	
- PILOMATRICOMA.	5.6								
- FIBROUS HISTIOCYTOMA	5.6		6.7		8.3				
- HISTIOCYTOMA.		8.3				n<10			
- MALIGN. HISTIOCYTOMA.									7.7
MAMMARY GLAND	18	12	15	8	12	7	14	13	
- FIBROADENOMA.		25.0		n<10		n<10			23.1
- ADENOMA.		8.3							
- CYSTADENOMA.		8.3							
- ADENOFIBROMA.		8.3				n<10			7.7
ADRENAL GLANDS	18	12	15	8	12	7	14	14	
- PHEOCHROMOCYTOMA.	5.6				8.3		7.1		
THYROID GLAND	18	12	15	8	12	7	14	14	
- FOLLIC. CEL. ADENOMA.	5.6								
LUNGS	18	12	15	8	12	7	14	14	
- BRONCHIO-ALVE. CARCI.			6.7						
DOSE GROUP:	T		A		B		C		
SEX :	M	F	M	F	M	F	M	F	
NO. ANIMALS:	18	12	15	8	12	7	14	14	
VAGINA		12		8		6		13	
- HAEMANGIOMA.								7.7	
SEMINAL VESICLES	18		15		12		14		
- BRON. ALVEO. CARC. .ME.			6.7						
LYMPH NODES	6	1	4	1	2		3	3	
- MALIGN. HISTIOCYT. .ME.									n<10
- BRON. ALVEO. CARC. .ME.			n<10						
OTHER ORGANS/TISSUES	3	2	4	2	2		1	1	
- LIPOMA. ADIPOSE TIS.				n<10					
- PAPILOMA, TONGUE.			n<10						

Table 36. e. Days 601-738

NUMBER OF ANIMALS WITH NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX IN A STATUS AT NECROPSY: → ONLY DAYS : 601- 738 NEOPLASTIC FINDINGS PER 200 DAYS INTERVALS										
DOSE GROUP:	T		A		B		C			
SEX :	M	F	M	F	M	F	M	F	M	F
NO. ANIMALS:	17	18	10	21	15	18	18	11		
HEMOLYMPHRET. SYS.										
- MYELOID LEUKEMIA.					2	2	1			
- MALIGN. LYMPH., IMPUN.					n<10	n<10	n<10			
- MALIGN. LYMPH., LYM-C.						n<10				
.....										
HEART										
- HAEMANGIOENDOTHELIO.	17	18	10	21	15	18	18	11		
							5.6			
.....										
BRAIN										
- EPENDYMOA.	17	18	10	21	15	18	18	11		
		5.6								
.....										
DUODENUM										
- LEIOMYOMA.	14	18	8	19	12	16	17	11		
							5.9			
.....										
LIVER										
- HEPATOCELLULAR ADEN.	17	18	10	21	15	18	18	11		
- DUCTUL. ADENOC., META.					4.8		5.6			7
- HEPATOCELLULAR CARC.						5.6				
.....										
PITUITARY GLAND										
- ACIDO. CHROMO. CEL. AD.	17	18	9	21	15	18	18	11		
	47.1	94.4	n<10	81.0	46.7	61.1	33.3	54.6		
- CHROMOPHOBE CEL. ADE.	29.4		n<10	9.5	20.0	16.7	16.7	18.2		
- ACIDOPHYL CELL ADE.	17.7	5.6	n<10	4.8		11.1		9.1		
.....										
SKIN										
- PILOMATRICOMA.	17	18	10	21	15	18	17	11		
- FIBROUS HISTIOCYTOMA					4.8	6.7	5.6	11.8		9.1
- LIPOMA.	5.9									
- FIBROMA.					6.7					
- TRICHO FOLLICULOMA.	11.8				6.7		5.9			
- BASAL CELL TUMOUR.	5.9	5.6								
- FIBROSARCOMA.							5.9			
.....										
MAMMARY GLAND										
- FIBROADENOMA.	17	18	10	21	15	18	18	11		
		16.7		33.3		55.6	5.6	27.3		
- ADENOMA.		16.7		14.3		5.6		9.1		
- CYSTADENOMA.		12.2		19.0		16.7				
- ADENOFIBROMA.				4.8				16.2		
- DUCTULAR ADENOCARCI.				9.5						

F. Summary Tables From Rat Pathology Reports Evaluating Status of Neoplastic Lesions (Page 150 to 160)

Dose Groups:

T=Controls, A=0.25 mg/kg/day, B=2 mg/kg/day, C=8 mg/kg/day

Sex: M=Male, F = Female

Rat Pathology References: Vol. 49, pp. 168-175

(Pages 174-175 are Repeats of 170-171)

Tables: Appendix 1, pp. 110-159

CODES FOR ANIMALS' STATUS AT NECROPSY:

K0=Terminal Sacrifice:

Incl. = Indicates inclusion of all sacrificed and moribund animals;

+ Includes all animals which are moribund or which have died.

Table 37. Evaluation of the Number and Nature of Neoplastic Lesions at Necropsy Following Terminal Sacrifice (K0) in Rat Carcinogenicity Study (Vol. 49, pp.168-169)

a. Number of Animals with Neoplasms

DOSE GR:	T		A		B		C		TOTAL	
SEX :	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	22	27	31	26	25	25	25	30	104	109
NO. AFF.:	21	27	23	20	19	1	19	29	82	95
% :	95.5	100.0	74.2	76.9	76.0	73.1	73.1	96.7	78.9	87.2

b. Number of Animals with Greater than One Primary Neoplasm

DOSE GR:	T		A		B		C		TOTAL	
SEX :	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	22	27	31	26	25	25	25	30	104	109
NO. AFF.:	11	17	12	9	9	9	12	21	44	51
% :	50.0	63.0	38.7	34.6	36.0	34.6	48.2	70.0	42.3	46.4

c. Number of Animals with Metastases

DOSE GR:	T		A		B		C		TOTAL	
SEX :	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	22	27	31	26	25	26	26	30	104	109
NO. AFF.:	0	1	0	0	0	0	0	0	0	1
% :	0.0	3.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9

d. Number of Benign, Unclassified, Malignant, Neoplasms/Group/Sex

DOSE GR:	T		A		B		C		TOTAL	
SEX :	M	F	M	F	M	F	M	F	M	F
BENIGN :	36	45	39	34	28	31	35	56	138	156
UNCLASS.:	0	0	0	0	0	0	0	0	0	0
MALIGN.:	0	2	1	1	0	0	0	1	1	4

Table 38. Evaluation of the Number and Nature of Neoplastic Lesions at Necropsy Following Inclusion of all Sacrificed and Moribund Animals (KO, Incl., and +). This is shown on pp. 170-171 of Vol. 49.

a. Number of Animals with Neoplasms

DOSE GR:	T		A		B		C		TOTAL	
SEX :	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	60	60	57	57	57	57	60	60	234	234
NO. AFF.:	53	55	43	49	43	45	49	56	188	205
% :	88.3	91.7	75.4	86.0	75.4	79.0	81.7	93.3	80.3	87.6

b. Number of Animals with Greater than One Primary Neoplasm

DOSE GR:	T		A		B		C		TOTAL	
SEX :	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	60	60	57	57	57	57	60	60	234	234
NO. AFF.:	22	30	17	26	17	23	22	31	78	110
% :	36.7	50.0	29.8	45.6	29.8	40.4	36.7	51.7	33.3	47.0

c. Number of Animals with Metastases

DOSE GR:	T		A		B		C		TOTAL	
SEX :	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	60	60	57	57	57	57	60	60	234	234
NO. AFF.:	0	1	1	1	0	0	0	1	1	3
% :	0.0	1.7	1.8	1.8	0.0	0.0	0.0	1.7	0.4	1.3

d. Number of Benign, Unclassified, Malignant, Neoplasms/Group/Sex

DOSE GR:	T		A		B		C		TOTAL	
SEX :	M	F	M	F	M	F	M	F	M	F
BENIGN :	80	89	59	83	57	76	74	95	270	343
UNCLASS.:	0	0	0	0	0	0	0	0	0	0
MALIGN.:	2	2	6	4	4	3	3	3	15	12

Table 39. Evaluation of the Number and Nature of Neoplastic Lesions at Necropsy Following Inclusion of Animals Which had Died or Become Moribund (+) as shown in Vol. 49, pp. 172-173.

a. Number of Animals with Neoplasms

DOSE GR:	T		A		B		C		TOTAL	
	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	38	33	26	31	32	31	34	30	130	125
NO. AFF.:	32	28	20	29	24	26	30	27	106	110
%	84.2	84.9	76.9	93.6	75.0	83.9	88.2	90.0	81.5	88.0

b. Number of Animals with Greater than One Primary Neoplasm.

DOSE GR:	T		A		B		C		TOTAL	
	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	38	33	26	31	32	31	34	30	130	125
NO. AFF.:	11	13	5	17	8	14	10	10	34	54
%	29.0	39.4	19.2	54.8	25.0	45.2	29.4	33.3	26.2	43.2

c. Number of Animals with Metastases.

DOSE GR:	T		A		B		C		TOTAL	
	M	F	M	F	M	F	M	F	M	F
NO. EXAM:	38	33	26	31	32	31	34	30	130	125
NO. AFF.:	0	0	1	1	0	0	0	1	1	2
%	0.0	0.0	3.9	3.2	0.0	0.0	0.0	3.3	0.8	1.6

d. Number of Benign, Unclassified, Malignant, Neoplasms/Group/Sex.

DOSE GR:	T		A		B		C		TOTAL	
	M	F	M	F	M	F	M	F	M	F
BENIGN:	44	44	20	49	29	45	39	39	132	177
UNCLASS:	0	0	0	0	0	0	0	0	0	0
MALIGN.:	2	0	5	3	4	3	3	2	14	8

Appendix 1B

Week of Appearance of Individual Palpable Masses

Dose Group (mg/kg/d)	Sex	First Week Observed	Microscopic Examination Results
Mice			
Control	M	67	Fibrohistiocyoma
Control	M	75	Fibrohistiocyoma
-	F	-	-
1	M	74	Suppurative cellulitis
1	F	45	Histiocyoma
5	M	45	Not found at Autopsy
5	F	43	Disappeared in wk 51
Rat			
Control	M	39	Histiocyoma
Control	F	41	Disappeared
0.25	M	41	Disappeared
0.25	F	49	Adenofibroma, mammary glands
2	M	84	Disappeared
2	F	57	Adenofibroma, mammary glands
8	M	45	Disappeared
8	F	18	Disappeared
25	M	-	-
25	F	49	Disappeared

References:

Mice: Vol. 45:pp. 63-65

Rats: Vol. 50:pp. 73-91

Appendix 1 C

Numbers of Animals Alive at Termination by Sex and by Dose

Number of Animals Alive at Experiment Termination, by Sex and by Dose Group					
	Sex	Dose (mg/kg) Groups			
Doses		0	1	5	25
	Male	53	52	51	49
Mice	Female	43	46	48	45
	Both Sexes	96	98	99	94
Doses		0	0.25	2	8
	Male	22	34	28	26
Rats	Female	27	29	29	30
	Both Sexes	49	63	57	56

References:

Mice: Vol. 46:pp. 32-61. Control n = 60.

Rats: Vol. 50:pp. 48-70. Control n = 30.

III. D. REPRODUCTION TOXICITY

III. D. I. Introduction

It is to be noted that, unless otherwise specified, the following conventions will be followed in this subdivision:

(Trandolapril), the ethyl ester prodrug of the angiotensin converting enzyme inhibitor will be referred to as TRAN.

(Trandolaprilat) will be referred to as TRAN-AT.

ACEI refers to Angiotensin Converting Enzyme Inhibitor (s).

Sponsor, for whom experiments were conducted, and to whom the data were reported:

3'

The experiments were conducted by:

GLP Compliance: Yes, for all experiments

Data Retention and Storage: Yes, 10 yr. at laboratory site

III. D.2. TRAN: Oral (Gavage) Fertility Study in the Rat:

Location of Data: Vol. 55 of 418, dated June 1988.

Testing Facility:

Study No./Dates: 5661-552/9. Study from 26 February through 27 October 1987.

Animals: Charles River Crl:CD(SD)BR strain weighing 238 to 280 g.

Mode of Administration:	Orally, by gavage
Dose Levels:	0, 1, 10, and 100 mg/kg/day TRAN
No. of Animals:	30 animals of each sex were allocated to each of the 3 dose groups prior to pairing for mating (60 days for males, 14 days for females), during pairing, gestation, and lactation, through to termination. The control group (0 mg/kg/d) received vehicle, 0.5 mg/ml methyl cellulose. These were the P, or parental, generation.

The objective of this study was to assess the effects of TRAN on fertility and general reproductive performance of the rat, when administered orally, by gavage, prior to pairing for mating, during the pairing, mating, gestation, and lactating periods.

Approximately 50% (15) of the females in each group were killed on day 20 of gestation (gd 20) and the pups were removed by cesarean section for evaluation of litter parameters. The remainder of the P generation females were allowed to litter and rear their offspring (F_{1a}) to weaning. From each dosing group, 15 males and 15 females of the weaned offspring were selected from as many litters as possible to form the first filial (F₁) generation. The F₁ generation was reared to maturity and allowed to mate and rear offspring (F_{2a}) to weaning.

Body weight gains of the mid and high dose P generation males were significantly lower than control during weeks 0-13. Food consumption data were not provided for males. Although food consumption of females was not reduced during pregnancy, food consumption of all treated dams and litters was significantly reduced throughout the post-partum period. There were no significant effects on P generation mortality rates, clinical or postmortem observations, mating performance, pregnancy rate, or duration of gestation.

Post-cesarean data indicated no effects on the number of corpora lutea, implantations, pre-or post-implantation losses, number and sex of fetuses, litter and mean fetal weight, or major fetal defects. A higher incidence of minor external/visceral defects in the mid and high dose groups (no evidence of statistical evaluation) was due to the significantly higher incidence of increased renal pelvic cavitation and dilated ureters (see Table 13), a selective compilation from sponsor's page B16 in Table 5 of Volume 55, showing the only significant post-cesarean fetal defects in the P generation).

Table 13
Segment I Study in Rats: Post-Cesarean Defects

Group Mean Caesarian Data-P Generation

Group incidence of defects

Type of defect	Classification	Group 1	Group 2	Group 3	Group 4
<u>Heart and great vessels</u>					
Atrium/atria enlarged	minor	72 (43.1)	60 (37.5)	80 (41.7)	77 (39.7)
Atrium/atria reduced	minor	1 (0.6)		2 (1.0)	2 (1.0)
Abnormal common carotid	minor	1 (0.6)		2 (1.0)	5 (2.6)
<u>Abdomen</u>					
Gonadal	major			1 (0.5)	
Haemorrhagic adrenal(s)	variant	4 (2.4)	10 (6.3)	11 (5.7)	4 (2.1)
Dilated ureter(s)	minor	72 (43.1)	90 (56.3)	128 (66.7) [*]	147 (75.0) ^{**}
Increased renal pelvic cavitation (bilateral)	minor	1 (0.6)	5 (3.1)	4 (2.1)	18 (9.3) ^{**}
Increased renal pelvic cavitation (unilateral)	minor	8 (4.8)	9 (5.6)	21 (10.9) [*]	21 (10.8)
Combined bilateral and unilateral increased renal pelvic cavitation	minor	9 (5.4)	14 (8.8)	25 (13.0) [*]	39 (20.1) [*]
Kidney reduced in size (unilateral)	minor			2 [†]	1 (0.5)
<u>SKELETAL DEFECTS</u>					
<u>Skull</u>					
Eye socket(s) reduced in size	major	2 (2.4)			
All skull bones incompletely ossified	minor		2 (2.4)		1 (1.0)
All skull bones except zygomatic arch incompletely ossified	minor	3 (3.5)	1 (1.2)	2 (2.0)	2 (2.0)
Frontal cleft	minor		1 (1.2)		
Frontal(s) incompletely ossified	minor	20 (23.5)	15 (18.3)	23 (23.2)	31 (30.4)
Interparietal cleft	minor	2 (2.4)	1 (1.2)	3 (3.0)	2 (2.0)
Interparietal fissure	minor	1 (1.2)	1 (1.2)		
Interparietal incompletely ossified	minor	20 (23.5)	13 (15.9)	19 (19.2)	14 (13.7)
Nasal(s) fused	major		1 (1.2)		
Nasal(s) incompletely ossified	minor	18 (21.2)	12 (14.6)	14 (14.1)	14 (13.7)
Occipital incompletely ossified	minor	14 (16.5)	14 (17.1)	10 (10.1)	16 (15.7)
Occipital cleft	minor	2 (2.4)			
Parietal(s) incompletely ossified	minor	5 (5.9)	2 (2.4)	2 (2.0)	1 (1.0)
Sutural bone(s)	minor		1 (1.2)		

Figures in brackets denote the percentage of affected fetuses/fetuses examined

A fetus with multiple defects will appear more than once in this tabulation

Statistical analysis: Intergroup difference from the control group significant at: Fisher's Randomisation test:

p<0.05 *

p<0.01 **

Litter data showed no effects on pup numbers, weight, or physical and functional development. Necropsy of pups found dead or culled prior to weaning showed higher incidence (significance not stated) of pups with renal pelvic cavitation, referred to as "kidney changes" in the table below (reproduced from sponsor's Vol. 55, p. A 30):

Incidence of kidney changes at weaning (F _{1a})	Group			
	1	2	3	4
Number of affected litters	1	2	4	7
Number of litters at weaning	12	12	13	13
Number of affected offspring	1	2	11	21
Number of offspring at weaning	90	77	84	79

N.B. Groups 1, 2, 3, and 4 refer to groups receiving control, 1, 10, and 100 mg/kg/day, respectively.

The F₁ generation was selected from weaned offspring (formerly called the F_{1a} generation) on, or shortly after, day 21 post partum. There were no effects on mortality, clinical observations, body weight, body weight gain, or on food consumption. High-dose derived F₁ males and females drank approximately twice as much water as did the control-derived animals. High-dose derived males showed increased (significance was not stated) swimming maze times compared with controls. Mating performance was slightly reduced in the high dose-derived group. At terminal necropsy, high dose-derived F₁ adults showed a greater incidence of macroscopic (principally increased renal pelvic cavitation) and microscopic (lesions of the adrenal medulla and /or papilla) changes considered to be the result of abnormal development and collectively termed papillary/medullary dystrophy. The F₁ findings are shown below in a table of group incidences of necropsy findings abstracted from page A 33 of Vol. 55:

Necropsy finding F ₁ adults	Group derivation			
	1	2	3	4
<u>Males:</u>				
Group size	14	15	15	15
Number showing:				
increased renal pelvic cavitation	3	2	4	12
renal cyst(s)	0	0	0	1
pale kidneys	0	0	0	1
<u>Females:</u>				
Group size	15	15	15	15
Number showing:				
increased renal pelvic cavitation	3	5	1	8

Litter data demonstrates that there were no significant differences from control in duration of gestation or pup numbers, weights, observations, and necropsy findings for the F_{2a} generation. The lack of effect on kidney changes is shown in the following Table derived from the table on page A 33, Vol.55:

Incidence of kidney changes at weaning (F _{2a})	Group derivation			
	1	2	3	4
Number of affected litters	6	4	4	2
Number of litters at weaning	15	13	15	13
Number of affected offspring	14	13	7	4
Number of offspring at weaning	145	126	142	125

N.B. Group derivation was as described for the preceding table.

III. D. 3. TRAN: Oral (Gavage) Teratology Study in Rat:

Location of Data: Vol. 25 of 418, dated December 1986.

Testing Facility:

Study No. /Dates: 5385-552/5 Study dates from 19 Jun to 31 Jul 1986.

Animals: Sexually mature, virgin rats of the Crl:CD(SD)BR strain from Charles River
Females within weight range of 205-274 g. at mating; 10-12 week old.

Mode of Administration: Orally by gavage

Dose Levels: 0, 100, 300, and 1000 mg/kg/day TRAN

No. of Animals: 24 timed-pregnant originally sexually mature, virgin, females.

The objective of the study was to evaluate the effects of TRAN administered orally, by gavage, to pregnant rats during the period of organogenesis.

The pregnant female animals were dosed with either the drug or the vehicle (0.5% methyl cellulose) from gestation days 6 to 15 (gd 6 to gd 15). On gd 20, the animals were killed, the uteri examined, and the litter parameters determined.

The dams' mortality was unaffected by TRAN; there were no remarkable clinical or post-mortem macroscopic observations. At cesarean section, there were no significant differences between treated and control groups in terms of implantations, post-implantation losses, number and sex of fetuses, litter weight, mean fetal weight, and major fetal defects.

Due to an increase in the number of fetuses with dilated ureters and increased renal pelvic cavitation (Table 14, Sponsor's Table 4, B7), the overall incidence of minor visceral defects was significantly increased in the high-dose group and to a lesser extent in the low dose group (Table 15, Sponsor's Table 3, B-5). No other visceral or skeletal defects were significantly increased above control levels.

It should be noted that the treated groups' incidence of renal pelvic cavitation, but not dilated ureters, was within the range of the mean historical control values (for 6 teratology studies prior to the present study at laboratories. This can be seen by comparing the two studies above with Table 16 (Sponsor's Appendix 8.2.1) which follows:

Table 14
Segment II Study in Rats: Group Incidence of Defects

Key	Type of defect	Classification	Group 1	Group 2	Group 3	Group 4
<u>Thorax</u>						
13	Atrium/atria enlarged	minor	47(14.9)	18(6.6)	39(14.0)	36(13.1)
14	Atrium/atria reduced in size	minor		4(1.5)	3(1.1)	1(0.4)
<u>Abdomen</u>						
16	Bilateral increased renal pelvic cavitation	minor	4(1.3)	15(5.5)	10(3.6)	26(9.5)
17	Unilateral increased renal pelvic cavitation	minor	6(1.9)	12(4.4)	10(3.6)	15(5.5)
	Combined bilateral and unilateral increased renal pelvic cavitation	minor	10(3.2)	27(9.9)	20(7.2)	41(14.9)
18	Dilated ureter(s)	minor	110(34.8)	158(58.1)	140(50.4)	185(67.3)
19	Decrease in kidney size	minor	1(0.3)			
20	Gaseous distension of stomach	minor	1(0.3)			
a	Haemorrhagic adrenal(s)	variant	8(2.5)	11(4.0)	15(5.4)	16(5.8)
<u>SKELETAL DEFECTS</u>						
<u>Skull</u>						
21	Nasal(s) incompletely ossified	minor	19(8.7)	15(8.0)	7(3.6)	13(6.8)
22	Frontal(s) incompletely ossified	minor	60(27.4)	57(30.5)	60(31.1)	62(32.6)
23	Interparietal incompletely ossified	minor	26(11.9)	16(8.6)	14(7.3)	23(12.1)
24	Occipital incompletely ossified	minor	40(18.3)	24(12.8)	21(10.9)	27(14.2)
25	Parietal(s) incompletely ossified	minor	4(1.8)	1(0.5)		3(1.6)
26	Cleft in frontal	minor	2(0.9)		1(0.5)	
27	Cleft/fissure in interparietal	minor	9(4.1)	4(2.1)	6(3.1)	10(5.3)
28	Cleft/fissure in parietal	minor			1(0.5)	
29	Sutural bone between frontals	minor			1(0.5)	
30	All skull bones except zygomatic arch incompletely ossified	minor	1(0.5)			
31	Zygomatic arch incompletely ossified	minor		1(0.5)	1(0.5)	1(0.5)

Figures in brackets denote the percentage of affected foetuses/foetuses examined.
A foetus with multiple defects will appear more than once in this tabulation.

Table 15
Group Mean Cesarean Data

3.3 Fetal defect data

	Group 1	Group 2	Group 3	Group 4
EXTERNAL AND VISCERAL DEFECTS				
Number of foetuses examined	316	272	278	275
Number showing major defects	1	1	0	1
% of foetuses examined	0.3	0.4	0.0	0.4
Number showing minor defects	166	185	175	212
% of foetuses examined	52.5	68.0*	62.9	77.1**
Number showing variants	9	15	22	17
% of foetuses examined	2.8	5.5	7.9	6.2
SKELETAL DEFECTS				
Number of foetuses examined	219	187	193	190
Number showing major defects	0	1	0	0
% of foetuses examined	0.0	0.5	0.0	0.0
Number showing minor defects	120	87	91	101
% of foetuses examined	54.8	46.5	47.2	53.2
Number showing variants	190	171	174	171
% of foetuses examined	86.8	91.4	90.2	90.0
Total number showing major defects	1	1	0	1
% of foetuses examined	0.3	0.4	0.0	0.4

* statistically significant from control values p<0.05 (Fisher's Randomisation test)
 ** statistically significant from control values p<0.01 (Fisher's Randomisation test)

Table 16
Historical Control Mean Defect Data

B.2 Defect data

B.2.1 Control group mean incidence of specific defects from the 6 rat teratology studies preceding the present study

Study	Number of litters	Number of foetuses	Foetuses showing:			Dilated ureter(s)	
			Bilateral No. (%)	Unilateral No. (%)	Combined No. (%)	No. (%)	
1	17	192	16 (8.3)	20 (10.4)	36 (18.8)	40 (20.8)	
2	22	278	14 (5.0)	13 (4.7)	27 (9.7)	79 (28.4)	
3	23	282	15 (5.3)	16 (5.7)	31 (11.0)	102 (36.2)	
4	24	260	13 (5.0)	9 (3.5)	22 (8.5)	66 (25.4)	
5	21	259	14 (5.4)	6 (2.3)	20 (7.7)	84 (32.4)	
6	20	234	5 (2.1)	4 (1.7)	9 (3.8)	73 (31.2)	
$\bar{x}$ =	21	251	13 (5.2)	11 (4.7)	24 (11.4)	74 (29.1)	

Furthermore, the laboratory performing the studies considers that these defects are known to appear in control group gd 20 fetuses but are rare in weaned offspring.

In summary, sponsor considers these defects to be of doubtful functional significance.

III. D.3. TRAN: Oral (Gavage) Teratology Study in The Rabbit:

Location of Data:	Vol. 55 of 418, Dated April 1987.
Testing Facility:	
Study No./Dates:	5379-552/7 19 Jun to 20 Aug 1986
Animals:	Sexually mature virgin New Zealand White rabbits were obtained from 64 were used initially, and more later for replacements. Male rabbits of the same strain were used for fertilization without dosing.
Mode of Administration:	Orally, by gavage
Dose Levels:	0, 0.2, 0.4, and 0.8 mg/kg/day of TRAN
No. of Animals:	16 timed-pregnant originally sexually mature, virgin females were used. Due to reduced pregnancy rate and the number of deaths, 5 replacement females were added to each group, for a total of 21 per group.

The objective of the study was to evaluate the effects of TRAN administered orally by gavage, to pregnant rabbits during the period of organogenesis. The dose groups were administered from gd 6 through gd 18. On gd 28, the animals were killed, the uteri examined, and the litter parameters were determined.

Mortality of dams was observed in all groups, but was most severe within the highest dose group. There were 5, 6, 7, and 12 of the 21 animals (from the control vehicle, 0.2, 0.4, 0.8 mg/kg dosing groups, respectively) which died or were killed for humane

reasons before the end of the 28 day dosing period. Clinical observations were most severely modified in the high dose group, but the intensity was dose-related. The observations were hunched posture, loss of appetite, decreased body weight and decreased body weight gain, with the clinical condition of group 2 being essentially the same as control. There were no consistent treatment-related macroscopic findings on gd 28.

Data at the time of cesarean delivery (see Table 17, sponsor's Table 3, part 3.1) revealed that there was an apparent reduction in the mean number of corpora lutea in all treated groups compared with the concurrent controls. However, these values were within the "background range" (historical control range based on the previous 6 studies done in the laboratories) of 8.0-13.4, as shown in Table 18 (copied from Sponsor's Table 8.1.1, Appendix 8). Pre-implantation loss was increased in all treated groups compared to the concurrent and historical controls. Consequently, the mean number of implantations was reduced in all groups, but the values of groups 2 and 4 were the only values outside the range seen in historical controls. Sponsor concludes that "Although dosing commenced at around the time of implantation, TRAN involvement in the increased pre-implantation loss could not be excluded".

Post-implantation loss was increased in group 4 compared to the concurrent control value; this resulted in a marked reduction in the mean number of viable fetuses. Post-implantation loss in groups 2 and 3 was similar to the controls, but the mean number of fetuses in these groups were reduced. These reductions were principally the result of the increased pre-implantation loss.

The ratio of male to female fetuses showed some intergroup variation, but no treatment-related trends were apparent. Mean fetal weight was not adversely affected by TRAN treatment. (See Table 17, sponsor's Table 3, part 3.2, p. 3.3, Vol. 26).

The totals of fetuses with major defects were 2, 1, 4, and 5, seen in the control, low, intermediate, and high dose groups, respectively; these represented 1.6%, 1.2%, 4.0%, and 10.9% of the total fetuses examined. (See Table 19, Sponsor's Table 3.3, p. B 5).

To provide the reader with a sensing of the distribution of defects, sponsor's Table 4, pp. B 6 through B 10 was condensed to summarize the Group Incidence of Defects as Tables 20, Parts A-D. As can be seen in these tables, the defects are classified as major (rare and /or possibly lethal defects) minor (commonly occurring non-lethal effects, or variants (abnormal common carotid, retarded skull, sternebral or phalangeal ossification, asymmetric insertion of the pelvic girdle, or the presence of supernumerary ribs).

Table 20, Parts A and B had the incidences of defects concentrated in one of the 4 columns (dosing groups) so that it was easy to summarize the data without crowding the data together. Using the key numbers to identify the types of defects we find that

there were single incidences of defects 1 and 2 seen in the first dosing group, one incidence of defect 3 seen in the second dosing group, one incidence each of defects 4 through 7 seen in dosing group 3, and one incidence each of defects 8 through 12 seen in group 4.

From sponsor's Table 3, p. B 3, it can be seen that the values in parentheses () = "Mean values of pregnant females surviving to day 28 of gestation".

To review, a total of 2, 1, 4, and 5 fetuses with major defects were seen in the control, low, intermediate, and high dose groups, respectively. The high incidence of major defects in group 4 suggests the possibility of a treatment effect of TRAN. Dosing group 3 was slightly increased compared with controls, however, this level of defect has been seen in control group litters. TRAN involvement cannot be completely ruled out but it seems unlikely in view of the historical control data (sponsor's Appendix 8). The incidence of major defects in group 2 was similar to that of controls. The incidence of fetuses with external and visceral minor defects in group 3 was greater than in the control group. This increase could be identified as due to 10 fetuses with enlarged atrium or atria. This finding appears to be strong, but the absence of a similar finding in dosing group 4 suggests that this may be a false positive.

There were no significant differences between control and treated groups relative to minor external and visceral defects in groups 2 and 4. There was no effect on external and visceral variants by TRAN. There were no significant differences in ossification parameters of fetuses without defects.

In conclusion, oral administration of TRAN, by gavage, at a dose of 0.8 mg/kg/day, during the interval of gd 6 to gd 18 was associated with maternal toxicity accompanied by death in 12/21 females. Treatment with the drug had an adverse effect on maternal physical condition which was accompanied by a marked reduction in body weight gain. Pre- and post-implantation losses were increased with a net effect of a marked decrease in the mean number of viable fetuses. The overall incidence of major malformations was also increased, but no other adverse effects on fetal development were observed at this dose.

Physical condition deteriorated and body weight gain was reduced during treatment with 0.4 mg/kg/day treatment. No other significant changes were seen at this dose.

The 0.2 mg/kg/day dosing was accompanied by a decrease in early body weight gain and a slight increase in pre-implantation loss. There were no other adverse maternal or fetal effects.

Table 17 Group Mean Cesarean Data

Part 3.1 Uterine/Implantation Data

	Group 1	Group 2	Group 3	Group 4
Females with live foetuses at day 28	14	13	14	8
Number of corpora lutea	160	121	135	76 (83)
Mean ² number per female	11.4	9.3	9.6	9.5 (9.2)
Number of implantations	138	92	109	55 (61)
Mean ² number per female	9.9	7.1	7.8	6.9 (6.8)
% pre-implantation loss	13.8	24.0	19.3	27.6 (26.5)
Number of early intrauterine deaths	2	1	5	5 (11)
Mean ² number per female	0.1	0.1	0.4	0.6 (1.2)
Number of late intrauterine deaths	8	7	3	4 (4)
Mean ² number per female	0.6	0.5	0.2	0.5 (0.4)
% post-implantation loss	7.2	8.7	7.3	16.4 (24.6)
Number of foetuses	128	84	101	46 (46)
Mean ² number per female	9.1	6.5	7.2	5.8 (5.1)
% of implantations	92.8	91.3	92.7	83.6 (75.4)

() - Mean¹ values of pregnant females surviving to day 28 of gestation

Mean² = pregnant females surviving to day 28 of gestation with live foetuses

Table 17. Group Mean Cesarean Data (Continued)
Part 3.2 Fetal Data

	Group 1	Group 2	Group 3	Group 4
Number of male foetuses	62	50	53	23
Number of female foetuses	66	34	48	23
Sex ratio males : females	1 : 1.06	1 : 0.68	1 : 0.91	1 : 1.00
Mean litter weight (g)	316.2	238.8	252.4	197.5
Mean foetal weight (g)	35.2	37.6	36.2	36.4
Mean foetal weight (g) - males only	35.4	37.8	36.3	34.9
Mean foetal weight (g) - females only	34.4	36.2	35.9	37.9

3'

Table 18
Sponsor's Appendix 8: Laboratory Standard Data For New Zealand White Rabbits
Used in Teratology Studies at

Caesarian data

...1 Control group mean values from the 6 rabbit teratology studies preceding the present study

Study	Pregnancy %	Pregnant females surviving to term	Corpora lutea	Implantations	Pre-implantation loss %	Intrauterine deaths		Post-implantation loss %	Foetuses	Litter weight g	Foetal weight g	Sex ratio males : females 1 :
						Early	Late					
1	100.0	15	10.2	9.3	8.5	0.0	0.0	8.6	8.5	313.2	37.6	1.36
2	93.8	15	8.0	7.3	9.2	0.1	0.5	8.3	6.7	233.1	34.7	0.92
3	93.8	15	9.0	7.8	13.3	0.1	0.3	6.0	7.3	277.0	39.4	0.72
4	100.0	16	9.5	8.6	9.2	0.5	0.2	8.0	7.9	294.4	37.7	1.05
5	100.0	14	10.9	10.4	4.6	1.3	0.4	16.6	8.6	310.6	36.0	0.95
6	100.0	15	13.4	11.3	15.9	0.1	1.0	9.5	18.2	353.2	35.1	0.84

Table 19
Segment II Study in Rabbits: Group Mean Cesarean Data
Part 3.3 Fetal Defect Data

	Group 1	Group 2	Group 3	Group 4
Number of fetuses examined	128	84	101	46
<u>EXTERNAL AND VISCERAL DEFECTS¹</u>				
Number showing major defects	2	0	3	4
% fetuses examined	1.6	0.0	3.0	8.7
Number showing minor defects only	3	4	14	2
% fetuses examined	2.3	4.8	13.9	4.3
Number showing variants	23	13	19	10
% of fetuses examined	18.0	15.5	18.8	21.7
<u>SKELETAL DEFECTS¹</u>				
Number showing major defects	1	1	1	5
% fetuses examined	0.8	1.2	1.0	10.9
Number showing minor defects only	60	47	40	19
% fetuses examined	46.9	56.0	39.6	41.3
Number showing variants	110	80	94	43
% fetuses examined	85.9	95.2	93.1	93.5
Total number of fetuses showing major defects	2	1	4	5
% fetuses examined	1.6	1.2	4.0	10.9

¹ fetuses with multiple defects are recorded more than once

Table 20, Part A.
Group Incidence of Defects

Key	Type of defect	Classification
MAJOR MALFORMATIONS		
1	Umbilical hernia	major
2	Multiple defects including: spina bifida; parietals incompletely ossified; interparietal incompletely ossified; occipital incompletely ossified; interparietal fused to occipital; lumbar vertebral arches 4 - 7R fused; lumbar vertebral centra 4 and 5 fused; distance between lumbar vertebral arches 4, 5 and 6 increased	major
3	Thoracic vertebral centrum 10 bipartite and asymmetrically ossified	major
4	Thoracic vertebral centra 10 and 11 fused	major
5	Bilateral arthrogryposis and malrotated hindlimbs	major
6	Internal microphthalmia; right lens reduced in size and opaque	major
7	Distended aortic arch; left common carotid and innominate artery arising from same point on aortic arch and ductus arteriosus closed	major
8	Frontals fused	major
9	Multiple defects including: domed head; hydrocephalus; unilateral arthrogryposis; malrotation of hindlimbs; absence of filtrum; absence of upper incisors; ventral surface of tongue fused to oral cavity; post-caval lung lobe agenesis, left and right lobes not differentiated into lobes; bilateral increased renal pelvic cavitation; interrupted aortic arch; large and small sutural bones between nasals; nasals reduced in size; fontanelle increased in size; frontals incompletely ossified/mal med; parietals incompletely ossified/malformed; interparietal incompletely ossified	major
10	Multiple defects including: domed head; hydrocephalus; bilateral arthrogryposis; absence of filtrum; ventral surface of tongue fused to oral cavity; absence of upper incisors; incomplete closure of muscular layer; heart increased in size; gross distension of great vessels; aorta and pulmonary trunks with common origin from heart; interrupted aortic arch with stenosis of ductus arteriosus; post-caval lung lobe agenesis, left and right lobes not differentiated into lobes; heart with single ventricle and common exit for aortic/pulmonary trunk; large sutural bone between nasals; nasals reduced in size; fontanelle increased in size; frontals incompletely ossified/malformed/fused; parietals incompletely ossified/malformed; interparietal incompletely ossified	major

Table 20, Part B.
Group Incidence of Defects

Key	Type of defect	Classification
<u>MAJOR MALFORMATIONS (continued)</u>		
11	Multiple defects including: domed head; hydrocephalus; foreshortening of face; absence of filtrum; absence of upper incisors; ventral surface of tongue fused to floor of oral cavity; oedema to right side of head; incomplete closure of muscular layer; heart increased in size; left auricle reduced; gross distension of great vessels; aorta and pulmonary trunks with common origin from heart; interrupted aortic arch; left subclavian artery originating at base of common carotid; post caval lung lobe agenesis; left and right lobes not differentiated into lobes; liver showing abnormal lobulation; agenesis of bile duct and gall bladder; heart showing large ventricular septal defect with common exit from ventricles to combined aortic/pulmonary trunk; large and small sutural bone between nasals; nasals reduced in size; fontanelle increased in size; frontals incompletely ossified/malformed/fused; parietals incompletely ossified/malformed	major
12	Single external nare mid-line; absence of filtrum; absence of upper and lower incisors; palate with abnormal longitudinal ridge in mid-line; encephalocele; nasals malformed; frontals incompletely ossified/malformed; parietals incompletely ossified; fontanelle increased in size	major

Table 20, Part C.
Group Incidence of Defects

Key	Type of defect	Classification	Group 1	Group 2	Group 3	Group 4
<u>EXTERNAL/VISCERAL DEFECTS</u>						
<u>Thorax</u>						
13	Atrium/atria increased in size	minor	1(0.0)	1(1.2)	10(9.9)	
14	Atrium/atria reduced in size	minor		2(2.4)	1(1.0)	
15	Post-caval lung lobe agenesis	minor	1(0.0)			
a	Abnormal common carotid	variant	23(18.0)	13(15.5)	19(18.8)	10(21.7)
<u>Body</u>						
16	Caseous distension of stomach	minor	2(1.6)		1(1.0)	1(2.2)
17	Gall bladder reduced in size	minor		1(1.2)		1(2.2)
18	Unilateral increased renal pelvic cavitation	minor			1(1.0)	
19	Gall bladder agenesis	minor			1(1.0)	
<u>SKELETAL DEFECTS</u>						
<u>Skull</u>						
20	Occipital incompletely ossified	minor	4(3.1)	3(3.6)	4(4.0)	3(6.5)
21	Parietal(s) incompletely ossified	minor			1(1.0)	
22	Cleft in parietal(s)	minor		1(1.2)	1(1.0)	
23	Interparietal incompletely ossified	minor	31(24.2)	26(31.0)	16(15.0)	5(10.9)
24	Fissure through interparietal	minor		1(1.2)		
25	Interparietal bipartite	minor			1(1.0)	
6	Cleft in interparietal	minor	1(0.8)			1(2.2)
7	Nasal(s) incompletely ossified	minor	1(0.8)		1(1.0)	
28	Cleft in nasal(s)	minor			2(2.0)	
29	Small sutural bone between frontals	minor	1(0.8)	1(1.2)		
30	Large/small sutural bones between parietals	minor		1(1.2)	3(3.0)	
31	Large sutural bone between parietal and frontal	minor	1(0.8)			
32	Small sutural bone between nasals	minor		1(1.2)	1(1.0)	1(2.2)
b	Frontal(s) incompletely ossified	variant	40(31.3)	37(44.0)	43(42.6)	20(43.5)
c	Cleft in frontal(s)	variant	33(25.8)	16(19.0)	19(18.0)	10(21.7)

Table 20. Part D.
Group Incidence of Defects

Key	Type of defect	Classification	Group 1	Group 2	Group 3	Group 4
SKELETAL DEFECTS (continued)						
Vertebral arches/centra						
33	Any thoracic vertebral centrum incompletely ossified	minor	1(0.0)			1(2.2)
34	Any cervical vertebral centrum incompletely ossified	minor		2(2.4)		
35	Any cervical vertebral centrum asymmetrically ossified	minor				1(2.2)
Ribs						
36	Spatulate rib(s)	minor	8(6.3)	6(7.1)	2(2.0)	2(4.3)
37	Clubbed rib(s)	minor	6(4.7)	9(10.7)	4(4.0)	4(8.7)
38	12R reduced in size	minor		1(1.2)		
d	Extra pair of ribs	variant	47(38.7)	51(60.7)	40(39.6)	13(28.3)
e	Extra rib	variant	19(14.8)	6(7.1)	14(13.9)	9(19.6)
Sternebrae						
39	Any sternebra(e) in the region 1 - 4 incompletely ossified	minor	2(1.6)		3(3.0)	2(4.3)
40	Sternebra(e) asymmetrically ossified	minor	10(7.0)	6(9.5)	5(5.0)	3(6.5)
41	Sternebra(e) bipartite	minor	4(3.1)	4(4.8)	3(3.0)	3(6.5)
42	Minor fusion of sternebrae	minor	3(2.3)	5(6.0)	2(2.0)	3(6.5)
43	Additional ossification site(s) between 5 and 6	minor	1(0.8)	2(2.4)		
44	Additional ossification site above 1	minor			2(2.0)	
	Additional ossification site fused to 5	minor	1(0.8)			
r	Sternebra 1 malformed	minor			1(1.0)	
	Sternebra 5 incompletely/not ossified	variant	13(10.2)	5(6.0)	12(11.9)	9(19.6)
g	Sternebra 6 incompletely/not ossified	variant	31(24.2)	17(20.2)	22(21.8)	11(23.9)
Pelvic girdle						
47	Pubis incompletely/not ossified	minor	6(4.7)	3(3.6)	2(2.0)	5(10.9)
h	Unilateral insertion on 2nd sacral vertebra	variant	6(4.7)	9(10.7)	5(5.0)	2(4.3)
j	Bilateral insertion on 2nd sacral vertebra	variant	41(32.0)	33(39.3)	30(29.7)	9(19.6)
Limbs						
48	Metacarpals incompletely ossified	minor	2(1.6)	3(3.6)	4(4.0)	4(8.7)
49	Metatarsals incompletely ossified	minor	1(0.8)			
50	Astragali incompletely/not ossified	minor	9(7.0)	3(1.2)	2(2.0)	7(15.2)
k	Forelimbs phalanges not ossified	variant	37(28.9)	23(27.4)	20(27.7)	31(67.4)
a	Hindlimbs phalanges not ossified	variant	3(2.3)	1(1.2)	3(3.0)	10(21.7)

III. D. 4. Embryotoxicity Study in the Rabbit:

Location of Data: Vol. 56 of 418, Dated September of 1988

Testing Facility:

Study No./Dates: 88/930/TX 25 May to 29 October 1987

Animals: HYLA females supplied by Charles River weighed 3.2-4.5 kg at 19-20 wk. Males at 7-23 months weighed about 4 kg. Supplier was from

Mode of Administration: Orally, by gavage.

Dose Levels: Two successive tests were performed; one group, received vehicle and the other received TRAN at 0.8 mg/kg. In the second test, vehicle, 0.1, 0.2, or 0.4 mg/kg doses were administered. Vehicle was, in all instances, 0.5% methyl cellulose in distilled water.

No. of Animals: 15 females (presumably virgin) were each mated twice with the same male of known fertility. The males had no further part in the experiments.

The purpose of the study was to determine the potential effects of TRAN on gestation in the rabbit and, in particular, the teratogenic risk to the fetus. The study was undertaken to confirm these facts and to attempt specifically to define the limits of maternal toxicity attributable to TRAN.

After mating, the females were distributed homogeneously among the different groups in such a way that those bred by the same male were not grouped together. TRAN was administered in a volume of 2 ml/kg, by stomach tube, once daily throughout the period of organogenesis (gd 6 to gd 18, inclusive). The dosing schedule is shown in Table 21, which also shows the mortality of the various groups. The study covered the course of gestation and the examination of the fetuses delivered by Cesarean section at about full term.

In general, the majority of the animals treated with the two high doses (0.4 and 0.8 mg/kg/day) showed clear signs of toxicity during and after treatment, e.g., tremors on handling, diarrhea, prostration, and loss of balance. The high dose animals showed the greatest decrease in body weight (did not recover), while the other treated groups

all showed a decrease. Marked mortality was seen in the treated groups which followed an approximate dose-effect relationship (see Table 21, Sponsor's Table 4., p.160, Vol. 56.).

Table 21
Mortality in Dams Treated with TRAN

Group	Dose in mg/kg/day	Number of dead females/ Number of pregnant females
1	0	0/12
2	0.8	9/14
3	0	0/12
4	0.1	1/13
5	0.2	1/12
6	0.4	7/14

Macroscopic examination at autopsy confirmed the liquid nature of the intestinal contents. However, histopathology of the principal organs (lungs, liver, or kidneys) did not reveal any evidence of organ specific lesions. Several females had no living fetuses in the uterus and only expulsion scars, resorptions, or dead fetuses. Other treated females had evidence of only partial interruption of gestation, with mean fetal losses ranging from 22 to 44%, compared with 16 to 10% in each of the control groups.

As a reflection of the interruptions of gestation, the mean number of living fetuses per litter decreased from a range of 7.33 to 8.08 in the controls to 5.17 to 6.40 in the treated animals. The mean weights of the surviving fetuses were approximately normal. External examination of the treated groups' fetuses revealed no malformations or abnormalities. Internal examination of the treated fetuses' offsprings revealed a few "fairly common abnormalities distributed randomly among the groups and with no apparent relationship to treatment" As seen in the previous rabbit experiment, dilation of the renal pelvis and ventricular septal defects occur in what appear to be random fashion even in control groups. Sponsor reported that no properly defined skeletal malformations were observed.

Sponsor's conclusions from this study were that: 1). TRAN was highly toxic to pregnant female rabbits. 2). There were no treatment related malformations during the period of embryogenesis, according to the usual examinations.

III. D. 5.RU 44 570 and Electrolyte Supplement: Oral (Gavage) Teratology Study in The Rabbit:

Location of Data: Vol. 56 of 418, Dated April of 1990.

Testing Facility:

Study No./Dates: 552/20: 23 July to 6 August 1989

Animals: Sexually mature, virgin New Zealand White rabbits were obtained from . 64 healthy mated females were mated for the study. Untreated male rabbits of the same strain were used for mating.

Mode of Administration: The test article and the control (s) were administered orally by gavage.

Dose Levels: The dose levels and nomenclature of dose groups are shown in Table 22, (from sponsor's page A 5.) A constant dose volume of 2 ml/kg body weight was used.

Table 22, Dosing Schedule

Group number	Group description	Dose level mg/kg/day
1	control	0
2	low	2
3	intermediate	4
4	high	8

Experimental Design: The test or control articles were administered to the mated females once daily from days 6 to 18 of gestation (gd 6 to gd 18), inclusive. The day of mating was designated as gd 0. The test and control articles were suspended in 0.5% w/v aqueous methyl cellulose. The electrolyte supplement, Ringer's Solution (1/2 strength), was provided as the drinking beverage (replacing water), and was administered on

gd 6 to gd 24, inclusive. Mains water was available from gd 0 to 6 and from gd 24 to 29; the animals were allowed free access to a pelleted diet.

The animals were killed on gd 29 and examined macroscopically. The fetuses were removed, killed and examined.

Proof of absorption was obtained by evaluating 2 ml blood samples drawn from the jugular vein of the first 8 animals in each group on gd 4, 8, and 17. The samples were processed and sent to sponsor for analysis.

The objective of this study was to assess the effects of TRAN when administered orally, by gavage, in conjunction with an electrolyte supplement in the drinking water, to pregnant New Zealand White rabbits during organogenesis. The rabbit was selected because it is a readily available non-rodent species which is accepted by regulatory agencies and which has reproductive characteristics which are well established.

Analysis of the liquid formulations used to deliver the doses were satisfactory. Analysis of the gd 4, 8, and 17 jugular vein blood samples showed a marked reduction of ACE activity in all test animals which was not dose-related since the high dose group values were only marginally greater than in the low and intermediate doses groups.

Table 23
Summary of Females' Performance

Number of animals:	Group 1	Group 2	Group 3	Group 4
In group	16	16	16	16
Not pregnant	0	0	2	0
Pregnant (%)	16 (100.0)	16 (100.0)	14 (87.5)	16 (100.0)
Died/killed	0	1	0	0
Aborted and killed*	0	0	0	4
With total resorption	0	0	1	0
With live fetuses on day 29	16	15	13	12

* animal 64 aborted and found dead in cage

Table 23, above (sponsor's Table 1, p. B 1, Vol. 56) shows the mortality and morbidity in the females of this study, the reproductive performance, and the effect on the fetuses. Four high dose group animals aborted between gd 26 and 29 and were killed. All four animals had shown body weight loss and low food intake during the dosing period. Two animals showed gut pathology and two showed kidney pathology. There were no unusual clinical signs in treated animals surviving to gd 29; all groups showed minor common clinical signs such as rough coat or fur staining. No gross maternal findings at necropsy were reported.

Group mean body weight gain of all treated groups was lower than mean controls during the treatment period; but, was slightly greater than controls from the end of the treatment period through to termination. The group values for animals surviving to day 29 showed an apparent lack of dose response, the high dose group apparently being the least affected. This was due to the most severely affected animals in the high dose group aborting and being excluded from the group values. Food was similarly affected.

Pregnancy rate was 100% in the control, low, and high dose groups and 87.5% in the intermediate group; these are considered satisfactory. Pre-implantation loss was increased, but only marginally so compared with recent historical controls and not different from concurrent controls. The group values for post-implantation loss were outside the control range from recent studies but the value for the high dose group was lower than that of the intermediate dose group; again this was due to the high dose females which aborted being excluded from the data group. One female in the intermediate dose group (#44) had total resorption with the majority of the intrauterine deaths in this female being late embryonic deaths.

Sponsor concluded: "a single female with total resorption cannot be conclusively associated with treatment, but this female showed apparently treatment-related body weight loss and low food intake, which together with the increased post-implantation losses seen in both the intermediate and high dose groups, means that the possibility of a relationship with treatment cannot be ruled out." This is a reasonable conclusion.

The mean number of fetuses was lower than controls in all treated groups. The difference from the control group was statistically significantly different from the low and the intermediate dose groups only ($p < 0.01$ and $p < 0.001$, respectively).

The following fetal data observations can be made: The proportion (%) of males in the litter was not adversely affected by treatment, nor was mean fetal weight. Differences from the control group for the above parameters were not statistically significant. Differences in group mean litter weight reflected differences in mean number of fetuses per litter.

The incidence of fetuses with malformations was not adversely affected by treatment; the values were 1, 1, 4, and 1, respectively, in groups 1 to 4. The malformations observed were considered unlikely to have been an effect of treatment since they showed no common etiology, no dose-response relationship in incidence, and the malformations observed are known to occur spontaneously in this strain of rabbit.

The incidence of external/visceral variations was slightly greater than controls in the high dose group. This was due primarily to an increased incidence of fetuses with abnormal common carotid; the overall incidence was not statistically significantly different from controls.

Sponsor concludes: "Administration of TRAN plus electrolyte supplement to the pregnant rabbit at 2, 4, or 8 mg/kg/day resulted in maternal toxicity characterized by maternal inappetence and reduced body weight gain and was also associated with increased post-implantation losses and fetal skeletal variations at all dose levels. Despite these findings, the incidence of fetal malformations was not adversely affected at any of the dose levels investigated."

These conclusions appear to be in harmony with the data presented.

III. D. 6 Oral (Gavage) Teratogenicity Study in The Cynomolgus Monkey.
Study No. 1:

Location of Data: Vol. 57 of 418, Dated April of 1990

Testing Facility:

Study No./Dates: 553-012: Report Date, July 15, to December 1, 1988.

Animals: Sexually mature female, weighing 2.8 to 4.1 kg, Cynomolgus monkeys (*Macaca fascicularis*) obtained from

These

 females were mated with untreated fertile male animals 1 or 2 days before the theoretical middle of the menstrual cycle or on the basis of consistency of the menstrual cervical mucus. The max. mating duration was 18 hr. The 0 day of gestation (gd 0) was taken as the day sperm was in the vaginal smear, post-mating. Ultrasonic examination verified pregnancy.

Mode of Administration: The test article and the control vehicle (methylcellulose) were administered orally by gavage.

Dose Levels: The dose levels and nomenclature of dose groups are shown in Table 24 (Sponsor's table on page 15 of Vol. 57).

Table 24
Dose Level Characteristics

Group number	Group description	Dose level mg/kg/day	Concentration mg/ml	Dose volume ml/kg/day
1	Control	0	0	10
2	Low	10	1	10
3	Intermediate	50	5	10
4	High	250	25	10

Experimental Design:

The test or control articles were administered to the mated females once daily from days 20 to 50 of gestation (31 consecutive days). On gd 20, pregnant females were randomly allocated to dosage groups. Table 25 lists the animals which began and which completed the trial due to false positive pregnancy tests. The net participating numbers were 10, 10, 11, and 10 for groups 1, 2, 3, and 4, respectively. (From Sponsor's Vol. 57, p18.).

Table 25

Group number	Test article	Number of inseminated animals
1	Vehicle	11*
2		11*
3		16*
4		12*

*) For one, one, five, and two animals in groups 1, 2, 3, and 4, respectively, the pregnancy test was found to be false positive.

All of the animals were examined twice daily from day 20 post-coitus until cesarean section on gd 100 (+/- 1) for changes in clinical signs; all changes were recorded. Blood samples were taken at approximately 7 day intervals and stored as frozen serum for future use. Body weight was recorded at the same time as blood sampling over the same time interval (gd 20 to gd 100). The animals were

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anesthetized, examined, and the fetuses removed by cesarean section and evaluated.

"The objective of this study was to assess the potential of TRAN to affect the embryonic and fetal development in *Cynomolgus* monkeys when administered daily from gd 20 to gd 50. The non-human primate was chosen for this study because of its similarities with reproduction physiology during pregnancy to that of man." Furthermore, it was hoped that this third species would improve risk assessment.

The potential external abnormalities at necropsy upon which sponsor focused were the following: (Table 26, Sponsor's section 8.3, p. 20, Vol. 57):

Table 26
Foci of Terminal Examination

- distance from coccyx to head	
- distance from tip of nose to back of head	
- distance from os frontale to back of head	
- width of head	
- distance between the eyes	- liver
- symmetry of head	- spleen
- facial form	- kidneys
- formation of the lower jaw	- adrenals
- eyebrows, eyes, and eyelids	- lungs
- hair on head	- heart
- body form	- thymus
- nipple formation	- testes
- anus	- uterus
- finger, toes, and nails of fingers and toes	- ovaries
- ears	- eyes
- tail	- brain
- upper and lower extremities	
- external genitals	
- palpation of the vertebral column	
- umbilical cord	
- palate	

Each fetus was photographed. The placentae were also weighed and examined macroscopically. The organs above, plus various levels of the gut were weighed, fixed in 10% formalin, and retained for possible histopathological examination. The ossified skeleton was stained, examined for possible defects, and retained in 99.5% glycerine. Where appropriate, data were processed to calculate mean, deviations from mean and standard deviation. One factor analysis of variance was used to evaluate body weight, body weight gain, placental weight, fetal weight, fetal body measurements, and fetal

organ weights followed by the Student-Newman-Keuls test for multiple group comparison.

The effect on the dams and fetuses of dosing the dams is shown in Table 27. This is adapted from sponsor's Table 1, page 37, Vol. 57.

Table 27
Fate of Dams and Fetuses

Parameter	Group 1 - 0 mg/kg/day	Group 2 - 10 mg/kg/day	Group 3 - 50 mg/kg/day	Group 4 - 250 mg/kg/day
Number of pregnant females	10	10	11	10
Number of females with false positive pregnancy test	1	1	5	2
Number of females which died	1*	0	1*	0
Number of females which were killed	0	0	0	0
Number of females which aborted	3*	6	5*	7
Number of females with dead fetus at cesarean section	0	0	0	0
Number of females with live fetus at cesarean section	7	4	6	3

* one of these females died after abortion

All groups demonstrated a reduction in food consumption and diarrhea during the study. These changes were frequently seen in the mid and the high dose groups, with the result of a slightly lower body weight gain during treatment. This is considered to be a treatment-related event. Incidences of abortions were 3/10, 6/10, 5/11, and 7/10 in the control, low, mid, and high dose groups, respectively. The higher than control values for treated animals indicates to sponsor that these events were likely related to treatment. I concur.

All abortions occurred during or shortly after termination of treatment. A kinked tail was observed in one of three viable fetuses of the high dose group. Sponsor states that the single occurrence would ordinarily be considered incidental, but since similar defects have not been observed in control fetuses of the same type in *Macaca fascicularis*, they cannot rule out the possibility of a treatment-related effect.

Several external, visceral, and skeletal findings were similar in occurrence to control and other treated groups.

Sponsor concludes that under the conditions of this experiment (doses of 10, 50, and 250 mg/kg/day of TRAN from gd 20 to gd 50) the drug induced slight maternal toxicity (clinical signs and reduced body weight gain) and abortions. After administration of the highest dose (250 mg/kg/day), one of 3 cesarean-obtained fetuses showed a unique to control group malformation. Due to the occurrence of numerous abortions,

teratogenic potential could not be properly assessed under the conditions of this study. I concur with their conclusion.

III. D. 7. Oral (Gavage) Teratogenicity Study in The Cynomolgus Monkey, Study No. 2:

Location of Data: Vol. 57 of 418, Dated April of 1990.

Testing Facility:

Study No./Dates: 552-018: Dates, January 4, 1989 to May 18, 1989.

Animals: Approximately as in the above study #1 with Cynomologous monkeys.

Mode of Administration: As in study #1.

Dose Levels: Three dose levels, plus the control group were used, as in study #1. The doses and their characteristics are shown below in Table 28.
(From page 198, Vol. 57.)

Table 28
Dose Characteristics

Group number	Group description	Dose level mg/kg/day	Concentration mg/ml	Dose volume ml/kg/day
1	Control	0	0	10
2	Low	5	0.5	10
3	Intermediate	25	2.5	10
4	High	125	12.5	10

Experimental Design: Table 29 below, in conjunction with Table 28 above define the experimental design in this study.

Table 29
Experimental Design

Group number	Test article	Number of pregnant animals
1	Vehicle	10
2	RU 44570	10
3	RU 44570	10
4	RU 44570	11*

*) One of these animals was eliminated due to a severe injury on the

The objective of this study was the same as the previous study.

The foci of necropsy and terminal examination were the same as the previous study.

Table 30 (from Sponsor's Table 1. p. 221 (38) Vol. 57) which shows the fate of the test animals is shown below. This corresponds with the similar table in the first report .

Table 30
Fate of Test Animals

Parameter	Group 1 - 0 mg/kg/day	Group 2 - 5 mg/kg/day	Group 3 - 25 mg/kg/day	Group 4 - 125 mg/kg/day
Number of pregnant females	10	10	10	11 *
Number of females with false positive pregnancy test	0	0	0	0
Number of females which were found dead	0	0	0	0
Number of females which were killed	0	0	0	0
Number of females which aborted	1	4	4	8 *
Number of females with dead fetuses at cesarean section	0	0	0	0
Number of females with live fetuses at cesarean section	9	6	6	3

* one of these animals eliminated due to an injury on the left hindlimb

Sponsor summarized the study as follows:

- a. At 5, 25, and 125 mg/kg/day body weight gain was slightly reduced.
- b. Group 3 (mid dose) and group 4 (high dose) had a higher frequency of clinical changes (e.g. vomiting).
- c. The incidence of abortions increased in the high dose TRAN group.
- d. TRAN treatment did not affect the fetal, placental, and organ weights, nor were the sizes of the fetuses affected.
- e. The nature and incidence of external, visceral, and skeletal findings were not different from the control group.

Sponsor's conclusions are as follows:

Slight maternal toxicity (reduced body weight gain) was apparently induced by the administration of TRAN at doses of 5, 25, and 125 mg/kg/day to Cynomolgus monkeys during gd 20 to gd 50, inclusive.

Vomiting was more frequent in the mid and high dose groups.

The abortion rate increased in the high dose group. The remaining low numbers of fetuses did not permit the assessment of the teratogenic potential of the test drug at the doses tested. These conclusions are reasonable and match those of the first test.

III. D. 8. Oral (Gavage) Pre and Post Natal Study in the Rat:

Location of Data: Vol. 58 of 418, Dated January of 1988.

Testing Facility:

Study No./Dates: 5590-552/8: Dates 19 February 1987 to 29 April 1987.

Animals: Four groups of 24 timed pregnant (originally sexually mature, virgin females) Crl:CD(SD)BR strain rats were dosed. Untreated male rats of the same strain were used for mating.

Mode of Administration: The test article and the control were administered orally by gavage.

Dose Levels: The dose levels and the nomenclature of the dose groups are shown in Table 31 (From sponsor's Table 3.2.3, p. A 5.

Table 31. Dose Levels.

Group number	Group description	Dose level mg/kg/day	Dose volume ml/kg
1	control	0	10
2	low	1	10
3	intermediate	10	10
4	high	100	10

Experimental Design: The test or control articles were administered to the mated females once daily from gestation day 14 (gd 14) until through weaning on post-partum day 21 (ppd 21), inclusive. The animals were mated using 1 or 2 females per male per night. The day on which sperm was observed in the vaginal smears was designated as gd 0.

The test and control articles were suspended in 0.5 % w/v aqueous methyl cellulose. The females were allowed to litter and rear their offspring to weaning. On ppd 7, litters were culled to 10 pups maximum, by

random card draw, with equal sex distribution where possible. On ppd 21 approx., dams and surviving offspring were killed and necropsied. Mated females failing to litter were killed and necropsied on day 25 post-coitum.

The objective of this study was to evaluate the effect TRAN when administered orally, by gavage, to pregnant rats during late gestation and throughout lactation.

There were two deaths which were apparently unrelated to treatment, but rather were due to a dosing error. A summary of the performance of the females is shown in Table 32 which was taken from sponsor's Table 1, p. B 1, Vol. 58.

Table 32
Summary of Female's Performance

Number of animals	Group 1	Group 2	Group 3	Group 4
In group	24	24	24	24
Not pregnant	4	1	0	1
Pregnant (%)	20 (83.3)	23 (95.8)	24 (100.0)	23 (95.8)
Died during parturition	0	0	0	1
Killed post-partum (litter subsequently killed)	0	0	1	0
With total litter loss post-partum	0	0	2	2
With live pups at day 21 post-partum	20	23	21	20

Clinical observations, such as fur staining, fur loss, or chromodacryorrea, were not altered by exposure to the drug.

Group mean maternal body weight gains were not adversely affected by treatment, as is shown in Table 33 (from Sponsor's Table 2, p. B 2, Vol. 58). Pregnancy rate and mean duration of gestation were similar in all groups. Litter size at birth was not adversely affected by treatment with the drug.

In dosing group 4 (100 mg/kg/day), there was increased pup mortality prior to cull on day 7; thus, there was slight reduction in day 4 and 7 post-partum litter size compared with mean litter size at birth. One female (#80) showed total litter loss on ppd 0.

Sponsor shows in their Table 3 (p. B.5 [p.34]), which we reproduce here as Table 34 that there was a statistically significant difference in the intergroup numbers of deaths compared with the control groups' dead pups. At the bottom of Table 34, sponsor shows that there is a $p < 0.01$ value for the comparison by Fisher's Randomization test.

Table 33
Effects on Group Mean Body Weight

Day of gestation	Group 1	Group 2	Group 3	Group 4
0	250	247	249	248
6	269	263	269	265
10	286	279	287	280
14	307	299	309	303
17	334	328	339	333
20	371	368	371	371
% body weight change days 0 - 20	48.4	49.0	49.0	49.6
% body weight change days 14 - 20	20.8	23.1	20.1	22.4

Table 34. Group Mean Litter Data

3.1 Pup numbers
3.1.2 Females rearing young to weaning

	Group 1	Group 2	Group 3	Group 4
Number of females with live pups at day 21 post-partum	20	23	21	20
Mean duration of gestation (days)	21.5	21.3	21.3	21.2
Number of pups born	272	329	295	297
Mean number per female	13.6	14.3	14.0	14.9
Number of pups alive day 1	263	319	288	287
Mean number per female	13.2	13.9	13.7	14.4
Number of pups alive day 4	257	304	277	269 **
Mean number per female	12.9	13.2	13.2	13.5
Number of pups alive day 7 (before culling)	256	301	272	264 **
Mean number per female	12.8	13.1	13.0	13.2
Number of pups culled day 7	60	72	70	65
Mean number per female	3.0	3.1	3.3	3.3
Number of pups alive day 7 (after culling)	196	229	202	199
Mean number per female	9.8	10.0	9.6	10.0
Number of pups alive day 14	187	209	194	194
Mean number per female	9.4	9.1	9.2	9.7
Number of pups alive day 21	185	201	192	189
Mean number per female	9.3	8.7	9.1	9.5

Statistical analysis: number of pups deaths

Intergroup difference from the control statistically significant at:

Fisher's Randomisation test:

p<0.01 **

In late lactation, three of twenty-one females in group 4 had reduced litter size and markedly reduced mean pup weight from ppd 14. Of these, one female (animal # 93) had total litter loss on ppd 16. Occasionally, litters in the control group also had reduced litter size, but control litters did not show reduced mean pup weight.

In group 3 (10 mg/kg/day), one female (#59) had a total litter loss on ppd 2, and another female (#64) had reduced mean pup weight at ppd 14, plus total litter loss by ppd 17. However, in most of the group 3 litters, treatment did not adversely affect litter size.

Pups in group 2 were not adversely affected by treatment from birth through weaning.

There was no effect of the treatment on the sex-ratio of pups. Occasional malformed offspring were seen at birth, but the incidences and types of malformation were not related to treatment. Similar malformations have been seen in control litters from previous studies in their laboratories. Some dead pups, weak pups or small and weak pups were seen as is usual in studies of this type.

The pups from animal #82, which died in incomplete parturition, were found dead. Necropsy findings from the pups included renal and/or urinary bladder changes. Sponsor notes that the rat requires physical stimulus prior to excreting. The changes in this litter were most likely due to the absence of physical stimuli from the moribund dam thereby preventing excretion.

Mean pup weights in group 4 were marginally lower than those in the control; at weaning there were occasional groups which were notably lighter than control. Group 3 animals were slightly lighter than control, while group 2 animals were essentially the same as the controls.

At the necropsy of weaned offspring, litters in group 4 (100 mg/kg), and to a lesser extent, in group 3 (10 mg/kg) showed increased incidence of pups with kidney changes taking the form of increased renal pelvic cavitation. Table 35 (sponsor's table on page A 8 [p. 26] Vol. 58) shows the numbers of offspring affected. The numbers of litters affected increase in a dose-related manner, as do the numbers of affected offspring.

Table 35
The Incidence of Kidney Changes

Incidence of kidney changes	Group			
	1	2	3	4
Number of affected litters	2	4	6	13
Number of litters at weaning	20	23	21	20
Number of affected offspring	5	5	14	45
Number of offspring at weaning	185	201	192	189

Maternal necropsy did not reveal any macroscopic findings. Histopathology of mammary tissue was assessed for the degree of alveolar development. Generally, alveolar development paralleled pup survival.

Sponsor concluded as follows:

a. 100 mg/kg/day of TRAN in late gestation and during the post partum period was associated with total litter loss in a small number of female rats, slightly increased pup mortality, reduced mean pup weight, and increased incidence of kidney changes in weaned offspring.

b. 10 mg/kg/day caused similar but less marked effects.

c. 1 mg/kg/day caused no adverse effects.

These are reasonable conclusions.

III. D. 8. And Electrolyte Supplement Pre- And Postnatal Study in the Rat:

Location of Data: Vol. 58 of 418, Dated December 16, 1991.

Testing Facility:

Study No./Dates: 91/3264/TX October 21, 1990 to February 21, 1991

Animals: Sprague Dawley OFA rats, Specific Pathogen Free, were used; supplied by the

On day 0, the males were aged 13 to 14 weeks; the females were aged 11 to 12 weeks and weighed about 260 grams. Only the females were dosed; the males were solely used for breeding.

Mode of Administration: The test article and the control were administered orally by gavage.

Dose Levels: The doses of TRAN were 0, 10, and 100 mg/kg/day. The top dose had caused renal abnormalities in the offspring during a peri- and postnatal study in the rat.

Experimental Design: Table 36 below shows, in a graphic manner, the core of the experimental design for this study.

Table 36
Dose Allocation in Experimental Design

GROUPS	Treatment RU 44570 mg/kg/day	Ringer's solution (drinking water)	Number of females	Numbers
I	0	without	12	1 - 12
II	0	with	12	13 - 24
III	10	without	12	25 - 36
IV	100	without	12	37 - 48
V	10	with	12	49 - 60
VI	100	with	12	61 - 72

Previous studies have shown that p.o. administration of TRAN to rats may cause renal abnormalities, such as dilation of the renal pelvis in young developing offspring. Using Ringer's Solution as an electrolyte source, replacement was tested as a method of preventing development of these abnormalities. Two doses (10 and 100 mg/kg) were tested, with and without electrolytes, for the prophylactic effect of electrolytes on kidney formation. Although it has not yet been mentioned by sponsor, the relationship, if any, of the structural anomalies to any functional abnormalities needs to be explored before too much effort is expended on exploration of the anomalies.

The protocol was pretty much as used in the pre- postnatal study analyzed above. The doses were administered once daily from gd 14 to ppd 21 (inclusive) using a constant volume of 10 ml/kg to contain the doses and to serve as the controls. The usual observations were made, the females were allowed to go to full term by littering spontaneously. At the end of the lactation period (ppd 21), all of the females were sacrificed and autopsied. All of the offspring were sacrificed at the end of maternal treatment (ppd 21); special attention was given to the urinary tract, which was examined *in situ*. Later the kidneys were removed and sectioned. The kidneys were later examined microscopically (dissecting scope?) and subjected to an histopathological examination.

The objective of this study was to determine whether the addition of electrolytes (as Ringer's solution) to the drinking water might reduce or prevent the appearance of the renal abnormalities identified in some of the reproductive toxicity experiments as dilation or cavitation of the renal pelvis in young offspring.

The animals' general status was satisfactory; behavior was normal. Variations in body weight were no greater for treated than for control animals. No animals died during the study.

The effects of TRAN, administered with and without Ringer's Saline, on food consumption are shown in Table 37 (from sponsor's Table 1, p 170, Vol. 58).

Table 37
Mean Food Consumption of Dams Fed TRAN With and Without Ringer's Solution

	FOOD CONSUMED (g/animal/day)						
	Gestation (days)				Lactation (days)		
	0 - 6	6 - 14	14 - 18	18 - 21	0 - 7	7 - 14	14 - 21
Without Ringer's solution							
CONTROLS Mean $\pm$ S.E.M.	26.7 $\pm$ 1.0	27.1 $\pm$ 0.8	30.9 $\pm$ 1.1	29.4 $\pm$ 0.8	39.1 $\pm$ 0.9	72.7 $\pm$ 1.5	84.1 $\pm$ 2.4
10 mg/kg/day Mean $\pm$ S.E.M.	26.1 $\pm$ 0.5	27.2 $\pm$ 0.4	28.8 $\pm$ 0.6	25.1** $\pm$ 1.0	36.3 $\pm$ 1.6	66.6* $\pm$ 2.7	76.7** $\pm$ 2.9
100 mg/kg/day Mean $\pm$ S.E.M.	25.7 $\pm$ 0.8	26.5 $\pm$ 0.6	27.8* $\pm$ 0.8	21.8** $\pm$ 1.2	32.4** $\pm$ 1.1	60.0** $\pm$ 1.9	75.3** $\pm$ 3.2
With Ringer's solution							
CONTROLS Mean $\pm$ S.E.M.	26.4 $\pm$ 0.5	26.9 $\pm$ 0.7	29.2 $\pm$ 1.1	28.4 $\pm$ 0.9	41.4 $\pm$ 0.9	74.6 $\pm$ 1.6	87.7 $\pm$ 1.0
10 mg/kg/day Mean $\pm$ S.E.M.	24.6 $\pm$ 0.5	27.3 $\pm$ 0.7	29.5 $\pm$ 0.8	28.2 $\pm$ 0.9	40.9 $\pm$ 0.7	73.1 $\pm$ 1.5	84.3 $\pm$ 1.5
100 mg/kg/day Mean $\pm$ S.E.M.	25.7 $\pm$ 1.1	28.2 $\pm$ 1.2	30.4 $\pm$ 1.0	28.8 $\pm$ 1.3	37.5** $\pm$ 1.0	69.4* $\pm$ 1.3	81.5* $\pm$ 1.7

* $p \leq 0.05$ ** $p \leq 0.01$ Mann-Whitney and Dunnett's tests.

The indicated significances are for the treated groups compared with the negative control. The groups treated with TRAN alone (no Ringer's) evidenced a significant reduction in mean food consumption by both dosing groups in the 18 to 21 gestational days interval ($p < 0.01$). The high dose group showed significant food consumption reduction during the 14 to 18 gd period ($p < 0.05$), thereby indicating a dose-related phenomenon. During the 0 to 6 and the 6 to 14 gd periods the treated groups were not significantly different from control. Food consumption by the 100 mg/kg TRAN alone dosing group during lactation was significantly less (at the $p < 0.01$ level) during the 0-7, 7-14, and 14-21 day measuring periods. The 10 mg/kg dosing group was

significantly less at the $p < 0.05$ and $p < 0.01$ significance levels during the 7-14 and 14-21 sampling periods, respectively.

When Ringer's Saline was added to the doses administered, none of the food consumption values was significantly different from control during any of the gestational measuring periods. The high dose of TRAN, combined with Ringer's Solution, significantly decreased food consumption during the three lactation intervals.

Although sponsor does not indicate that they made the comparison, it is quite obvious that the food consumption by the control saline drinkers was not significantly different from the consumption by the control water drinkers.

The effects on water consumption by the same dosing groups are shown in Table 38 (Sponsor's Table 2, p. 171, Vol. 58).

Table 38
Mean Water Consumption of Dams Fed TRAN With and Without Ringer's Solution

	WATER CONSUMED (g/animal/day)							
	Gestation (days)		Lactation (days)					
	14 - 18	18 - 21	1 - 4	4 - 7	7 - 10	10 - 14	14 - 18	18 - 21
Without Ringer's solution								
CONTROLS Mean $\pm$ S.E.M.	48.9 $\pm$ 2.0	49.0 $\pm$ 3.3	53.9 $\pm$ 2.2	72.4 $\pm$ 3.1	90.8 $\pm$ 3.2	97.3 $\pm$ 3.1	105.2 $\pm$ 3.6	127.2 $\pm$ 5.2
10 mg/kg/d Mean $\pm$ S.E.M.	54.7 $\pm$ 3.0	57.6* $\pm$ 3.2	53.8 $\pm$ 3.2	74.8 $\pm$ 3.5	87.4 $\pm$ 2.8	95.1 $\pm$ 2.2	101.2 $\pm$ 2.6	118.8 $\pm$ 2.9
100 mg/kg/d Mean $\pm$ S.E.M.	61.9 $\pm$ 1.5	40.7** $\pm$ 1.0	50.1 $\pm$ 2.2	64.5* $\pm$ 2.5	78.3** $\pm$ 2.0	83.0** $\pm$ 3.2	95.9 $\pm$ 4.0	121.9 $\pm$ 6.3
With Ringer's solution								
CONTROLS Mean $\pm$ S.E.M.	69.4 $\pm$ 3.8	48.0 $\pm$ 3.4	62.6 $\pm$ 2.6	91.4 $\pm$ 8.4	106.8 $\pm$ 8.0	118.0 $\pm$ 5.7	123.2 $\pm$ 3.9	159.2 $\pm$ 8.0
10 mg/kg/d Mean $\pm$ S.E.M.	80.9 $\pm$ 7.0	97.4** $\pm$ 9.6	69.7 $\pm$ 4.8	97.9 $\pm$ 5.1	112.7 $\pm$ 2.6	122.6 $\pm$ 2.6	123.6 $\pm$ 2.6	162.5 $\pm$ 2.2
100 mg/kg/d Mean $\pm$ S.E.M.	67.8 $\pm$ 3.8	67.9 $\pm$ 3.8	83.4 $\pm$ 2.5	90.6 $\pm$ 3.4	110.5 $\pm$ 4.2	122.3 $\pm$ 6.3	128.0 $\pm$ 9.0	191.7 $\pm$ 12.3

* $p \leq 0.05$ ** $p \leq 0.01$ Mann-Whitney or Dunnett's tests.

Water consumption was measured for the same dosing groups previously described. In the groups treated with TRAN alone, mean water consumption of the high dose group was generally less (usually significantly so) than the mean control values for both the gestation and lactation days. The low dose group was slightly greater than control during the 14-18 and 18-21 gestation days, but the lactation day outputs were generally approximately equal to control. When Ringer's Solution was combined with the two TRAN dosages, neither of the dose groups caused marked dose-related changes in water consumption.

The outcomes of gestation and parturition were normal, as was the behavior of the dams during lactation.

The neonates had no external malformations. There were statistically fewer young per litter from the 100 mg/kg/day dose group which was without Ringer's Solution in its dosing solution. All other groups had normal mean litter sizes, sex ratios, and viability indices. The mean offspring weights of dams treated with the high and low doses of TRAN without Ringer's Solution were statistically significantly less than the corresponding controls. Conversely, the mean weight on day 21 was approximately the same as that of the controls. The groups dosed with TRAN plus Ringer's Solution had normal mean body weights.

Sponsor reported that, relative to the kidneys, "on microscopic examination morphological abnormalities were apparent in all the groups in the experiment, including the controls."

a. The offspring of dams treated with TRAN without Ringer's Solution:

At the 10 mg/kg/day dose the incidence of individuals affected was slightly greater than the controls; the difference was not statistically significant.

At the 100 mg/kg/day dose these values were markedly increased: all of the litters were involved and the incidence of affected individuals was 50%.

The abnormalities seen (Tables 37 and 38) were classified in terms of 3 degrees of severity (mild, moderate and severe), and were generally unilateral. The renal pelvis was dilated to a greater or lesser extent and the papilla sometimes appeared atrophied. The parenchyma was often a brownish color in the cortical and medullary regions. The appearance of the ureters was more or less normal." (From sponsor's p.164, Vol. 58).

The table which follows describes the structural abnormalities seen after dosing with TRAN without Ringer's Solution. This will be compared with the effects of TRAN with Ringer's Solution in the following discussion and Tables 37 and 38 (sponsor's Tables 4 and 6, pp. 173 and 175, respectively, in

Vol.58).

Table 37
Examination of Offsprings' Kidneys Following TRAN Without Ringer's Solution

Group	1	3	4
Doses of RU 44570 (mg/kg/day). Without Ringer's solution	0	10	100
Total number of offspring	148	158	147
Number of litters examined	10	11	12
Number of litters affected (%)	6 (60)	8 (73)	12 (100)
Number of offspring examined	148	158	147
Number of offspring affected (%)	12 (8.1)	16 (10.1)	74 (50.3)*
Number of offspring with unilateral or bilateral abnormalities of the same degree	12	16	68
Number of offspring with a bilateral abnormality of different degrees	0	0	6
Abnormalities (dilatation of renal pelvis)			
Total number of abnormalities	12	16	80
1. Number of mild abnormalities	8	7	27
Unilateral	6	6	20
Bilateral	2	1	7
2. Number of moderate abnormalities	2	8	38
Unilateral	2	6	28
Bilateral	0	2	10
3. Number of severe abnormalities	2	1	15
Unilateral	0	1	13
Bilateral	2	0	2

* * p ≤ 0.01 (Chi-square test with Yates' correction)

b. The offspring of dams treated with TRAN containing Ringer's Solution:

Following the 10 mg/kg/day dose, the incidence of young displaying dilation of the renal pelvis was comparable to that of the controls (12 % and 16%, respectively): the difference was not statistically significant.

Following the 100 mg/kg/day dose, the incidence of young displaying dilation of the renal pelvis increased to 64% and all the litters were affected.

c. A comparison of the effect of Ringer's Saline on renal structures:

The effect in the latter instance (with 50% Ringer's Saline) on renal structures were the same as in the former instance (without 50% Ringer's Saline).

Thus, supplementing the electrolytes did not reduce the abnormalities in the renal pelvis or in the ureters.

Histopathological findings summarized in the following section confirmed the gross findings.

Necropsy of the individuals found dead did not reveal any new information.

Table 38

Examination of Offsprings' Kidneys Following TRAN Containing Ringer's Saline:

Group	2	5	6
Doses of RU 44570 (mg/kg/day), With Ringer's solution	0	10	100
Total number of offspring	124	155	141
Number of litters examined	6	11	10
Number of litters affected (%)	5 (6%)	9 (82)	10 (100)
Number of offspring examined	124	155	141
Number of offspring affected (%)	15 (12.1)	25 (16.1)	91 (64.5)**
Number of offspring with unilateral or bilateral abnormalities of the same degree	15	23	85
Number of offspring with a bilateral abnormality of different degrees	0	2	6
<u>Abnormalities (dilatation of renal pelvis)</u>			
Total number of abnormalities	15	27	97
1. Number of mild abnormalities	6	15	29
Unilateral	6	13	21
Bilateral	0	2	8
2. Number of moderate abnormalities	9	8	39
Unilateral	9	7	22
Bilateral	0	1	17
3. Number of severe abnormalities	0	4	29
Unilateral	0	3	18
Bilateral	0	1	11

** $p \leq 0.01$ (Chi-square test with Yates' correction)

d. Sponsor's Conclusions From the Histopathological Examination:

"Administration of TRAN to rats revealed dose-dependent renal abnormalities in the offspring of treated dams:

Dilatation of the renal pelvis of varying intensity, both in the controls and in the treated animals.

Papillary atrophy, the extent and incidence of which appeared dose-dependent but unrelated to the intensity of the pyelic dilatation.

Atrophy and dilatation of the cortico-medullary tubules, also dose-dependent.

The topography of these tubular lesions suggests general damage to one or more nephrons, but there were no visible glomerular lesions or arteriolar or arterial lesions.

An electrolyte supplement caused no significant improvement in the abnormalities associated with treatment with . (Sponsor's conclusions, p 228 (p 79) Vol. 58).

e. Sponsor's Examples of the Varying Degrees of Abnormality Examined Histopathologically:

(Each letter corresponds to one rat; the degrees of lesion are + mild, ++ moderate, +++ severe)

TRAN Alone (No Ringer' Saline):

Degree of lesion	Controls			10 mg/kg			100 mg/kg		
	+	++	+++	+	++	+++	+	++	+++
Dilatation of pelvis	B	C	D	G	H	I	M	N,O	P,Q
Papillary atrophy		D						O,Q	M,N,P
Tubular atrophy				G				Q	H,P

e. Sponsor's Examples.....(continued):

TRAN Combined with Ringer's Saline:

Degree of lesion	Controls			10 mg/kg			100 mg/kg		
	+	++	+++	+	++	+++	+	++	+++
Dilatation of pelvis	E	F		J	K	L	R	S,T	U,V
Papillary atrophy				L			T,V	R	S,U
Tubular atrophy							V	R,T	S,U

f. Appendix: Photographs of Offspring's Kidneys Examined During Histopathological Studies:

Kidney of a 21-day old rat from a control dam.
 Transverse section, stained with haematoxylin, eosin and saffron.
 Magnification: x 16 approx.
 Normal appearance (1).



(1)

f/ Appendix # 2:

Kidney of a 21-day old rat from a control dam.
Transverse section, stained with haematoxylin, eosin and
saffron.
Magnification: x 16 approx.
Atrophy of the parenchyma and dilatation of the renal pelvis:
mild (2), moderate (3) and severe (4).



f. Appendix #3:

Kidney of a 21-day old rat from a dam treated with RU 44570 at a dose of 10 mg/kg/day. Transverse section, stained with haematoxylin, eosin and saffron. Magnification: x 16 approx. Atrophy of the parenchyma (6, 7). Dilatation of the renal pelvis: mild (5), moderate (6) and severe (7).



f. Appendix # 4:

Kidney of a 21-day old rat from a dam treated with RU 44570 at a dose of 100 mg/kg/day. Transverse section, stained with haematoxylin, eosin and saffron. Magnification: x 16 approx. Atrophy of the parenchyma (10). Dilatation of the renal pelvis: mild (8), moderate (9) and severe (10). Presence of



8



9



10

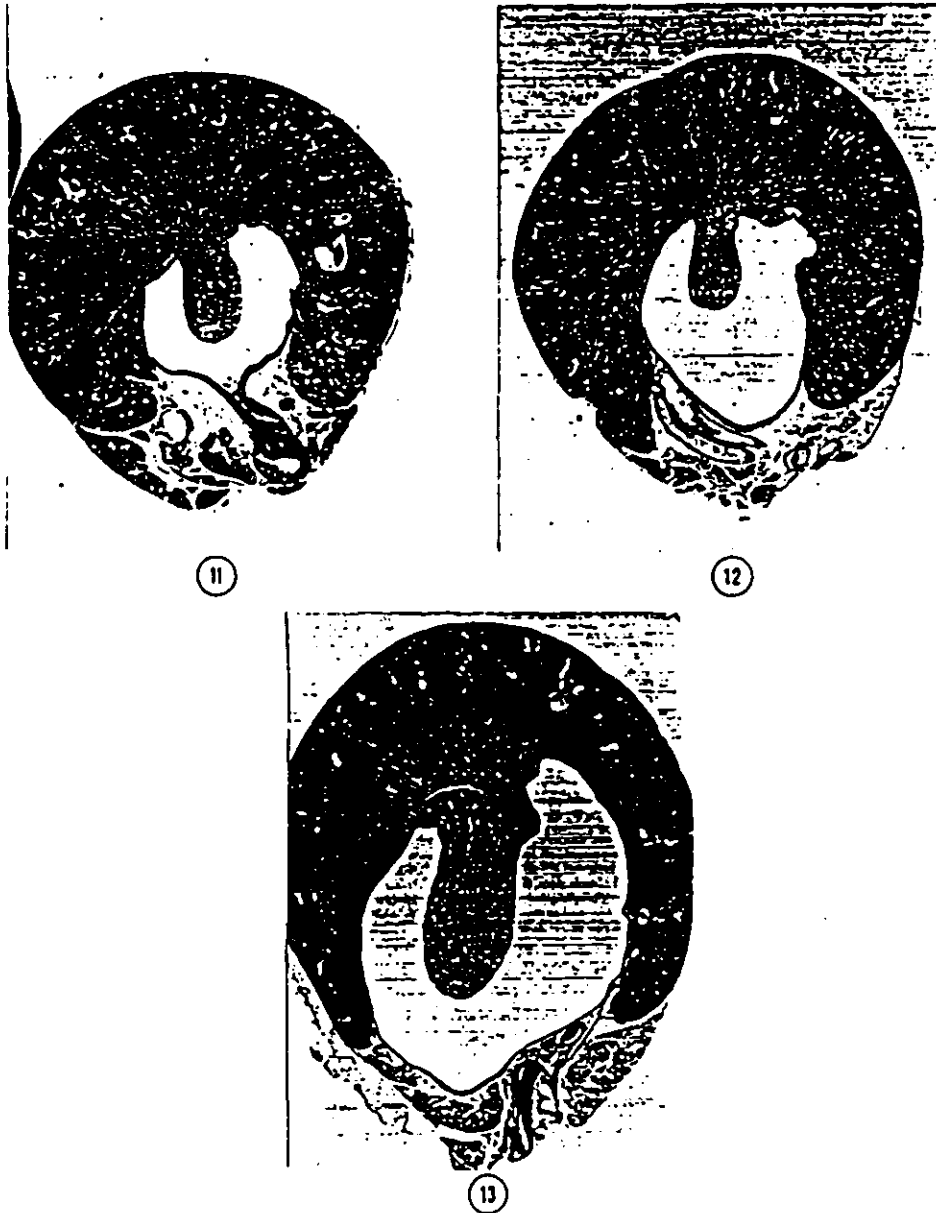
f. Appendix # 5:

Kidney of a 21-day old rat from a dam treated with RU 44570 at a dose of 100 mg/kg/day with Ringer's solution in the drinking water.

Transverse section, stained with haematoxylin, eosin and saffron.

Magnification: x 16 approx.

Atrophy of the parenchyma and dilatation of the renal pelvis: mild (11), moderate (12) and severe (13). Presence of dilated tubules (11, 12).



III. D.9 TRAN: Reproductive Toxicity Summary and Conclusions:

Reproductive toxicity studies were performed to satisfy FDA Segments I, II, and III. In addition to the usual studies in the rat, teratogenicity was studied in the rabbit and the cynomolgus monkey. The effect of electrolyte supplementation was studied in the rabbit teratogenicity and the rat peri-postnatal models.

Fertility and general reproductive performance were studied using rats orally dosed with 0, 1, 10, and 100 mg/kg/day of RU 44 570 (Trandolapril), coded in this review as TRAN. The parental (P) generation animals were not adversely affected by treatment except for slightly decreased mean body weight gain and food consumption. Litter parameters at gestation day 20 (gd 20) were not adversely affected by treatment except for slightly lower fetal weights and litter size. Mean pup weights post-partum were slightly decreased. The incidence of renal abnormalities (pelvic cavitation and dilated ureters) was increased in fetuses, neonates, weaned offspring, and untreated F₁ adults derived from the 100 mg/kg/day dose group. Additionally, the 10 mg/kg/day P dose group had evidence of the same abnormalities, but to a lesser degree.

The increased incidence of renal pelvic cavitation may have resulted from reduction of the relative papillary size while the renal pelvis expands due to the continued normal growth of the cortex. Sponsor cites the investigation of a similar phenomenon in rats following the administration of methyl salicylate by Woo and Hoar (Teratology 6:191-196, 1972) which led sponsor to postulate that drug-related inhibition of papillary growth resulted in "delayed renal development" characterized by renal cavitation. It is not known whether the rat fetal renal effects are direct or indirect, since radiolabeled studies indicate that TRAN does not cross the placenta.

The potential for teratogenesis was studied in orally-dosed timed-pregnant female rats receiving 100, 300, and 1000 mg TRAN /kg/day and similarly treated rabbits receiving 0.2, 0.4, and 0.8 mg TRAN/kg/day. Both dose regimens included control vehicle administrations. TRAN had marked adverse effects on both the pregnant rabbits and their fetuses, and less severe effects on rat dams and fetuses. There were increased incidences of pelvic cavitation and dilated ureters, especially in the fetuses derived from the high dose groups of both species. This indicates a dose-related response at these high doses. Sponsor considers these defects to be of doubtful significance; if one takes into account the high doses used in rats in these experiments and the known sensitivity of rabbits to teratogenicity testing, sponsor's conclusions appear reasonable.

The high-dose rabbits (0.8 mg/kg/day) had maternal toxicity which apparently resulted in 12/21 deaths compared with 5/21 deaths in the control group. The signs of physical deterioration seen to some degree (depending on dose) were loss of appetite, hunched posture, and reduction in body weight gain. Pre- and post-implantation losses and the overall incidence of major malformations were also increased, but the

major malformations were also present at similar frequencies in control rabbits from other Segment II studies. The low-dose treated rabbit dams had some deterioration in maternal condition after displaying loss of appetite and reduction in body weight gain without evidence of other adverse effects on the dams or the fetuses; the low dose group changes were approximately equivalent to those seen in the control group.

Two successive tests were done using female virgin rabbits. The purpose of the tests was to determine the potential effects of TRAN on gestation in the rabbit, and in particular, the teratogenic risk to the fetuses. Confirmation of previous experimental data was sought, as well as some definition of TRAN's limits of maternal toxicity.

In sponsor's ascending order of study from 1 to 6, the doses tested in the **first rabbit study** were (in mg/kg/day) **0, 0.8, 0, 0.1, 0.2, and 0.4**. Generally, the majority of the animals treated with the two high doses (0.4 or 0.8 mg/kg/day) showed clear signs of toxicity during and after treatment (e.g., tremors upon handling, diarrhea, prostration, and loss of balance). High dose animals showed the greatest decrease in body weight (did not recover) and the lower dose groups had dose-related severity of their toxic effects. Mortality was also dose-related. Sponsor concluded that TRAN was toxic to pregnant female rabbits, and that there were no treatment - related malformations during the period of organogenesis by the usual methods.

The **second rabbit study** was to determine if the toxicity of TRAN, which seems to have the kidney as one of its target organs, could be ameliorated by administration of an electrolyte supplement, 1/2-strength Ringer's Solution orally by gavage. At the end of the study, sponsor concluded that "Administration of TRAN plus electrolyte supplement to pregnant rabbits at **0, 2, 4, or 8 mg/kg/day** resulted in maternal toxicity characterized by maternal inappetence and reduced body weight gain and was also associated with post-implantation losses and fetal skeletal variations at all dose levels. In the present study, the use of an electrolyte supplement increased the tolerance of the test system, allowing the use of higher dose levels."

Sexually mature female **Cynomolgus monkeys** were mated with untreated fertile male animals at the appropriate times in the menstrual cycle for Segment II studies. The test article and the control vehicle were administered orally by gavage using methylcellulose as the vehicle (also the negative control). The test and control articles were administered to the mated females from gd 20 to 50 as doses of **0, 10, 50, and 250 mg/kg/day**. The groups consisted of 10 subjects, except for the 50 mg/kg dose which consisted of 11. All animals suffered from food intake reduction and adverse gastrointestinal events. Incidences of abortions were dose-related at 30, 60, 45, and 70 percent, respectively, in increasing order of dosage groups. These events are considered by sponsor to be related to the treatment of the animals with the test articles. I agree. Furthermore, teratogenic potential could not be assessed at the doses tested because of the mortality in the high-dose group.

A second teratogenicity study using *Cynomolgus* monkeys was done using the same protocol as the first study, except that the doses used were decreased by 1/2 (i.e., 0, 5, 25, and 125 mg TRAN/kg/day). Weight gain was slightly reduced by all doses, the incidence of abortions increased in the high dose group, the incidence of vomiting was elevated in the mid and high dose groups, TRAN treatment did not affect the fetal, placental, and organ weights, nor were the fetal sizes affected. The nature and incidence of external, visceral, and skeletal findings were not different from control. The low numbers of surviving fetuses did not permit evaluation of teratogenic potential. This was a waste of monkeys since the first study clearly mandated much lower doses.

To evaluate the effect of TRAN orally administered by gavage to pregnant female rats during late gestation and throughout lactation (Segment III) four dosing groups of timed pregnant, sexually mature, virgin females were mated with untreated males. The doses used were 0 (methyl cellulose vehicle), 1, 10, and 100 mg/kg/day. The usual clinical observations were not modified by exposure to TRAN, nor were the rates of pregnancy, mean duration of gestation, or litter size at birth. In the high dose group, there was increased pup mortality just before cull on day 7. One female had total litter loss on ppd 0.

Sponsor concluded that the Segment III study of the peri- postnatal periods in female dams demonstrated that: 100 mg/kg/day of TRAN in late gestation during the post-partum period was associated with total litter loss in a small number of female animals, slightly increased pup mortality, reduced mean pup weight, and increased incidence of kidney changes in weaned offspring. The middle dose, 10 mg/kg/day, caused similar but less marked effects, while the low dose (1 mg/kg/day) animals experienced no adverse effects.

The last reproductive toxicity study done was to define the effects of supplemental electrolyte solution on the toxicity usually observed in a peri- postnatal (segment III) study using rats. The doses administered were 0 (negative control), 10, and 100 mg/kg/day; the latter dose had previously caused renal abnormalities in another study. When Ringer's Saline was added to the TRAN doses administered, none of the food consumption values were significantly different from control during any of the gestational periods of measurement. The high dose of TRAN, when combined with Ringer's Saline, significantly returned food consumption to control levels during the three lactating intervals.

The offspring of dams treated with 10 mg/kg of TRAN, but without the Ringer's Saline, had an incidence of affected individuals that was slightly greater than controls, but not statistically different from control. At 100 mg/kg, the values were markedly increased, and all the litters were involved; there were 50% affected individuals.

The offspring of dams treated with TRAN (10 mg/kg containing Ringer's Solution)

showed an incidence of renal pelvic dilation which was comparable to that of controls; there were no significant differences. Thus, supplementing the electrolytes did not reduce the abnormalities of the renal pelvis or the ureters.

Administration of TRAN to rats revealed the following dose-dependent renal abnormalities in the offspring of treated dams:

1. dilation of the renal pelvis in controls and in treated animals.
2. papillary atrophy (dose-related).
3. atrophy and dilation of the cortico-medullary tubules (dose-dependent).
4. the above suggest general damage to one or more nephrons, but no visible glomerular, arteriolar, or arterial lesions.

E. MUTAGENICITY

E.1. In vitro mutagenicity by the Ames test (Vol. 1.58 pp. 234-257):

(batch #1) was tested for mutagenic potential by the Ames test in 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) in both the presence and absence of metabolic activation by Aroclor 1254-induced rat liver fraction (S-9).

Experiments were performed according to standard procedures with negative and positive controls using (dissolved in DMSO) at concentrations of 0.05, 0.15, 0.5, 1.5, and 5.0 mg/dish (3 dishes per point) in duplicate experiments.

Results showed that when tested at concentrations of up to 5.0 mg/dish produced no increase in the levels of spontaneous reversion (no mutagenic activity) with or without metabolic activation in all five strains tested.

E.2. Gene conversion test in *Saccharomyces cerevisiae* D4 (Vol. 1.58 pp. 258-285):

batch 15, was tested for mutagenic potential in *Saccharomyces cerevisiae* D4 cells to screen gene conversion at the Ade 2 and Try 5 loci which test the ability of the cells to synthesize adenine and tryptophan in media deficient in these components.

Cells were exposed to test article at 100, 500, 1000, and 2000 µg/ml ± S-9 with negative and positive controls. in duplicate experiments.

Results showed that did not significantly increase the revertant frequencies in test article cultures when compared to negative controls with or without metabolic activation.

E.3. Chromosome aberrations in human lymphocytes cultured *in vitro* (Vol. 1.58 pp. 286-311):

batch 15, was tested for clastogenic activity in cultured human lymphocytes by examining for the presence of chromosome aberrations.

at 10, 100, 500, 1000, and 2000 µg/ml was added to phytohemagglutinin-stimulated normal human peripheral blood lymphocytes ± S-9 with appropriate positive and negative controls. After 4 hours, cells were treated with colchicine, fixed, stained, and examined microscopically for chromosomal aberrations at metaphase (fragment, gap, break, or exchange).

Results showed that no significant increase in the frequency of chromosomal aberrations was found at test substance concentrations up to 1000 µg/ml (the 2000 µg/ml concentration precipitated out of solution).

E.4. Chromosome aberration test on V79 cells *in vitro* with trandolapril (Vol. 1.58 pp. 312-351):

Trandolapril was studied for induction of structural and numerical chromosome aberrations in cultures of the V79 Chinese hamster lung fibroblast cell line *in vitro*.

Cells (2 independent experiments in duplicate dishes) were exposed to trandolapril at 200, 500, 2000, and 4000 µg/ml for 4 hours with and without S-9 mix. After 8 and 18 hours, cells were fixed, stained, and examined microscopically for chromosome aberrations (defined as gaps, breaks, deletions, acentric fragments, and exchanges).

Results showed that trandolapril decreased the mitotic index in a concentration-dependent manner beginning generally at concentrations above 1000 µg/ml. At the 8-hour time point, statistically significant ($P < 0.05$) increases in the mean aberration rates (including gaps) were found in cultures treated with trandolapril at concentrations ≥ 2000 µg/ml in the presence of S9 (2.8-6.8% of metaphases counted). At the 18-hour time point, statistically significant ($P < 0.05$) increases in the mean aberration rates (including gaps) were found in cultures treated with trandolapril at concentrations ≥ 500 µg/ml in the presence of S9 (2.3-6.8% of metaphases counted). No significant increases in numerical aberrations (polyploidy) were found in cells treated with trandolapril at concentrations up to 4000 µg/ml.

Because the increase in mean aberration rates (including gaps) was generally not consistent between experiments and generally did not display a dose-dependent effect, the results were not considered biologically relevant.

E.5. *In vitro* chromosome analysis in Chinese hamster CHO cells (Vol. 1.58 pp. 352-382):

The clastogenic potential of trandolapril was studied in Chinese hamster ovarian (CHO) cells. Cells were treated with test compound at 1, 3, 10, 30, 100, and 500 µg/ml (the stated limit of solubility) with and without S-9 for 4 hours. The medium was replaced, and the cells were cultured for an additional 20 hours. Two hours before collection, cells were treated with colcemid to block them in metaphase. Four and 22 hours after drug treatment, cells were fixed, stained, and examined in metaphase for chromosomal aberrations (gaps, breaks, exchanges, and multiple aberrations). A preliminary experiment determined the cytotoxicity of drug on the cells.

Results showed that the mitotic index of cells treated with the two highest doses (100 and 500 µg/ml) of trandolapril in the presence of S-9 was reduced by 40% when compared to controls, indicating moderate cytotoxicity. Chromosome analysis revealed no significant increase in morphological aberrations 4 and 22 hours after drug treatment.

It was concluded that trandolapril was not clastogenic in CHO cells when tested at concentrations up to 500 µg/ml (the solubility limit).

E.6. HPRT gene mutation assay in V79 Chinese hamster cells (Vol. 1.58 pp. 383-411):

Mutagenic activity of trandolapril was tested *in vitro* in V7889 Chinese hamster cells by testing the ability of trandolapril to induce mutations at the HPRT locus. Mutant cells survive in the presence of 6-thioguanine (a toxic precursor) because they must synthesize nucleosides-5'-monophosphates by a pathway different from that used by the mutated enzyme HGPRT. Non-mutated cells die due to incorporation of 6-thioguanine into nucleic acids.

Cells were treated with trandolapril at 1, 3, 10, 30, 100, and 500 µg/ml for 3 hours with and without S-9. The medium was replaced and the cells were cultured for an additional 6 days to allow expression of an altered HPRT phenotype. Cells were seeded and then treated with 6-thioguanine and cultured for an additional 10 days to allow surviving colonies to develop. Colonies were fixed, stained, and counted. Mutagenic activity was defined by the Sponsor as: a 3X increase in colonies when compared to controls, a dose-related effect, and reproducible in replicate experiments.

Results showed that trandolapril at concentrations up to 500 µg/ml did not increase cytotoxicity or mutagenicity when compared to control cultures.

It was concluded that trandolapril was not mutagenic in the Chinese hamster V79/HPRT gene mutation assay when tested at concentrations up to 500 µg/ml (the solubility limit).

E.7. Micronucleus test (Vol. 1.58 pp. 412-433):

The clastogenic activity of trandolapril was tested *in vivo* by examining micronucleus induction in mouse bone marrow cells. This test detects formation of micronuclei in polychromatic erythrocytes that result from acentric chromatids and chromosome fragments (damaged genetic material) remaining in the cell after the nucleus has been expelled from erythroblasts.

Mice were given a single oral dose of trandolapril at 1000 mg/kg. After 18, 42, and 66 hours, animals were sacrificed, and the bone marrow removed from the femurs. The cells were smeared onto microscope slides, fixed, stained, and the polychromatic erythrocytes examined for micronucleated cells. In addition, the ratio of polychromatic to normochromatic erythrocytes (PE/NE) were calculated to assess possible bone marrow toxicity.

Results showed that the micronucleus frequency in bone marrow cells from trandolapril-treated animals was not significantly increased when compared to cells

from control animals. No significant decrease was found in the PE/NE ratio.

It was concluded that trandolapril was not mutagenic when given orally to mice at a dose of 1000 mg/kg.

E.8. Micronucleus test in the mouse (Vol. 1.58 pp. 434-459):

This study examined the clastogenic activity of trandolapril in the mouse micronucleus test as described above, but used a higher oral dose (2000 mg/kg). Animals were sacrificed at 24, 48, and 72 hours, and the number of micronuclei in polychromatic erythrocytes determined.

Results showed that trandolapril, when compared to controls, did not increase the frequency of micronuclei in polychromatic erythrocytes when tested at an oral dose of 2000 mg/kg in mice. No significant decrease was found in the PE/NE ratio.

It was concluded, therefore, that trandolapril was not clastogenic under the conditions of the study.

III. PACKAGE INSERT

The reproductive system should be more completely covered in the package insert summation. Sponsor has done experiments covering the three segments; this should be the minimum subject matter summarized. Additionally, the CFR mandates that we express the toxicity in terms of the multiples of the maximum recommended human daily doses (MRHDD) used. Furthermore, this Office requires that the doses be calculated in terms of mass per unit of body surface area as well as per body weight. Sponsor should be requested to conform with these requirements.

The recommended changes to the package insert are as follows:

Carcinogenesis, Mutagenesis, Impairment of Fertility: Long-term studies were conducted with oral trandolapril hydrochloride administered by gavage to mice (78 weeks) and rats (104 to 106 weeks). No evidence of carcinogenic potential was seen in mice dosed up to 25 mg/kg/day and rats dosed up to 8 mg/kg/day. On a body-weight basis, these doses are 313 and 100 times, respectively, the maximum recommended human daily dose (MRHDD) of 4 mg, (or 0.08 mg/kg/day based on a 50 kg individual); on a body surface-area-basis, these doses are 29 times (mice) and 21 times (rats) the MRHDD. The mutagenic potential of trandolapril hydrochloride was evaluated in the microbial mutagenicity (Ames) test, the point mutation and chromosome aberration assays in Chinese hamster V79 cells, and the micronucleus test in mice. There was no evidence of mutagenic or clastogenic potential in these *in vitro* and *in vivo* assays.

Reproduction studies in rats did not show any impairment of fertility at doses up to 100 mg/kg/day of trandolapril hydrochloride, or 1250 and 257 times the MRHDD on the basis of body-weight and body-surface-area, respectively.

Pregnancy Category D:

See WARNINGS, Fetal/Neonatal Morbidity and Mortality.

IV. OVERALL SUMMARY AND EVALUATION

A. Background

Trandolapril (Mavik®) is the ethyl ester prodrug of the nonsulfhydryl angiotensin converting enzyme (ACE) inhibitor, trandolapril. After oral administration, the prodrug trandolapril is deesterified in the liver to its active diacid metabolite, trandolaprilat, which is about eight times more active than the prodrug as an inhibitor of ACE activity.

ACE catalyzes the conversion of angiotensin I to angiotensin II, which, in turn, functions to increase blood pressure by inducing vasoconstriction of vascular smooth muscle and aldosterone secretion by the adrenal cortex facilitating renal sodium and fluid reabsorption. In addition, angiotensin II possesses trophic effects on the heart resulting in cardiac and vascular hypertrophy. The antihypertensive activity of trandolapril appears to result primarily from inhibition of circulating (plasma) and tissue ACE activity resulting in reduced angiotensin II formation. The subsequent reduction in angiotensin II levels is responsible for peripheral vasodilation, reduced blood pressure and total peripheral resistance, and decreased renal sodium and fluid retention.

Trandolapril is intended for use as an antihypertensive agent with a recommended dose for patients not receiving a diuretic of 2 to 4 mg once daily. The diacid, trandolaprilat, is the active metabolite and possesses high lipophilicity when compared to the diacids of other ACE inhibitors. This property may contribute to good penetration into various tissues resulting in more efficient ACE inhibition in both tissue and in plasma, and may provide beneficial effects on reversing cardiac and vascular hypertrophy.

B. Pharmacodynamics

Pharmacodynamic studies examined the effects of trandolapril and trandolaprilat *in vivo* and *in vitro*. Primary effects studied included: ACE inhibition, antihypertensive effects, hemodynamic effects, and effects on vascular and cardiac hypertrophy. Secondary effects studied included: effects on ischemic hearts, effects on congestive heart failure, renovascular effects, and effects on physiological functions other than those related to the cardiovascular system. Additional studies compared the effects of trandolapril and trandolaprilat to other ACE inhibitors, particularly enalapril (MK 421) and enalaprilat (MK 422).

was shown to have activity as an ACE inhibitor when given either by the intravenous or oral routes. In the rat it selectively inhibited the pressor effects of Ang I, but not Ang II, and showed about 6 times more activity on a mg/kg basis in reducing an Ang I-mediated pressor response (diastolic BP) than a known ACE inhibitor enalapril (MK 421) when given IV, and about 3 times more active when given orally. Inhibition of

serum ACE activity was more sensitive to both ACE inhibitors, [redacted] and MK 421, than was inhibition of the Ang I-induced pressor response. However, the difference in inhibition was greater for [redacted] than for MK 421. The reason for this lack of correlation between inhibition of ACE activity and inhibition of the Ang I-mediated pressor effect by [redacted] was not known, but was thought to be linked to the importance of circulating ACE and Ang I levels when compared to their levels in tissues.

In the conscious dog, [redacted] after oral administration was more active and the effect more rapid to the pressor effect of Ang I than MK 421. The diacid of [redacted] showed similar activity to MK 421, which is in contrast to the diacid of MK 421, MK 422, which, according to the Sponsor, is not easily absorbed after oral administration.

Pretreatment with a thiazide has been shown to increase the antihypertensive activity of ACE inhibitors which may be due to the greater activity of the renin-angiotensin system following thiazide-induced sodium depletion. In the hydrochlorothiazide-pretreated GH rat, [redacted] was shown to have greater antihypertensive activity than in non-pretreated rats. This effect was also observed with MK 421.

In the dog, preliminary studies (not provided) showed that the hemodynamic effects of [redacted] were transitory. Therefore, pretreatment with a thiazide was chosen as the experimental model. Results showed that [redacted] caused a dose-dependent fall in BP, total peripheral resistance, and heart work. This was interpreted to mean that the compound acted like a vasodilator with no baroreflex activation accompanying the decreased BP.

Taken together, these studies demonstrated that [redacted] possessed significant antihypertensive activity which was active after oral administration, and with a long duration of action (up to 24 hours in the rat). Its mechanism of action was postulated to be via a decrease in Ang II production following ACE inhibition. Its most important effect is a vasodilation not accompanied by a baroreflex activation. In the dog studies, the activation state of the renin-angiotensin system was important for the final effect of the compound.

Additional studies showed the inhibitory effect of [redacted] and its diacid [redacted] against ACE. In the *in vitro* studies, [redacted] (the diacid) inhibited human, dog, and rat serum ACE at nanomolar concentrations (IC_{50} of 0.93 ± 0.35 nM and 7.06 ± 2.11 nM for [redacted] respectively in human serum). In general, the diacid was the more active form of the ACE inhibitors tested, except in rat plasma where they were equal. This was thought to be due to the rapid hydrolysis of [redacted] in rat plasma. Although in human plasma, [redacted] was not hydrolyzed to its diacid, it was still active.

In vivo, given IV to rats potentiated the hypotensive effects of bradykinin. It was not clear what role, if any, bradykinin played in the hemodynamic effects of 570. (Note: Although the induced release of the endothelium-derived vasodilator nitric oxide may be involved, no data were presented to support this view). In the anesthetized dog, was about 9X more active than MK 421 in inhibiting the Ang I-induced increases in blood pressure and about 4X more active in inhibiting the increase in dP/dt. The activities of the two diacids (and MK 422) were comparable. It was thought that the differences in activity between the two parent molecules (and MK 421) were possibly due to differences in their capacity to be hydrolyzed, since the activity of the diacids was similar both *in vitro* and *in vivo*. The abolishment of the drug's antihypertensive effect with bilateral nephrectomy in SH rats demonstrated that its activity is dependent upon the presence of renin which converts angiotensinogen to the ACE substrate angiotensin I. Also in SH rats, the hypotensive effect of was not modified by indomethacin, suggesting that prostaglandins were not involved in the antihypertensive activity of or other ACE inhibitors (MK 421) as well.

The drug had moderate effects on reducing blood pressure in rats and dogs with little effect on heart rates. In dogs the drug inhibited the increase in peripheral vascular resistance that resulted in response to the reduction in cardiac output. In hydrochlorothiazide-pretreated dogs, had a greater effect on decreasing blood pressure, peripheral vascular resistance, and cardiac output than in control animals. This indicated that these antihypertensive effects may have been related to the increased plasma renin and angiotensin I concentrations induced by hydrochlorothiazide. The main effect of was vasodilation without an associated reflex tachycardia. The magnitude of the response appeared to be dependent upon the activation state of the renin-angiotensin system, since the antihypertensive effect was low in animals with decreased (nephrectomized) renin levels, and higher in animals with increased (hydrochlorothiazide-pretreated) levels.

Hemodynamic studies were conducted to examine the effects oftrandolapril on plasma and tissue ACE activity, blood pressure, and vascular and cardiac hypertrophy in normotensive and hypertensive (genetic and spontaneous) rats. In spontaneously hypertensive rats, bothtrandolapril and enalapril at the doses used reduced serum ACE activity to a similar extent (about 90%). However, the persistence of inhibition varied between the two drugs. In the serum of animals treated withtrandolapril, the inhibition persisted for up to 8 days, whereas in animals treated with enalapril, serum ACE activity was at control values by day 3. Similar differences in the persistence of inhibition were obtained in tissue ACE activity (aorta, heart atrium and left ventricle), except in the kidney (internal cortex).

In normotensive rats, both and the diacid had hypotensive activity, particularly on diastolic blood pressure, in anesthetized normotensive rats at doses from 0.1 and 0.3 mg/kg, respectively. The degradation product was

inactive at 3 mg/kg.

In ageing spontaneously hypertensive rats, treatment with [redacted] at 3 mg/kg for 4 weeks decreased mean blood pressure by 18%. Cardiac hypertrophy was reduced by 35% on Day 0, and remained 19% less than controls on Day 15. Serum ACE activity was reduced 84% at the end of treatment, and remained 24% reduced two weeks after the end of treatment. [redacted]-treated animals when compared to untreated SH rats showed a slight improvement in the response to isoprenaline at the end of treatment which may have been related to the reduced cardiac hypertrophy. Histological examination of hearts from both treated and untreated rats showed degenerative lesions characteristic of hypertension and ageing with large intragroup variability. No morphological evidence was reported that [redacted] reduced these lesions.

[redacted] decreased serum ACE activity in both normotensive and spontaneously hypertensive rats to a similar extent. However, there was a greater effect on reducing mean blood pressure in SH rats than in normotensive rats. Heart rates were minimally affected. The mean serum concentration of the diacid trandolaprilat [redacted] was 78% greater in the SH rats than in the normotensive rats. The reason for this difference was not clear. Taken together, these results showed that SH rats were more sensitive to the hypotensive effects of trandolapril than normotensive rats, demonstrating the importance of the renin-angiotensin system in maintenance of blood pressure in the SH rat model of hypertension.

Comparative studies between trandolapril and enalapril in genetically hypertensive rats showed that trandolapril at 0.4 mg/kg/day and enalapril at 10 mg/kg/day for 4 weeks lowered blood pressure, reduced cardiac hypertrophy, and reduced vessel wall tension during the course of treatment. Upon termination of treatment, the effects were found to be reversible within 2 to 4 weeks with vascular compliance (decreased wall tension) showing a more rapid reversibility. Trandolapril showed a longer duration of action on blood pressure after termination of treatment than enalapril.

In spontaneously hypertensive rats, trandolaprilat and enalapril caused a dose-dependent inhibition of ACE in all the various sections of the heart and aorta. Trandolapril was much more active than enalapril in reducing ACE in ventricular tissue. Significant inhibition was achieved with doses of trandolapril between 0.3 and 1.0 µg/kg/day with near maximal activity at doses between 10 and 100 µg/kg/day. The minimum active dose of enalapril was between 10 and 100 µg/kg/day. Trandolapril was 250 to 1000X more active than enalapril on ACE in these tissues. It was concluded that trandolapril was more potent than enalapril in reducing ACE in the heart and aorta (minimum effective dose of between 0.3 and 1.0 µg/kg/day). This difference in potency between the two drugs was more pronounced on ventricular ACE activity where enalapril showed less activity.

Studies to determine the minimum effective dose in spontaneously hypertensive rats

showed thattrandolapril caused a dose-dependent reduction in mean blood pressure with a minimal effective dose of 0.03 mg/kg/day. The effect oftrandolapril on cardiac hypertrophy also showed a dose-dependent effect with the same minimal effective dose of 0.03 mg/kg/day. At this dose (0.03 mg/kg/day)trandolapril reduced serum ACE activity by 40.9% with a minimal effective dose of 0.003 mg/kg/day. When compared to the effects oftrandolapril, a 10 mg/kg/day dose of enalapril was equivalent to a 0.3 mg/kg/day dose oftrandolapril on mean blood pressure and cardiac hypertrophy, and to a 0.03 mg/kg/day dose oftrandolapril on serum ACE activity.

Effects oftrandolapril on tissue ACE activity showed an organ specific effect. There was a > 90% inhibition of the ACE activity in serum, atrium, and aorta. In the left ventricle and renal inner cortex the activity was reduced by 60-70%. Reversibility of the inhibition 8 days after the end of treatment also varied with the specific organ. In serum and ventricles, ACE activity approached 60-80% of control values, while in the aorta, atrium, and kidney, levels were 10%, 20-30%, and 40% of the control values, respectively. These results suggested that the inhibitory effects oftrandolapril on ACE activity have a longer duration in specific organs (aorta, atrium, and kidney) than in serum.

Trandolapril was examined *in vitro* to determine its effect on guinea pig smooth muscle ileum (with and without bradykinin), tissue ACE activity, isolated blood vessels, binding to purified human ACE and to hepatic microsomal proteins. Trandolapril potentiated the response of guinea pig ileum smooth muscle to bradykinin (EC_{50} of 1×10^{-7}), and inhibited the response to angiotensin I (EC_{50} of 5×10^{-8}). Since angiotensin I is less than 1% as potent as angiotensin II on smooth muscle, it was not clear whether the inhibitory effect oftrandolapril was on angiotensin I or on its conversion to angiotensin II. *In vivo*trandolapril potentiated the inflammatory reaction to carrageenin at doses shown in previous studies to inhibit ACE activity. Trandolapril had no effect on the edema caused by dextran which does not cause release of bradykinin. These results demonstrate thattrandolapril had effects *in vitro* and *in vivo* consistent with inhibition of angiotensin converting enzyme activity.

Examination of the effects oftrandolaprilat on the relaxation response of isolated segments of rat and dog arteries after contraction with prostaglandin showed thattrandolaprilat did not cause direct vessel relaxation in the absence of additional agents. However, when given in the presence of bradykinin at 10^{-9} , significant relaxation occurred. Since bradykinin is a vasorelaxant and is a substrate for ACE, inhibition of its degradation may be responsible for potentiation of the relaxation effects bytrandolaprilat. Significant relaxation also occurred whentrandolaprilat was given in the presence of angiotensin I at 10^{-8} to 10^{-9} M. In this case, inhibition of ACE activity may have prevented formation of angiotensin II, a vasoconstrictor. Similar results were obtained with adenosine diphosphate at 10^{-6} to 10^{-8} M. The relaxation

observed in the presence of bradykinin, angiotensin I, and adenosine diphosphate was shown to be endothelium-dependent (no relaxation was observed when vessels free of endothelium were used). The relaxation observed in the presence of bradykinin was reversed by the addition of a B2 kinin receptor antagonist, indicating that the effect was bradykinin-dependent, and not due to a direct effect oftrandolaprilat. These results support the view thattrandolaprilat reduces the degradation of bradykinin by ACE present on endothelial cells, resulting in potentiation of the relaxation response, and that this may be a major mechanism responsible for the vasodilator effects of ACE inhibitors, possibly through induction of nitric oxide by the endothelium.

Binding studies to purified human renal ACE showed that the diacidtrandolaprilat had the highest binding affinity with an IC_{50} of 3.2×10^{-9} M, while its monoestertrandolapril had an IC_{50} 4.7X greater ($IC_{50} = 1.5 \times 10^{-8}$ M). The IC_{50} of enalapril (3.4×10^{-8} M) was 1470X higher than that of its diacid enalaprilat (5.0×10^{-5} M) indicating a relative weaker binding of the monoester form to its diacid when compared to that oftrandolapril and its diacidtrandolaprilat. When comparing the diacids,trandolaprilat had about 11X higher binding affinity than enalaprilat (IC_{50} of 3.2×10^{-9} M vs 3.4×10^{-8} M fortrandolaprilat and enalaprilat, respectively).

Comparative binding affinities between various ACE inhibitors showed that none of the other inhibitors studies had significantly greater binding affinities thantrandolaprilat ortrandolapril when compared to their respective diacid and monoacid-monoester forms, respectively. Due to the variations in binding affinities between the various inhibitors and tissues studied, it was difficult to rank the inhibitors by overall affinity.

General pharmacology studies were conducted to examine the effects oftrandolapril andtrandolaprilat *in vivo*, *in vitro*, and in isolated organ systems in mice, rats, guinea pigs, and rabbits on physiological functions other than the cardiovascular system. No significant effects were noted on the central nervous system, bronchorespiratory system, diuresis, blood glucose, analgesic action, local anesthetic activity, platelet aggregation, coagulation, warfarin interaction, nictitating membranes, in isolated organs, and on the cough induced in antigen-sensitized guinea pigs after exposure to a mist of citric acid. However, significant effects were found on the digestive system consisting of increased gastric fluid volume and acidity at the high dose (30 mg/kg) only, as well as delayed gastric emptying at doses of ≥ 1 mg/kg. These effects have been noted with other ACE inhibitors.

Secondary effects oftrandolapril were examined on cardiovascular and renal systems. Trandolapril (5 mg/kg/day for 8 days) given by gavage to SH rats decreased mean blood pressure by -30.5% with a minimal effect on heart rate (-5.8%). This decreased blood pressure was accompanied by a 37.2% decrease in total peripheral vascular

resistance. There was also a decrease in cardiac hypertrophy (heart to body weight ratio of -8.0%) which may have resulted from the reduction in afterload in the heart. Examination of regional hemodynamics showed decreased regional vascular resistance and increased regional blood flows in all tissues examined. It was concluded that the fall in blood pressure after trandolapril administration was due to a decrease in peripheral vascular resistance (which was accompanied by increased regional blood flow), since there was minimal change in the cardiac index, heart rate, or stroke volume.

In spontaneously hypertensive rats, trandolapril reduced systolic, diastolic, and mean blood pressures by 25%. The blood pressure was still decreased 10% 8 days later. Heart rates also decreased by 12% on the last day of treatment. However, heart rates increased to 9% above control values on day 3, but returned to near control levels by day 8 (+4%). Ventricular heart to body weight ratios were 15% less in trandolapril-treated animals than controls on the last day of treatment. This decrease was still significant (-12%) 8 days after the end of treatment. It was concluded that trandolapril, when given at 3 mg/kg/day for 14 days, showed antihypertensive activity in the SH rat which was greater and more prolonged than enalapril at 10 mg/kg/day for 14 days (recall that previous comparative studies generally showed that a 10-fold higher dose of enalapril was required to reduce blood pressure to a similar extent as that for trandolapril). Also trandolapril induced a significant decrease (-15%) in ventricular hypertrophy that was sustained for up to 8 days after the end of treatment.

In rats with hypertension of renal origin trandolapril at 0.3 and 1.0 mg/kg/day for 4 weeks reduced blood pressure and regressed cardiac hypertrophy, with the 1.0 mg/kg/day dose reducing blood pressure and cardiac hypertrophy to levels found in normotensive rats.

In rats made ischemic by coronary artery ligation (model of heart failure), trandolapril improved survival for the first 6 months of treatment when compared to untreated infarcted animals. After 6 months there was no difference. The effects of trandolapril on systolic blood pressure were moderate (but significant), and the effects of drug treatment on sham or infarcted rats appeared to be similar. There was no effect on heart rates. There was an increase in urine output (diuresis) in trandolapril-treated animals. This effect was greater in the sham-operated animals (6.6X increase over controls at 6 months). In infarcted animals, the greatest effect was at 9 months (2.6X over controls). Minor effects were noted on excretion of sodium (natriuresis), urinary creatinine, and urinary cGMP. Infarct size was not reduced in trandolapril-treated animals. In fact, there was a moderate (54%) increase in infarct size at 9-12 months which may have been an artifact since there were reductions in left ventricular hypertrophy and fibrosis. Decreases in vascular resistance were found in most tissues examined. Conversely, regional (organ) blood flows were increased in trandolapril-treated animals. It was concluded that the increased survival of infarcted rats treated with 0.1 mg/kg/day trandolapril for 12 months could be explained by the decreased

cardiac hypertrophy (due mostly from reduced collagen synthesis) and reduced vascular resistances with corresponding increases in regional (organ) blood flows.

In rats surviving myocardial infarction due to coronary artery occlusion, trandolapril at 0.7 and 1.0 mg/kg/day increased survival from 68% (untreated controls) to 100% and 92%, respectively. In untreated controls, cardiac hypertrophy due to congestive heart failure (heart weight/body weight ratio) increased 46% when compared to sham-operated controls. Trandolapril at 0.3, 0.7, and 1.0 mg/kg/day reduced cardiac hypertrophy to 27%, 15%, and 12% increases over sham-operated controls, respectively. Heart rates were significantly increased (+9%) in only the 1.0 mg/kg/day dose group when compared to untreated infarcted controls where they approached the values seen in the sham-operated controls. Likewise, the PQ-intervals were normalized (decreased 13%) in only the 1.0 mg/kg/day group when compared to untreated controls where they approached the values seen in the sham-operated controls. Systolic and diastolic blood pressures were significantly reduced in the 0.7 and 1.0 mg/kg/day treatment groups when compared to the infarcted controls. Left ventricular end diastolic pressure (LVEDP) in the 0.3 and 1.0 mg/kg/day dose groups was significantly reduced when compared to infarcted controls and approached the values seen in sham-operated controls. Coronary reserve in infarcted controls decreased 33%. Trandolapril treatment at 0.7 and 1.0 mg/kg/day increased coronary reserve to values seen in sham-operated controls. These results suggest that trandolapril, when given orally at an optimum dose of 0.7 mg/kg/day for 24 weeks, increased survival and reduced cardiac hypertrophy resulting from congestive heart failure in rats surviving myocardial infarction due to coronary artery occlusion. These effects appeared to be related to its ability to reduce left ventricular end diastolic pressure and normalize coronary reserve (blood flow).

The effect of trandolapril on the cardiac hypertrophy induced in rats by isoproterenol was examined. Results showed that stimulation of β -adrenergic receptors in the heart by isoproterenol produced cardiac hypertrophy (increased weight in atria, and right and left ventricles) which was associated with increased angiotensin II levels in these tissues. Concomitant administration of trandolapril reduced tissue angiotensin II levels, but reduced tissue weights in only the left ventricle (atria and right ventricular weights were unchanged). Although trandolapril had no effect on heart rates, systolic blood pressure was reduced by 19%, suggesting that the reduced cardiac hypertrophy was associated with both reduced left ventricular angiotensin II levels and reduced blood pressure. The lack of an association between reduced tissue angiotensin II levels in atria and right ventricles and lack of a reduction in their weight (in contrast to the relationship of reduced left ventricular angiotensin II levels and reduced left ventricular weight) was left unresolved.

In spontaneously hypertensive rats, trandolapril reduced tissue angiotensin II levels (atria, right and left ventricles). These effects correlated with the corresponding tissue weights, except in the right ventricle where tissue weight was relatively unchanged.

These results showed that left ventricular angiotensin II levels correlated better than circulating (plasma) angiotensin II levels with respect to left ventricular hypertrophy in this rat model of hypertension.

In isolated perfused rabbit hearts, trandolaprilat at 10^{-5} M significantly reduced the leakage of CK and LDH caused by ischemia and reperfusion when compared to untreated control hearts. The reductions in enzyme leakage in hearts treated with trandolapril at 10^{-5} M or trandolaprilat at 10^{-6} M were not significant when compared to untreated control hearts. Coronary perfusion or myocardial contractile function were not improved when compared to untreated control hearts. These results showed that trandolaprilat was more effective than trandolapril in protecting the heart from ischemic damage in an isolated rabbit heart model.

In an attempt to determine the effect of trandolapril on oncogene expression as it relates to vascular and cardiac hypertrophy, results of Northern analyses were concluded by the Sponsor to indicate that angiotensin I and angiotensin II induced the expression of c-myc, c-fos, and HSP-68 in rabbit heart and aorta (the aorta data was not interpretable), and that trandolapril at 0.5 mg/kg inhibited these effects of angiotensin I. According to the Sponsor, the data supported their hypothesis that trandolapril might inhibit the development of cardiac hypertrophy and vascular hypertensive angiopathy resulting from arterial impedance, such as arterial hypertension and atherosclerosis. In this study, the data suggested an effect of trandolapril on inhibiting the expression of genes associated with cellular growth (c-myc, c-fos, and HSP-68) in the heart and vessels, although the aorta data was of poor quality.

The effects of trandolapril were studied on hemodynamic parameters and on intensity of electrical ischemia in healthy myocardium or myocardium made ischemic by coronary occlusion in anesthetized dogs. Results showed that trandolapril at doses up to 1.0 mg/kg IV reduced ACE activity and the hypertensive effects of angiotensin I in anesthetized dogs. These effects were likely due to decreased peripheral resistance, since similar doses of trandolapril had no effect on cardiac output or heart rate. Regional blood flows (endocardium/epicardium ratio) were slightly increased in healthy myocardium, but not in ischemic tissue.

In anesthetized dogs, doses of trandolapril (10 mg/kg IV) which had marginal hemodynamic effects (heart rate and blood pressure) increased salt (Na^+ , K^+ , and Cl^-) excretion with little effect on renal function (clearance).

In conclusion, the pharmacodynamic studies showed that trandolapril is an ACE inhibitor with a relatively long duration of action for its antihypertensive effect in animals. The antihypertensive activity of trandolapril appears to result primarily from inhibition of circulating (plasma) and tissue ACE activity resulting in reduced angiotensin II formation. The subsequent reduction in angiotensin II levels is

responsible for peripheral vasodilation, reduced blood pressure and total peripheral resistance, and decreased renal sodium and fluid retention. In addition, The diacid, trandolaprilat, possesses high lipophilicity when compared to the diacids of other ACE inhibitors. This property may contribute to good penetration into various tissues resulting in more efficient ACE inhibition in both tissue and in plasma, and may provide beneficial effects on reversing cardiac and vascular hypertrophy.

C. Pharmacokinetics

Pharmacokinetic studies in rats, dogs and monkeys demonstrated that trandolapril was rapidly but incompletely absorbed and excreted. Trandolapril had no affinity for melanin-containing tissues and did not penetrate the CNS. Trandolapril accumulates markedly in the kidney, liver and lung due to the binding to angiotensin converting enzyme which is particularly prevalent in these tissues. After an oral administration, trandolapril is rapidly converted into its diacid metabolite, trandolaprilat, in all animals including humans. The bioavailability values of trandolaprilat were $37.29 \pm 7.92\%$, $42.99 \pm 9.76\%$ and $59.8 \pm 5.2\%$ for rats, dogs and monkeys, respectively. Binding of trandolaprilat to plasma proteins was noticeably negatively concentration dependent in both dogs and humans. The binding of trandolaprilat to plasma proteins increased from 58.5% to 86% and 65.1% to 94.2% over the concentrations ranging from 10,000 to 0.1 ng/ml in dog and human, respectively. This negative concentration dependent binding of trandolaprilat in both dog and human plasma indicated saturation of the binding sites at low concentrations. Approximately 30% of administered drug was excreted in bile in rats and dogs; urine accounts for a slightly greater fraction of the excreted drug.

D. Toxicology

LD₅₀ values after an oral administration were 4875 (M), 3990 (F); > 5000 (both sexes) and >1000 (both sexes) mg/kg in mice, rats and dogs, respectively. After an I.P. administration LD₅₀ values ranged from 1010 to 1765 mg/kg in mice and rats. In acute studies in mice, rats and dogs, main adverse effects were piloerection, hypomotility, hypotonicity, gross hemorrhage in the gastric atrium and pancreas, thickened liver lobes and enlargement and thickening of the spleen and kidneys.

In two subchronic (up to 30 days) studies in rats an oral (gavage) administration of trandolapril (4 to 500 mg/kg/day) induced dose-dependent decrease in angiotensin converting enzyme associated with reduction in blood pressure. Clinico-chemical or histological abnormalities were observed, in a dose-dependent manner, including renal lesions (presence of basophilic tubules in the cortex, tubular dilatation and nephritis) and gastric ulcerations. Similarly, in two subchronic (up to 30 days) studies in dogs an oral (capsule) administration of trandolapril (10 to 250 mg/kg/day) induced dose-related decrease in angiotensin converting enzyme associated with fall in blood

pressure. A slight to moderate increase in serum cholesterol, urea and alkaline phosphatase levels with no concomitant histopathology of the kidneys and liver was detected at ≥ 50 mg/kg/day doses. A few minor renal lesions such as basophilic tubules, dilated tubules and cells with pyknotic nuclei in the distal convoluted tubules were observed at 250 mg/kg/day dose group.

Chronic oral (gavage) administration of trandolapril (0.25 to 25 mg/kg/day) to rats for 26 weeks induced dose-related significant decrease in angiotensin converting enzyme. A slight increase in mortality was noted in 2.5 and 25 mg/kg day dose groups. Reduction in food consumption associated with decrease in body weight gain was observed in all drug-treated groups, but was not dose-related. Increase in levels of plasma urea and creatinine were noted at mid (2.5 mg/kg) and high (25 mg/kg) doses in rats drinking tap water, and these changes were reversible on drug withdrawal. Furthermore, rats drinking Ringer's saline had no such marked increase in plasma urea and creatinine. This indicates that these renal effects may be due to hemodynamics rather than direct cytotoxicity to the kidney. Both absolute and relative kidney weights were elevated with increased incidence of glomerulonephritis. Statistically significant decrease in absolute and relative heart weights with no histopathology was seen at 25 mg/kg/day dose groups of either sex. All these drug-related adverse effects tended to normalize after drug free period of six weeks.

Administration of trandolapril orally (gavage) to rats for 78 weeks at 0.25, 1.5 or 9 mg/kg/day doses induced dose related decrease in body weight gain associated with food consumption. Mortality was higher in females than in males, but for each sex the distribution of mortality was normal and not related to the treatment. The mortality was mostly associated with pituitary adenoma, primary hepatic tumors, mammary adenocarcinoma and tumors of the endometrium and stroma. The rate of mortality and tumors and the factors contributing to them did not indicate any treatment or dose relationship as the incidence of total or malignant tumors in the treated animals was almost identical to those in the controls. Slight changes in hematology and blood chemistry were noted in the treated groups, but none was dose proportional. No drug-induced statistical changes in organ weights or histopathology were observed. In conclusion, the oral (gavage) administration of trandolapril was fairly well tolerated by rats at doses up to 9 mg/kg/day [225 times human (2 mg) dose based on 50 kg body weight] for 78 weeks as no histopathology or increase in the incidence of total or malignant tumors were different than those in the concurrent controls.

Trandolapril (2.5, 25, 250/125 or 125 mg/kg/day) was administered orally to beagle dogs for 26 weeks followed by 6 weeks of recovery period. The highest dose tested, 250 mg/kg/day [6250 times human dose (2 mg) based on 50 Kg body weight], in the 26 week study in dogs induced several clinical signs such as vomiting, diarrhea, hypotonia and dehydration which were responsible for some mortality and euthanasia. Subsequently, the high dose was reduced to 125 mg/kg/day. Dose-dependent mortality was detected at doses ≥ 25 mg/kg/day. Angiotensin converting enzyme

activity was reduced at 2.5 mg/kg dose and was completely inhibited by ≥ 25 mg/kg/day doses. Several changes in hematological parameters (Increases of fibrinogen levels, sedimentation rates, platelet counts, activated partial thromboplastin times, hemoglobin levels, erythrocyte counts and hematocrit values), blood chemistry (increases of urea, creatinine, alkaline phosphatase, 5'nucleotides, ornithine carbomyltransferase, glutamate dehydrogenase and leucine aminopeptidase activities) and urinalysis (decrease in urinary Na, K, Cl, Ca and Mg outputs) were detected at mid- and high-dose groups, but normalized after drug free period of 6 weeks. Some macroscopic and microscopic evidence of gastrointestinal irritation, erosion and ulceration, and renal tubulonephrosis was observed at 25 and 125 mg/kg/day doses. The alteration in blood parameters, BUN and creatinine levels, gastrointestinal and renal structural integrity have also been reported for other ACE inhibitors - captopril and enalapril. Secondly, oral (capsule) administration oftrandolapril to beagle dogs for 52 weeks was well tolerated by both sexes at doses up to 25 mg/kg/day [625 times human (2 mg) dose based on 50 kg body weight].

E. Carcinogenicity

A brief coversheet for the carcinogenesis study review is shown in Appendix 1A.

The carcinogenicity studies were carried out in France using animals supplied by Charles River Laboratories. The mouse studies were conducted over a period of 78 weeks using the Crl; CD-1 C-CRBR strain, and the rat studies were done using the Sprague Dawley Cri CD(SD)Br strain over a period of 104 weeks. The mean weights of the mice of both sexes were approximately 27 grams, and of the rats approximately 100 grams. The mode of drug and control vehicle administration was by oral gavage, using a 0.5% methylcellulose suspension dispensed via stainless steel dosing needles and plastic syringes. Mice received 10 ml/kg/d and rats 2.5 ml/kg/d of drug suspensions or control vehicle.

Individually housed mice were dosed for 78 weeks according to the schedule shown in Table 1. Rats were housed two animals per cage during the 104 weeks of dosing according to the dosing schedule shown in Table 2. The doses were chosen by the conventional method of a sub-chronic range-finding study to determine if a maximum tolerated dose could be defined. Failing that, other surrogate indicators of the selection of rational doses were used. Sponsor described how a 60-day dose-ranging study (doses of 2, 10, and 50 mg/kg/day) was used to choose the final mouse doses of 1, 5, and 25 mg/kg/d, and how a six-month chronic rat study was used to define the final rat doses of 0.25, 2, and 8 mg/kg/day. During the 17th week of the experiment, the original high dose of 16 mg/kg/day was decreased to 8 mg/kg/day because of the histopathological evidence of increased incidence of renal glomerulonephrosis in the high dose animals.

Table 1.
Schedule for Dosing Mice

Groups	Dose levels (mg/kg/d)	Sex	Animals Number	Cages Number
T	0	Males	C02130 to C02189	1 to 60
A	1	Males	C02190 to C02249	61 to 120
B	5	Males	C02250 to C02309	121 to 180
C	25	Males	C02310 to C02369	181 to 240
T	0	Females	C02370 to C02429	241 to 300
A	1	Females	C02430 to C02489	301 to 360
B	5	Females	C02490 to C02549	361 to 420
C	25	Females	C02550 to C02609	421 to 480 ²

Table 2.
Schedule for Dosing Rats

Groups	Dose levels (mg/kg/d)	Sex	Animals Number	Cages Number
T	0	Males	C00018 to C00077	1 to 30
A	0.25	Males	C00078 to C00137	31 to 60
B	2	Males	C00138 to C00197	61 to 90
C	8	Males	C00198 to C00257	91 to 120
T	0	Females	C00258 to C00317	121 to 150
A	0.25	Females	C00318 to C00377	151 to 180
B	2	Females	C00378 to C00437	181 to 210
C	8	Females	C00438 to C00497	211 to 240

During the in-life mouse and rat studies the following clinical examinations were carried out: once daily clinical signs; mass palpations each 2 wk after 6 mo; two examinations per day after which debilitated animals were sacrificed and blood analysis and macroscopic examinations were carried out; body weight was measured once per wk for 13 wk followed by 1 determination each 2 wk for the remainder of the experiment; food consumption was measured once/wk during the first 13 wk, on wk 18, then every 4 wk until the end of treatment; water consumption was measured during treatment weeks 4, 13, 26, 52, and 78 in the first 10 males and 10 females which survived from each group, except for the control groups which were measured in week 26.

Hematological laboratory examinations were done on blood samples from both species collected in weeks 52 and 78 from survivors and from all moribund animals before sacrifice (if possible). Blood smears were prepared, the white blood cell differentials were determined and the cells were examined. No other hematological evaluations appear to have been made. ▽

Pathological examinations were done after both species of animals were sacrificed by the usual methods. Approximately 45 major organs were harvested, plus tissues and masses which were worthy of investigation in the sponsor's judgement.

All tissues required from both species for microscopic examination were sectioned at approximately 4-5 microns thickness and stained with hemalum-eosin (hematoxylin-alum-eosin). Microscopic examination was done on all macroscopic lesions and the above cited tissues, all the animals from the high dose and control groups were sacrificed at the end of the treatment period, as were all animals dying or being sacrificed prematurely. Microscopic examination was also carried out on the intermediate and low dose groups.

Results of the carcinogenicity studies showed that the palpable masses in mice and rats were confirmed as tumors by histopathological examination. We are in agreement with sponsor's conclusions that the findings "showed no indication of treatment or dose relationship either in morphological type, size or multiplicity." Appendix 1 B also shows that there is very little evidence of latency shown by the weeks of appearance of the palpable masses.

Monitoring of rats' clinical signs revealed only soiled litter as a possible treatment related effect. Clinical signs of treated mice were hypothermia and/or tremors in males and females which appear to be dose-related and statistically significant. Female mice may also display dose related piloerection and hunched posture which are possibly statistically significant. Unfortunately, sponsor did not evaluate the statistical significance of these deviations from control.

Mortality distributions in mice and rats were similar for both sexes in treated and

control animals. Death and morbidity in rats of all groups were related by sponsor to the occurrence of a pituitary tumor. In mice, the usual contributing factors to animal mortality of all groups were amyloidosis and malignant lymphoma. Some female mice showed endometrial sarcoma with metastases in some organs as contributory factors to death in all dosing groups. Misdosing was also a factor in the death of animals from all groups. Appendix 1 C shows summary data for both species demonstrating that rates of mortality do not vary with drug dosages.

Mean food consumption changes were quite small in male and female mice, but there appeared to be greater consumption by both sexes dosed with the mid and high doses during the first 4 weeks of the study; this was followed by a gradual decline in consumption until the end of the study. There were several points of significant difference compared with control consumption. Food consumption by the treated rats was slightly less than by the controls, but similar in males and females at about 7 % for the first 13 wk of treatment. After that time interval they were only occasionally significant.

Mean water consumption by male mice appeared to show greater increases by the mid and high doses than did the females. However, significance was seldom reached. Water consumption by the low dose male rats did not vary significantly from control. The animals from the mid and high dose groups had increases in consumption during weeks 4 and 14 which were not dose related. These statistically significant differences were about 55% and 40% in weeks 4 and 14, respectively. Female rats were not reported to have any consistent pattern of change.

Body weights and body weight changes for all groups of animals of both sexes and species were monitored for the duration of the experiments. Figures 1 A and 1 B (formerly 63 A and B) show the curves for the male and female mice, respectively. The curves for the males show that most of the treated animals differ significantly from control. Sponsor has verified this by calculations showing that there were approximately 8 % differences between most curves and the control curve. For the females, the differences were not at all clear cut, and there appear to be few, if any, significant differences in any of the mean values on the plateaus.

Figures 1A and 1B.

BODY WEIGHT CHANGES IN MICE TREATED WITH TRANDOLAPRIL
 FIGURE 63 A: MALES

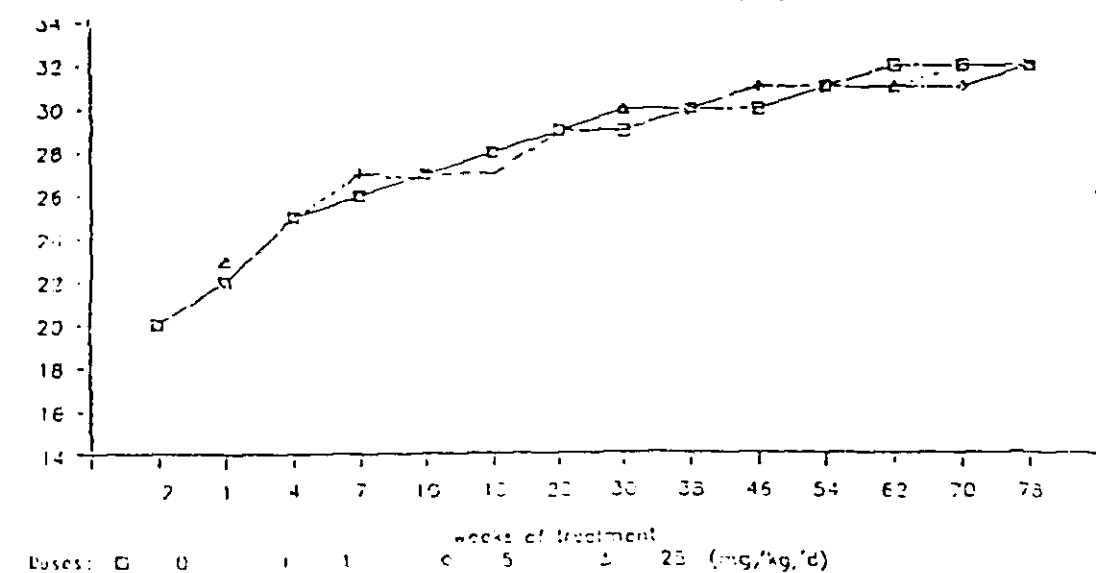
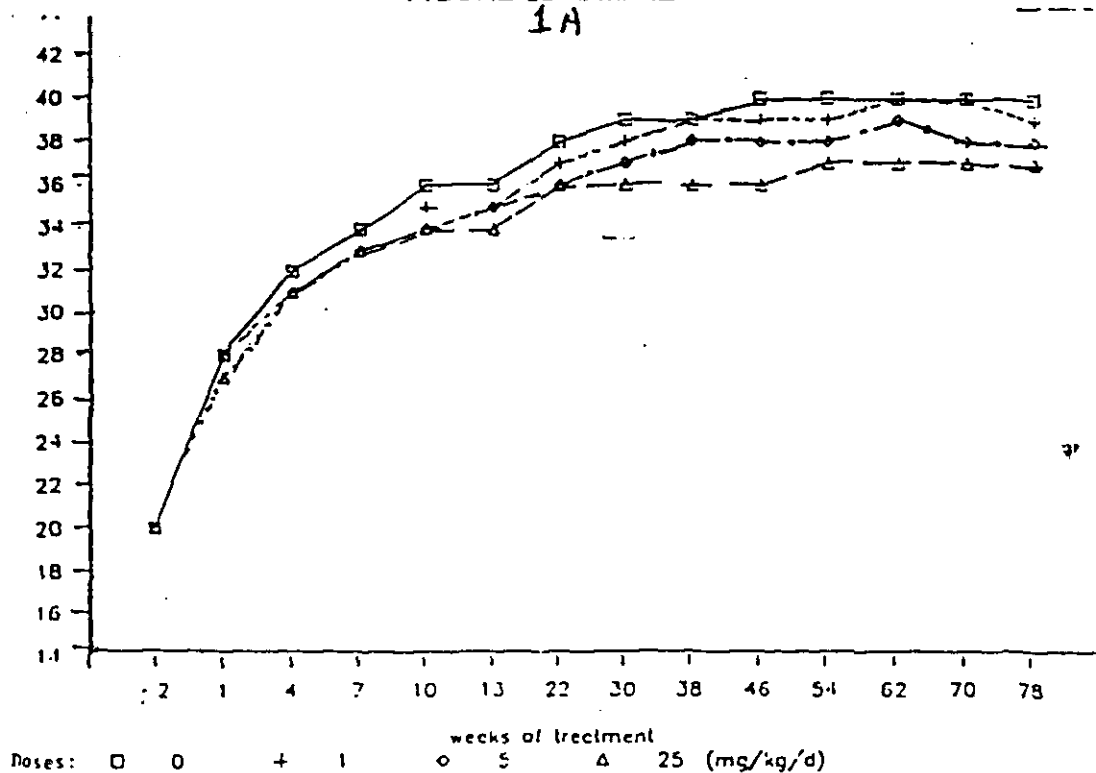


FIGURE 63 B: FEMALES
 1B

Figures 2 A and 2B

RAT BODYWEIGHT CHANGES (GRAMS) WITH TRANDOLAPRIL
FIGURE 65A: MALES

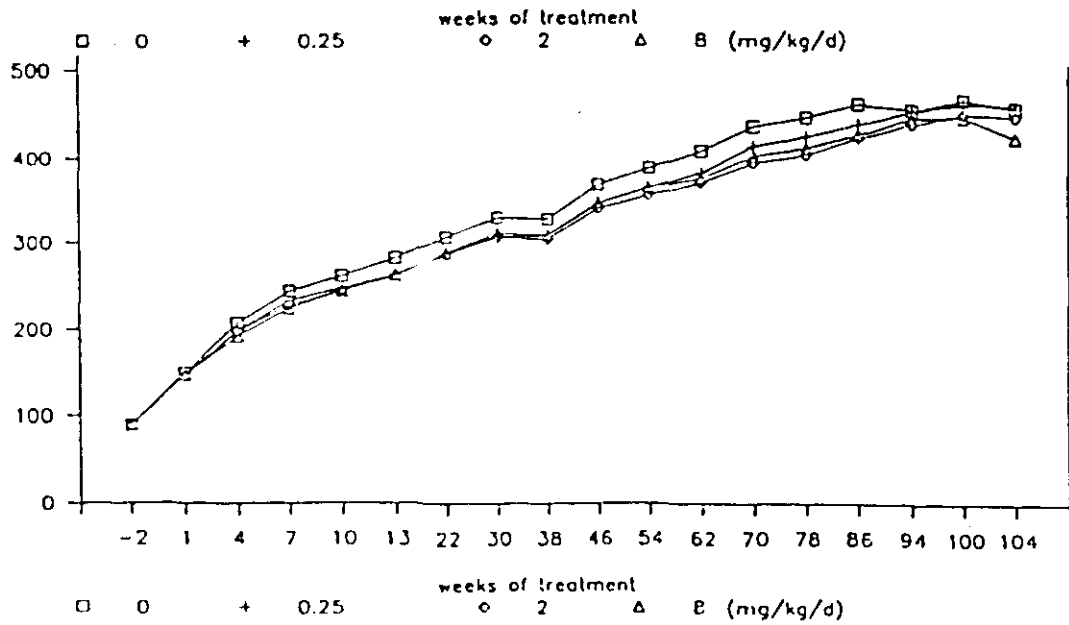
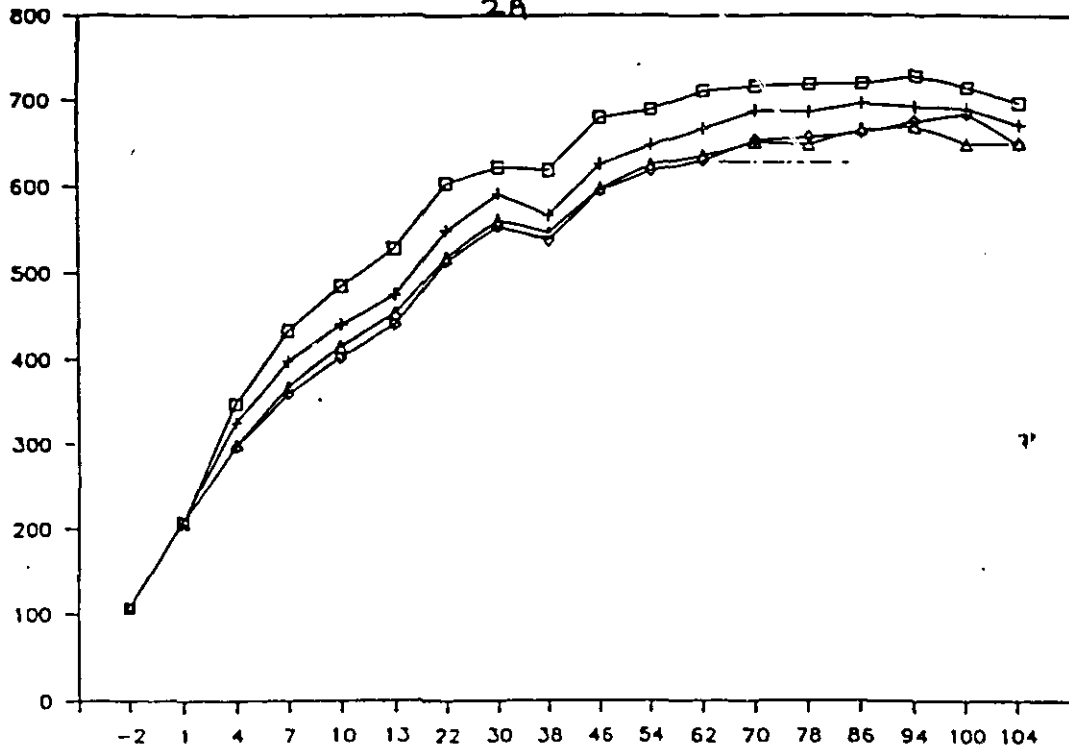


FIGURE 65B: FEMALES

The rat body weight changes shown in Figures 2 A and 2 B (formerly Figures 65 A and B, respectively) of the males and females are significantly different when one compares the controls with the treated animals. The animal body weight changes discussed above allow us to test for the achievement of a **Maximum Tolerated Dose (MTD)**. As can be seen from above, three of the four groups of tested animals (male and female rats and male mice) have clear cut separations of the curves, (i.e., 75%, minimum success). The female mice are not complete failures, however, since the curves seem to be sufficiently well separated at the very top to qualify as successful.

Laboratory investigations of hematology demonstrated only minimal changes in blood parameters of both species. Studies were limited to differentials of white blood cells.

The non-neoplastic and neoplastic findings in rats and mice were those that are typical for old animals. As such, the control and dosed animals presented approximately the same histopathological and general macroscopic findings. The incidences of focal nephropathy among female mice of the high (30 %) and mid-dose (42%) groups³ were increased compared with control animals (8%). Microscopic findings matched the general macroscopic observations in this and other organs for animals of advanced age. The above listed percentages are within the range of the spontaneous incidence (50-60%) for this type of lesion in old mice. However, sponsor feels that a relationship between the increased incidence of nephropathy and treatment with the compound cannot be ruled out.

Liver cell alterations (hepatocellular adenomas and carcinomas) were not dose related with respect to incidence or morphological character in either species. Again, the characteristics were those of cells from aged animals. Lymphomas were found in several major organs without any drug-related lymphoreticular distribution. Lungs were free of drug-influenced distribution in mice.

Sponsor reported that a major factor contributing to the death and morbidity of treated and control rat groups was the occurrence of pituitary tumors. Historical control data for 19 groups of female CD rats dosed over a 2-year period were provided by the supplier (Charles River Laboratories). The minimum to maximum percent incidence for the pituitary gland adenoma, pars distalis, was 31.4 to 88.8, respectively. This magnitude of variation indicated that there was no significant relationship between the doses administered and the tumor findings.

Sponsor reported no significant difference between the numbers of animals with neoplasms, the numbers of animals with more than one primary neoplasm, and the numbers of benign and malignant tumors among the various groups including the controls. Additionally, incidental tumor analysis did not indicate that there was a latency of tumor appearance in the treated animals compared with controls.

Kidney tubular cell adenomas, malignant lymphomas, perifollicular cell adenomas and carcinomas, and mammary gland tumors did not differ significantly between control and treated groups, nor was there evidence of dose-related response or treatment relationships. Furthermore, in a 78 week chronic rat study, TRAN did not cause even in a dose as high as 9 mg/kg/day, any histopathological evidence of toxicity or any change in incidence of tumors.

It was concluded by sponsor that there was no evidence of carcinogenic potential or increased incidence of spontaneously occurring tumors in the TRAN-treated mice and rats of these studies. We concur with sponsor's conclusion.

Dr. Daphne Lin was the mathematical statistician reviewing the Mavik submission. The potential issue of the validity of the tumor challenge presented to the female mice by the highest dose (25 mg/kg/day) was raised. As discussed above, the clinical signs of hypothermia and/or tremors seen in male mice were still increasing at the high dose and had achieved a level approximately sevenfold higher than control by the end of the experiment. The same parameter in female mice was still showing increased elevation at the end of the experiment as well showing increases in piloerection and hunched posture. Since clinical signs of toxicity should not be produced in the process of determining the MTD (NCI'S Guidelines for Carcinogen Bioassay in Small Rodents), this suggests that the MTD has been reached.

Additional evidence that the doses used were adequate was supplied by calculating the multiples of the maximum recommended human daily doses (MRHDD) in mg/kg and mg/m² provided by the two animal carcinogenicity studies. The results of these evaluations are shown in Appendix 1 D. This type of MTD evidence was sought because the AUC (Area Under the Curve) could not be calculated due to inadequate data for its calculation. The results shown in Appendix D indicate that the MRHDD values are reasonable multiples of control.

In summary, ACE inhibitors are known for the characteristic difficulty encountered when toxic manifestations of the compounds are sought. It is suggested that this is the sort of difficulty encountered with TRAN and not the lack of an MTD.

Table 3 summarizes the results of mutagenicity studies in which all tests were negative. This is also evidence supporting the conclusion that TRAN (trandolapril) is not carcinogenic.

In conclusion, trandolapril is not carcinogenic and does not show an increased incidence of spontaneously occurring tumors in mice and rats used in these experiments.

Appendix 1A
A Brief Coversheet for Carcinogenesis Study Review

	Rat	Mouse
1. No. of Study	203b TCR	203b TCS
2. Name of Laboratory		
3. Strain	Sprague Dawley CrI CD(SD)Br Rats	CrI:CD-1C-ICR BR Mice
4. No./sex/group	30	60
5. Doses (0,L,M,H)	0, 0.25, 2, 8	0, 1, 5, 25
6. Basis for dose selection stated	Yes	Yes
7. Interim Sacrifice	No	No
8. Total Duration (wks)	104	78
9. Week/Site for first tumor (0,L,M,H)	See Attached Appendix D	See Attached Appendix B
10. No. alive at termination (0,L,M,H)	See Attached Appendix C	See Attached Appendix C
11. Statistical Methods Used	Page 54 in Body of Review Plus Statistician's Review.	
12. Attach tumor and non-tumor data for each tissue	Attach Tumor and Non-Tumor Data for Each Tissue:	

Appendix 1B

Week of Appearance of Individual Palpable Masses			
Dose Group (mg/kg/d)	Sex	First Week Observed	Microscopic Examination Results
Mice			
Control	M	67	Fibrohistiocytoma
Control	M	75	Fibrohistiocytoma
-	F	-	-
1	M	74	Suppurative cellulitis
1	F	45	Histiocytoma
5	M	45	Not found at Autopsy
5	F	43	Disappeared in wk 51
Rat			
Control	M	39	Histiocytoma
Control	F	41	Disappeared
0.25	M	41	Disappeared
0.25	F	49	Adenofibroma, mammary glands
2	M	84	Disappeared
2	F	57	Adenofibroma, mammary glands
8	M	45	Disappeared
8	F	18	Disappeared
25	M	-	-
25	F	49	Disappeared

References:

Mice: Vol. 45:pp. 63-65

Rats: Vol. 50 pp. 73-91

Appendix IC

Number of Animals Alive at Experiment Termination, by Sex and by Dose Group					
	Sex	Dose (mg/kg) Groups			
Doses		0	1	5	25
	Male	53	52	51	49
Mice	Female	43	46	48	45
	Both Sexes	96	98	99	94
Doses		0	0.25	2	8
	Male	22	34	28	26
Rats	Female	27	29	29	30
	Both Sexes	49	63	57	56

References:

Mice: Vol. 46:pp. 32-61. Control n = 60.

Rats: Vol. 50:pp. 48-70. Control n = 30.

Appendix 1D

Calculation of Doses Used to Multiples of Maximum Recommended Human Daily Dose (MRHDD)
Based on Mg/Kg and Mg/M²

		Ave. Starting Wt. (gms)	Ave. Ending Wt. (gms)	Median Wt. (gms)	K	km	High Dose (mg/kg)	High Dose (mg/m ²)	Multiple of Human High Dose (MRHDD)	
									mg/kg	mg/m ²
Mouse	Male	20	39	30	9.0	3.46	25	86.5	313	32
	Female	20	33	27		3.34		83.5	313	31
Rat	Male	100	650	375	9.0	8.02	8	64.2	100	24
	Female	100	400	250		7.00		56.0	100	21
Human					10.8 (50 kg)	34.1	0.08 (4 mg/50 kg)	2.73		

Table 3
Results of Mutagenicity Studies

Assay	Cells	Conditions	Results	Interpretation
Ames Test	<i>S. typhimurium</i> TA 98, 100, 1535, 1537, 1538, +/- S9	Up to 5.0 mg/plate	Negative	Not mutagenic
Gene Conversion Test	<i>S. cerevisiae</i>	Up to 2.0 mg/ml +/- S9	Negative	Not mutagenic
Chromosome Aberration Test	Human lymphocytes	Up to 2.0 mg/ml +/- S9	Negative	Not clastogenic
Chromosome Aberration Test	V79 Chinese hamster lung fibroblasts	Up to 4.0 mg/ml +/- S9	Significant increase in mean aberration rate at $\geq 500 \mu\text{g/ml} + \text{S9}$, but not dose-dependent	Not biologically relevant
Chromosome Aberration Test	Chinese hamster CHO cells	Up to 500 $\mu\text{g/ml}$ +/- S9	Negative	Not clastogenic
HPRT Gene Mutation Assay	V7889 Chinese hamster cells	Up to 500 $\mu\text{g/ml}$ +/- S9	Negative	Not mutagenic
Micronucleus Test	Mouse bone marrow cells	Single oral dose of 1000 mg/kg	Negative	Not clastogenic
Micronucleus Test	Mouse bone marrow cells	Single oral dose of 2000 mg/kg	Negative	Not clastogenic

F. Reproductive Toxicology

Fertility and general reproductive performance in rats were not adversely affected by doses of trandolapril as great as 100 mg/kg/day, or 1250 times the maximum recommended human dose (MRHD) on a body weight basis, or 257 times the MRHD on the basis of body surface area. There is some evidence of modified renal growth patterns which result in apparently temporary renal structural abnormalities (pelvic cavitation and dilated ureters) in the 100 mg/kg/day untreated F₁ offspring; these abnormalities disappear as growth rates equilibrate.

In a study of trandolapril's possible effects on organogenesis, the overall incidence of minor visceral defects was significantly increased only in rats receiving the high dose of 1000 mg trandolapril/kg/day which, on the basis of body weight, is 12500 times the MRHD or 2570 times the MRHD on the basis of body surface area. Sponsor reasonably considers that the defects are of doubtful significance, therefore, there is no evidence of teratogenic potential under the conditions of this experiment in rats.

The teratogenic potential of trandolapril was also studied in rabbits orally dosed at 0.2 to 0.8 mg/kg/day, on the basis of body weight, and 2.7 and 10.8 mg/M², respectively, on the basis of body surface area (1.0 to 4.0 times the MRHD). Maternal toxicity was evidenced by 12/21 deaths in the high dose group compared with 5/21 deaths in the control group as well as clear signs of toxicity during and after treatment. Pre- and post-implantation losses and the incidences of major malformations were balanced by equivalent frequencies of these events in the control groups. Additionally, it is common experience that rabbits are very sensitive to agents of this type and are thereby poor models for these assays.

Sponsor used rabbits in another test to determine the potential of trandolapril (0.1 to 0.8 mg/kg/day) for maternal toxicity and treatment-related malformations during the period of organogenesis. There were clear signs of maternal toxicity during and after treatment with 4 mg/kg/day trandolapril, but there were no treatment-related malformations at and above 0.4 mg/kg/day by body weight or the equivalent of 5.4 mg/M²/day when calculated as surface area.

An electrolyte supplement (1/2 strength Ringer's Solution) administered to rabbits by gavage in doses of 2 to 8 mg/kg/day was tested for its ability to ameliorate the apparent renal toxicity of TRAN. Sponsors reported that the tolerance to TRAN was not adversely affected at any of the dose levels.

Sexually mature female Cynomolgus monkeys were dosed orally with 10 to 250 mg/kg/day TRAN. Gastrointestinal upset and incidences of abortions were present in dose-related increasing amounts which sponsor attributed to the drug. Teratogenic potential could not be calculated because of the high mortality in the high-dose group. Sexually mature Cynomolgus monkeys were dosed at 1/2 the dose levels used in the

above study (5 to 125 mg/kg/day) to determine potential teratogenicity. There were mild incidences of maternal and fetal toxicities, but teratogenic potential could also not be calculated in this instance because of the low numbers of surviving fetuses.

A segment III study of the peri-and post-natal periods of reproduction in pregnant female rats was done at oral doses of 1 to 100 mg/kg/day based on body weight, or 7 to 700 mg/M²/day based on body surface area. The 1 mg/kg/day dose was the only dose tested which caused no maternal, fetal, or pup adverse effects. Therefore, the multiple of the MRHD which is available for safe human use predicted by this test is approximately $1/0.08=12.5$ on the basis of body weight (mg/kg/day) and $1/2.72=0.37$ on the basis of body surface-area.

A rat segment III study was used to define the effects of a supplemental electrolyte solution on the usually observed toxicity of such a study. Doses of 10 and 100 mg/kg/day trandolapril were administered with and without 1/2 strength Ringer's saline; comparison with a negative vehicle control was also made. Without saline in the 10 mg/kg/day treatment solution, food consumption during the lactation days was significantly decreased from control. With saline in this dosing solution, food consumption was equivalent to control and the high dose was returned part-way back to the control level. However, supplementing the electrolytes did not reduce the dose-related abnormalities of the renal pelvis (dilated) or the ureters.

G. Mutagenicity

Several tests for possible mutagenic effects were performed. *In vitro* trandolapril was tested on bacteria, yeasts, and several mammalian cell types with or without metabolic activation. Results showed that trandolapril did not significantly increase: (1) the levels of spontaneous reversion in all five *Salmonella typhimurium* strains tested (Ames test) at concentrations of up to 5.0 mg/dish, (2) the revertant frequencies of *Saccharomyces cerevisiae* D4 cells when tested at concentrations of up to 2000 µg/ml, (3) the frequency of chromosomal aberrations in phytohemagglutinin-stimulated normal human peripheral blood lymphocytes when tested at concentrations of up to 2000 µg/ml, (4) the mean chromosome aberration rates (including gaps) in V79 Chinese hamster lung fibroblast cells when tested at concentrations of up to 4000 µg/ml, (5) the morphological chromosomal aberrations in Chinese hamster ovarian (CHO) cells when tested at concentrations of up to 500 µg/ml, and (6) the frequency of mutations at the HPRT locus in V7889 Chinese hamster cells when tested at concentrations of up to 500 µg/ml. The clastogenic activity of trandolapril was tested *in vivo* by examining micronucleus induction in mouse bone marrow cells in two separate experiments. Results showed that trandolapril did not increase the frequency of micronuclei in polychromatic erythrocytes when tested at an oral dose of up to 2000 mg/kg in mice. In conclusion, trandolapril was not mutagenic both *in vitro* and *in vivo* when tested under the conditions and concentrations examined.

H. Conclusions

The antihypertensive studies in animals showed that trandolapril had a relatively long duration of action. This was due primarily to inhibition of circulating (plasma) and tissue ACE activity resulting in reduced angiotensin II formation. The subsequent reduction in angiotensin II levels was responsible for peripheral vasodilation, reduced blood pressure and total peripheral resistance, and decreased renal sodium and fluid retention.

In genetically hypertensive (GH) and spontaneously hypertensive (SH) rats, significant reductions in mean blood pressures were achieved at oral doses of 0.3 mg/kg with a minimum effective dose of 0.03 mg/kg. This same dose resulted in a substantial decrease in serum ACE. Much lower doses (0.001 mg/kg) resulted in significant reductions in tissue (cardiac) ACE. Significant reductions in cardiac hypertrophy were seen at doses of 0.7 to 3 mg/kg.

The toxicology studies showed that, in general, adverse effects were seen only at oral doses much higher than that required to produce reductions in blood pressure, indicating a relatively wide margin of safety. In rats, body weights were reduced at doses of 4 mg/kg/day for 30 days, but no other adverse effects were noted at doses of up to 9 mg/kg/day for 78 weeks. At 20 mg/kg/day for 30 days, however, renal tubule lesions were observed.

In rats no effect on fertility was seen at doses as high as 100 mg/kg/day nor on teratogenicity at doses as high as 1000 mg/kg/day. However, some litter loss was observed at 10 mg/kg/day. In a segment-II study in rabbits, no teratogenicity or mortality was detected at the highest dose of 0.8 mg/kg/day. However, at this dose an overall increase in major nonstructural fetal malformations was observed. These non-teratogenic maternal and fetal toxic effects in rabbits have been noted with other ACE inhibitors. In a monkey teratology study, doses of ≥ 10 mg/kg/day resulted in clinical signs of toxicity with an increased abortion rate at 125 mg/kg/day.

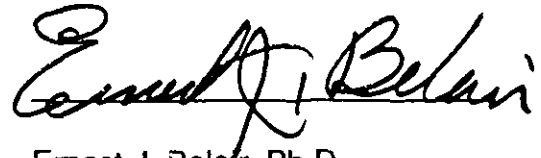
In dogs, no adverse effects were seen at doses of 2.5 mg/kg/day for 26 weeks, 10 mg/kg/day for 15 days, or at 25 mg/kg/day for 52 weeks. However, increased mortality and increased hematologic and histopathologic changes were observed in one study at doses of ≥ 25 mg/kg/day for 26 weeks.

The dose in rats that produced toxic effects (9 mg/kg) was about 30-300X higher than the dose that produced the pharmacologic effect (0.03 to 0.3 mg/kg), indicating a sufficiently wide margin of safety. In dogs, the dose that produced no adverse effects (25 mg/kg/day for 52 weeks) was about 300X higher than the maximum recommended human daily dose (MRHDD) of 4 mg/day (= 80 μ g/kg/day based on a 50 kg human). In monkeys, the margin of safety was about 125X when compared to the MRHDD.

From the preceding studies in animals, trandolapril appears to reduce blood pressure at doses much lower than those that produce toxic effects. However, at 10X the MRHDD nonstructural fetal malformations were detected in rabbits.

V. RECOMMENDATIONS

On the basis of the above pharmacology and toxicology studies in animals, trandolapril has shown antihypertensive activity without significant associated toxicity. Therefore, trandolapril may be approved for therapeutic use.



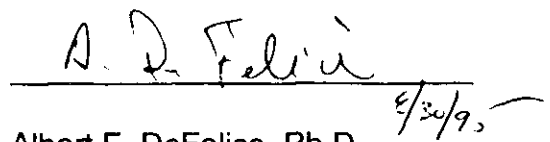
Ernest J. Belair, Ph.D.
Pharmacologist



Thomas Papoian, Ph.D.
Pharmacologist



D.G. Patel, Ph.D.
Pharmacologist



Albert F. DeFelice, Ph.D.
Supervisory Pharmacologist

- cc:
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HFD-110
HFD-110/ KBongiovanni
HFD-110/ EBelair
HFD-110/ TPapoian
HFD-110/ DPatel

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