CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 21-526

Pharmacology Review(s)

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

DATE: February 3, 2006

FROM: A.DeFelice, Ph.D.. Pharmacology Team Leader, DCarRP

TO: NDA 21-526. (Ranexa®, CV Therapeutics, Inc.) file

Through: Norman Stockbridge, Director, DCarRP.

SUBJECT: Rationale for Ranexa® labeling: pre-clinical sections

This is to provide some background and rationale for the **Warnings: Tumor Promotion; Carcinogenesis, Mutagenesis, Impairment of Fertility;** and **Pregnancy – Pregnancy Category C** sections of the final printed labeling for Ranexa® approved 1/27/06. It is being provided in the context of archived primary (Dr. Elizabeth Hausner) and secondary (Dr. Albert DeFelice) Pharmacology - Toxicology reviews.

WARNINGS Tumor Promotion.

The promotion by ranolazine is based on a study by Suckow *et al* [Cancer Letters 209 (2004) 165-1690] who report excess intestinal mucosal tumors - observed by investigators blinded to treatment - in male (females not tested) transgenic $APC^{(min/+)}$ mice after 30-day treatment at 30 mg/Kg twice daily starting at 40 days of age. Reportedly, overall intestinal tumor incidence nearly doubled (12 ± 2 vs. 20 ± 3 at the high dose; $p < 0.01$). Furthermore, both the adenocarcinoma/adenoma and invasive carcinoma /carcinoma in-situ ratios at autopsy (70 days of age) were increased (p-value, if calculated, was not provided) indicative, *prima facie*, of enhanced malignancy. No intestinal tumors were observed in either untreated or treated intact (wild-type) C57B16/J male mice. The article is silent on whether other tissues were examined for any excess in tumors whether background or *de novo*. It is observed that female min/+ mice were not evaluated, which is an important data gap as they are reported to be pre-disposed to mammary neoplasia (A. Shoemaker et al Bioch. Biophys. Acta 1332. 1997. F25-F48) as well as (presumably) intestinal mucosal neoplasia.

Suckow *et al* assert that length of administration was based on preliminary data showing that control tumor incidence was maximal at 70 days of age with

only size, and not number, of tumors increasing thereafter. It is noted that, even though a dissecting microscope was used to view the entire intestine, the mean control (background) incidence of intestinal mucosal tumors (ca. 12), which is interpreted to be the mean maximum incidence, is ostensibly appreciably less (ca. 50) than that implied in the publication by Shoemaker et al (*ibid).* It is noted, in passing, that the spontaneous background incidence reported by Shemaker et al would exceed by several fold the tumor incidence observed by Suckow et al in APC min/+ which received the high dose of ranolazine (30 mg/Kg, twice daily).

The labeling states that the clinical significance of the positive findings is unclear. Neither the Division, nor the FDA at large, has as much experience with this assay as with conventional lifetime assays and such cardinal associated features of the model as the critical p value criterion for statistical significance (based on spontaneous tumor incidence and power of detection) is lacking. Lacking also is an appreciation of false positive and negative rates based on behavior of chemicals in the model vs. patients. The clinical significance of this isolated positive finding is also unclear because of perceived deficiencies is study design, execution, and analysis, which render the positive findings equivocal at best and warrant further investigation according to our NTP consultant. The deficiencies of the Suckow et al study , and the need for independent confirmation of the positive findings in this and other models are stated in an external consult requested of Dr John French at NTP and appended to Dr. Hausner's review of 12/15/05 of Sponsors NDA 21-526 pharmacology/toxicology amendment of 12/6/05.

This **WARNINGS: Tumor promotion** section of the labeling is silent on the behavior of ranolazine in the standard lifetime rat and mouse tumorigenicity assays. Unlike the APC $^{min/+}$ model - which is unequivocally predisposed to a high early excess incidence of discrete intestinal neoplasia (and readily promoted, for example, by ethyl nirosourea) - it is not clear whether standard lifetime assays can reliably, if at all, detect tumor promoters. Nevertheless, spontaneous background tumors are routinely encountered in the control cohorts of standard assays (both species; both sexes), and at high enough incidences as to be expected *a priori* to be "promoteable". Indeed, Dr Hausner interprets (see below) a ranolazine-associated tendency to increase the incidence of certain common spontaneous background tumors - in some cases statistically significantly per FDA methodology/critical p-value - as possibly suggesting that ranolazine has the capacity to promote tumors.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis:

This section states that there was no evidence of carcinogenic potential in lifetime studies in either rats or mice tested at up to maximum tolerated dosage (MTD) in both species, and that the rat MTD and the mouse MTD are, respectively, approximately at or appreciably less than the MRHD on a mg/m2 basis. This reflects the determination (*a priori* of the findings in the APC $^{min/+}$ model) by The Executive Carcinogenicity Assessment Committee (ECAC) on January 15, 2002 – namely, that there were no noteworthy ranolazine - related findings. The ExecCAC is composed of senior FDA Center for Drug Evaluation and Research scientists , and is chaired by two recognized experts in toxicologic carcinogenesis. They are familiar with the vagaries of background tumor burden – both qualitative and quantitative - in lifetime rat and mice assays and detection of tumorigenic activity based on perceived strength of signal in the context of this variability in spontaneous tumor incidence. Exec CAC determinations are considered the official CDER position on tumorigenic potential of drug candidates and the adequacy of trials to identify such.

Dr. Elizabeth Hausner's review dated 12/15/2005, - which references DFS listing I 043735 N 162 IT25-Jul-2001 - asserts that, in retrospect, " The results from the required 2-year rodent studies can also be interpreted to suggest further support for tumor promotion" , an assertion based on species-specific and sex-specific behavior of ranolazine in these lifetime assays. Namely, that ranolazine significantly and dose -relatedly increased, by several-fold, the incidence of certain common benign tumors in <u>male</u> (thyroid follicular adenomas; testicular interstitial tumors), and <u>female</u> (adreno-cortical adenoma) rats (Statistical review and evaluation of carcinogenicity data: IND number 43,175. Biometrics Report: John Lawrence, Ph.D., dated 12/3/01). Viewed in a wider context, however, it should be recognized that an excess of such tumors occurred in only one of 4 "fields" i.e., no excess of thyroid or adrenal adenoma was encountered in the complementary rat sex, and no exacerbation of these – or any other neoplastic lesions – were encountered in either sex of the mouse. It is noted, in passing and without further comment, that the incidence of lymphoma, and mammary, liver pituitary, and lung tumors, – which were appreciably encountered in approx 10-70% of untreated rats and/or mice, and presumably "promoteable" – was not exacerbated by ranolazine, but rather were depressed at HD in some cases although not statistically significantly.

A further retrospective look at the standard 2-year mouse and rat assays for evidence that ranolazine may promote tumors – either in terms of extent of malignancy, or incidence of a tumor-bearing (whether malignant or benign) rodents – reveals:

a. no increase in *malignant* adrenal cortical, *malignant* thyroid, or *malignant* testicular neoplasia in ranolazine-treated rats (Biometrics report; *ibid*); and

b. no increase in incidence of either rats or mouse with one or more tumors in ranolazine-treated cohorts (Carcinogenicity Assessment Committee report and FDA-CDER rodent Carcinogenicity Database Factsheet; Elizabeth Hausner, dated 1/31/02). The incidence of (any) tumor-bearing animal at autopsy was as follows:
Mice, female: 61% control....vs. 52% at High Dose (50 mg/Kg) of ranolazine (HD)
Mice, male: 66% control..... Vs. 58% at HD
Rats, female: 97% control.... vs. 95% at High Dose (150 mg/Kg) ranolazine (HD)
Rats, male: 83% control..... Vs. 72% at HD.

Since the control incidence of a tumor –bearing animal is so high in the rat, it might be more informative to look at incidence of malignant-tumor bearing animals - namely, male: 23% control ...vs. 23 % at HD; and female: 22% control vs. 15% at HD; as well as the relative incidences of rats with a metastatic neoplasm - namely, male: 3% control...vs. 2% at HD; and female: 5% control...vs. 2% at HD. Accordingly there is no evidence of tumor promotion in the rat or mouse from the perspectives of extent of malignancy or incidence of rodents with at least one of *any* type of benign or malignant tumor.

Impairment of Fertility, and Pregnancy –Pregnancy category C:
The basis for the assertions that there are no adequate studies assessing the effect of ranolazine on fertility or reproductive capacity, or on the developing fetus are described in Dr Hausner's review of September 2, 2003. In it are described deficiencies in the rat fertility and rabbit teratogenicity studies - including, most prominently, egregiously high dam mortality at the HD (rat:35%: rabbit:25%). There is an inadequate number of litters to reliably confirm and extend ostensible effects apparent at lower dosages, and to identify any *dose-related* selective reproductive toxicity. Clearly, ranolazine is maternotoxic in rat, and probably rabbit as well, at or close to clinical AUC exposures. This confounds identification of selective reproductive toxicity. With these caveats, embryotoxicity was apparent in both rats and rabbits, and included retarded fetal body weight, delayed ossification; cranial dysmorphia in the rat (which may or may not be secondary to the excess delayed ossification); and retarded neo-natal development in the rat (per standard landmarks e.g., time to eye and vaginal opening). Accordingly, Pregnancy C - which acknowledges evidence of anatomic and/or behavioral embryotoxicity, - was recommended by the primary reviewer, and accepted.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Albert Defelice
2/6/2006 03:27:42 PM
PHARMACOLOGIST

Norman Stockbridge
2/6/2006 03:36:34 PM
MEDICAL OFFICER

Memo on ranolazine labeling for carcinogenesis, N21526
From A. Jacobs 1/27/06 1/2 → low

It seems appropriate that a description of the positive results of the studies in APC $^{min+/-}$ mice with ranolazine be included in the labeling.

APC $^{min+/-}$ is an autosomal dominant mutation that predisposes mice to develop adenomas throughout the intestinal tract. (In humans familial adenomatous polyposis (FAP) affected individuals develop as many as several thousand intestinal adenomas, often by the second decade of life. FAP results from germline mutation of the adenomatous polyposis coli (APC) tumor suppressor gene).

In a literature report, ranolazine in this APC model of promotion of neoplasms (tumor promoters) in the intestines in mice clearly caused an increase in intestinal neoplasms. Although this model has not been studied as well as some other transgenic mouse models accepted by CDER, the results from this study should not be ignored. The negative results seen in the 2-year studies in Sprague-Dawley rats and CD-1 mice do not alleviate the concern for several reasons. First, the 2-year studies in the rat and mouse strains used in the 2-year studies are not thought to be sensitive to the effects of chemicals without initiating potential, and a number of chemical/drugs known to be and clearly recognized as tumor promoters give negative results in those 2-year studies. Second and even more importantly, the 2-year studies were conducted at exposures that were only 0.8 the human exposure at the maximum recommended dose (MHRD) for rats and were only 0.1 the human exposure at the MRHD for mice, so the test was relatively insensitive to evaluate any neoplastic effects at or above human exposure.

Nevertheless, the APC $^{min+/-}$ model has not been well-validated, and the clinical implications of a positive result in this assay are not clear and likely do not warrant a boxed warning. It is noted that folate at 8 ppm and a number of other pharmaceutical product components have given positive results in this model under some experimental conditions. This further suggests that the relevance to humans of positive results in this model needs to be better understood.

Appears This Way
On Original

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Abby Jacobs
1/27/2006 12:19:03 PM
PHARMACOLOGIST

Memo To The File

NDA21526
Drug: Ranexa® (Ranolazine)
Sponsor: CV Therapeutics
Indication: angina refractory to other treatments
Reviewer: Elizabeth Hausner, D.V.M.

Date: December 20, 2005

This memo is to clarify the change in the reviewer's opinion regarding the approvability status of this drug from "Approvable depending upon clinical benefit" to "Approvable pending resolution of the issue of potential tumor promotion." Experts in the area of carcinogenesis both in the FDA and in the NTP have stated that the findings of tumor promotion in the Cancer Letters publication require further study to be either confirmed or refuted but that they cannot be dismissed as they stand.

The original review of the standard rodent carcinogenicity studies occurred in 2001, several years prior to the Cancer Letters publication. The required mouse carcinogenicity study was conducted 1989-1991 and the rat study in 1992-1993. The datasets were provided to CDER statisticians in 2001. The Executive CAC met to discuss the studies January 15, 2002.

In my original review of the studies, I put the following summary of findings on the first page of the review:

From the original 2002 Carcinogenicity review.

Comment: CDER statistical analysis indicated a trend toward decreased survival in both male mice and rats. Significant neoplastic findings are summarized in the table below.
Summary of tumor incidences

species	sex	Tumor type	Incidence per dose group				P value	
			C	LD	MD	HD	sponsor	FDA*
Rat	m	Thyroid, follicular adenoma	3/120	1/60	2/58	6/60	<0.001 **	0.0027
rat	f	Adrenal, cortical adenoma	0/120	0/60	1/60	2/60	0.032[4]	0.0225
Rat	m	Testes, interstitial (b)	5/120	3/60	4/60	8/60	0.001[3]	0.0030
mouse	m	Testes, interstitial	5/100	1/50	2/50	5/49	0.019**	0.0276

*p value from Exact test, **one-tailed prevalence trend-test
[3] one-tailed combined trend p-value, [4] one-tailed exact trend test

From the original 2002 review

The sponsor reported significant findings of malignant sarcoma of subcutaneous tissue and benign pheochromocytoma of the adrenal gland, both tumor types in female rats. The p values do not reach significance per the FDA standard.

Summary of tumor incidences

species	sex	Tumor type	Incidence per dose group				P value	
			C	LD	MD	HD	sponsor	FDA*
Rat	F	Adrenal pheochromocytoma	1/120	1/60	1/60	3/60	0.013**	0.0549
Rat	f	Malignant sarcoma,SC	0/120	0/60	0/60	2/60	0.040[4]	0.0380
rat	m	Malignant histiocytic sarcoma	2/120	1/60	1/60	3/60	0.037[5]	0.059

*p-value from exact test, [4] one-tailed exact trend test,[5] one-tailed time to tumor trend p-value

Relative human exposure: Based on AUC comparison, total exposure at the highest dose tested in rats was 0.7-4X the human plasma exposure. The highest mouse dose tested produced ≤ 0.25X the human therapeutic exposure based on AUC.

As stated in that original review, a true maximally tolerated dose was achieved in males based upon body weight changes and decreased survival. The rat study was stopped at 90 weeks instead of the protocol-designated 104 weeks due to declining numbers of animals. In female mice, there were no effects upon body weight and no significant toxicological findings. The CDER statistician questioned the adequacy of the female mouse study for those reasons. However, the sensitivity of the rodent to the central nervous system effects of ranolazine precluded the use of higher doses.

As stated in the review at that time, I considered several possible interpretations (see review below):

- Species specific metabolites causing a species specific effect
- Adequate representation of human metabolites and the possibility of carcinogenic potential

- Another possibility was the coincidental appearance of the same tumor type in two separate species

The summary of the original carcinogenicity review is reproduced below.

SUMMARY

Given the sensitivity of the two rodent species for the CNS effects of the drug, the studies done were adequate. The mouse had on average <0.25X the human exposure while the rat had <4X the human exposure, yet higher doses were probably not feasible. The lack of metabolism data for the mouse is of some concern. Based upon the presence in murine urine of several metabolites, the assumption appears to have been made that there was adequate representation of the human metabolites. One may consider the question from the other direction and ask if there were murine-specific metabolites, perhaps that were genotoxic.

The appearance of the same tumor type (benign interstitial cell tumor of the testes) in both rats and mice at low multiples of human exposure is suggestive of carcinogenic potential. This material will be submitted to the Executive CAC for their deliberation.

When the Executive CAC examined the findings of the rodent carcinogenicity studies, due consideration was given to the use of two separate but identically treated control groups for each sex of both rodent species. Depending upon whether those control groups are combined for statistical analysis or left as individual units influences the level of statistical significance of the findings. Based upon the disparity of the tumor load in the control groups, the ExecCAC concluded that there were no noteworthy findings (see minutes of the ExecCAC meeting).

When the published Cancer Letter report about the APC $^{(min/+)}$ mouse was found in July of 2004,several years after the carcinogenicity review, one of the first actions I took was to contact Joseph Contrera, Ph.D. of the ExecCAC and ask the regulatory experience with and opinion of this transgenic mouse model. This is recorded in the review Aug_15_05.doc. After discussions with several of the authors of the Cancer Letters publication and various efforts within CDER (summarized in the Aug_15_05.doc review) the sponsor was asked to provide some explanation of the publication. The response that CV Therapeutics generated appeared to this reviewer to be superficial and based on scant and somewhat unconvincing science. Background material on the transgenic mouse model itself, the particular publication and the full response from CV Therapeutics was given to the PTCC. The PTCC convened September 15, 2005 to discuss the situation. This is reviewed in Aug_15_05.doc. Part of the discussion is summarized here:

1. It was acknowledged that this model is not typically required of sponsors.
2. It is not uncommon for one company to synthesize a competitor's compound and use it as a comparator compound in their own non-clinical studies. Adverse effects in these studies still require explanation even though the sponsor did not conduct the studies.
3. The two-year studies primarily find complete carcinogens. It is possible that a promoter will not be identified in these studies.

4. It is easy to miss a tumor in the intestinal tract depending upon the method of tissue handling and processing.

CV Therapeutic's response to the Cancer Letters publication was not perceived as adequate. The PTCC also felt that although there were data gaps in the publication and the study was not optimal, the findings could not be dismissed out of hand. The ideal situation was judged to be a repeat of the study, using both sexes of mice and conducted to GLP standards with complete reporting. Another recommendation from the PTCC was to seek the opinion of the National Toxicology Program (NTP) as the APC $^{(min/+)}$ mouse has been a subject of study for them.

A teleconference was held December 2, 2005 with Dr Jef French from the NTP transgenics program. Details have been summarized and recorded by Meg Pease-Fye the project manager . Among other things, Dr French was asked if there should not have been some signal in the standard 2 year rodent carcinogenicity studies that would support the Cancer Letters contention that ranolazine is a tumor promoter. Dr French replied that very sensitive transgenic animals were used for the Cancer Letters study and much less sensitive outbred animals were used for the standard studies. Therefore, the transgenic animals might show effects of promotion after 1 month of exposure while the outbred animals may not necessarily show anything outside of the usual background tumors. Dr French did note that decreased survivability and increased incidence of background tumors may be signs of tumor promotion activity. It was reiterated that the 2-year studies do not necessarily identify promoting agents.

In the group discussion immediately after the December 2 telecon, it was decided to revisit the original studies and see if there were any findings that had been overlooked, such as decreased survivability or increases in common tumor types. After the meeting, Ms. Pease-Fye contacted CV Therapeutics with a request for an electronic version of the original carcinogencity study reports.

Based upon the recommendations of an outside expert in the area of transgenic models of carcinogenesis, I re-examined the original rodent carcinogenicity studies for ranolazine. As mentioned earlier in this memo, decreased survival and increased common tumor types were present in both species of rodent and had in fact been identified over 4 years ago during the original review although the Executive CAC concluded that there were no noteworthy excess tumor findings. This re-evaluation was made in the light of the unrepeated Cancer Letter publication. One could also ask if the findings in the standard rodent carcinogenicity studies are simply coincidental.

Summary

1. We are faced with a study in a transgenic model that is not required in the regulatory toxicology assessment of new drugs. This un-required study produced a signal for tumor promotion.
2. The study was not conducted or reported according to GLP standards. Both the PTCC and the NTP consultant have acknowledged that there are data gaps and the results may perhaps be equivocal. Dr French's written critique (see APC_NDA.doc) provides a detailed listing of the shortcomings of the publication.

3. When the standard 2-year rodent studies were re-evaluated according to the recommendations of the NTP consultant, the findings of decreased survival and increases in a common tumor type as identified in the original review were consistent with what Dr French had cited as possible evidence of tumor promotion. Another possible interpretation is that the findings are purely coincidental or simply further evidence of reaching a maximally tolerated dose.

The fact that the $APC^{(min/+)}$ mouse is not a regulatory requirement does not negate the safety flag that has been raised. The published scientific literature is supportive of the APC(min/+) mouse as an established model for the investigation of carcinogenesis. As both internal consultants (the PTCC) and an external consultant (the NTP) have stated, the results cannot be dismissed without further data.

The CFR 312.32(a) Definitions does not specify that safety issues disclosed by non-required studies may be ignored. Rather, the statement is simply that

> (B) Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity. Each notification shall be made as soon as possible and in no **event** later than 15 calendar days after the sponsor's initial receipt of the information. Each written notification may be submitted on FDA Form 3500A or in a narrative format (foreign events may be submitted either on an FDA Form 3500A or, if preferred, on a CIOMS I form; reports from animal or epidemiological studies shall be submitted in a narrative format) and shall bear prominent identification of its contents, i.e., ``IND Safety Report.'' Each written notification to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility
>
> [[Page 64]]
>
> for review of the IND. If FDA determines that additional data are needed, the agency may require further data to be submitted.

As the matter stands now, there is insufficient data to accept the premise that ranolazine is a tumor promoter. There is also insufficient data to dismiss the possibility that ranolazine is a tumor promoter. As is the case with any data set, multiple interpretations may be given to the material in hand. One interpretation of the existing information is supportive of ranolazine as a tumor promoter. This situation did not arise until after the original decision on the ranolazine NDA. On the basis of the above material, I feel that in the interests of the public safety, the sponsor should generate data that will address the issue of possible tumor promotion. Ranolazine should therefore be approvable contingent upon the resolution of this safety concern.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-**526**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**12/6/2005**
PRODUCT:	ranolazine
INTENDED CLINICAL POPULATION:	angina patients
SPONSOR:	CV Therapeutics
DOCUMENTS REVIEWED:	**Vol.**
REVIEW DIVISION:	**Division of Cardio-Renal Drug Products(HFD110)**
PHARM/TOX REVIEWER:	**Elizabeth Hausner, D.V.M.**
PHARM/TOX SUPERVISOR:	**Al DeFelice, Ph.D.**
DIVISION DIRECTOR:	**Norman Stockbridge, M.D., Ph.D.**
PROJECT MANAGER:	**Meg Pease-Fye**

Date of review submission to Division File System (DFS):

TABLE OF CONTENTS

Appears This Way
On Original

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: Based upon the most recent information, including the opinions of the Pharmacology/Toxicology Coordinating Committee and consultation from the National Toxicology Program, this reviewer recommends that ranolazine be approvable contingent upon the sponsor satisfactorily addressing the issue of cancer promotion.

B. Recommendation for nonclinical studies: Studies need to be performed that will address the issues raised by the 2year rodent carcinogenicity studies and the APC+/-min mouse study.

C. Recommendations on labeling: Should ranolazine be approved without resolution of this matter, a black box warning should be included to let potential users know of the possibility of promotion of pre-cancerous lesions or existing cancerous lesions.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings: In July 2004, the Division became aware of a publication in which ranolazine was tested in a murine model of cancer promotion. In this publication (Suckow et. al., Cancer Letters 209 (2004)165-169), ranolazine was administered for 30 days to male $APC^{(min/+)}$ mice. When compared to control animals, the ranolazine-treated animals showed a dose-related trend to more tumors. The tumors also tended to be larger and show more of the characteristics associated with malignancy than did the tumors in the control mice.

B. Nonclinical safety issues relevant to clinical use. While this animal model is not typically asked of sponsors, the fact remains that the study was conducted and a safety issue raised. Both an internal consult (with the Pharmacology Toxicology Coordinating Committee) and an external consult (with the national Toxicology Program) produced the same comments: the results cannot be dismissed out of hand but warrant further investigation.

C. The sponsor did not inform us in a timely manner of the findings. Rather they waited approximately 1.5 years after receiving a pre-publication copy of the manuscript. The Division asked the company to address the issues raised by the mouse study. The Sponsor's response and the Pharmacology Toxicology Coordinating Committee (PTCC)'s subsequent discussion of the response was the subject of a previous review for this NDA (Listing in DFS: "APC mice" N 021526 N 000 BP 15-Aug-2001). To briefly recapitulate, the subject of the APC mouse model in general and the specific study were presented to the PTCC. The PTCC acknowledged that sponsors are not usually asked to

conduct studies in this model. However, the results of the study could not be ignored and the sponsor's written dismissal of both the study and the model was found by the PTCC to be an inadequate investigation of the safety concerns. One of the recommendations made by the PTCC was to gather more information from the scientific community, in particular, from the National Toxicology Program (NTP).

A teleconference was held Friday December 2, 2005 in which the outside participants were John Edgar French(NTP), Nicholas Paoni (senior author of the Cancer Letters paper) and Mark Suckow (first author of the Cancer Letters paper). The details of that teleconference can be found in DFS authored by Meg Pease-Fye, the project manager for this NDA. One of the points of discussion was what might appear in the two-year rodent studies if ranolazine actually is a tumor promoter. The two main points were that one would expect to see decreased survivability and possibly an increase in some background tumors, but with the caveat that it is possible to miss tumor promoters in the standard 2-year studies. After the teleconference, Dr French from NTP sent the following email:

From: Jef French [french@niehs.nih.gov]
Sent: Friday, December 02, 2005 2:30 PM
To: Pease-Fye, Margaret
Cc: Hausner, Elizabeth A
Subject: conference call

Based upon my review and todays conference call I will write a brief analysis of the papers discussed and options that I conceive. For certain and at minimum, the B6-min/+ mouse studies should be repeated and a thorough report prepared. There are several data gaps in the Suckow et al. paper in Cancer Letters, but their hypothesis is legitimate. It is an interesting model and result (tumor promotion) that warrants further evaluation. I am reminded of the trials with the dietary intervention with carotenoids in the US and Finland that had to be stopped due to an increase in lung tumors in individuals (treated groups) who had a previous history of smoking and/or working in the shipyards (asbestos?). Promotion of existing preneoplasia is not impossible, so it all depends upon the associated risk and the treatment of the primary disease in question.

I expect to send my analysis by the end of next week.

jefrench

John E. French, Ph.D.
Groups Leader
Transgenic Carcinogenesis, LMT
NIEHS, NIH

P. O. Box 12233, MD F-05
Research Triangle Park, NC 27709
919-541-2569 Tel 919-541-1460 Fax
french@niehs.nih.gov

Dr French's summary statement and recommendations are attached as Appendix 1.

The sponsor was then asked to submit electronic copies of the original study reports for the 2-year mouse and rat studies. This reviewer found no further useful information in the mouse report. However, re-visiting the CDER statistician's report and my carcinogenicity summary (written in 2002 . Listing in DFS: I 043735 N 162 IT 25-Jul-2001), the following points were apparent and are reproduced here from the original review:

From the original 2002 Carcinogenicity review.

Comment: CDER statistical analysis indicated a trend toward decreased survival in both male mice and rats. Significant neoplastic findings are summarized in the table below.
Summary of tumor incidences

species	sex	Tumor type	Incidence per dose group				P value	
			C	LD	MD	HD	sponsor	FDA*
Rat	m	Thyroid, follicular adenoma	3/120	1/60	2/58	6/60	<0.001**	0.0027
rat	f	Adrenal, cortical adenoma	0/120	0/60	1/60	2/60	0.032[4]	0.0225
Rat	m	Testes, interstitial (b)	5/120	3/60	4/60	8/60	0.001[3]	0.0030
mouse	m	Testes, interstitial	5/100	1/50	2/50	5/49	0.019**	0.0276

*p value from Exact test, **one-tailed prevalence trend-test
[3] one-tailed combined trend p-value, [4] one-tailed exact trend test

The results from the female mice and rats are also reproduced below:

Reproduced from original 2002 carcinogenicity review

The sponsor reported significant findings of malignant sarcoma of subcutaneous tissue and benign pheochromocytoma of the adrenal gland, both tumor types in female rats. The p values do not reach significance per the FDA standard.

Summary of tumor incidences

species	sex	Tumor type	Incidence per dose group				P value	
			C	LD	MD	HD	sponsor	FDA*
Rat	F	Adrenal pheochromocytoma	1/120	1/60	1/60	3/60	0.013**	0.0549

Rat	f	Malignant sarcoma,SC	0/120	0/60	0/60	2/60	0.040[4]	0.0380
rat	m	Malignant histiocytic sarcoma	2/120	1/60	1/60	3/60	0.037[5]	0.059
*p-value from exact test, [4] one-tailed exact trend test,[5] one-tailed time to tumor trend p-value								

The rat study was stopped at 90 weeks due to decreased survival. The statement from the original report is shown below.

1.3.1 Treatment Deviation

It was intended to dose the animals for two years (104 weeks) however because of poor survival, especially in the females, the study was terminated at 21 months. The post mortem examinations commenced after 89 weeks of dosing was complete and lasted for approximately 3 weeks.

The numbers of animals surviving until the terminal sacrifice is tabulated below:

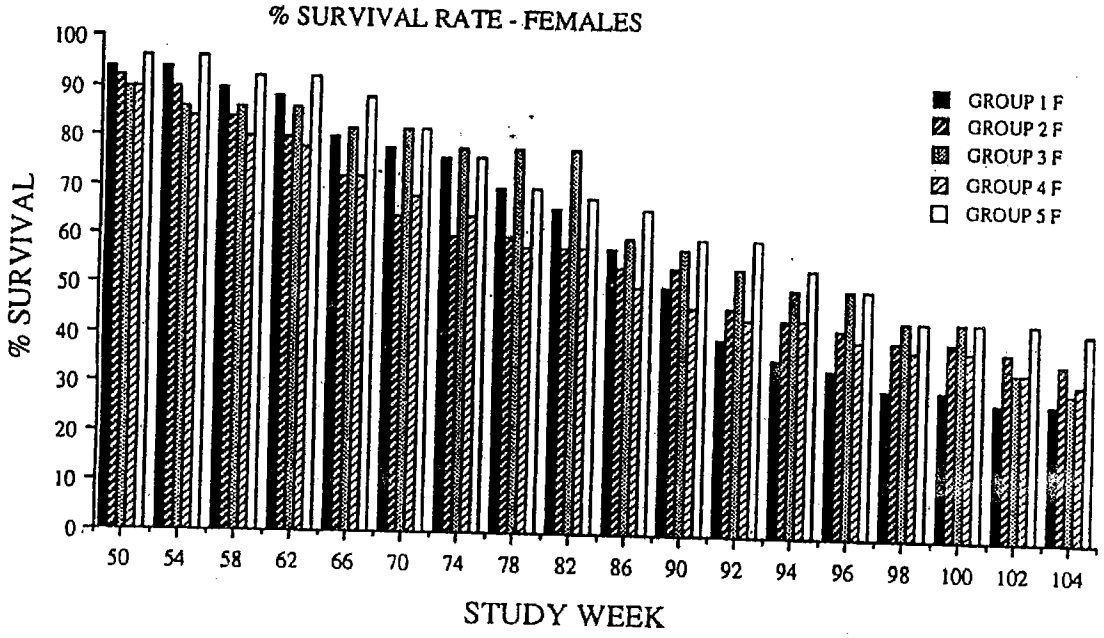
Group	Males	Females
1	34	27
2	27	22
3	37	29
4	28[1]	17
5	24	18[2]

[1] Excludes animal 4089 which was found dead during week 90

[2] Excludes animals 5096 and 5112 which died under anaesthesia during the terminal bleed

The female mice might also have shown decreased survival except that the control groups showed relatively lower survival from the beginning of the study.

Appears This Way
On Original



In addition, the multiples of human exposure were low:

Relative human exposure as reproduced from the original 2002 Carcinogenicity report

Relative human exposure: Based on AUC comparison, total exposure at the highest dose tested in rats was 0.7-4X the human plasma exposure. The highest mouse dose tested produced ≤ 0.25X the human therapeutic exposure based on AUC.

Two separate but identically treated control groups were used in both rodent studies. The disparity in tumor incidence in the control groups led the Executive CAC to consider the study findings as non-significant.

To summarize, the 2-year rodent carcinogenicity studies were not perfectly clean. Both decreased survival and increased incidences of common tumor types were in fact seen as indicated in the recent teleconference with NTP.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Although the APC(min/+) mouse model is not usually asked of sponsors in a new drug application, a study in this model was conducted using ranolazine. Both the Pharmacology/Toxicology Coordinating Committee and an outside consultant from the National Toxicology Program have given their opinions that the safety issue raised cannot be dismissed out of hand and that further laboratory investigation is warranted. The results from the required 2-year rodent studies can also be interpreted to suggest further support for tumor promotion. It is inconceivable that a responsible company, concerned for the safety of potential users of their product would not investigate this safety issue. The fact that CV Therapeutics

1) did not inform us of the results of the study in a timely fashion is at best irresponsible.
2) In 1.5 years has done nothing to investigate this safety question is deeply disturbing.

Unresolved toxicology issues (if any): Is ranolazine a tumor promoter?

Recommendations: This reviewer recommends that the drug be approvable based upon the investigation and resolution of this safety issue.

Suggested labeling: Should this drug be approved without resolution of this issue, ranolazine should carry a black box warning to the effect that use of this product may accelerate the growth and transformation of precancerous lesions and existing cancerous lesions.

Other points for the labeling include:

Description: Should be changed to read "The mechanism of pharmacologic action of ranolazine is not certain."

Clinical Pharmacology, Mechanism of action: Should be changed to read "The mechanism of action is[ ] The next 3 paragraphs should be removed.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

Appendix I Dr French's Summary Statement

Ranolazine: Review and Analysis of *Suckow MA, Gutierrez LS, Risatti CA, Wolter WR, Taylor RE, Pollard M, Navari RM, Castellino FJ, Paoni NF. The anti-ischemia agent ranolazine promotes the development of intestinal tumors in APC (Min/+) mice. Cancer Lett. 209(: 165-9, 2004*

John E. French, Ph.D., Laboratory of Molecular Toxicology, DIR, NIEHS, RTP, NC

The authors of this research article describe their studies on intestinal tumor promotion in C57BL/6-*Apc*^{MinMlcr}/+ mice by ranolazine, an experimental anti-angina agent. The authors report that ranolazine promotes hypoxic adenomas and carcinomas under the experimental conditions employed. Although the hypothesis (see below) is legitimate, the research result presented may be flawed and, thus, may only represent equivocal evidence of the tumor promoting properties of the test agent. Nonetheless, individuals with inherited germ line or sporadic mutations in tumor suppressor genes and/or activating mutations in proto-oncogenes may develop pre-neoplastic lesions that may be promoted and transformed to the malignant phenotype when under increased clonal selection pressure based on caloric intensity, diet, medications, and/or other environmental or occupational exposures. Further studies are warranted to determine the potential risk of ranolazine to promote pre-existing lesions that may give rise to a cancer with decreased latency and early onset of morbidity.

Review Summary

Test agent: Ranolazine dihydrochloride (no CAS No available for the dichloride) in PBS, pH 5.8 (0, 10, or 30 mg/kg BW ip, 2×/day.

Ranolazine (CAS No. 95635-55-5): 1-[3-(2methoxyphenoxy)-2-hydroxypropyl]-4-{2,6-dimethylphenyl)aminocarbonylmethyl]piperazine (Kluge et al. US Patent 4,567,264; 1986) used was custom synthesized and purified.

Hypothesis: Sporadically arising intestinal epithelia derived preneoplastic lesions in the C57BL/6-*Apc*^{min}/+ mouse[1] heterozygous for the mutated and wildtype allele of the mouse homolog of the adenomatous polyposis coli gene are hypoxic[2] and exposure to ranolazine enhances energy metabolism conversion from fatty acids to glucose that provides a clonal selection advantage, thus promoting tumor growth and conversion to malignancy.

Animal Model: C57BL/6J-*Apc*^{MinMlcr}/J heterozygous (for mutant and wild type allele), i.e. haploinsufficient for *Apc* protein, and C57BL/6 male mice (control) were 40 days of age at the start of treatment. This is a well-described model that is accepted as a valuable tool for experimental research on molecular genetics of cancer and chemoprevention. There are at least 6 mouse gene knockouts of the *Apc* locus that result in truncation of *Apc* protein carried on different genetic backgrounds. A number of these lines show a slightly different phenotype with differences in the distribution of intestinal and colonic tumors observed, but the number of tumors and spectrum of malignancies are similar. *Apc* is required for proper beta-catenin regulation in the *Wnt* signaling pathway and

This review is based on my personal opinions and expertise, and does not and should not be construed or interpreted as NIH opinion or policy. JEFrench

control of epithelial cell proliferation. Mechanism of action is similar and the phenotype of loss of function is similar between mouse and human.

Diet: undefined standard rodent diet (specific diet and manufacturer and the amount of fat and protein source along with phytoestrogen content must be specified)

Exposure Period: 30 days (mice 70 days of age at the termination of exposure)

Observations Reported: B6-*Apc*^{min}/+ mice: 1) the number of intestinal tract tumors per animal increased in a dose related manner and the number of tumors in the 30 mg/kg BW was significantly different ($P<0.01$) from control. 2) The number, degree of dysplasia and malignancy increased with dose in the B6-*Apc*^{min}/+ mice. However, there was no difference in average tumor diameter between the controls and the two-ranolazine dose groups.

Ancillary data showing the up-regulation (5×) of the *Hif1a* mRNA supports the potential for hypoxia in the tumors in question (see GES422 http://www.ncbi.nlm.nih.gov/projects/geo/query/acc.cgi) as described in Paoni et al. (2003). Pooling cells captured by laser microdissection from 6 different wildtype mice or 5 different *Apc*$^{Min/+}$ mice to form biological replicate samples for cRNA preparation (pooled) for hybridization to the Affymetrix array does not allow individual variability to be ascertained for controlling other variables. This procedure introduces uncertainty in the results.

Critical Data Absent:

1) Quality control and quality assurance specifications for the test agent were not described.
2) Randomization procedure was not described.
3) Dose selection was inadequately described (study references were for rats)
4) Animal housing conditions and specifications were not described.
5) Diet specifications and manufacturer were not stated.
6) Body weights at the beginning, intermediate, and at termination of the study were not presented.
7) Clinical observations (daily) were not evaluated.
8) Method of statistical analysis for tumor multiplicity was not described.
9) The number of animals with tumor or a statement that all mice were tumor bearing was not presented.
10) No microscopic histopathology was presented in detail.
11) No evidence that the tumors showed differences in either cell proliferation or rates of apoptosis, or vascularization was presented.

Factors other than ranolazine treatment that may have affected the results

1) Stress (housing conditions)
2) Dietary fat
3) Dietary phytoestrogens (e.g. genistein)

This review is based on my personal opinions and expertise, and does not and should not be construed or interpreted as NIH opinion or policy. JEFrench

4) Unknown dietary contaminants

Data that might strengthen the evidence that ranolazine promotes tumorigenesis through hypoxia and conversion of energy metabolism from fatty acids to glucose include the following parameters, but not necessarily limited to these parameters:

- *Hif1a* immunohistochemistry of adenomas and carcinomas of sporadically arising with and without ranolazine exposure to demonstrate cell specificity
- Evidence of conversion from FA to glucose (gycolysis) metabolism by ranolazine by enzyme up regulation and substrate utilization
- Random selection of untreated and ranolazine treated Min/+ mice at selected intervals to demonstrate latency, number of tumor bearing mice in sample and tumor multiplicity
- Biomarkers of cell proliferation (BrdU, PCNA, Ki67, etc.), apoptosis (Bax, Bcl2, ApoTag Plus, i.e. TUNEL, etc.) associated with clonal growth advantage in cultured preneoplastic cells and in situ tumors
- Expression array or quantitative PCR analysis of laser micro-dissected from ranolazine treated adenomas and/or carcinomas or cell culture systems
- Intracellular pH and pO_2 changes in cultured pre-neoplastic cells treated with and without ranolazine at appropriate concentrations and test any transformed clones by transplantation to nude mice

Recommendation:

1) Repeat the ranolazine promotion studies in the C57BL/6J-*Apc*^{MinMlcr}/J[1] inbred heterozygous (for mutant and wild type allele model with additional parameters as described above.
2) Use B6.129-*Pten*+/-^{tm1Ppp}*p27Kip1*-/-^{tm2Ako} mouse[3,4] prostate model to test for short-term (30 day) promotion in another pre-neoplastic tissue model. The allelotype of this mouse model is undefined (unknown backcross generation, but the line may not be inbred).
3) Use SD/Jcl-TgN^{Hras128Ncc} rat model[5,6] for DMBA initiated mammary tumorigenesis to test ranolazine promotion in another short-term model in an appropriate species. This line may also be available on the inbred F344 rat background.

Basis: Each of the proposed models has demonstrated rapid induction of preneoplasia and neoplasia that is reasonably tissue specific based upon genetic susceptibility and/or tissue specificity of an organotropic carcinogen and, thus, should allow testing of promotion of preneoplastic lesions in vivo by ranolazine.

This review is based on my personal opinions and expertise, and does not and should not be construed or interpreted as NIH opinion or policy. JEFrench

References:

[1] Moser AR, Pitot HC, Dove WF. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science. 247:322-4, 1990.

[2] Paoni NF, Feldman MW, Gutierrez LS, Ploplis VA, Castellino FJ. Transcriptional profiling of the transition from normal intestinal epithelia to adenomas and carcinomas in the $APC^{Min}/+$ mouse. Physiol Genomics. 15(3): 228-35, 2003. (GES422; http://www.ncbi.nlm.nih.gov/projects/geo/query/acc.cgi)

[3] Di Cristofano A, De Acetis M, Koff A, Cordon-Cardo C, Pandolfi PP. Pten and p27KIP1 cooperate in prostate cancer tumor suppression in the mouse. Nat Genet. 27:222-4, 2001.

[4] Kiyokawa H, Kineman RD, Manova-Todorova KO, Soares VC, Hoffman ES, Ono M, Khanam D, Hayday AC, Frohman LA, Koff A. Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27(Kip1). Cell. 85:721-32, 1996.

[5] Asamoto M, Ochiya T, Toriyama-Baba H, Ota T, Sekiya T, Terada M, Transgenic rats carrying human c-Ha-ras proto-oncogenes are highly susceptible to N-methyl-N-nitrosourea mammary carcinogenesis. Carcinogenesis. 243-9, 2000.

[6] Tsuda H, Fukamachi K, Ohshima Y, Ueda S, Matsuoka Y, Hamaguchi T, Ohnishi T, Takasuka N, Naito A. High susceptibility of human c-Ha-ras proto-oncogene transgenic rats to carcinogenesis: a cancer-prone animal model. Cancer Sci. 96:309-16, 2005.

Appears This Way
On Original

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Elizabeth Hausner
12/15/2005 07:55:40 AM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
12/15/2005 02:05:10 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-526
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**8/15/05**
PRODUCT:	ranolazine
INTENDED CLINICAL POPULATION:	anigina patients
SPONSOR:	CV Therapeutics
DOCUMENTS REVIEWED:	**Vol. 4,5 and 6 out of 80**
REVIEW DIVISION:	**Division of Cardio-Renal Drug Products (HFD-110)**
PHARM/TOX REVIEWER:	**E. Hausner, D.V.M.**
PHARM/TOX SUPERVISOR:	**A. DeFelice, Ph.D.**
DIVISION DIRECTOR:	**N. Stockbridge, M.D., Ph.D.**
PROJECT MANAGER:	**M. Pease-Fye**

Date of review submission to Division File System (DFS)

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

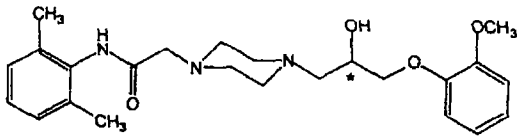
NDA number: 21-526
Review number: 1
Sequence number/date/type of submission: 000/July 26, 2005/AZ
Information to sponsor: Yes ( ) No ( )
Sponsor and/or agent: CV Therapeutics
Manufacturer for drug substance:

Reviewer name: Elizabeth Hausner, D.V.M.
Division name: Division of Cardio-Renal Drug Products
HFD #: 110
Review completion date:

Drug:

Trade name: Ranexa
Generic name: ranolazine
CAS registry number:
Molecular formula/molecular weight: $C_{24}H_{33}N_3O_4$/427.54

1-Piperazineacetamide, N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy) propyl]-, (±)-



* chiral center

Relevant INDs/NDAs/DMFs: IND 43,735

Drug class: Anti-anginal

Intended clinical population: people who have not achieved an adequate response with other anti-anginal drugs

Clinical formulation: extended release tablets with carnauba wax, hypromellose, magnesium stearate, methacrylic acid copolymer (Type C), microcrystalline cellulose, polyethylene glycol, polysorbate 80, sodium hydroxide, titanium dioxide and FD&C Yellow #6 Lake

Route of administration: oral

The material in this amendment was submitted in response to the Division's letter of July 20, 2005 asking for a response to the publication of the article "The anti-ischemia agent ranolazine promotes the development of intestinal tumors in $APC^{(min/+)}$ mice" M.A.Suckow, L.S. Gutieerrez, C.A. Risatti, W.R. Wolter, R.E. Taylor, M. Pollard, R.M. Navari, F.J. Castellino, N.F.Paoni. Cancer Letters 209(2004): 165-169.

The history of this request will be summarized here.

Thursday July 1, 2004
John Koerner, Ph.D. saw this article in a literature search conducted for other purposes. Dr Koerner asked this reviewer if the sponsor had brought this article to my attention or included a reference to it in any submission. The answer was no. We considered the possibility that CV Therapeutics was unaware of the publication and to that end called several of the authors of the publication.

Telephone conversation with Mark A. Suckow (7/1/2004): Dr Suckow informed us that the sponsor did not know that the work was being done. However, July 29, 2003 the sponsor was presented with a copy of the manuscript, prior to publication. There was some contact before that time as CVT suggested that the investigators use the dichloride salt form of ranolazine. The material that the authors synthesized was offered to CV Therapeutics for analysis. CV Therapeutics declined that offer but saw the results of the chemical analysis conducted by the sponsors and agreed that the material was acceptable. The purity was reported as [ ]. A portion of the synthesized material was also sent to [ ] for in vitro pharmacology testing. At the time of this conversation the authors were still in possession of [ ] of material.

Telephone conversation with Nicholas Paoni (7/1/2004 and thereafter): Dr Paoni had tested ranolazine in this model based upon the proposed mechanism of modulation of fatty acid oxidation. Tumors have glycolytic metabolism and Dr Paoni hypothesized that inhibition of fatty acid oxidation would make the tumors more sensitive to chemotherapy. In the mouse model used, ranolazine was associated with dysplastic adenomas and invasive carcinomas. The author did not say that ranolazine was carcinogenic but that it stimulated existing tumors and had no effect in the wild type mice. Dr Paoni said that CV Therapeutics acknowledged the receipt of the manuscript. The author also offered CVT a patent opportunity for a new use (adjunct to cancer therapy).

Email to Joe Contrera: It was then decided to obtain information from a member of the Executive Carcinogenicity Assessment Committee. The emails contacting Joe Contrera, Ph.D. and his reply are shown below.

-----Original Message-----
From: Hausner, Elizabeth A
Sent: Thursday, July 01, 2004 3:27 PM
To: Contrera, Joseph F
Cc: Koerner, John E
Subject: FW:

Hi Joe,

we have a situation where there was a published report for a drug in the clinic using APC(min/+) mice. The citation is Cancer letters 209 (2004) 165-169. "The anti-ischemia agent ranolazine promotes the development of intestinal tumors in APC (min/+) mice." The conventional carcinogenicity studies submitted with the IND went through the CAC review and there was determined to be no evidence of carcinogenic potential.

We have several questions.
1. what experience does the CAC have with this model and how much weight can it be given?
2. Has this kind of situation happened before where a non-sponsor-generated published report appears about carcinogenicity in a drug in the NDA stage ?
Any suggestions?

Thanks
Elizabeth

From: Contrera, Joseph F
Sent: Friday, July 02, 2004 11:10 AM
To: Hausner, Elizabeth A
Cc: Koerner, John E
Subject: RE:
Elizabeth

This is an interesting situation. The APC mouse is a tumor promotion model for a specific type of human cancer. Transgenic models are tumor induction models and ICH policy indicates that we would consider new models that are adequately supported by sufficient scientific data and experience. Tumor promotion models have never been accepted by the Center or used as the basis of regulatory action. Tumor promotion involves entirely different scientific and policy issues. There are a few examples in the past where drugs were evaluated in other rodent tumor promotion models and they have caused regulatory problems. In addition, primary issues for all new models are sensitivity, specificity and validation. Has the model been applied to many diverse compounds? Is the model consistent,can these animals be produced in large enough numbers and are they genetically uniform enough for regulatory use. Can the results be duplicated in other laboratories. At this point it is hard to judge the significance of the findings or to support regulatory action without considerably more research and experience with the model and the acceptance of tumor promotion models for regulatory use by the agency, scientific community and the pharmaceutical industry.

I hope this helps.

Regards,

Joe

2 Page(s) Withheld

✓ § 552(b)(4) Trade Secret / Confidential

___ § 552(b)(5) Deliberative Process

___ § 552(b)(4) Draft Labeling

Dr Paoni also provided copies of the paper correspondence with CV Therapeutics. These are reproduced below.

DEPARTMENT OF
CHEMISTRY & BIOCHEMISTRY

PHONE 574/631-7068
FAX 574/631-6652

UNIVERSITY OF NOTRE DAME
251 NIEUWLAND SCIENCE HALL
NOTRE DAME, INDIANA 46556-5670

July 29, 2003

Brent K. Blackburn, Ph.D.
Senior Vice President
Drug Discovery and Preclinical Development
CV Therapeutics, Inc.
3172 Porter Drive
Palo Alto, CA 94304

Dear Dr. Blackburn: Brent

My colleagues and I have been working for the past several months with ranolazine that was synthesized at the University of Notre Dame. We believe that we have uncovered a possible safety issue regarding the use of ranolazine in patients that have a history of cancer. Our findings are detailed in the enclosed manuscript entitled "The anti-ischemia agent ranolazine promotes the development of intestinal tumors in $APC^{(Min/+)}$ mice". As a professional courtesy we are sending you the manuscript to review and comment on for a week prior to our submitting it for publication. We are also willing to provide a sample of the ranolazine used in the study for you to examine for identity and purity. Please feel free to phone or e-mail me if you have any questions regarding our study.

Sincerely,
Nick

Nicholas F. Paoni, Ph.D.
Associate Director, W.M. Keck Center for Transgene Research
Research Professor, Department of Chemistry and Biochemistry
Phone: 574.631.0199
e-mail: npaoni@nd.edu

cc: Andrew A. Wolff, M.D., F.A.C.C

Dr Blackburn's response is shown below.

CVT | CV Therapeutics

August 11, 2003

Nicholas F. Paoni, Ph.D.
251 Neiuwland Science Hall
University of Notre Dame
Notre Dame, Indiana 46556

Dear Dr. Paoni:

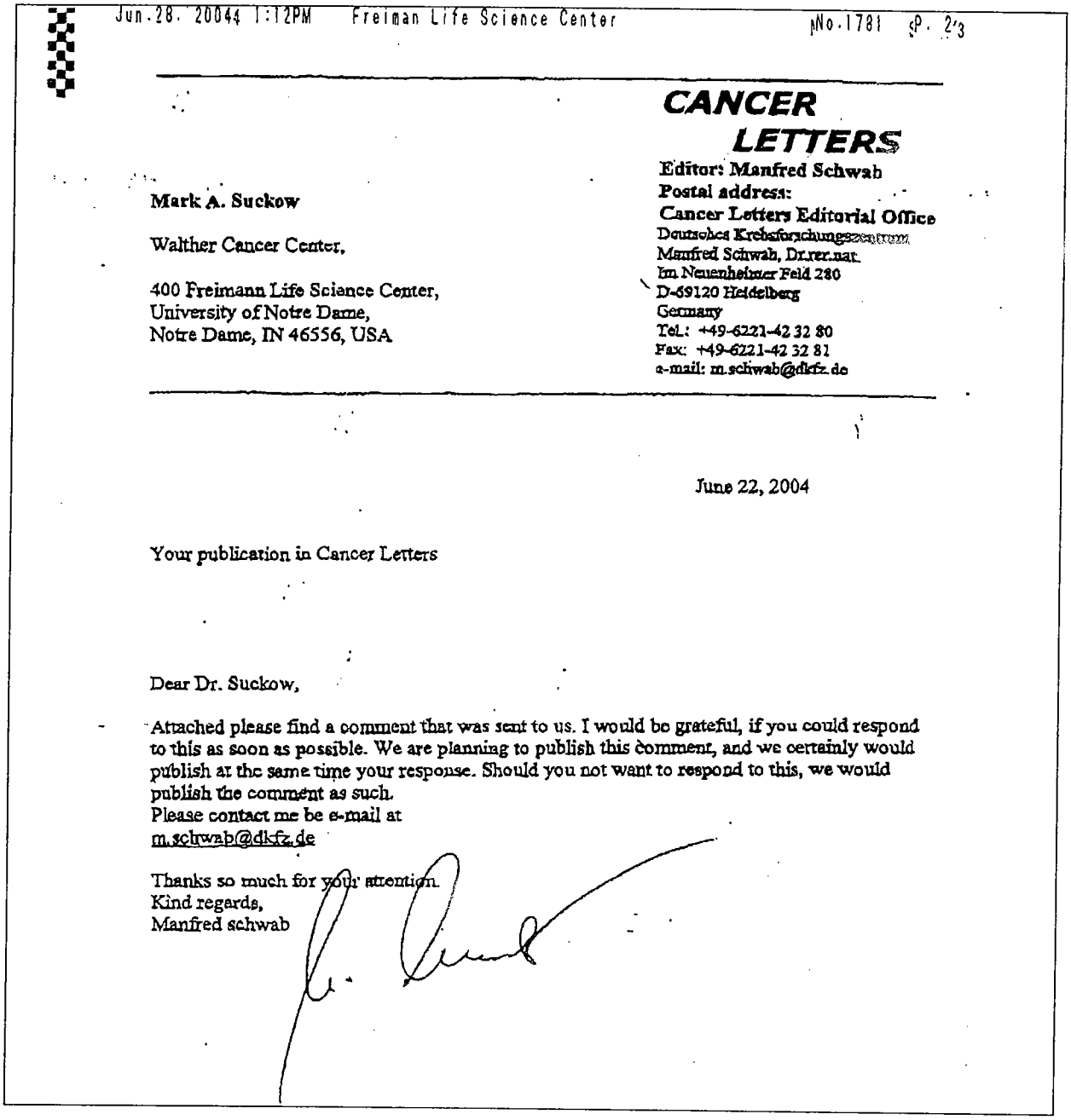
Thank you for sharing your draft manuscript on the study you have conducted in $APC^{(min/+)}$ mice with your own ranolazine product. We are always interested in reviewing study results for compounds related to Ranexa, and appreciate your having shared the manuscript with us.

Sincerely,

Brent Blackburn
Senior Vice President
Drug Discovery & Preclinical Development

CV Therapeutics, Inc. | 3172 Porter Drive Palo Alto, CA 94304 | Tel 650.384.8500 Fax 650.858.0390 | www.cvt.com

Another dimension was added to this story when Stuart Aaronson, M.D., a member of CV Therapeutics science advisory board, wrote to Cancer Letters regarding the original article. The correspondence is shown below.



Jun.28. 2004 1:12PM Freiman Life Science Center No.1781 P. 2/3

CANCER LETTERS

Editor: Manfred Schwab
Postal address:
Cancer Letters Editorial Office
Deutsches Krebsforschungszentrum
Manfred Schwab, Dr.rer.nat.
Im Neuenheimer Feld 280
D-69120 Heidelberg
Germany
Tel.: +49-6221-42 32 80
Fax: +49-6221-42 32 81
e-mail: m.schwab@dkfz.de

Mark A. Suckow

Walther Cancer Center,

400 Freimann Life Science Center,
University of Notre Dame,
Notre Dame, IN 46556, USA

June 22, 2004

Your publication in Cancer Letters

Dear Dr. Suckow,

Attached please find a comment that was sent to us. I would be grateful, if you could respond to this as soon as possible. We are planning to publish this comment, and we certainly would publish at the same time your response. Should you not want to respond to this, we would publish the comment as such.
Please contact me be e-mail at
m.schwab@dkfz.de

Thanks so much for your attention.
Kind regards,
Manfred schwab

MOUNT SINAI
SCHOOL OF
MEDICINE

Stuart A. Aaronson, M.D.
Jane B. and Jack R. Aron
Professor and Director
Dorald H. Ruttenberg Cancer Center

One Gustave L. Levy Place, Box 1130
New York, NY 10029-6574

Phone 212.659.5400
Facsimile 212.987.2240
Stuart.aaronson@mssm.edu

arch
pidemiology, Experimental Therapeutics

RE: M.A. Suckow et al. "The Anti-ischemic Agent Ranolazine Promotes the Development of Intestinal Tumors in APC$^{(Min/+)}$ Mice."

[redacted]

The manuscript by Suckow et al (1) suggests that ranolazine may be associated with increased development of intestinal carcinoma in the APC$^{Min/+}$ mouse model. Unfortunately, there are a number of methodological issues that raise serious concerns about the results presented and conclusions drawn. First, the model used by the authors performs differently in their laboratory compared to the published literature. It is well documented that the APC$^{Min/+}$ mouse model produces adenomas and only rarely invasive adenocarcinomas. (2,3). This is in striking contrast to the findings of Suckow et al indicating that invasive carcinomas occurred in around 15% of control animals of this same strain (1). In fact, other studies have shown that even with genetic manipulation causing loss of function of a major tumor suppressor gene, p53, only a small fraction of resulting APC$^{Min/+}$ mice develop invasive adenocarcinomas (3).

The authors' APC$^{Min/+}$ mouse model also seems to differ from the literature concerning frequency of tumors. For example, Suckow et al (1) report a tumor incidence (combined adenoma and carcinoma) in their APC$^{Min/+}$ controls at least a 2-3 fold lower than the incidence of adenomas in this same strain reported by others (2,3,4) or reported elsewhere by these same authors (5). Thus, Suckow et al report both qualitative and quantitative differences from other studies utilizing the same mouse model.

The mechanism proposed by Suckow et al for the effect of ranolazine in promoting tumor growth under oxygen-poor conditions is also not consistent with the results presented. The APC$^{Min/+}$ model allows analysis of early adenomas under conditions where the tumor is small enough to require a dissecting microscope for detection. In this situation there is

unlikely to be compromise in the vascular supply. In fact, Suckow et al report more tumors in "ranolazine"-treated mice but no increase in the size of these tumors, contrary to what would be expected if ranolazine acted to increase tumor growth by their postulated mechanism.

Finally, one must question the quality and purity of the "ranolazine" synthesized by the authors. It cannot be ruled out that an uncontrolled impurity in the author-synthesized compound may also have affected their results.

It should be noted that chronic, well-controlled toxicity studies of ranolazine have been conducted in rats and dogs, and carcinogenicity studies have been conducted with ranolazine in rats and mice. None of the chronic toxicity or carcinogenicity studies has shown any pathologic changes suggestive of carcinogenic potential of ranolazine. In addition, a battery of mutagenicity studies has been conducted, and ranolazine has not shown any mutagenic potential. Indeed, contrary to the Suckow et al claims, these data provide strong support for the safe use of ranolazine in humans.

Sincerely,

Stuart Aaronson, MD,
Member, CV Therapeutics Scientific Advisory Board

References

1. Suckow MA, Gutierrez LS, Risatti CA, Wolter WR, Taylor RE, Pollard M, Navari RM, Castellino FJ, Paoni, FF. The anti-ischemia agent ranolazine promotes the development of intestinal tumors in APC (Min/+) mice, Cancer Letters 2004: in Press.
2. Boivin GP, Washington K, Yang K, Ward JM, Pretlow, TP, Bussell R, Besselsen DG, Godfrey VL, Doetschman T, Dove WF, Pitot, HC, Halberg RB, Itzkowitz SH, Groden J, Coffey RJ. Pathology of mouse models of intestinal cancer: Consensus report and recommendations. Gastroenterology 2003:124: 762-777.
3. Halberg RB, Katzung DS, Hoff PD, Moser AR, Cole CE, Lubet RA, Donehower LA, Jacoby RF, Dove WF. Tumorigenesis in the multiple intestinal neoplasia mouse: Redundancy of negative regulators and specificity of modifiers, PNAS 2000: 97: 3461-3466. mice killed at 90 days.
4. Takeda H, Sonoshita M, Oshima H, Sugihara K, Chulada PD, Langenbach R, Oshima M, Taketo MM. Cooperation of cyclooxygenase 1 and cyclooxygenase 2 in intestinal polyposis. Cancer Res. 2003: 53(16): 4872-7.
5. Gutierrez LS, Suckow M, Lawler J, Ploplis VA, Castellino FJ. Thrombospondin 1 – a regulator of adenoma growth and carcinoma progression in the APC Min/+ mouse model. Carcinogenesis 2003:24(2): 199-207.

our study 25 days.

The authors' response to Dr Aaronson's letter is shown below.

June 29, 2004

Manfred Schwab, Ph.D
Cancer Letters Editorial Office
Deutsches Kregsforschugszentrum
Im Neuenheimer Feld 280
D-69120 Heidelberg
Germany

Dear Dr. Schwab:

We wish to thank you for the opportunity to provide clarification to issues raised by Dr. Aaronson regarding our manuscript, "The Anti-Ischemic Agent Ranolazine Promotes the Development of Intestinal Tumors in $APC^{(Min/+)}$ Mice."

Dr. Aaronson correctly notes that we found fewer tumors in our control mice compared to data reported by other authors. It is important to note, however, that those studies examined mice of 90 days of age or older. One study cited by Aaronson (Takeda, *et al*) specifically used $APC^{Min/+}$ mice which were also COX-1 (-/-), and that report did not include data related to enumeration of tumors. In contrast, the mice in our study were sacrificed at 70 days of age, the earlier time point being used in order to better discern possible differences in the rates of tumor development. As with most scientific investigations, comparisons are most meaningful when made between age-matched groups.

With respect to the relatively large number of lesions classified as carcinomas in our study, it is notable that Aaronson does not question our criteria for tumor classification as stated in the paper. These criteria were used by a pathologist (L. Gutierrez) in a blind fashion to classify tumors. In any case, the total number of tumors was increased in mice dosed with ranolazine by approximately 70%, and the odds of that happening by chance were less than 1 in 100.

We believe the mechanism for promotion of tumor growth by ranolazine is wholly consistent with our results. We found a trend for increased tumor size with greater dosages of ranolazine and the largest tumor in the study, 3.74 mm, was found in the high dose ranolazine group (compared to 2.1 mm for the largest tumor in the control group). We further believe that the increased number of tumors in ranolazine-treated mice may reflect the promotion of growth of small tumors which might not otherwise be discernible. Though we do not have data demonstrating these tumors to be hypoxic,

Aaronson's assertion that they are likely to be normoxic due to uncompromised vascularization is likewise unsupported.

As for questioning the purity of the ranolazine used in the studies, the route used to synthesize ranolazine was essentially identical to the patent, and the results of the chemical analysis of the material were provided in the paper. The dose range studied was consistent with those used in animal models of angina and heart failure. While it is conceivable that a uniquely powerful contaminant might explain our observed results, the final compound was pure within consistent with guidelines set forth by the American Chemical Society. Intermediates in the synthesis were consistent with spectroscopic data in the patent.

Finally, readers are directed to Section 3.1 of Results, where we clearly indicate that no intestinal tumors were observed in wild-type mice undergoing the same ranolazine treatments as $APC^{(Min/+)}$ mice. The suggestion by Aaronson that we claim our data to indicate carcinogenic potential for ranolazine is errant. We instead explicitly state that our data support the development of pre-existing neoplastic lesions, a process distinct from carcinogenesis by most definitions. Furthermore, the toxicology described by Aaronson was performed in normal animals and does not address the potential to promote the development of pre-existing neoplastic lesions, which is the subject of this work.

In summary, we are confident in our data which show that ranolazine promotes the development of existing tumors in the $APC^{(Min/+)}$ mouse model.

Regards,

Mark A. Suckow
Linda Gutierrez
Christina Risatti,
William R. Wolter
Richard E. Taylor
Morris Pollard
Rudolph M. Navari
Francis J. Castellino
Nicholas F. Paoni

University of Notre Dame
Notre Dame, IN 46556

2 Page(s) Withheld

✓ § 552(b)(4) Trade Secret / Confidential

___ § 552(b)(5) Deliberative Process

___ § 552(b)(4) Draft Labeling

This reviewer re-examined the material that the sponsor had submitted to the NDA to that time and found no reference to this study. Meg Pease-Fye, the project manager subsequently did an exhaustive search of material submitted both to the NDA and to the IND, including correspondence. Again, no mention was made of this study in material submitted up to the summer of 2004.

Norman Stockbridge, M.D., Ph.D., Acting Division Director for the Cardio-Renal Division, consulted with Robert Temple, M.D. as well as the Division of Scientific Investigation and FDA's legal counsel. Details of those discussions can be obtained from Dr Stockbridge. Eventually, the letter of July 20, 2005 was sent to CV Therapeutics. The response was to be part of the complete response to the Approval Letter.

The Sponsor's cover letter for the response to the July20, 2005 letter from the Division, states that

> At the time the Suckow et. al. article was published, CVT had reviewed the publication and had concluded that the study provided no new information relevant to the safety of ranolazine. The citation for this article was submitted to the ranolazine IND Annual Progress Report (IND43,735; Serial No. 0248, dated 22 December 2004), as part of the summary of information from published reports of ranolazine.

A response to this:

1. The wording used in the above statement is somewhat ambiguous as to timing. The sponsor was provided with a copy of the manuscript prior to its submission for publication.
2. The sponsor waited 1.5 years before reporting this information to the FDA.
3. It is not for the sponsor to make the assessment of safety, but rather to provide the information to the FDA.

According to the Code of Federal Regulations, 312.32 (a) Definitions

> (B) Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity. Each notification shall be made as soon as possible and in no **event** later than 15 calendar days after the sponsor's initial receipt of the information. Each written notification may be submitted on FDA Form 3500A or in a narrative format (foreign events may be submitted either on an FDA Form 3500A or, if preferred, on a CIOMS I form; reports from animal or epidemiological studies shall be submitted in a narrative format) and shall bear prominent identification of its contents, i.e., ``IND Safety Report.'' Each written notification to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility
>
> [[Page 64]]
>
> for review of the IND. If FDA determines that additional data are needed, the agency may require further data to be submitted.

The Division should have been informed within 15 days of finding out the results of the study. They still have not reported the work done in athymic nude mice.

The body of the reply contains the following points to discredit the published report:
1.lack of validation of the APC(Min/+) model for the evaluation of the carcinogenic potential of a drug;2)issues pertaining to tumor burden and study conduct; 3)weakness of the metabolic hypothesis; 4) questions regarding the purity of the ranolazine synthesized by the authors and tested in the study. It is interesting to note that points 2,3 and 4 raised by the sponsor are the same points in the same order raised by Dr Aaronson in his letter to Cancer Letters.
Only two of the sponsor's comments will be addressed here.

3. Weakness of the metabolic hypothesis

The sponsor protests that the hypothesis of the paper was based upon one of the discarded hypotheses for the mechanism of action of ranolazine. That is, the proposal that ranolazine worked by partial inhibition of fatty acid oxidation. The sponsors say that

> In fact, recent experiments suggest that inhibition of the pathologically-enhanced late sodium current in ventricular muscle cells by ranolazine may be responsible for its anti-anginal and anti-ischemic effects.

The material for the late sodium current hypothesis has been reviewed and found to be unconvincing (see review of 7-26-2005 material). However, ranolazine has long been shown to have a multiplicity of biological effects as well as many poorly characterized metabolites. The precise mechanism of this potential promotion of carcinogenicity effect is arguably of less importance than the reproducibility of the event.

4. Questions regarding the purity of the ranolazine synthesized.

The sponsors could have requested a sample of material and analyzed it in response to this request for information. If the information provided to me verbally by one of the authors is accurate, then the sponsors have not addressed that 1)they were the ones who suggested the salt form of ranolazine to be used in the study ; 2)that they were given the opportunity to analyze the material prior to publication

The sponsors also have not shown that the data is not reproducible.
The sponsor does not raise issues with the sample sizes used or the use of only male mice, route of administration (intraperitoneal vs oral), or criteria for classification of tumors.

Summary

The Pharmacology/Toxicology Coordinating Committee (PTCC) was asked to give the Division their opinion on this study. The subject was discussed at the September 15, 2005 meeting.

To address this strictly on the basis of the science, the necessary background information the following material was submitted to the PTCC for consideration:

1. A copy of this document in draft form (Aug_15_05.doc)
2. A complete copy of the sponsor's response (CVT response to published report.doc)
3. A summary of the background of the drug (Overall_Background.doc)
4. A copy of the Cancer Letters publication "The anti-ischemia agent ranolazine promotes the development of intestinal tumors in $APC^{(min/+)}$ mice." M.A. Suckow, L.S. Gutierrez, C.A. Risatti, W.R. Wolter, R.E. Taylor, M. Pollard, R.M. Navari, F.J. Castellino, N.F. Paoni. Cancer Letters 209(2004)165-169.
5. A copy of "Studies of Neoplasia in the Min mouse." A.R. Shoemaker, K.A. Gould, C. Luongo, A.R. Moser, W.F. Dove. Biochimica et Biophysica Acta 1332(1997) F25-F48.
6. A copy of "APC^{Min}: A mouse model for intestinal and mammary tumorigenesis." A.R.Moser, K.A. Gould, M.K. McNeley, A.R. Shoemaker and W.F. Dove. European Journal of Cancer, Vol 31A, Nos7/8, pp1061-1064, 1995.
7. "Point: From animal models to prevention of colon cancer. Systematic review of chemoprevention in Min mice and choice of the model system." D.E. Corpet and F.Pierre. Cancer Epidemiology, Biomarkers&Prevention. Col. 12, 391-400, May 2003.
8. "Counterpoint: From animal models to prevention of colon cancer. Criteria for proceeding from preclinical studies and choice of models for prevention studies." W.R. Bruce. Cancer Epidemiology, Biomarkers&Prevention. Col. 12, 401-404, May 2003.

We sought the expertise of the Exec CAC in helping us to address the following questions:

1. Is there any reason to disregard results from this study?
2. What level of concern should we have about positive results in this study?
3. What work would be necessary to alleviate concern?
4. What should the regulatory action be?

The reviewer's comments and summary of the meeting are provided here.

Summary of discussion: This is a larger issue than just one drug. Another Division (Metabolic and Endocrine) has also seen positive results in this model for a particular class of drugs. The findings are occurring post-approval and the Division is uncertain at the moment what course of action to take. A representative of another Division raised the point that pharmaceutical companies will frequently synthesize a competitor's compound and use it as a comparator drug in their own non-clinical studies. Adverse effects in these studies are still required to be explained or investigated by the sponsor of the drug even though they did not generate the original results.

Other discussion involved the fact that the 2-year standard rodent studies had not produced noteworthy findings and shouldn't this provide some level of reassurance? It was reiterated in the group that:

1. The two year studies primarily find complete carcinogens. It is possible that a promoter will not be identified in the standard studies.

2. It is easy to miss a tumor in the intestinal tract depending upon the method of tissue handling and processing.

In general, the response provided by the sponsor was not perceived as adequate.

The answers to the questions were:

1. There is no reason to dismiss the findings in this study.
2. The level of concern for the general population is moderate. However, for people who have been treated for cancer or for FAP patients, the level of concern is higher. There was one member of the PTCC who had a low level of concern overall.
3. It was felt that the ideal situation would be for the study to be re-done, conducted properly. That is, using both sexes of animals, oral administration of the drug, adequate sample numbers and GLP procedures. There was some discussion as to the timing of when this be completed and if a Phase IV commitment was appropriate.
4. It was also felt that if action was to be taken in this matter, that the sponsor should be given the opportunity to present to the PTCC and state their own case.
5. The PTCC is advisory to the Divisions. The final assessment of risk/benefit remains with the individual Division.

As this model is also the subject of work by the National Toxicology Program, it was recommended to contact them for information and also a consult on this particular study

Appears This Way
On Original

2 Page(s) Withheld

✓ § 552(b)(4) Trade Secret / Confidential

___ § 552(b)(5) Deliberative Process

___ § 552(b)(4) Draft Labeling

Appendix II From IND 43735 Annual Report

2.6.2 Published Reports

The following are published articles of nonclinical studies with ranolazine from April 2003 through 19 October 2004.

1. Antzelevitch C, Belardinelli L, Wu L, et al. Electrophysiologic Properties of Ranolazine: A Novel Anti-Anginal Agent. J Cardiovasc Pharmacol Therapeut 2004;9 (Supplement 1):S65-S83.

2. Antzelevitch C, Belardinelli L, Zygmunt AC, et al. Electrophysiological Effects of Ranolazine, A Novel Antianginal Agent with Antiarrhythmic Properties. Circulation 2004;110:904-910.

3. Liu XD, Xie L, Liang Y, Li L, Lu T. Gender Difference in Ranolazine Pharmacokinetics in Rats. Eur J Drug Metab Pharmacokinet. 2003;28:119-123.

4. Schram G, Zhang L, Derakhchan K, Ehrlich JR, Belardinelli L, Nattel S. Ranolazine: Ion-Channel-Blocking Actions and In Vivo Electrophysiological Effects. Br J Pharmacol. 2004;142:1300-1308.

5. Suckow MA, Gutierrez LS, Risatti CA, et al. The Anti-Ischemia Agent Ranolazine Promotes the Development of Intestinal Tumors in APC(Min/+) Mice. Cancer Lett. 2004;209:165-169.

6. Song Y, Shryock JC, Wu L, Belardinelli L. Antagonism by Ranolazine of the Pro-Arrhythmic Effects of Increasing Late INa in Guinea Pig Ventricular Myocytes. J Cardiovasc Pharmacol. 2004 Aug;44(2):192-199.

7. Wu L, Shryock JC, Song Y, Li Y, Antzelevitch C, Belardinelli L. Antiarrhythmic Effects of Ranolazine in a Guinea Pig in Vitro Model of Long-QT Syndrome. J Pharmacol Exp Ther. 2004 Aug;310(2):599-605.

The following are published Abstracts of nonclinical studies with ranolazine from the reporting period of 20 October 2003 through 19 October 2004.

1. Song Y, Wu L, Shryock JC, Belardinelli L. Ranolazine attenuates increased variability of action potential duration and afterdepolarizations caused by augmentation of late sodium current. Journal of the American College of Cardiology, 2004.

2. Undrovinas AI, Undrovinas NA, Belardinelli L, Sabbah HN. Ranolazine Inhibits Late Sodium Current in Isolated Left Ventricular Myocytes of Dogs with Heart Failure. Journal of the American College of Cardiology, 2004.

3. Wu L., Santiku[illegible] M., Belardinelli L. Reversal by Ranolazine of Ventricular Tachycardias induced by Cisapride, DPI 201-106, and Moxifloxacin. Keystone, 2004.

4. Dhalla AK, Wang WQ, Belardinelli L. Effect of Ranolazine on Ischemia-Induced Ventricular Arrhythmias in Rats In Vivo. International Society for Heart Research, 2004.

Appears This Way
On Original

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Elizabeth Hausner
9/29/2005 08:12:04 AM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
9/30/2005 01:42:31 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-526
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**7/26/05**
PRODUCT:	ranolazine
INTENDED CLINICAL POPULATION:	anigina patients
SPONSOR:	CV Therapeutics
DOCUMENTS REVIEWED:	**Vol. 4,5 and 6 out of 80**
REVIEW DIVISION:	**Division of Cardio-Renal Drug Products (HFD-110)**
PHARM/TOX REVIEWER:	**E. Hausner, D.V.M.**
PHARM/TOX SUPERVISOR:	**A. DeFelice, Ph.D.**
DIVISION DIRECTOR:	**N. Stockbridge, M.D., Ph.D.**
PROJECT MANAGER:	**M. Pease-Fye**

Date of review submission to Division File System (DFS):

TABLE OF CONTENTS

Appears This Way
On Original

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: see original NDA review

B. Recommendation for nonclinical studies: see original NDA review

C. Recommendations on labeling:

Description: Should be changed to read "[illegible]

Clinical Pharmacology, Mechanism of action: Should be changed to read "The mechanism of action [illegible] The next 3 paragraphs should be removed.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings: the studies presented do not present convincing evidence of a new mechanism of action.

B. Pharmacologic activity: uncertain, probably a beta-adrenergic antagonist

C. Nonclinical safety issues relevant to clinical use: see previous NDA reviews

[Please limit to 1-3 pages]

Appears This Way
On Original

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

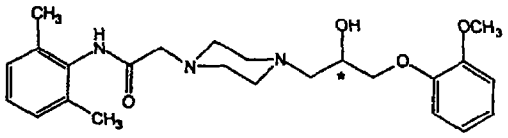
NDA number: 21-526
Review number: 1
Sequence number/date/type of submission: 000/July 26, 2005/AZ
Information to sponsor: Yes ( ) No ( )
Sponsor and/or agent: CV Therapeutics
Manufacturer for drug substance:

Reviewer name: Elizabeth Hausner, D.V.M.
Division name: Division of Cardio-Renal Drug Products
HFD #: 110
Review completion date:

Drug:

Trade name: Ranexa
Generic name: ranolazine
CAS registry number:
Molecular formula/molecular weight: $C_{24}H_{33}N_3O_4$/427.54

1-Piperazineacetamide, N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy) propyl]-, (±)-



* chiral center

Relevant INDs/NDAs/DMFs: IND 43,735

Drug class: Anti-anginal

Intended clinical population: people who have not achieved an adequate response with other anti-anginal drugs

Clinical formulation: extended release tablets with carnauba wax, hypromellose, magnesium stearate, methacrylic acid copolymer (Type C), microcrystalline cellulose, polyethylene glycol, polysorbate 80, sodium hydroxide, titanium dioxide and FD&C Yellow #6 Lake

Route of administration: oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

[For (b)(2) applications:

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of 21-526 are owned by CV Therapeutics or are data for which CV Therapeutics has obtained a written right of reference. Any information or data necessary for approval of 21-526 that CV Therapeutics does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that CV Therapeutics does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of 21-526.

Studies reviewed within this submission:

CVT303.067-P: Effects of ranolazine on systemic hemodynamics and coronary circulation in conscious dogs

CVT303.080-P: Effects of ranolazine on sustained current carried by disease-associated mutant sodium channels.

CVT303.087-P: Ranolazine: In vitro effect on hERG current (IKr) expressed in human embryonic kidney (HEK) cells.

CVT303.019-T: Mammalian Erythrocyte Micronucleus Test: ranolazine

Studies <u>not</u> reviewed within this submission:

CVT303.040-N: Binding of ranolazine metabolites CVT-2514, CVT-4786 and CVT-2537 to human plasma in vitro

CVT303.045-N: Binding of a ranolazine metabolite CVT2738 to human plasma in vitro

CVT303.060-P: A study of the effects of ranolazine and E-4031 on T-wave morphology in guinea pig isolated Langendorff-perfused hearts and effects of ranolazine on T-wave morphology in a guinea pig isolated heart model of long QT3 (ATX-II).

CVT303.066-P: Effects of ranolazine, its R and S enantiomers, and eleven metabolites on rat left atrial contractility.

CVT303.073-P: Effect of ranolazine on ischemia-induced ventricular arrhythmias in rats in vivo

CVT303.074-P: Pro-arrhythmic effects of increasing late INa in guinea pig ventricular myocytes. Antagonism by ranolazine.

CVT303.075-P: A study of the hemodynamic effects of compound A and compound B prior to ischemia and during reperfusion in the isolated heart of the male rabbit.

CVT303.076-P: High resolution optical mapping study of the electrophysiological effects of ranolazine in guinea pig isolated hearts: models of long QT-3 and -2 syndrome.

CVT303.077-P: Reversal by ranolazine of the Proarrhythmic effects of cisapride, DPI 201-106 and moxifloxacin in female rabbit isolated hearts.

CVT303.082-P: Effects of ranolazine on the Na+/H+ exchanger

CVT303.088-P: Ranolazine decreases ATX-II-induced contracture in isolated left atria from rats

CVT303.093-P: Assessment of ranolazine's effects on mechanical function and [Ca2+]I accumulation in ejecting rat hearts

CVT303.094-P: ranolazine improves the contractile dysfunctions associated with ATX-II in rat isolated ejecting hearts

CVT303.105-P:effects of ranolazine on isosorbide dinitrate- or sildenafil-induced changes in blood pressure and heart rate in unanesthetized dogs

CVT303.085-P Electrophysiological effects of ranolazine in ventricular myocytes from dogs with chronic heart failure

CVT303.086-P Preferential lengthening of right ventricular repolarization by ranolazine—No indication for ventricular proarrhythmia

Appears This Way
On Original

The material in this submission was submitted in response to the approvable letter.

2.6.2 PHARMACOLOGY

2.6.2.1Brief summary : The sponsor is proposing ranolazine as the first in a new mechanistic class. The two studies reviewed below were those cited in the sponsor's annotated labeling to support the proposed mechanism of action.

For the reasons cited in the reviews the studies themselves are suboptimal. If one accepts that ranolazine in the μM range does have effects on the I_{Na} there are several questions that logically follow:

1. What is the in vivo biological significance of this effect?
2. How many other drugs can modulate this channel?
3. What distinguishes this from the Na+ channel blockers used as anti-arrhythmics, e.g. encainide (no longer available) and flecainide? "State-dependent block of wild-type and inactivation-deficient Na+ channels by flecainide." GK Wang, C Russell, S-Y Wang. J Gen Physiol. 122: 365-374 (2003).

The sponsor tries to draw a theoretical bridge in Item 3, Volume 1, page 33 where it is stated that:

> In ischemic conditions the late sodium current is increased, leading to intracellular sodium overload. Sodium overload leads to calcium overload as a result of either a decrease in the efflux of calcium ions via the forward mode of the Na-Ca exchanger (NCX) or an increase in the efflux of calcium ions via the reverse mode of the NCX, or both. Calcium overload during the ischemia causes slowing of the left ventricular relaxation and an increase of left ventricular diastolic stiffness. Ranolazine by inhibiting this pathologically-enhanced late sodium current, reduces intracellular sodium and calcium loading of ventricular muscle cells, and thereby improves ionic homeostasis. The reduction in cellular calcium overload is expected to reduce myocardial stiffness, oxygen consumption and ATP utilization.

In Item 5, Volume 1, page 9, the sponsor begins another discussion of the relevance of the late sodium channels. On page 40, the sponsor cites that:

> During ischemia, sodium influx via late I_{Na} appears to be a major contributor to the rise of [Na+]i.[53] Sodium channel blockers (e.g. tetrodotoxin, lidocaine) have been shown to reduce the rise in $[Na+]_i$ in rat ventricular myocytes and isolated hearts during hypoxia and ischemia respectively. [53-55] This reduction in the rise

in $[Na]_i$ is associated with a reduction in the rise in intracellular calcium concentration ($[Ca^{2+}]_i$) and improvement in contractile function.[56,57]

However, the sponsor goes to state that in addition to the peak and late sodium currents, the sodium/hydrogen exchanger and the sodium calcium exchanger are two important pathways responsible for the regulation of sodium entry into cells, which contribute to maintenance of cardiac intracellular sodium and calcium homeostasis. Therefore, there is no clear statement of mechanism but the implication is that complex electrophysiological interactions make up the overall mechanism.

Although not all of the submitted studies were reviewed, they were examined to see if the sponsor had used any comparator compounds. Given the proposed mechanism of sodium channel inhibition, the most appropriate comparators would seem to be Class I anti-arrhythmics such as encainide, flecainide or quinidine. This kind of comparison is apparently lacking in the material provided by the sponsor. Lidocaine would not be a reasonable comparator due to the differences in rate of recovery. As defined in Goodman and Gilman's 9th Edition (Ch.35) $\tau_{recovery}$, the time required for 63% of an experimentally determined process to be complete, is very short for lidocaine. The recovery time for lidocaine is <<1 second, so that substantial Na channel block only occurs in rapidly driven tissues, especially in ischemia. Drugs such as flecainide have $\tau_{recovery}$ >10seconds. Due to this long recovery time, marked slowing of conduction occurs even in normal tissues at normal conduction rates.

However, the sponsor has also shown in vivo that ranolazine antagonizes the positive inotropic effects of isoproterenol. This is generally considered indicative of a β1 adrenergic antagonism. This would also explain the loss of contractility and negative inotropy seen in the studies conducted in dogs.

Using the information in Goodman and Gilmans' as a reference, the information about flecainide may be summarized as follows:

The pharmacologic effect is attributed to the drug's long $\tau_{recovery}$ from Na+ channel block. Flecainide blocks Na+ current and Ikr current at concentrations of 1-2μM. It also blocks Ca2+ channels in vitro and can prolong the duration of PR, QRS and QT even at normal heart rates. Flecainde does not cause EADs in vitro or torsade de pointe. In atrial tissue, there is a disproportionately long prolongation of action potentials at fast rates, desirable in antiarrhythmics. The drug can however exacerbate congestive heart failure, cause heart block in patients with conduction system disease and can provoke or exacerbate potentially lethal arrhythmias.

According to the PDR, flecainide does not alter heart rate although bradycardia and tachycardia have been reported. Negative inotropic effects have been reported.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: unknown

Drug activity related to proposed indication: unknown, possibly beta adrenergic blockade

CVT303.080-P Effects of ranolazine on sustained current carried by disease associated mutant sodium channels. August 19, 2004

The studies were designed to 1) test the effects of ranolazine on normal (WT) and mutant sodium channels 2)determine relative potency of ranolazine for peak vs sustained (I_{sus}) sodium channel currents and 3) determine whether or not ranolazine activity is affected by mutations of residues that define the binding site for local anesthetics (sodium channel blockers).
The two LQT-3 mutations chosen were those previously shown to induce I_{sus} which is associated with fatal arrhythmias in patients carrying the gene mutations. These are the Y1795C mutation in the carboxy tail of the sodium channel and ΔKPQ that results in a deletion mutation in the sodium channel inactivation gate.

Na+ channels were expressed in HEK293 cells. CD8 was transfected as the reporter gene. The CD8 positive cells were patch-clamped 48 hours after transfection. Na+ channels were also measured in myocytes isolated from transgenic mouse hearts (for the ΔKPQ channel) . The transgenic mice were heterozygous for the *Scn5a* gene with deletion of residues 1505-1507.

Membrane currents were measured using whole cell and single channel patch clamp procedures. The composition of the internal and external solutions are summarized below.

Summary of Internal and External Solutions

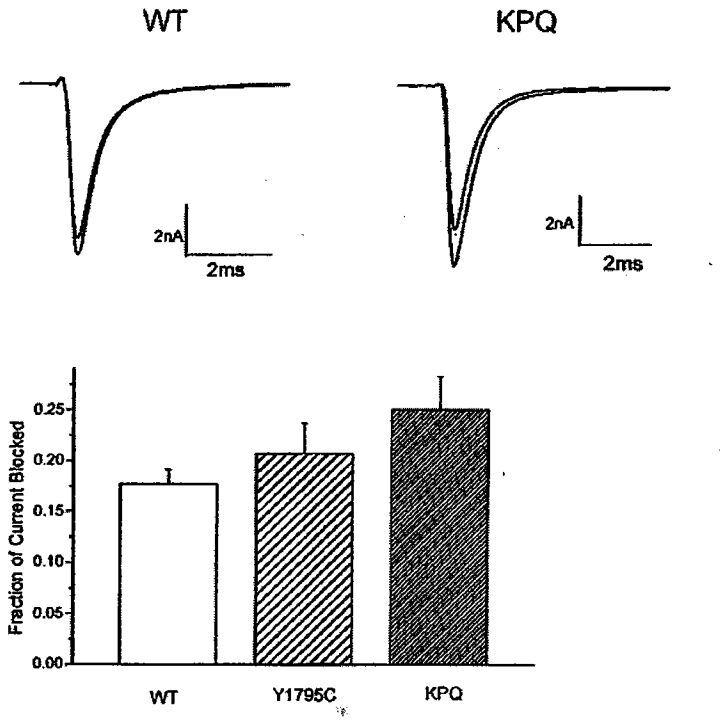
	Internal solution (mmol/l)	External solution (mmol/l)
Aspartic acid	50	
NaCl		130
CsCl	60	5
Na_2ATP	5	
EGTA	11	
Hepes	10	10
$CaCl_2$	1	2
$MgCl_2$	1	1.2
pH	7.4	7.4
pH adjustment	CsOH	CsOH
glucose		5

In experiments designed to test for sustained currents, tetrodotoxtin (TTX) was applied at 30 μM to block expressed Na+ channel currents and to reveal background currents that were then subtracted.

Holding potential	100 mV with a test pulse of -10mV for 200 ms
Use dependent block (UDB)	Conditioning trains of 100-300 pulses (-10mV, 25ms) from a -100 mV holding potential at a frequency of 5 Hz.UDB was measured as the ratio of peak current at -10 mV after and before application of a conditioning train and is reported as % block of peak current
Tonic block (TB)	Measured at 0.1 Hz after steady state was achieved in the presence of drug

Results

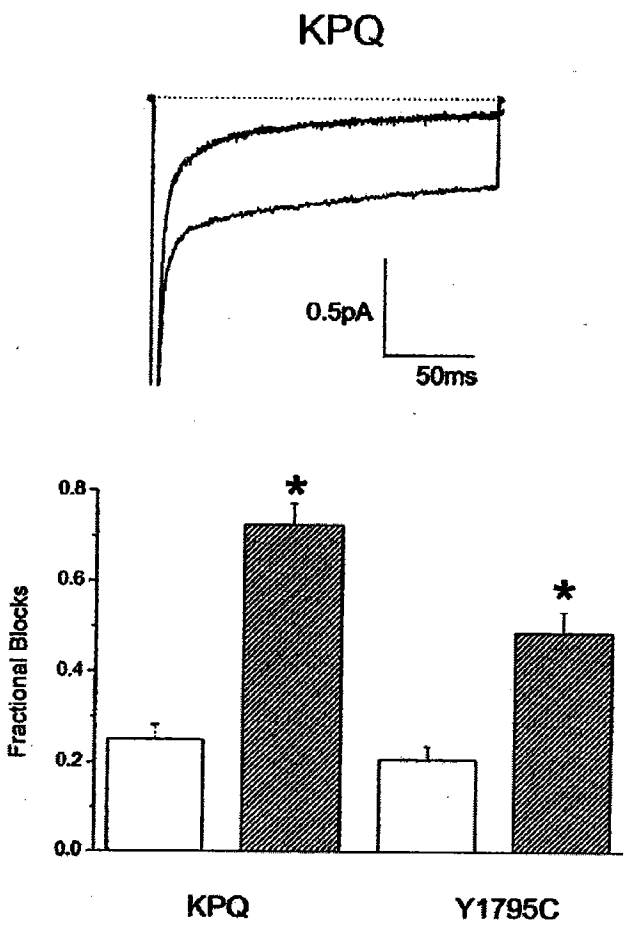
The sponsor presents bar graphs to show the effects of ±50 μM ranolazine on whole cell recordings of WT, and the mutant channels Y1795C and ΔKPQ recorded at low stimulation rates (tonic block). While there was a trend towards block of current, there was no significant difference between peak current block for the constructs.



6.1 Figure 1. Effects of ranolazine on peak current (I_{peak}) carried by wild type (WT) and two mutant human sodium channels

Traces show currents from HEK 293 cells expressing wild type (left) and ΔKPQ sodium channel currents before and after steady state block of peak current by ranolazine (50 μM). Bar graphs summarize results from multiple cells. The number of experiments was WT (open bar, n=3); Y1795C channels (middle bar, n=4); ΔKPQ channels (right bar, n=7). Shown are mean +/- S.E.M. data. There is no significant difference between block of peak currents for any construct.

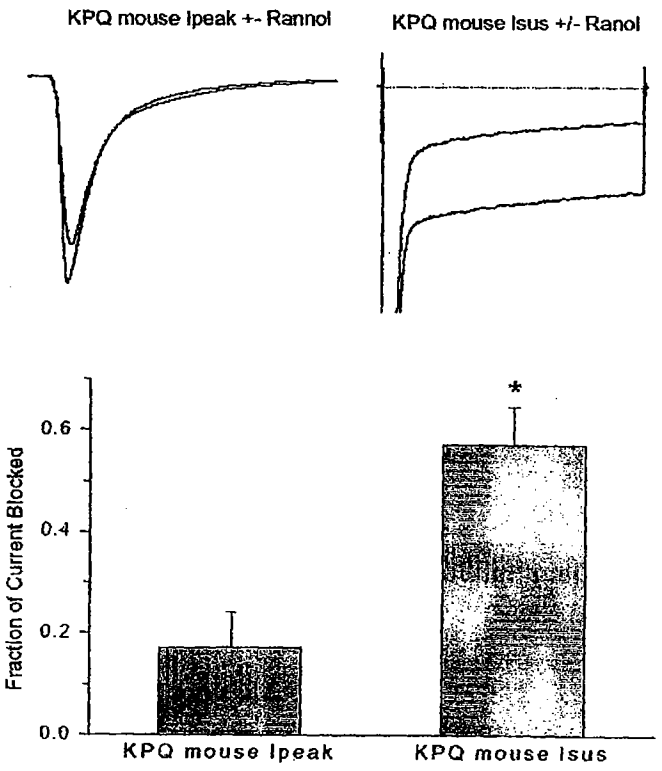
The second bargraph showed a statistically significant difference in fractional block of I_{sus} versus I_{peak} by 50 μM ranolazine in the mutant sodium channels. The WT cells are not shown and there is no comparator substance.



6.2 Figure 2. Effects of ranolazine on sustained current (I_{sus}) in mutant Na channels

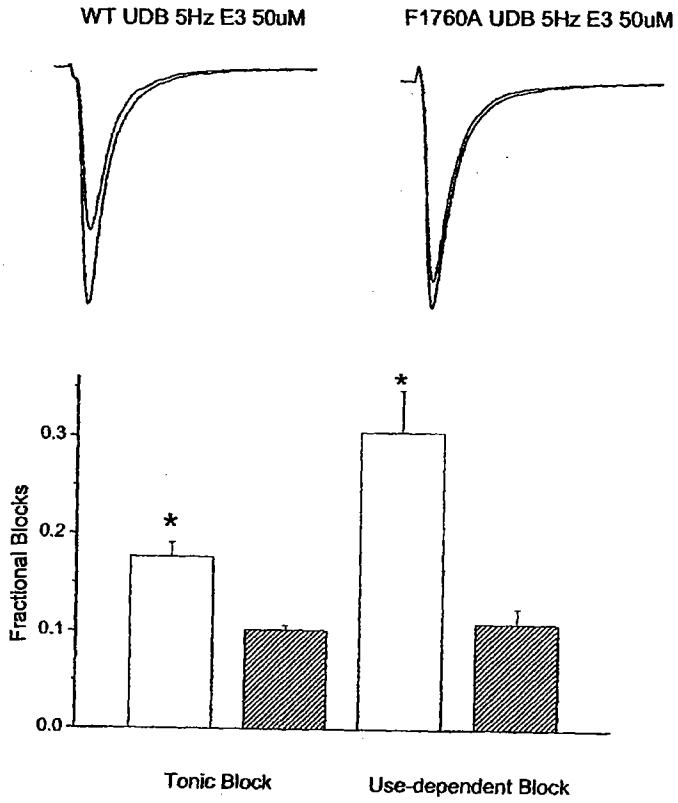
High gain recordings show sustained current and its block by ranolazine (50 μM) carried by ΔKPQ channels. Bars summarize experiments in multiple cells and plot fractional block of I_{sus} by ranolazine. Pulse frequency was 0.33 Hz, pulse voltage, -10 mV, pulse duration 200 ms. Open bars are drug-free; filled bars indicate results with ranolazine. P<0.01 for KPQ I_{peak} (0.25 ± 0.03, n=7, open) vs. KPQ I_{sus} (0.72 ± 0.05, n=7, filled); p<0.01 for YC I_{peak} (0.21 ± 0.03, n=4, open) vs. YC I_{sus} (0.49 ± 0.04, n=4, filled).

Next the sponsor presented low and high gain recordings of currents from cardiomyocytes isolated from mice expressing ΔKPQ channels ±50μM ranolazine. The purpose of this was to show consistency of effect between HEK cells expressing the channel of interest and the channels isolated from transgenic mice. Control data was not shown. The fraction of current blocked for KPQ mouse I_{peak} was shown as less than 0.2 while the fraction of current blocked for KPQ mouse I_{sus} was slightly less than 0.6 ($p<0.01$).



gure 3. Effects of ranolazine on Na currents in KPQ mouse cardiomyocytes

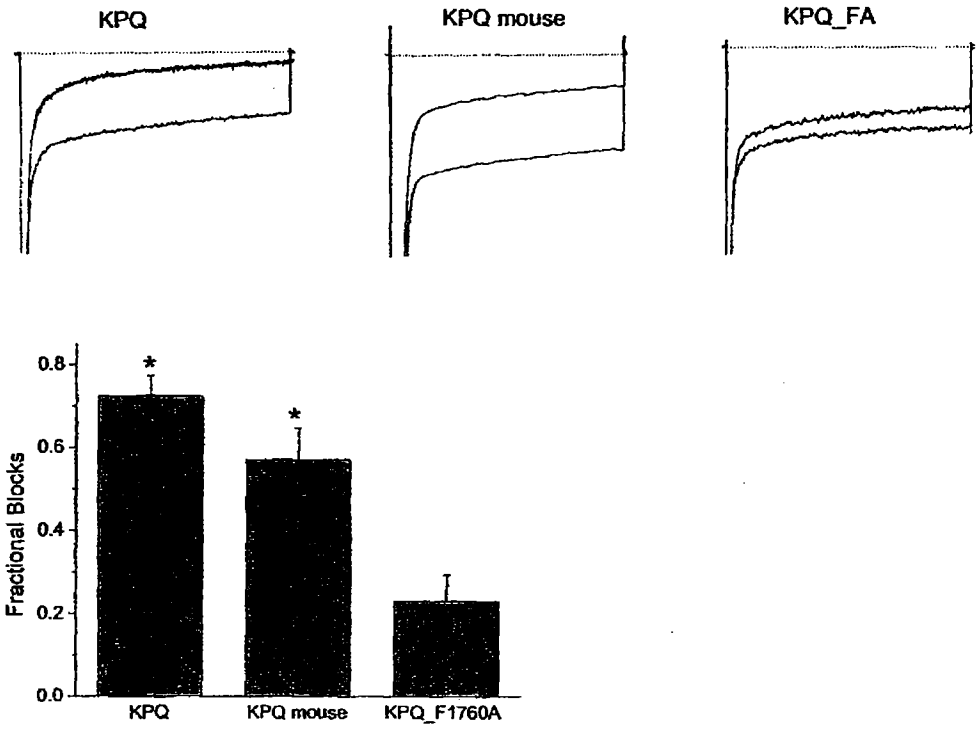
The sponsor then presents 2 additional pieces of data. The first was a bar graph to indicate that mutating the local anesthetic receptor site residue F1760A decreased the tonic and use-dependent block of sodium channels by a single concentration of ranolazine. It has been shown in other work that mutation of this site also reduces binding of the anti-arrhythmic flecainide.



6.5 Figure 5. Mutation of the local anesthetic receptor site residue (F1760A) diminishes tonic and use-dependent block of sodium channels by ranolazine

The mutation F1760A was engineered into the WT sodium channel alpha subunit template and the effects of ranolazine (50 μM) were measured. Shown are currents recorded at 0.33 Hz (tonic block, left traces) and 5 Hz (use-dependent block, UDB, right traces). Bar graphs summarize multiple experiments and plot fraction of current blocked for tonic block (left) and UDB (right). Open bars are drug-free and filled bars are drug-containing conditions. Block of F1760A mutated channels is significantly less than block of channels with F1760 intact. *P<0.01 for WT I_{peak} (0.18 ± 0.01, n=3, open) vs. F1760A I_{peak} (0.1 ± 0.005, n=6, filled); p<0.01 for WT UDB (0.3 ± 0.04, n=3, open) vs. F1760A UDB (0.11 ± 0.02, n=5, filled).

The final bargraph was to show that mutation of the same local anesthetic receptor binding site decreased ranolazine block of the mutant channel sustained current. This final graph compared effects in HEK293 cells to myocytes from transgenic mice.



6.6 Figure 6. Mutation of the local anesthetic receptor binding site (F1760A) diminishes ranolazine block of mutant channel sustained current

Current traces at high gain illustrate ranolazine block of sustained current in HEK 293 cells (left) and murine myocytes (middle) expressing ΔKPQ channels and HEK 293 cells expressing ΔKPQ mutant channels in an alpha subunit construct harboring the F1760A mutation (right). The F1760A mutation greatly reduces the sensitivity of sustained ΔKPQ channel activity to ranolazine. The bar graph summarizes multiple experiments as described for earlier figures. *P<0.01 for KPQ_F1760A (0.23 ± 0.06, n=6) vs. KPQ (0.72 ± 0.05, n=7) and KPQ mouse (0.57 ± 0.07, n=3).

The text of the report states that "We have previously shown that mutation of these and nearby residues to alanine greatly reduces block of LQT-3 and WTchannels by the local anesthetic flecainide (28) and, in particular, the mutation F1760A potently reduces block." Since it is known that flecainide, an anti-arrhythmic, is active in this model, it would be very useful to see how ranolazine compares to a known drug, i.e. a positive control.

A consistency of result is demonstrated between HEK cells and cells from transgenic mice, but relationship of concentration to effect has not been demonstrated. It could also be commented that achieving an effect at 50 μM does not indicate impressive potency.

The sponsor identifies somewhat obliquely a safety concern also. They state on page 162, vol 2, item 5, that

> Our data indicate that ranolazine is approximately 2.3 to 3.3-fold more potent in blocking sustained than peak inward current, and this is an important distinction. Peak current underlies impulse conduction and sustained current underlies QT prolongation. Preferential block of sustained current suggests pharmacologic targeting of the mechanism underlying QT prolongation, which is associated with arrhythmias in this disorder, with minimal side effects due to conduction slowing through the ventricles.

The sponsor has shown a distinction between the effects of ranolazine on peak versus sustained sodium current. This has been 1) without demonstration of concentration dependence 2) without benefit of a positive control and 3) at a concentration outside the sponsor's stated therapeutic plasma concentration range of 1-10μM. Furthermore, the sponsor draws several similarities of ranolazine to flecainide, a drug that can be pro-arrhythmic.

CVT303.085-P Electrophysiological effects of ranolazine in ventricular myocytes from dogs with chronic heart failure. November 2, 2004

The goal of this study was to determine the effects of ranolazine on the electrophysiological and contractile function of ventricular myocytes from canine failing hearts. Specific assessment was made of the effect of ranolazine on :

1. action potential duration (APD)
2. peak transient and late Ina (I_{NaT} and I_{NaL})
3. early after depolarizations (EAD)
4. twitch contraction (TC) including aftercontractions and contracture

Ventricular myocytes were isolated from the left ventricle of dogs with chronic HF induced by multiple sequential intracoronary microembolizations.

Ion currents were recorded using whole cell patch-clamp technique. Ion currents were recorded at room temperature (22-24°C).

APD	β-escin perforated patch clamp at frequencies of 0.25 and 0.5 Hz
Twitch contractions	TC or myocytes shortenings. Recorded using an edge movement detector at frequencies of stimulation from 0.5Hz to 2.0 Hz.
I_{NaL}	Elicited by 2 second long depolarization from holding potential of -140mV at pacing freq of 0.1 Hz leak current determined after TTX(25μM) or after inactivating I_{NaL} using 2 sec long pre-pulses to -40mV and was subtracted from the current traces
I_{NaL}	Determined from averaged current measured 200 ms to 220 ms after onset of 2 sec-long membrane depolarization to -30mV. The time chosen to avoid the peak current (I_{NaT})
I_{NaT}	Measured at symmetrical Na+ concentration of 5mM
Inhibition of I_{NaL}, I_{NaT}	Ranolazine added to solution. 2-6 minutes later inhibition measured
A holding potential of -140 mV was listed as used to have maximal availability of Na+ channels.	

Action Potential Recordings: were recorded in current-clamp mode using β-escin perforated patch clamp. The membrane patch was considered intact when the evoked APs were associated with visually discerned twitch contractions of the myocytes. APs were recorded at 35° C. Myocytes were stimulated using current pulses of 0.2 ms. Effects of ranolazine were determined at 2 pacing rates: 0.25 Hz (15 bpm) and 0.5 Hz(30 bpm).

Myocyte Contraction Recordings: Myocyte contraction was elicited using electrical field stimulation by applying electric pulses of 16 ms duration and a voltage that was 1.5-2 fold greater than the threshold. The experiments were carried out at 37°C in Tyrodes solution with 1.2 mM $CaCl_2$.

K+ concentrations used are summarized in the sponsor's table alongside.

7.1 Table 1: Extracellular (Bath) and intracellular (Pipette) solutions used in the study

	Pipette solutions mM		Bath solutions mM			
	I_{NaT}, I_{NaL}	AP Recordings, Perforated Patch	I_{NaT}	I_{NaL}	AP	Contraction
β-escin		50μM				
NaCl	5	10	5	140	140	140
KCl		10	5.4		5.4	5.4
CsCl	133		133	5.4		
Glucose		5			5	5
$CaCl_2$			1.8	1.8	1.8	1.2
$MgCl_2$		2	2	2	2	2
MgATP	2					
TEA	20					
Nifedipine			0.002	0.002		
KF		125				
EGTA	10					
HEPES	5	5	5	5	5	5
pH	7.3	7.3	7.3	7.3	7.3	7.3
	CsOH	KOH	NaOH	NaOH	NaOH	NaOH

Results

Ranolazine decreased the duration of APD90, amplitude of aftercontraction and duration of contraction. The effects were not completely reversible after washout.

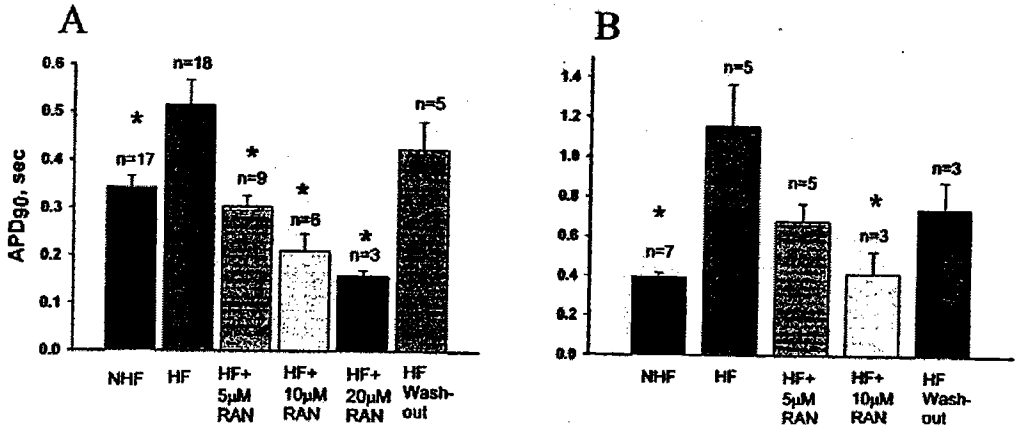
7.3 **Table 3. Effects of ranolazine on various contractile parameters in canine LV myocytes from failing hearts.**

Condition	APD_{90} [1] (msec)	Amplitude of Aftercontraction [2] (% D/S)	Duration of Contraction (msec)	Contracture (%) [3]		
				Stimulation Frequency (Hz)		
				1.0	1.5	2.0
Control	516 ± 51 (18)	18.3 ± 2.1 (17)	542 ± 13 (11)	10.80 ± 2.45 (9)	13.70 ± 1.06 (11)	15.40 ± 1.16 (11)
Ranolazine (10 μM)	212 ± 34* (6)	7.0 ± 0.7* (16)	403 ± 28* (11)	3.46 ± 0.64* (9)	5.17 ± 1.02* (11)	6.36 ± 0.94* (10)
Washout	424 ± 57 (5)	20.9 ± 1.8 (16)	482 ± 15 (11)	9.97 ± 2.41 (6)	15.92 ± 3.15 (8)	13.68 ± 2.77 (8)

Values are mean + SEM. Numbers in parentheses are the number of myocytes studied.

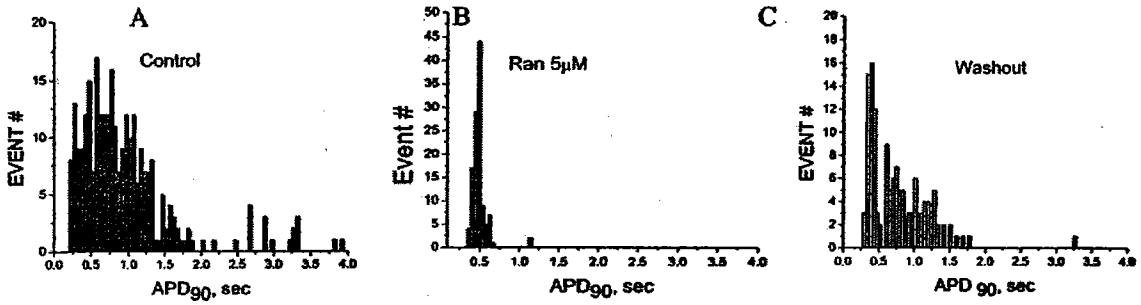
A concentration dependence was also demonstrated for the APD90 effect.

8. FIGURES



A 10μM concentration of ranolazine shortened the APD, and according to the sponsor abolished beat to beat variability and suppressed EADs. This effect appeared to be reversible as shown in the sponsor's figure 3 .

Figure 4 showed histograms of distribution of APD_{90} duration ±5μM ranolazine and after washout of drug. The histogram of APD90 in the presence of 1 concentration of ranolazine shows far fewer histogram bars, suggesting that there is a decreased distribution of APD_{90} durations. The sponsor's graphs are shown below.



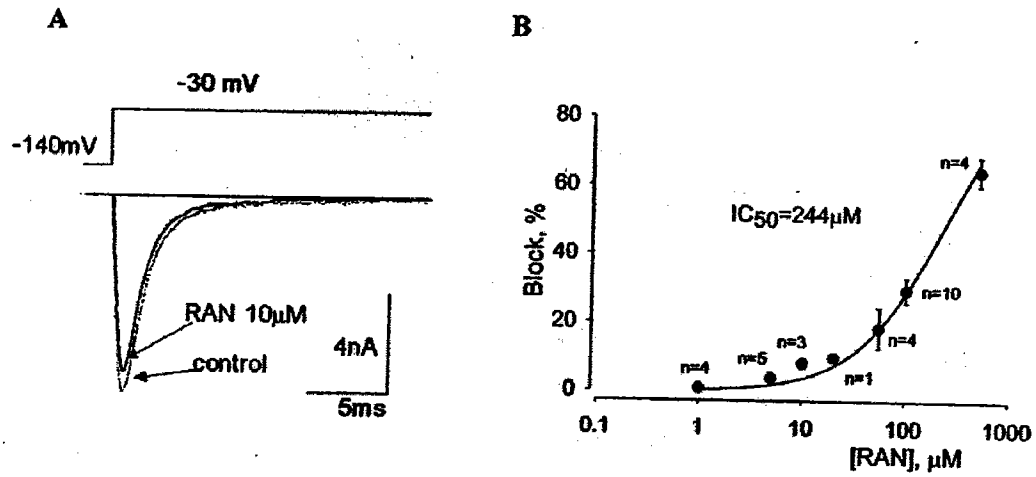
8.4 Figure 4. Ranolazine (RAN) reversibly reduces the action potential duration dispersion of left ventricular myocytes isolated from canine failing hearts.

Histograms of the distribution of the AP durations measured at 90% repolarization (APD_{90}). APs were recorded in control (A), in the presence of 5 μM RAN (B), and after the drug washout (C). Each bin size=0.05 sec for all histograms. Action potentials were recorded at the pacing rate of 0.25 Hz, at a temperature of 35°C, using the β-escin perforated patch. Data were obtained in 5 cells of 3 failing dog hearts.

Appears This Way
On Original

Experiments to determine the IC50 values for ranolazine to inhibit peak and late I_{Na} during low frequency stimulation (0.1Hz) showed results demonstrated in the figures below.

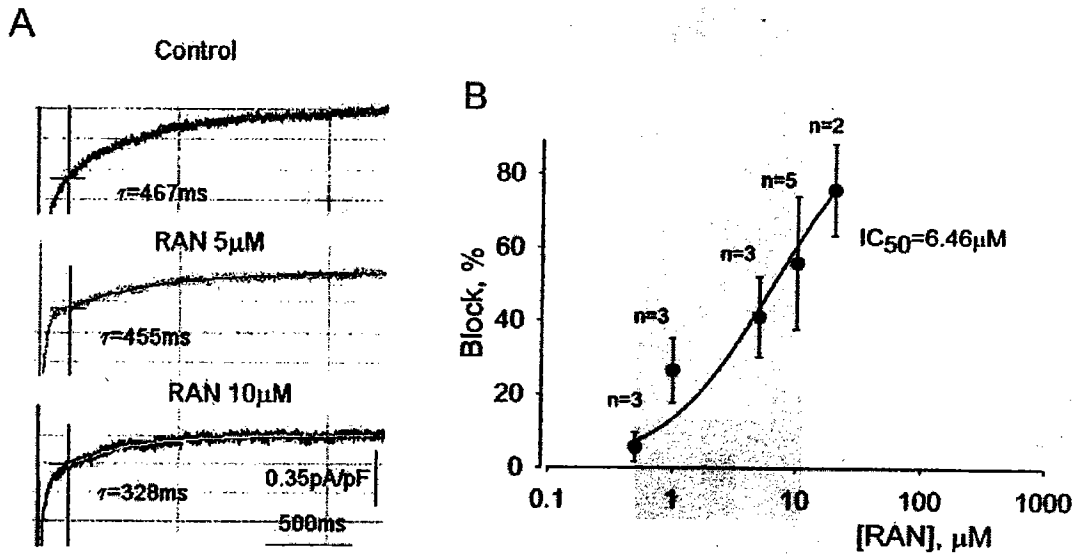
The figure immediately below shows a concentration-related increase in percent block of peak sodium I_{Na}. The IC50 on the graph is 244μM.



8.5 Figure 5. Ranolazine (RAN) weakly inhibits peak transient sodium current (I_{NaT}).

Effect of RAN on I_{NaT} recorded in left ventricular myocytes isolated from failing canine hearts. Panel A. Superimposed membrane sodium current traces recorded in control and in the presence of 10 μM RAN. The voltage-clamp protocol is depicted above the traces.
Panel B. Concentration-response relationship for the inhibition of I_{NaT}. Points represent experimental data obtained in 14 myocytes from four failing hearts. Data points are fitted to a single-site binding model (Eq. 1, Methods). The IC_{50} value is the concentration of ranolazine that causes 50% of inhibition of the current, a value derived from the model fit. Experimental conditions were: frequency of depolarizing steps = 0.1 Hz; temperature = 23°C; V_h = -140mV; V_m = -30mV; $[Na]_o$ = 5 mM and $[Na]_i$ = 5 mM. Error is SEM unless n<3, then SD is used.

The late I_{Na} also showed a concentration-related increase in percent block as depicted below. The IC50 is 6.46 μM.



8.6 Figure 6. Inhibition by ranolazine (RAN) of the late I_{Na} of left ventricular myocytes isolated from canine failing hearts.

Panel A. Traces of membrane sodium current (I_{Na}) recorded in control, and in the presence of 5 and 10 μM RAN. The peak transient inward sodium current is truncated. The black solid lines represent single exponential fit (Eq. 3, Methods). Decay time constant for I_{NaL} (τ values) is given in the traces.

Panel B. Concentration-response relationship for the inhibition of I_{NaL} by RAN. Points represent experimental data obtained in six myocytes from two hearts. Data points are fit to a single-site binding model (Eq.1, Methods). The IC_{50} value is the concentration of RAN that caused 50% reduction, a value derived from the model fit. Experimental conditions were: frequency of depolarizing steps = 0.1 Hz; temperature = 23°C; holding potential (V_h) of –140mV; test potential (V_m) of –30 mV; $[Na]_o$ = 5mM, and $[Na]_i$ = 5mM. The amplitude of I_{NaL} was determined as an averaged current within 220-220 ms after the onset of a 2 second long depolarization step. Data are mean ± SD.

The text of the report notes that LV myocytes isolated from healthy dogs also demonstrated a susceptibility to concentration related decrease in peak I_{Na} with an IC50 of 294 μM. Based upon the above data, the sponsor concluded that ranolazine had 38 fold more potency in inhibiting late versus peak Na channels. Amiodarone was reported to have a potency ratio of 12.9 for these parameters.

Summary: The study indicated that there were distinguishable properties of ranolazine on peak versus late sodium flow in canine cardiac myocytes.

1. Ranolazine shows activity at the micromolar level, this time within the proposed therapeutic concentration range. How well do the in vitro concentrations correlate to the in vivo concentrations?
2. There are no concurrent comparator compounds.
3. What is the biological significance of these findings? Is this a situation similar to that seen when the sponsor was certain that ranolazine modulated the enzymes of fatty acid oxidation? That is, many drugs can be demonstrated in vivo to cause measurable alterations of specific enzymes of fatty acid oxidation, but in whole animals, the mechanism by which any of those drugs produces a pharmacologic effect is entirely different.

The text of the report contains the statement that:

> Recently our group reported the presence of a novel ultraslow inactivating and reactivating late Na+ current (I_{NaL}) in human myocytes from the mid-myocardium of both normal and failing human hearts (21) that could contribute to the ventricular repolarization abnormalities of HF. We demonstrated a significant contribution of I_{NaL} to the AP duration in ventricular myocytes of normal and failing human hearts.

While it is not unheard of for a proprietary drug to be used to describe specific pharmacologic mechanisms, it is fortuitous for the sponsor that after exploration of many possible mechanisms of action of ranolazine beyond the straightforward beta adrenergic or calcium channel blockade, they have not only identified a unique ion channel but that the new ion channel is also the one upon which their proprietary compound acts.

A publication of interest is "State-dependent block of wild-type and inactivation-deficient Na+ channels by flecainide." GK Wang, C Russell, S-Y Wang. J Gen Physiol. 122: 365-374 (2003). In this publication the authors demonstrate that flecainide also shows distinguishable effects on the late and peak sodium channels, with IC50 values in the same range as those for ranolazine. How would these two agents compare if tested concurrently?

Appears This Way
On Original

CVT303.087-P In vitro effect on hERG current (Ikr) expressed in human embryonic kidney (HEK) cells. December 22, 2004.

This was an amendment to the report dated 10/14/04. The IC50 of 13.7μM reported originally has been recalculated to be 14.1μM. This does not alter the interpretation of the study.

Safety Pharmacology

CVT303.066-P <u>Amendment</u> Effects of ranolazine, its R and S enantiomers and eleven metabolites on rat left atrial contractility. Amendment date January 21, 2004

The amendment corrects the original study dates and also examined the ability of ranolazine to antagonize the positive inotropic effect of isoproterenol in rat isolated left atria with K_B value of 2.4 μM. A concentration-dependent effect was demonstrated over the limited concentration range tested. This is indicative of β1-adrenergic antagonistic effects, the originally proposed mechanism of action for ranolazine. These results are also consistent with previous studies including ranolazine competitively inhibiting the specific binding of ^{125}I-pindolol to rat ventricular membranes with a Ki of 5.4μM (CVT303.030N).

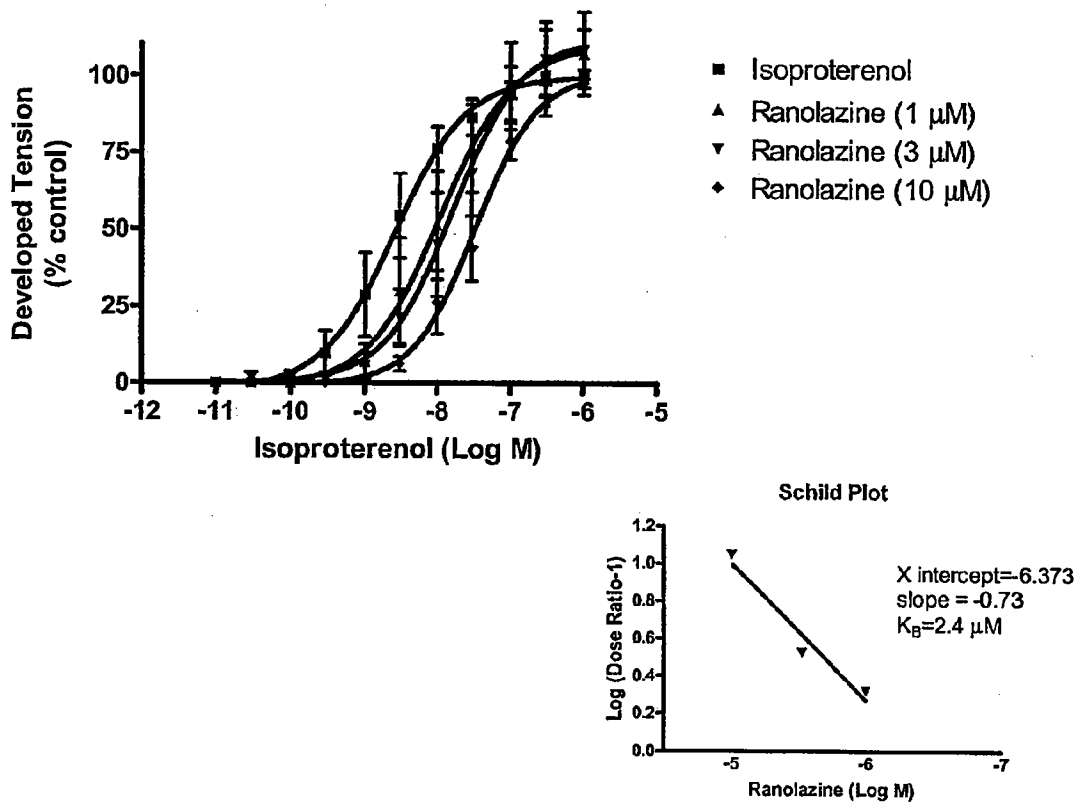


Figure A1. Effect of ranolazine on the concentration-response curves for the effect of isoproterenol on left atrial tension development and Schild analysis (inset). Data are means ± SEM for 3 hearts per concentration.

CVT303.067-P ***Amendment Effects of ranolazine on systemic hemodynamics and coronary circulation in conscious dogs. Amendment date August 17, 2004***

This amendment was submitted to provide time points that were previously unreported: the 5 and 10 minute exposure data and 5, 15 and 30 minutes after stopping the infusion. The report originally provided only 15 minutes post injection data.

The data as shown do not indicate a detectable effect on heart rate or mean arterial pressure. However, the figure legends indicate that doses of ranolazine causing plasma concentrations of 1,3 and 14μM caused <12% increase in HR and <5% change in MAP. At a plasma concentration of 18μM, ranolazine increased HR by 20% ($p<0.05$) at 10 minutes during the infusion. MAP was reported to be unchanged.

At the plasma concentrations of 1,3 and 14 μM there was <5% decrease in LVSP or LV dP/dt. At a concentration of 18μM, there was a 7% decrease in LVSP and a 15% decrease in LV dP/dt ($p<0.05$).

The next question is how this compares with the desired pharmacology. The sponsor claims that a plasma concentration range of 1 to 10 μM is the human therapeutic range. In this study, using conscious dogs, there was no significant effect on coronary blood flow. This is shown in the sponsor's data shown below.

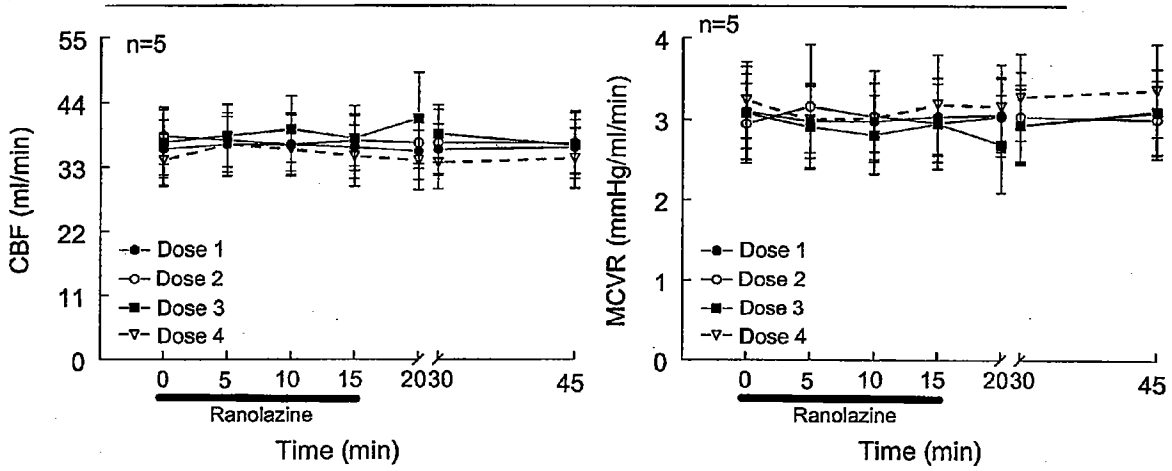


Figure A3. Lack of effect of ranolazine on coronary blood flow (CBF) and coronary vascular resistance (CVR) in awake dogs. Shown is the time course of changes in CBF and CVR in dogs treated with four different doses of ranolazine that resulted in steady-state plasma concentrations of 1, 3, 14, and 18 μM, respectively. The changes in CBF and CVR observed in the presence of ranolazine (1 to 18 μM) were ≤ 8%. These changes were transient and random. For results of the statistical analyses, see the tables.

Therefore, in this model, there is a negative inotropic effect before reaching the plasma concentration where there is the desired pharmacologic effect.

The sponsor then provided a tabular summary of data including uncorrected QT intervals.

Table A1. Effects of Ranolazine (Dose 1) on Hemodynamics, Coronary Circulation and R-R and Q-T Intervals in Awake Dogs

		ranolazine					
	N	Baseline	5 min	10 min	15 min	20 min	30 min	45 min
LVSP (mmHg)	5	130±6	125±6*	130±7	128±6	127±5	125±5	125±6
LV dP/dt (mmHg/s)	5	2669±360	2528±337*	2606±338	2571±332	2580±279	2512±268	2711±296
MAP (mmHg)	6	103±5	100±4*	100±4	101±4*	102±5	100±5	103±5
HR (bpm)	6	98±8	99±8	99±6	95±7	101±5	90±7	94±7
CBF (mL/min)	5	36±5	37±5	37±5	36±5	36±5	36±4	36±5
MCVR(mmHg/mL/min)	5	3.1±0.46	3.0±0.46	3.0±0.47	3.0±0.48	3.1±0.45	2.9±0.45	3.1±0.53
R-R Intervals (ms)	5	661±72	588±66	581±51	669±75	601±42	680±67	667±64
Q-T Intervals (ms)	5	239±7	220±7	229±5	229±14	230±8	227±10	232±7

LVSP: Left ventricular systolic pressure, MAP: Mean arterial pressure, HR: Heart rate, CBF: Coronary blood flow, MCVR: Mean coronary vascular resistance. Dose 1: 0.4mg/kg bolus injection followed by infusion at 22.5 μg/kg/min for 15 min. * P<0.05, compared with the baseline using Paired t-Test. Data are mean ± SEM.

Table A2. Effects of Ranolazine (Dose 2) on Hemodynamics, Coronary Circulation and R-R and Q-T Intervals in Awake Dogs

		ranolazine					
	N	Baseline	5 min	10 min	15 min	20 min	30 min	45 min
LVSP (mmHg)	5	126±6	123±8	124±7	124±8	127±9	127±7	125±6
LV dP/dt (mmHg/s)	5	2538±311	2403±312	2415±295	2446±277	2498±293	2416±296	2478±290
MAP (mmHg)	6	104±5	100±5	102±5	103±6	106±6	104±5	103±5
HR (bpm)	6	95±7	96±7	99±7	99±8	96±5	89±7	91±6
CBF (mL/min)	5	38±5	38±6	37±5	38±4	37±4	37±6	37±5
MCVR(mmHg/mL/min)	5	3.0±0.49	3.2±0.76	3.0±0.56	3.0±0.48	3.0±0.49	3.0±0.55	3.0±0.48
R-R Intervals (ms)	5	637±52	634±54	625±58	632±61	645±42	699±78	686±65
Q-T Intervals (ms)	5	232±6	236±6	237±8	236±8	235±8	243±7*	237±12

LVSP: Left ventricular systolic pressure, MAP: Mean arterial pressure, HR: Heart rate, CBF: Coronary blood flow, MCVR: Mean coronary vascular resistance. Dose 2: 1.2 mg/kg bolus injection followed by infusion at 67.5 μg/kg/min for 15 min. * P<0.05, compared with the baseline using Paired t-Test. Data are mean ± SEM.

Appears This Way
On Original

Table A3. Effects of Ranolazine (Dose 3) on Hemodynamics, Coronary Circulation and R-R and Q-T Intervals in Awake Dogs

		ranolazine					
	N	Baseline	5 min	10 min	15 min	20 min	30 min	45 min
LVSP (mmHg)	5	126±6	122±6*	119±7	123±7	122±6	122±7	130±6
LV dP/dt (mmHg/s)	5	2304±200	2218±189	2198±217	2213±209*	2241±214	2351±259	2393±213
MAP (mmHg)	6	103±5	101±5	99±4	100±5	98±5	103±5	105±3
HR (bpm)	6	92±7	103±7*	96±7	97±7	93±9	97±9	91±9
CBF (mL/min)	5	37±6	38±5	39±6	38±6	41±8	39±5	37±6
MCVR(mmHg/mL/min)	5	3.1±0.57	2.9±0.53	2.8±0.49*	3.0±0.56	2.7±0.61	2.9±0.49	3.1±0.52
R-R Intervals (ms)	5	678±68	610±51	655±62	647±55	693±94	669±72	723±105
Q-T Intervals (ms)	5	238±10	237±10	243±9	240±8	236±14	233±11	229±14

LVSP: Left ventricular systolic pressure, MAP: Mean arterial pressure, HR: Heart rate, CBF: Coronary blood flow, MCVR: Mean coronary vascular resistance. Dose 3: 3.6 mg/kg bolus injection followed by infusion at 200 μg/kg/min for 15 min. * P<0.05, compared with the baseline using Paired t-Test. Data are mean ± SEM.

Table A4. Effects of Ranolazine (Dose 4) on Hemodynamics, Coronary Circulation and R-R and Q-T Intervals in Awake Dogs

		ranolazine					
	N	Baseline	5 min	10 min	15 min	20 min	30 min	45 min
LVSP (mmHg)	5	130±6	121±6*	126±4	122±4*	124±4	124±5	135±3
LV dP/dt (mmHg/s)	5	2570±148	2175±71*	2273±89*	2231±129*	2362±172*	2463±169	2637±102
MAP (mmHg)	6	104±3	103±4	103±3	101±3	100±3	102±3	108±4
HR (bpm)	6	87±8	100±8*	104±9*	102±9*	99±10	94±9	99±9
CBF (mL/min)	5	34±5	37±5	36±4	35±5	34±5	34±5	35±5
MCVR(mmHg/mL/min)	5	3.2±0.47	3.0±0.41	3.0±0.42	3.2±0.61	3.2±0.52	3.3±0.53	3.4±0.57
R-R Intervals (ms)	5	748±96	617±58	623±63	608±61*	638±73	701±67	651±71
Q-T Intervals (ms)	5	242±10	236±10	229±12	237±8	229±13	235±13	228±15

LVSP: Left ventricular systolic pressure, MAP: Mean arterial pressure, HR: Heart rate, CBF: Coronary blood flow, MCVR: Mean coronary vascular resistance. Dose 4: 6.0 mg/kg bolus injection followed by infusion at 337.5 μg/kg/min for 15 min. * P<0.05, compared with the baseline using Paired t-Test. Data are mean ± SEM.

No NOAEL was identified for the decrease in LV dP/dt seen during the first 15 minutes (when there was exposure to drug). At the highest plasma concentration identified in this study , 18μM, there was a 20% increase in HR. There was no significant increase in coronary blood flow or mean coronary vascular resistance. The human therapeutic plasma concentration range is reported by the sponsor to be 1-10μM. The concentrations reported in this study were 1-18μM.

Toxicology

CVT303.019-T Mammalian erythrocyte micronucleus test: ranolazine. November 17, 2004
Study location: [ ]
Lab study number: AA99VB.125.BTL
Study dates: September28, 2004
GLP Compliance: statement included
QA: yes
Test article: ranolazine lot # SD1701AU01, purity [ ],

Male and female Sprague-Dawley rats were given oral doses of 25, 75 and 250 mg/kg body weight. Water was used as the vehicle/negative control and cyclophosphamide at 40 mg/kg was the positive control. The study design is summarized below:

Summary of study design

group	Treatment mg/kg	# of animals per sex dosed and euthanized at	
		24 hours	48 hours
Vehicle	Water 0	5	5
LD	Ranolazine 25	5	5
MD	Ranolazine 75	5	5
HD	Ranolazine 250	5	5
Positive control	Cyclophosphamide 40	5	0

No unscheduled mortality was seen at any dose. Lethargy was reported for the day of dosing in the HD group. Bone marrow cells (PCEs and NCEs) collected 24 and 48 hours after treatment were examined microscopically for the presence of micronuclei. 2000 PCE for each rat were evaluated for micronuclei. The number of micronucleated NCE in the field of 2000 PCEs per animal was enumerated but was not used to evaluate the response of the test article.

Under the conditions of the study, there was no increase in micronuclei apparent in the data as presented.

Appears This Way
On Original

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Elizabeth Hausner
10/31/2005 12:06:03 PM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
11/2/2005 03:06:09 PM
PHARMACOLOGIST

MEMO TO THE FILE

NDA 21,526 Reviewer: Elizabeth Hausner, D.V.M.
Drug:Ranolazine
Sponsor: CVTherapeutics
Correspondence Date: December 1, 2003
Center Receipt Date: December 2, 2003
Reveiwer Receipt Date: December 4, 2003
Review completed: January 29, 2004

The current information was submitted in response to the October 30 ,2003 FDA action letter where testicular toxicity was identified as a potential safety concern for ranolazine. On November 6, 2003, Dr John Koerner and myself had a telecom with representatives from CV Therapeutics. In this discussion, CVT agreed to have a board certified pathologist review the histopathology slides of the testes from the chronic rat and dog studies with ranolazine.

The sponsor submitted statements from L.T.Pulley D.V.M., Ph.D. as to the evaluation of the histopath slides. Dr Pulley is a former employee of Syntex and Roche Bioscience.

Material Submitted
Peer histopathologic evaluation of testes from repeat dose oral toxicology studies with ranolazine in dogs

Peer histopathologic evaluation of testes from repeat dose oral toxicology studies with ranolazine in rats

Material Reviewed
Peer histopathologic evaluation of testes from repeat dose oral toxicology studies with ranolazine in dogs

Peer histopathologic evaluation of testes from repeat dose oral toxicology studies with ranolazine in rats

For the studies for both species, Dr Pulley noted adequate quality of fixation and staining and did specify methods of fixation and thickness of sectioning. He reported a detailed qualitative examination using the guidelines set forth in the Society for Toxicologic Pathology Position Paper on the Recommended Approaches for the Evaluation of Testicular and Epididymal Toxicity (Lanning et.al, 2002).

Summary of studies for which slides were evaluated

species	duration	Doses(mg/kg/day)	Numbers per group
dog	3 month	0 and 80	Control n=3 HD n=3
dog	6 month	0, 5,25,60	N=4 per group
dog	12 month	0, 10, 25, 60	N=5 per group
rat	3 month	0, 500	N=12 per group
rat	6 month	0, 200	N=20
rat	12 month	0, 200	N=20

Results of dog studies: There were no significant dose-related findings.

Results of rat studies: There were no significant dose-related findings.

The doses used in the fertility study were 5, 40 and 300 mg/kg/day. The 3 month rat study exceeded that dose for 90 days without evidence of dose-related findings. The lack of any histological findings of significance in these 6 studies provides some evidence that diminishes the likelihood of clinical significance of the findings in the fertility study.

Appears This Way
On Original

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Elizabeth Hausner
2/3/04 12:17:07 PM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
2/3/04 01:51:43 PM
PHARMACOLOGIST

NDA 21526

MEMORANDUM

To: Douglas Throckmorton, M.D., Division Director
HFD 110, Division of Cardio-Renal Drug Products

From: John Koerner, Ph.D., Pharmacologist
HFD 110, Division of Cardio-Renal Drug Products

Through: Charles Resnick, Ph.D., Supervisory Pharmacologist
HFD 110, Division of Cardio-Renal Drug Products

Subject: Nonclinical Electrophysiological Effects of Ranolazine

Date: 23 October 2003

The sponsor (CV Therapeutics, Inc.) has submitted an amendment to this NDA[1] providing nonclinical study reports to address Pharmacology/Toxicology issues discussed in the DISCIPLINE REVIEW LETTER. Among other things, the sponsor attempts to prove that QT prolongation with ranolazine is not a concern. This memo addresses study reports describing ranolazine's electrophysiological properties in nonclinical studies. Other study reports provided in the amendment were addressed by Dr. Elizabeth Hausner in her memo dated 29 September 2003.

Although some of the evidence provided in this amendment is consistent with ranolazine induced QT prolongation and repolarization disturbances, other evidence suggests antiarrhythmic rather than proarrhythmic potential. Nevertheless, these study results do not alter the previous conclusion (see original memorandum dated 4 September 2003) that the nonclinical electrophysiology findings do not preclude human risk, in large part due to lack of comprehensive information addressing the sensitivity and specificity of the in vitro assays cited. Findings are summarized below.

The sponsor addressed effects of ranolazine, its enantiomers and metabolites on several ionic currents that modulate ventricular repolarization.

- Ranolazine did not inhibit the slowly activating delayed rectifier potassium current (I_{Ks}) through human channels (KvLQT1/minK) expressed in *Xenopus* oocytes. Hence, the sponsor concluded that ranolazine at concentrations up to 900 μM does not inhibit I_{Ks}. However, these data are not convincing since the *Xenopus* oocyte expression system greatly underestimates potency. The sponsor argued that results of previously submitted studies showing that ranolazine inhibited native I_{Ks} in isolated canine ventricular myocytes were due to current run-down and therefore an artifact of the test system. Given the contradictory study reports and assay limitations, the effects of ranolazine on I_{Ks} are presently unclear.

- Ranolazine enantiomers, like racemic ranolazine, inhibited I_{Kr} and late I_{Na} with similar potencies in canine ventricular myocytes. Additionally, several ranolazine metabolites inhibited late I_{Na} when evaluated at a concentration of 10 μM. The sponsor argues that late I_{Na} inhibition attenuates ranolazine's effects on action potential duration in M-cells and other ventricular tissue, thereby preventing proarrhythmic activity. However, the torsadogenic drugs terfenadine and cisapride also inhibit late I_{Na}, and terfenadine, like ranolazine, showed similar potencies on I_{Kr} and late I_{Na}. Therefore, inhibition of late I_{Na} does not appear to preclude risk of drug-induced *torsade de pointes*.

- Ranolazine increased the decay of I_{Ca} and therefore inhibited what the sponsor called late I_{Ca}. Although this activity theoretically could shorten action potential duration, the actual significance of this finding is unknown.

---

[1] Amendment dated 13 September 2003; received by Center on 15 September 2003.

The sponsor addressed proarrhythmic and antiarrhythmic potential of ranolazine in vitro. The relevance of these findings is unknown since the sensitivity of these in vitro models has not been sufficiently well characterized.

- Ranolazine lengthened epicardial monophasic action potential duration in isolated female rabbit hearts. Ranolazine did not increase apex-base dispersion of action potential duration nor induce ventricular arrhythmias. Additionally, ranolazine, at concentrations that inhibit I_{Kr} and late I_{Na} in canine ventricular myocytes, exerted actions consistent with antiarrhythmic activity, since it prevented pause-dependent ventricular arrhythmias induced by positive controls. The specificity of these effects was not evaluated with other QT prolonging drugs that inhibit multiple ionic currents, e.g. terfenadine and cisapride.

- Ranolazine prevented isoproterenol-induced delayed afterdepolarizations in isolated guinea pig ventricular myocytes. This effect is consisted with ranolazine's ability to block β1 adrenergic receptors at the concentration evaluated.

- Ranolazine lengthened epicardial monophasic action potential duration in isolated guinea pig hearts, while showing effects consistent with antiarrhythmic activity. The sensitivity and specificity of this model has not been thoroughly characterized.

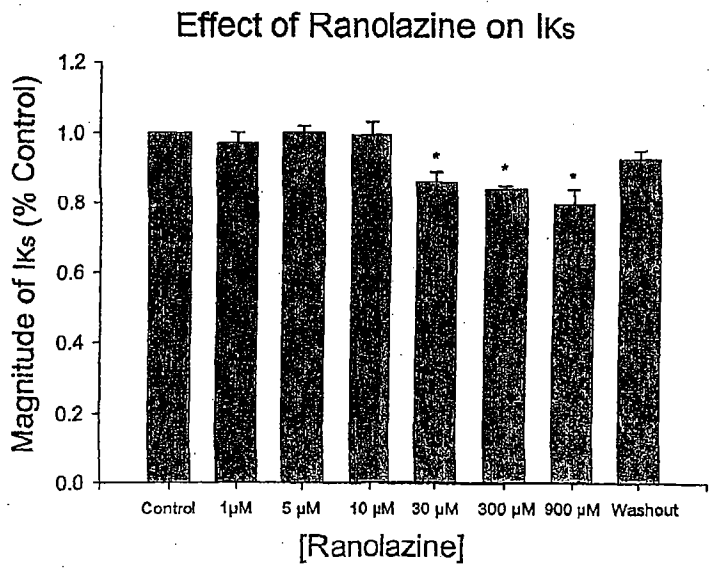
- This sponsor argues that ranolazine lacks proarrhythmic activity since it does not induce early afterdepolarizations, or increase M-cell APD90 and transmural dispersion of repolarization in isolated canine cardiac wedge preparations. Ranolazine was additionally negative for in vitro proarrhythmic effects in this preparation, since *torsade* like arrhythmias did not occur spontaneously and could not be elicited with a single extrastimulus in the drug's presence. Epicardial stimulation was utilized for these studies since stimulation at this site was necessary to capture cisapride's proarrhythmic activity, presumably due to increased transmural dispersion of repolarization at baseline.

  In contrast to the present study results, a previous study showed ranolazine to lengthen M-cell action potential duration and increase transmural dispersion of repolarization, but only in the presence of 2 mM potassium. The lowest potassium concentration evaluated in the present studies was 3 mM. The difference in findings can be explained by the known enhancement of drug-induced I_{Kr} inhibition by hypokalemia.

The individual studies addressed in this memorandum are listed below.

CVT303.069-P: Effect of Ranolazine on I_{Ks} in Isolated Canine Left Ventricle Myocytes

Ranolazine did not inhibit current (I_{Ks}) through human channels (KvLQT1/minK) expressed in *Xenopus* oocytes. Hence, the sponsor concluded that ranolazine at concentrations up to 900 μM does not inhibit I_{Ks}. However, these data do not convincingly show lack of effect of ranolazine on I_{Ks} since the *Xenopus* oocyte expression system greatly underestimates potency.



Effect of Ranolazine on I_{Ks}. Bar graph shows the average change of I_{Ks} tail current under control conditions and after application of increasing concentrations of ranolazine. Bars are mean ± SEM. * Significantly different at $p<0.05$ vs. control.

CVT303.063-P: Effects of Ranolazine Enantiomers on I_{Ks}, I_{Kr}, and Late I_{Na}, and Ranolazine Metabolites on Late I_{Na}

Ranolazine enantiomers were evaluated for effects on ionic currents modulating ventricular repolarization in isolated canine ventricular myocytes. Both enantiomers inhibited I_{Kr} and late I_{Na}, but only the S-enantiomer inhibited I_{Ks}, and then only weakly.

Test Substance	IC50 (μM)		
	I_{Kr}	I_{Ks}	Late I_{Na}
R-Ranolazine	28	no inhibition^	8
S-Ranolazine	10	>100	5

^ tested at concentrations up to 100 μM

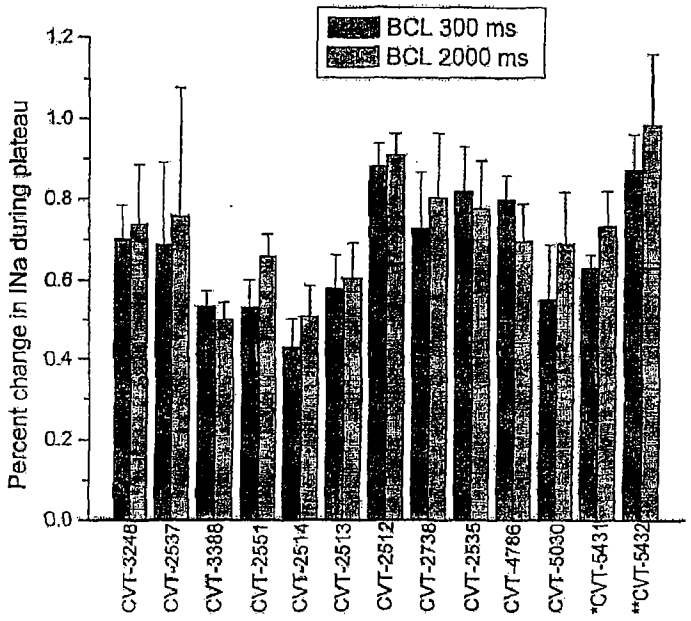
The following table summarizes effects of racemic ranolazine and its enantiomers in the context of positive control drugs. Racemic ranolazine and positive control drugs and were not evaluated concurrently. Note that terfenadine and cisapride, both torsadogenic drugs, inhibit both I_{Kr} and late I_{Na}, similar to ranolazine. Furthermore, terfenadine, like ranolazine, is similarly potent on late I_{Na} and I_{Kr}. Therefore, blockade of late I_{Na} does not appear to preclude torsadogenic risk.

Ion Channel Current Inhibition by Ranolazine, Its Enantiomers and Several Comparator Drugs.

	I_{Kr}	I_{Ks}	Late I_{Na}	I_{Ca}	Late I_{Ca}	I_{Na-Ca}	I_{to}
RS-Ranolazine	11	>100	5.9	296	50	91	
R- Ranolazine	28	-	8				
S-Ranolazine	10	>100	5				
Verapamil	3.51	>50	0.33	0.11			
Terfenadine	0.79	-	1.44	1.16			
Risperidone	4.2	>50	13.6	32.5			
Cisapride	0.46	3.5	6.2				
Erythromycin	~80						
HMR 1556	12.6	0.011		27.5			33.9

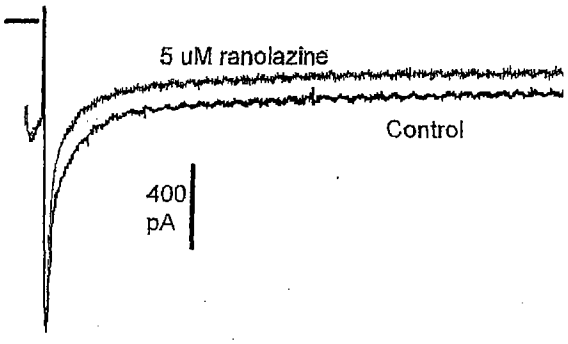
IC_{50} values are shown in μM. All currents were measured in canine ventricular M or epicardial cells.

The following figure illustrates effects of several ranolazine metabolites on late I_{Na}. At a concentration of 10 μM, several ranolazine metabolites inhibited late I_{Na}. Inhibition was independent of basic cycle length (BCL).



CVT303.059-P: Electrophysiologic Effects of Ranolazine on Late I_{Ca} in Isolated Canine Left Ventricular Myocytes

Ranolazine increased the rate of decay of I_{Ca} in isolated left ventricular myocytes. Late I_{Ca}, defined as current measured 300 ms after the start of the test pulse, was inhibited with an IC50 of 50 μM. The figure below, which shows the I_{Ca} current trace vs time, illustrates that 5 μM ranolazine inhibited late I_{Ca} while only minimally affecting peak I_{Ca}.

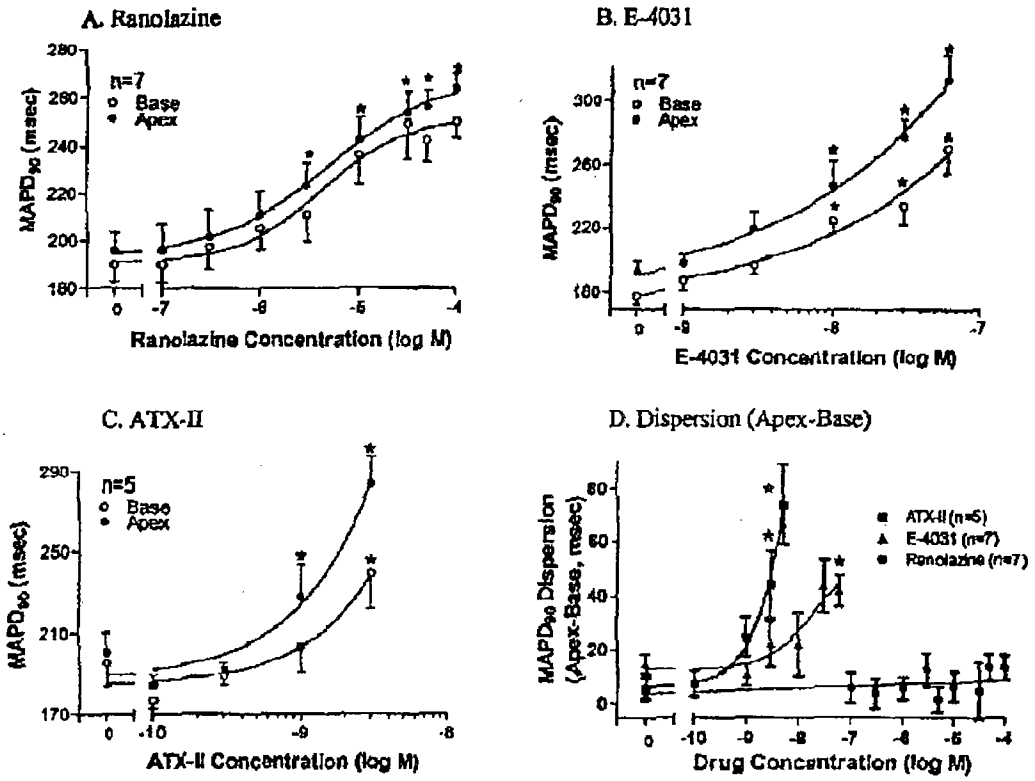


Appears This Way
On Original

CVT303.065-P: Effects of Ranolazine on Ventricular Repolarization in Isolated Rabbit Hearts

Ranolazine and its enantiomers were evaluated for effects on monophasic action potential duration (MAPD) in isolated female rabbit hearts paced at a constant rate of 1 Hz. Epicardial MAPDs were monitored in base and apex of the heart for determination of spatial dispersion. E-4031, which inhibits I_{Kr}, and ATX-II, which enhances the late I_{Na}, were utilized as concurrent control test substances.

Ranolazine at concentrations of 1-100 μM increased MAPD in both base and apex in a concentration-related manner. Effects were similar in base and apex, such that the difference between these two sites, or what the sponsor refers to as dispersion, was not altered. Potencies (EC50s) were similar for both sites (4.3 and 4.8 μM for base and apex, respectively). In comparison, E-4031 and ATX-II increased MAPD and dispersion in a concentration dependent manner. Ranolazine enantiomers also increased MAPD at a single site in a concentration dependent and manner (the site at which MAPD was monitored was not provided). The enantiomers'potencies on MAPD were similar (6.4 and 5.9 M for R and S enantiomers, respectively).



Concentration-response relationships for ranolazine (*A*), E-4031 (*B*) and ATX-II (*C*) to increase the duration of the basal and apical ventricular monophasic action potential ($MAPD_{90}$) and regional (Apex-Base) differences in $MAPD_{90}$ prolongation (Dispersion; panel *D*) in rabbit isolated hearts. Hearts were paced at a rate of 1 Hz. Baseline values for basal and apical $MAPD_{90}$ were 189±7 and 196±7 msec (n=7) for ranolazine; 178±6 and 191±7 msec (n=7) for E-4031; and 188±15 and 190±10 msec (n=5) for ATX-II treated hearts, respectively. Asterisks indicate values significantly different from those in control of individual group ($p<0.05$).

Ranolazine, in contrast to E-4031, did not induce ventricular ectopic beats at any concentration evaluated (data not shown). Ranolazine at concentrations of 5 and 10 μM reduced ventricular ectopic beats induced by E-4031 (data not shown). Additionally, ranolazine at 5 μM attenuated pause dependent ventricular arrhythmias induced by E-4031 and ATX-II (data not shown). The specificity of this in vitro antiarrhythmic effect was not evaluated using other QT prolonging drugs that inhibit multiple ionic currents, e.g. terfenadine or cisapride.

CVT303.070-P: Effects of Ranolazine on Isoproterenol, Forskolin, and Ouabain Induced Delayed Afterdepolarizations and Triggered Activity of Guinea Pig Ventricular Myocytes

Ranolazine at a concentration of 10 μM reduced the amplitude of isoproterenol-induced delayed afterdepolarizations (DADs) and triggered activity in isolated guinea pig ventricular myocytes. Ranolazine did not alter DADs induced by forskolin and ouabain. The effect on isoproterenol-induced DADs is likely due to ranolazine's β1 adrenergic receptor blockade at the concentration evaluated.

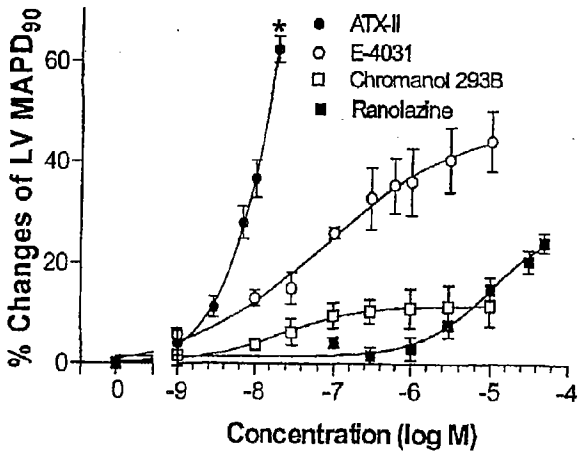
Amplitude of DADs (mV) induced by isoproterenol, forskolin or ouabain before and after addition of ranolazine.

	Isoproterenol (0.1 μM)	Forskolin (3 μM)	Ouabain (20 μM)
Before Ranolazine	11.6±1.1	9.0±1.2	10.1±1.0
After Ranolazine (10 μM)	1.1±0.5	8.5±0.9	9.8±1.4
n	11	6	8
p	< 0.001	0.203	0.840

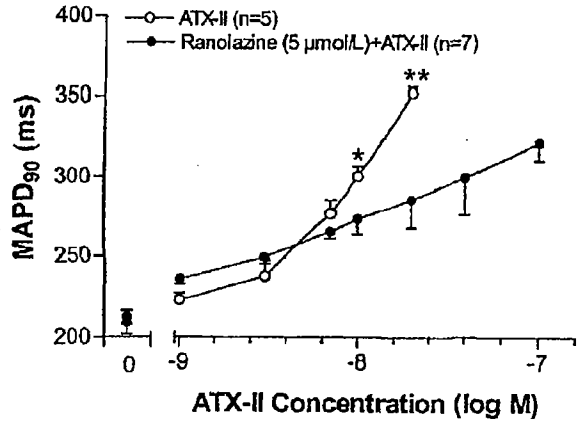
CVT303.061-P: Antiarrhythmic Effects of Ranolazine in a Human LQT Model: The In Vitro Guinea Pig Heart Perfused with the Proarrhythmic Sea Anemone Toxin ATX-II

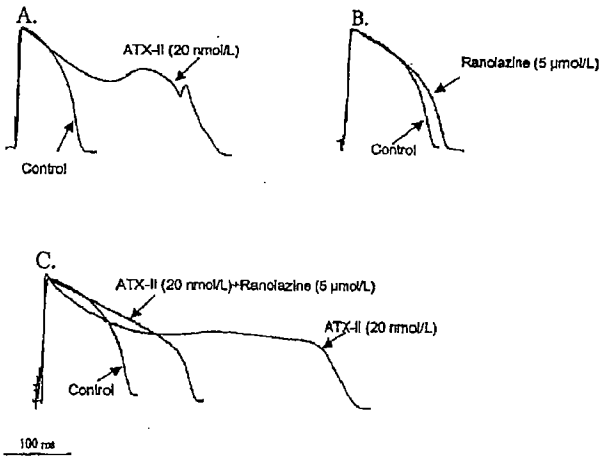
Ranolazine was evaluated for effects on epicardial monophasic action potential duration ($MAPD_{90}$) in isolated guinea pig hearts (gender not provided) paced at a constant rate of 1.5 Hz. Acute AV block was induced by infusion of N^6-cyclypentyladenosine, which blocks adenosine receptors. E-4031, which selectively inhibits I_{Kr}, ATX-II, which selectively enhances the late I_{Na}, and chromanol 293, which inhibits I_{Ks}, were utilized as concurrent control test substances. In an additional experiment, rate dependence of ranolazine was compared to that of the positive control test substances.

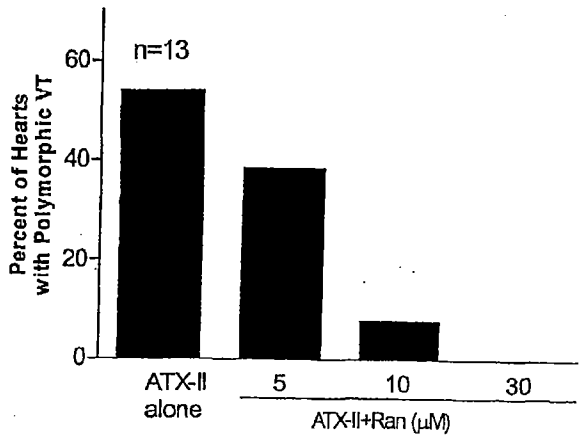
Ranolazine at concentrations of 1-100 μM lengthened epicardial $MAPD_{90}$ in a concentration dependent manner, similar to positive control substances. Ranolazine's effects on $MAPD_{90}$ were independent of pacing cycle length, ranging from 400 to 1000 ms (data not shown). In contrast, E-4031 and ATX-II but not chromanol 293B showed inverse rate dependence, with greater percentage increases at a basic cycle length of 1000 ms than at 400 ms.



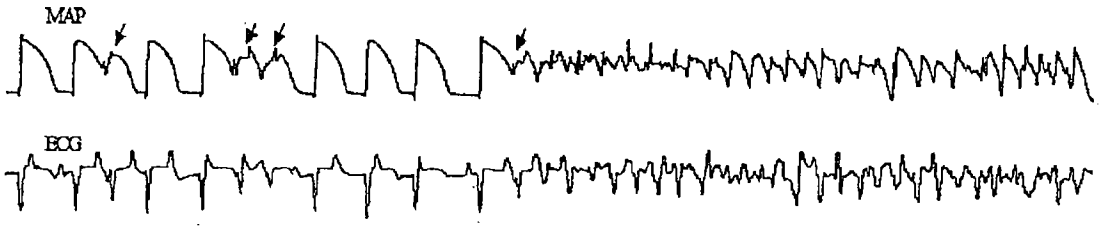
Ranolazine attenuated MAPD lengthening and prevented early afterdepolarizations and polymorphic ventricular tachycardia induced by ATX-II in a concentration related manner. Effects of positive control drugs that inhibit multiple ionic currents (including the late I_{Na}), e.g. terfenadine and cisapride, were not evaluated.



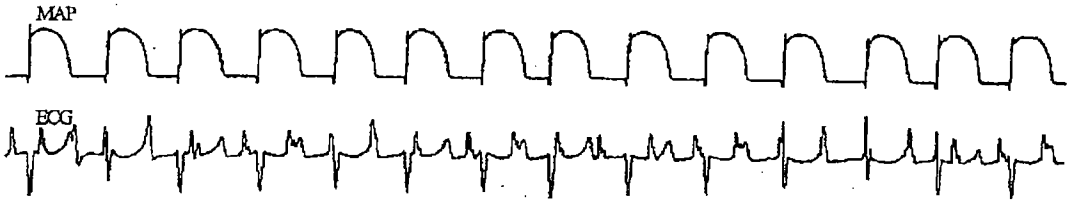




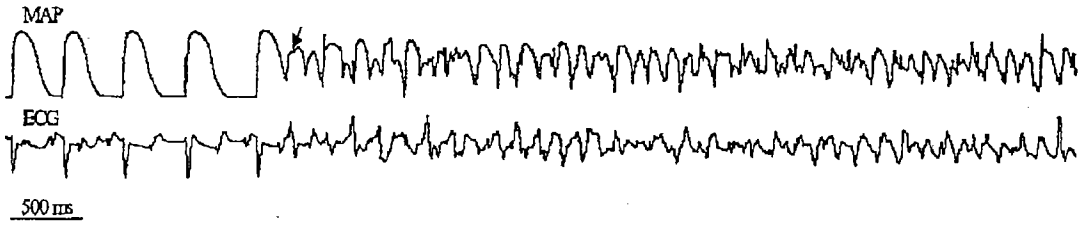
A. ATX-II (20 nmol/L)



B. ATX-II (20 nmol/L)+Ranolazine (5 μmol/L)



C. ATX-II (20 nmol/L after washout ranolazine



CVT303.068-P: Electrophysiologic Effects of Ranolazine in Arterially Perfused Wedge Preparations from the Canine Left Ventricle: A comparison Between Epicardial and Endocardial Stimulation

Endocardial stimulation appears to sensitize the isolated canine left ventricular wedge preparation to arrhythmias induced by QT prolonging drugs, since cisapride's proarrhythmic effect in this model was captured with epicardial, but not with endocardial stimulation. Ranolazine was previously evaluated using endocardial stimulation; however, effects with epicardial stimulation have not yet been evaluated. The purpose of the present study was to evaluate the in vitro electrophysiologic effects of ranolazine in canine wedges stimulated from the epicardial surface. Results were compared to those using endocardial stimulation in the same experiment. Both normokalemic (4 mM potassium) and hypokalemic (3 mM potassium) conditions were utilized, as was pacing the preparations at both short and long basic cycle lengths (500 and 2000 ms) to address rate dependence of effects.

With epicardial stimulation and normokalemia, ranolazine at concentrations of 1-100 μM did not lengthen M-cell or epicardial action potential duration (APD90). With epicardial stimulation and hypokalemia, ranolazine at 100 μM lengthened epicardial but not M-cell APD90.

While ranolazine prolonged transmural QT interval and increased Tpeak –Tend in a concentration dependent manner, it did not lengthen M-cell action potential duration or transmural dispersion of repolarization (TDR) under any conditions. Indeed, ranolazine decreased TDR when evaluated with hypokalemia, likely due to a proportionately greater increase in epicardial vs M-cell APD90.

Canine Left Ventricular Wedge: 4 mM $[K]_o$, BCL=2000 msec, epicardial stimulation

	Epicardium		M region					
Concentration	**APD50 ± SE**	**APD90 ± SE**	**APD50 ± SE**	**APD90 ± SE**	**QT_{end}**	**Tpeak-Tend Area**	**$T_{peak} - T_{end}$**	**TDR**
control	193.5 ± 9.7	231.8 ± 10.0	218.8 ± 10.6	275.3 ± 12.0	291.3 ± 7.5	20.1 ± 2.3	47.5 ± 1.6	63.7 ± 4.0
1 μM	194.4 ± 8.1	238.2 ± 9.8	221.0 ± 2.6	279.5 ± 7.9	297.4 ± 7.5	17.2 ± 2.2	47.4 ± 2.5	58.6 ± 5.7
5 μM	204.4 ± 10.1	245.8 ± 9.4	226.0 ± 6.7	290.2 ± 8.3	303.4 ± 7.6*	16.3 ± 2.0	47.1 ± 2.7	63.7 ± 4.3
10 μM	202.5 ± 8.2	252.3 ± 7.3	224.8 ± 3.3	292.4 ± 8.4	311.7 ± 6.0*	14.7 ± 2.3	51.8 ± 3.0	59.9 ± 6.3
100 μM	179.4 ± 7.5	244.2 ± 4.1	193.4 ± 3.4	285.4 ± 4.6	330.2 ± 6.9*	16.3 ± 3.2	77.4 ± 8.3*	65.2 ± 5.9

* $p<0.05$ vs. control n=5

Canine Left Ventricular Wedge: 3 mM $[K]_o$, BCL=2000 msec, epicardial stimulation

	Epicardium		M region					
Concentration	**APD50 ± SE**	**APD90 ± SE**	**APD50 ± SE**	**APD90 ± SE**	**QT_{end}**	**Tpeak-Tend Area**	**$T_{peak} - T_{end}$**	**TDR**
control	190.1 ± 10.4	237.7 ± 10.7	224.9 ± 13.0	282.1 ± 13.4	302.6 ± 12.4	17.8 ± 3.8	50.9 ± 2.7	60.6 ± 1.9
1 μM	199.5 ± 8.9	245.4 ± 10.7	228.5 ± 12.2	290.4 ± 12.9	310.7 ± 12.1	18.9 ± 4.5	60.5 ± 5.1	62.6 ± 4.6
5 μM	205.0 ± 7.4	253.2 ± 11.0	223.2 ± 11.4	295.5 ± 13.6	322.2 ± 11.2*	15.3 ± 2.5	56.2 ± 3.9	63.7 ± 4.4
10 μM	209.5 ± 6.5	269.5 ± 7.8	234.1 ± 9.5	307.2 ± 7.3	337.8 ± 13.0*	15.1 ± 2.0	61.8 ± 5.8*	59.3 ± 4.3
100 μM	192.6 ± 14.2	294.7 ± 11.5*	194.4 ± 10.5	307.8 ± 8.4	356.3 ± 18.5‡*	12.3 ± 1.5‡	70.6 ± 5.6‡*	32.4 ± 8.3*

* $p<0.05$ vs. control n=5 (unless otherwise noted) ‡n=3

With endocardial stimulation, ranolazine at concentrations of 1-100 μM did not lengthen M-cell or epicardial action potential duration (APD90). Effects were independent of potassium concentration. Ranolazine prolonged transmural QT interval in a biphasic manner, with peak effects at 5-10 μM. Ranolazine increased Tpeak –Tend over control values, but only at the highest concentration evaluated. Ranolazine did not significantly lengthen M-cell action potential duration or increase transmural dispersion of repolarization (TDR). Ranolazine's electrophysiologic effects were similar at basic cycle lengths of 500 and 2000 ms (only effects at 2000 ms are shown).

Canine Left Ventricular Wedge: 4 mM $[K]_o$, BCL=2000 msec, endocardial stimulation

	Epicardium		M region					
Concentration	APD50 ± SE	APD90 ± SE	APD50 ± SE	APD90 ± SE	QT_{end}	Tpeak-Tend Area	$T_{peak} - T_{end}$	TDR
control	187.0 ± 7.3	227.6 ± 9.4	218.0 ± 10.2	275.4 ± 11.6	282.5 ± 8.4	6.3 ± 1.5	24.9 ± 1.9	29.5 ± 2.1
1 μM	192.4 ± 6.3	236.6 ± 8.4	220.0 ± 1.4	279.4 ± 7.3	290.2 ± 8.5	6.4 ± 2.6	26.0 ± 2.9	26.4 ± 2.7
5 μM	198.0 ± 8.2	241.8 ± 8.3	223.8 ± 6.3	288.5 ± 8.2	301.8 ± 8.7*	5.8 ± 2.4	29.1 ± 3.4	29.4 ± 5.2
10 μM	199.7 ± 8.6	249.8 ± 6.4	225.2 ± 2.1	292.0 ± 8.6	307.2 ± 7.4*	6.0 ± 2.4	30.8 ± 2.5	26.0 ± 4.1
100 μM	171.0 ± 4.8	238.5 ± 2.8	192.9 ± 4.4*	284.8 ± 4.3	318.8 ± 1.3‡	8.3 ± 3.2‡	47.1 ± 3.8‡	28.5 ± 7.6

* p<0.05 vs. control. n=5 (unless otherwise noted) ‡n=3 In this and all other tables, measurements are in the following units APD, QT, Tpeak-Tend and TDR are in msec, whereas Tpeak-Tend Area is in mV*msec.)

Canine Left Ventricular Wedge: 3 mM $[K]_o$, BCL=2000 msec, endocardial stimulation

	Epicardium		M region					
Concentration	APD50 ± SE	APD90 ± SE	APD50 ± SE	APD90 ± SE	QT_{end}	Tpeak-Tend Area	$T_{peak} - T_{end}$	TDR
control	186.4 ± 8.6	234.9 ± 10.3	228.6 ± 12.9	285.9 ± 14.3	293.0 ± 12.6	5.3 ± 2.4	33.1 ± 4.0	29.5 ± 7.7
1 μM	192.2 ± 9.2	240.0 ± 11.0	229.8 ± 13.9	289.7 ± 14.6	300.3 ± 12.8	5.2 ± 1.7	33.7 ± 2.7	28.7 ± 7.2
5 μM	193.6 ± 9.1	250.6 ± 10.8	226.5 ± 11.7	298.4 ± 13.7	314.4 ± 11.5*	3.3 ± 1.0	34.8 ± 3.0	28.0 ± 5.4
10 μM	198.9 ± 7.7	262.1 ± 8.2	236.4 ± 8.9	307.9 ± 7.0	318.8 ± 8.8	3.9 ± 1.1	32.6 ± 1.6	25.0 ± 5.6
100 μM	181.6 ± 14.7	285.3 ± 10.1*	198.4 ± 12.0	312.2 ± 9.6	347.7 ± 16.2‡*	3.6 ± 0.5‡	47.0 ± 4.8‡	12.9 ± 3.7

* p<0.05 vs. control n=5 (unless otherwise noted) ‡n=3

Ranolazine did not induce *torsade de pointes* - type arrhythmias in vitro in canine left ventricular wedges using epicardial stimulation.

Test Substance	Potassium (μM)	Spontaneous Arrhythmia	Stimulation-induced Arrhythmia^
Ranolazine (1-100 μM)	4	0/5	0/5
Ranolazine (1-100 μM)	3	0/5	0/5

^ The sponsor attempted to induce ventricular arrhythmias using a single extrastimulus applied to the epicardial surface at progressively shorter intervals until refractoriness was reached. This methodology was successful in eliciting ventricular arrhythmias in 2 of 6 wedge preparations exposed to cisapride (0.2 μM). Spontaneous arrhythmias were not observed with cisapride. Cisapride's proarrhythmic effects were limited to a single concentration, with slightly higher and lower concentrations yielding no arrhythmias.

Assessing Predictors of Drug-Induced *Torsade de Pointes*

The sponsor provided a manuscript arguing that delayed ventricular repolarization and proarrhythmia are separable, i.e. drugs that do not induce early afterdepolarizations or increase dispersion of repolarization are unlikely to cause TdP, even in the setting of prolonged QT.

Sponsor's Abstract

Torsades de Pointes (TdP) is a malignant polymorphic ventricular tachyarrhythmia that can be caused by drugs that induce electrophysiological changes. Although the number of drugs known to cause TdP has increased in recent years, there is no cell-based assay, *in vitro* heart preparation, or animal model that predicts a drug's potential to induce TdP in humans. Nevertheless, certain electrophysiologic events are known to be associated with the development of TdP. A drug that prolongs action potential duration, induces early afterdepolarizations and ectopic beats, and increases dispersion of ventricular repolarization is very likely to cause TdP. By contrast, a drug that does not induce these changes is unlikely to cause TdP. The exact relationship between prolonged action potential duration, early afterdepolarizations, ectopic beats, increased dispersion of ventricular repolarization, and the development of TdP has not been defined, but the potential of a drug to elicit these events might predict its pro-arrhythmic risk.

The sponsor supports the hypothesis that torsadogenic drugs are always associated with early afterdepolarizations and spatial dispersion of repolarization with data from several in vitro models. The sponsor did not provide a comprehensive assessment of the sensitivity and specificity of any single model.

Appears This Way
On Original

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

John Koerner
10/23/03 12:09:20 PM
PHARMACOLOGIST

Charles Resnick
10/23/03 01:18:11 PM
PHARMACOLOGIST

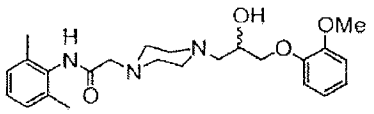
Memo to the File

NDA number:21-526 Elizabeth Hausner, D.V.M.
Sequence number/date/type of submission: N(000)B2 September29, 2003
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: CVT Therapeutics
Manufacturer for drug substance :
Division name: Division of Cardio-Renal Drug Products
HFD #: 110
Drug:

Trade name: Ranexa
Generic name (list alphabetically): ranolazine
Code name: RS-43285-193, RS-43285-003, CVT-303, RAN D, Ran4
Chemical name:

IUPAC:	N-(2,6-dimethylphenyl)-2-{4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]piperazinyl}acetamide
CAS[1]:	1-piperazineacetamide, N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-
Other:	(±)-4-[2-hydroxy-3-(*o*-methoxyphenoxy)propyl]-1-piperazineaceto-2',6'-xylidide

CAS registry number: 95635-55-5
Mole file number:
Molecular formula/molecular weight: $C_{24}H_{33}N_3O_4$/427.54
Structure:



Ranolazine

Relevant INDs/NDAs/DMFs: IND 43,735
Drug class: anti-anginal
Indication: angina
Route of administration: oral
Proposed use: angina for those patients in whom all other anti-anginals are inadequate or not tolerated

The current submission was provided to try to address questions raised in the Discipline Review Letter. The sponsor has submitted the following:

Cerep 951003: In Vitro Pharmacology - Study of Several Compounds. Binding of Ranolazine, Its Enantiomers and Eleven Metabolites to the alpha1-adrenergic Receptors and Opiate Receptors. Effects of Ranolazine, Its R- and S-enantiomers on phenylephrine-induced Contraction in Isolated Rabbit Aorta.

Cerep 951006 and 951009: Binding of Ranolazine, Its Enantiomers and Eleven Metabolites to Human Serotonin 5-HT1A Receptors

CVT303.029-N: Binding of Ranolazine, Its Enantiomers and Three Metabolites (CVT -2514, CVT -2551 and CVT -3388) to the alpha-adrenergic Receptors in Rat Tissues (Addendum to Cerep 951003)

CVT303.030-N: Binding of Ranolazine, Its Enantiomers and Eleven Metabolites to the

beta-adrenergic Receptors and their Antagonism of Isoproterenol-induced Increase in cAMP

CVT303.059-P: Electrophysiologic Effects of Ranolazine on Late ICa in Isolated Canine Left Ventricular Myocytes

CVT303.061-P: Ant arrhythmic Effects of Ranolazine in a Human LQT Model: The In Vitro Guinea Pig Heart Perfused with the Proarrhythmic Sea Anemone Toxin ATX-II

CVT303.062-P: Functional Evidence of Anti-alpha Adrenergic Activity of Ranolazine in Awake Rats

CVT303.063-P: Effects of Ranolazine Enantiomers on IKs, IKr, and Late INa, and Ranolazine Metabolites on Late Ina

CVT303.064-P: Functional Evidence of Anti-beta Adrenergic Activity of Ranolazine in Awake Rats

CVT303.065-P: Effects of Ranolazine on Ventricular Repolarization in Rabbit Isolated Hearts

CVT303.066-P: Effect of Ranolazine, Its Rand S Enantiomers, and Eleven Metabolites on Rat Left Atrial Contractility

CVT303.067 -P: Effects of Ranolazine on Systemic Hemodynamics and Coronary Circulation in Conscious Dogs

CVT303.068-P: Electrophysiologic Effects of Ranolazine in Arterially-perfused Wedge Preparations from the Canine Left Ventricle: A Comparison Between Epicardial and Endocardial Stimulation

CVT303.069-P: Effect of Ranolazine on IKs in Isolated Canine Left Ventricle Myocytes

CVT303.070-P: Effects of Ranolazine on Isoproterenol-, Forskolin-, and Ouabain-induced Delayed Afterdepolarizations and Triggered Activity of Guinea Pig Ventricular Myocytes

MDS 1011172: Effect of the R- and the S-enantiomers of Ranolazine on the Inhibition of Neurogenic Twitch in Isolated Guinea Pig Ileum (a Serotonin 5-HT1A Receptor-mediated Response)

MDS 1011220: Binding of Ranolazine, Its Enantiomers and Eleven Metabolites to the Benzothiazepine, Dihydropyridine and Phenylalkylamine-binding Sites of the L-type Calcium Channels

MDS 1033853: Effects of Ranolazine on Serotonin 5-HT1A, 5-HT2A and 5-HT2B Receptor-mediated Responses in Isolated Tissues

CVT303.013-R: Pharmacokinetics of Enantiomers of CVT -303 (Ranolazine), CVT -3758 (S-Enantiomer), and CVT -3759 (R-Enantiomer) in Male Beagle Dogs Following Intravenous Administration of CVT -303, CVT -3758 and CVT-3759

CVT303.014-R: Pharmacokinetics of Enantiomers of CVT -303 (Ranolazine), CVT -3758 (S-Enantiomer), and CVT -3759 (R-Enantiomer) in Male Sprague Dawley Rats Following Intravenous and Oral Administration of CVT -303, CVT -3758 and CVT -3759 CVT303.015-R: Pharmacokinetics of Enantiomers of CVT -303 (Ranolazine), CVT -3758 (S-Enantiomer), and CVT -3759 (R-Enantiomer), in Male Beagle Dogs Following Oral Administration of CVT -303, CVT -3758 and CVT -3759

Attachment 5-1: Assessing Predictors of Drug-induced Torsade de Pointes
Attachment 5-2: Ophthalmology Data
Attachment 5-3: Pathology Data
Attachment 5-4: Summary of Results. Organ Weights and Microscopic Findings for Male and Female Reproductive Organs in Individual Repeated Dose Oral Studies in Rats and Dogs

This memo is not so much a review as a statement and brief commentary on the material provided.

Cerep 951003 Binding of ranolazine, its enantiomers and eleven metabolites to the α1-adrenergic receptors and opiate receptors. Sept 10, 2003: Ranolazine and the R and S enantiomers showed < 20% inhibition of specific 3H-naloxone binding to opiate receptors in membranes prepared from rat cerebral cortex. Two of the metabolites (CVT-3388 and CVT5030) showed significant binding to the opiate receptors with Ki values of 2.7 and 2.3 μM respectively. Both of these metabolites produced 68% inhibition of specific tritiated naloxone binding. Ranolazine, both enantiomers and several metabolites showed significant α-adrenergic receptor binding as summarized in the reviewer's table below.

Ki values (μM) of test compounds for α-adrenergic and opiate receptors in rat cerebral cortex

	α-adrenergic receptors	Opiate receptors
Ranolazine	1.9	n.d.
R-enantiomer	2.1	n.d.
S-enantiomer	1.7	n.d.
CVT-2514	4.9	n.d.
CVT-2551	2.6	n.d.
CVT-3388	1.5	2.7
CVT-5030	>30	2.3

n.d.= not done

Ranolazine and both enantiomers were also effective in the phenylephrine-induced contraction in rabbit aorta, supporting α-adrenergic ability.

Cerep 951006 and 951009 Summary: Binding of ranolazine, its enantiomers and eleven metabolites to human serotonin 5-HT_{1A} receptors. Sept 10, 2003
The report lists Ki values for ranolazine and its enantiomers for opiate receptors as well as $5HT_{1A}$ binding. These are summarized in the reviewer's table below.

	Ki value (μM) for opiate receptors	%inhibition of specific binding (10μM)human serotonin 5HT1A in mammalian cells (Ki μM)
Ranolazine	2.1	71
R-enantiomer	13.0	32
S-enantiomer	1.0	81
CVT-3248		6
CVT-2537		13

CVT-3388		52
CVT-2551		80
CVT-2514		64
CVT-2513		27
CVT-2512		13
CVT-2738		20
CVT-2535		-10
CVT-4786		12
CVT-5030		39

CVT303.029-N : Addendum to Cerep 951003, Binding of ranolazine, its enantiomers and 3 metabolites to α-adrenergic receptors. The study confirmed the previous findings that ranolazine and several of the metabolites showed α-adrenergic binding. The sponsor's table is shown below.

Table 1. IC_{50} values (μM) of test compounds for α-adrenergic receptors in rat tissues.

tissues (receptor subtype)	gland (α1A)	liver (α1B)	brain (α1A/B)
compound		IC50 values, μM	
Ran	13.9	34.2	19.0
Ran-R	30.4	39.6	30.8
Ran-S	8.3	27.7	14.8
CVT-3388	12.8	25.4	18.9
CVT-2514	30.2	18.9	26.5
CVT-2551	14.2	28.7	21.2

Table 2. K_i values (μM) of test compounds for α-adrenergic receptors in rat tissues.

tissues (receptor subtype)	gland (α1A)	liver (α1B)	brain (α1A/B)
compound		Ki values, μM	
Ran	5.6	18.9	10.2
Ran-R	12.3	21.9	16.5
Ran-S	3.4	15.3	7.9
CVT-3388	5.2	14.1	10.2
CVT-2514	12.2	10.5	14.2
CVT-2551	5.7	15.9	11.4

The affinities of test compounds (i.e. K_i values) were calculated based on the Cheng Prusoff equation, $K_i=IC_{50}/(1+(L/K_D))$, where L is the concentration of radioligand in the assay and K_D is the affinity of ^{3}H-prazosin for a-adrenergic receptors. The K_D values for a-adrenergic receptors in rat salivary gland, rat liver and rat brain are 0.17 nM, 0.31 nM and 0.29 nM, respectively (1-4).

Electrophysiology studies:

The electrophysiology findings provided do not supersede the clinical findings of QT prolongation and cannot be extrapolated to provide human safety information

CVT303.064-P Functional evidence of anti-beta adrenergic activity of ranolazine in awake rats. July-August 2003. The study provides evidence that ranolazine has functional β-adrenergic antagonistic activity in conscious rats. Atenolol was used as a comparator compound in attempts to modify the dose-response curve of isoproterenol. A less specific β-blocker such as carvedilol might have been a more appropriate comparator. The sponsor does not show time course data for the cardiovascular effects

CVT303.067-P Effects of ranolazine on systemic hemodynamics and coronary circulation in conscious dogs. August 11, 2003-September 5, 2003. Chronically instrumented dogs were given increasing intravenous doses of ranolazine with a 30 minute interruption of infusion between each dose (bolus + infusion). The experimental protocol for each dose lasted 45 minutes with recordings of the measured parameters obtained at 0, 5,10, 15, 20, 30 and 45 minutes and blood samples collected after each recording except for 0 minutes. However, the only data presented or discussed was the 0 and 15 minute data. This "snapshot" view of the results is unhelpful and unacceptable. As reported, the study is inconclusive.

MDS 1011172 Effect of the R- and S- enantiomers of ranolazine on the inhibition of neurogenic twitch in isolated guinea pig ileum (a serotonin 5-HT_{1A} receptor-mediated response). August 28, 2003 Apparently significant agonism was seen as demonstrated by a ≥ 50% decrease in field stimulated contractile responses by CVT3758 (S-enantiomer, 68% agonism at 10 μM)and CVT-3759(R-enantiomer, 157% agonism at 10 μM) at concentrations of ≥10μM. A selective serotonin $5HT_{1A}$ antagonist WAY-100635 produced minimal reversal of the enantiomers effects. Was there a problem in the assay or are the effects due to factors other than serotonin antagonism? A dose response was shown in the results.

MDS1033853 Effects of ranolazine on serotonin 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2B} receptor-mediated responses in isolated tissues. Ranolazine inhibited the neurogenic twitch in isolated guinea pig ileum (a serotonin 5-HT_{1A} receptor mediated response) with an EC_{50} value of 5.3μM. No activity was reported for $5HT_{2A}$ and $5HT_{2B}$ receptors as measured by contraction or inhibition of agonist-induced contraction in rat aorta or stomach respectively.

MDS 1011220 Binding of ranolazine, its enantiomers and eleven metabolites to the benzothiazepine, dihydropyridine and phenylalkylamine binding sites of the L-type calcium channels. August 14, 2003 Very minimal calcium channel binding was demonstrated.

CVT303.013-R Pharmacokinetics of enantiomers of CVT-303(ranolazine), CVT-3758(S-enantiomer) and CVT-3759(R-enantiomer) in male Beagle dogs following intravenous administration of CVT-303, CVT-3758 and CVT-3759. June 4- June17, 2003
Three male dogs were used in this pharmacokinetic comparison of the enantiomers when given in the racemic mix and when given individually. The plasma analysis procedure used produced baseline resolution of the enantiomers with retention times of 9.6 (CVT-3758) and 15.5 (CVT-3759) minutes. The sponsor's results are shown below.

Pharmacokinetic parameters of the two enantiomers are summarized in the following t

Compound Dosed	CVT-303 (Racemate)		CVT-3758 (S)	CVT-3759 (R)
Dose	0.5 mg/kg total 0.25 mg/kg each enantiomer		0.5 mg/kg	0.5 mg/kg
Enantiomer	CVT-3758 (S)	CVT-3759 (R)	CVT-3758 (S)	CVT-3759 (R)
$AUC_{(0-t)}$ (ng•hr/mL)	93.1±5.85	57.3±5.90	168±15.2	138±10.1
$AUC_{(0-\infty)}$ (ng•hr/mL)	100±7.03	67.2±6.14	180±20.0	143±11.8
CL_p (mL/min/kg)	41.7±2.91	62.4±5.75	46.8±5.42	58.4±4.98
Vd_β (L/kg)	1.92±0.104	2.00±0.303	2.09±0.154	2.21±0.126
$t_{1/2}$ (hr)	0.53±0.03	0.37±0.03	0.52±0.08	0.44±0.05

Values represent mean±SD of three dogs.

There appear to be slight differences in the AUC and clearance values for the enantiomers where the S enantiomers appears in the plasma at a ratio of 1.1 to 1.7 compared to the R- enantiomers. The clearance for the R-enantiomer is slightly higher than that for the S-enantiomer. AUC for the S-enantiomer was also slightly greater than AUC for the R-enantiomer. While the results are consistent between the administration of the racemic mix and the single isomers, the sample size is small. Also, it was reported that both compounds were below the limits of detection by 1(R-enantiomer) to 2(S-enantiomer) hours after intravenous dosing. Therefore, the sampling times of 2,5,15 and 30 minutes followed by 1,2,4,6,8,10 12 and 24 hours post-dose were not optimal for characterization. The report noted that the R enantiomer was not found after intravenous dosing of the S-enantiomer and vice versa.

CVT303.014R Pharmacokinetics of enantiomers of CVT-303, CVT-3758 and CVT-3759 in male Sprague-Dawley rats following intravenous and oral administration of CVT303, CVT3758 and CVT3759. August7-18, 2003. Oral and intravenous doses of the racemic mix and the enantiomers were tested. Quantification was by the same chiral separation methods as used for the dog study. Blood samples were collected from the rats at 2.5, 5, 15.30 minutes and 1, 2, 4, 6, 8 and 24 hours after intravenous dosing and at 5, 15, 30 minutes and 1, 1.5, 2, 4, 6, 8 and 24 hours after oral dosing.

The sponsor's results are shown below.

Summary Table 1: Pharmacokinetics of CVT-3758 and CVT-3759 Following Intravenous and Oral Administration of CVT-303

Compound Dosed	CVT-303 (Racemate)		CVT-303 (Racemate)		CVT-303 (Racemate)	
	Intravenous Doses (mean ±SD, n=3)					
Dose[a]	5 mg/kg		10 mg/kg		25 mg/kg	
Enantiomer	CVT-3758 (S)	CVT-3759 (R)	CVT-3758 (S)	CVT-3759 (R)	CVT-3758 (S)	CVT-3759 (R)
$AUC_{(0-t)}$ (ng•hr/mL)	536±72.8	464±57.5	1,168±64.9	953±42.7	3,263±418	2,781±418
$AUC_{(0-\infty)}$ (ng•hr/mL)	571±31.9	479±46.2	1,181±64.5	961±42.6	3,275±425	2,792±415
CL_p (mL/min/kg)	73.1±4.04	87.5±8.11	70.7±3.95	86.8±3.90	64.3±8.29	75.7±10.9
Vd_β (L/kg)	4.85±0.804	4.95±0.32	4.43±0.373	4.74±0.290	4.44±0.275	4.75±0.29
$t_{1/2}$ (hr)[d]	0.76±0.09	0.66±0.056	0.72±0.06	0.63±0.01	0.80±0.07	0.73±0.09
	Oral Doses (mean ±SD, n=4)					
Dose	5 mg/kg		20 mg/kg		50 mg/kg	
$AUC_{(0-t)}$ (ng•hr/mL)	187±56.0	120±46.3	1,536±365	1,133±306	4,607±896	3,524±614
$AUC_{(0-\infty)}$ (ng•hr/mL)	203±62.0	133±48.7	1,782±453	1,253±345	4,824±1,129	3,580±772
C_{max} (ng/mL)	120±18.7	91.5±17.4	834±195	739±184	1,933±837	1,847±869
T_{max} (hr)	0.44±0.13	0.44±0.13	0.31±0.13	0.31±0.13	0.33±0.20	0.208±0.08
F (%)	35.7±10.9	27.6±10.1	78.2±19.9	65.2±18.0	84.6±19.8	74.7±16.1

[a] Values represent dose of CVT-303 (racemate). The dose of the individual enantiomers would be half.

Summary Table 2: Pharmacokinetics of CVT-3758 and CVT-3759 Following Administration of the Individual Enantiomers

Route	Intravenous (mean ±SD, n=3)		Oral (mean ±SD, n=4)	
Enantiomer Dosed	CVT-3758 (S)	CVT-3759 (R)	CVT-3758 (S)	CVT-3759 (R)
Dose[a]	5 mg/kg	5 mg/kg	5 mg/kg	5 mg/kg
$AUC_{(0-t)}$ (ng•hr/mL)	812±36.4	876±182	400±97.7	277±110
$AUC_{(0-\infty)}$ (ng•hr/mL)	823±42.1	895±169	457±20.5	297±115
CL_p (mL/min/kg)	101±5.1	95.7±20.2	nc[b]	nc
Vd_β (L/kg)	6.00±0.56	4.92±0.170	nc	nc
$t_{1/2}$ (hr)	0.69±0.09	0.61±0.10	nc	nc
C_{max} (ng/mL)	nc	nc	227±97.7	199±65.0
T_{max} (hr)	nc	nc	0.438±0.24	0.25±0.00
F(%)	nc	nc	55.4±12.5	33.2±12.9

[a] Values represent dose of the individual enantiomers CVT-3758 and CVT-3759.
[b] nc = Not calculated.

At each dose by both routes of administration, the S-enantiomer produced non-significantly higher plasma levels than did the R-enantiomer. Clearance was also slightly higher for the R-enantiomer. Bioavailability was slightly greater for the S-enantiomer. The results are consistent with the dog study. The report noted that the R enantiomer was not found after either oral or intravenous dosing of the S-enantiomer and vice versa.

CVT303.015-R Pharmacokinetics of enantiomers of CVT-303, CVT-3758 and CVT-3759 in male Beagle dogs following oral administration of CVT-303, CVT3758 and CVT3759. August 11-August 27, 2003. Four male Beagles received oral doses of either the racemic mixture or the enantiomers of ranolazine. Blood samples were collected at 2,5, 15, and 30 minutes and at 1,2,4,6,8,10, 12 and 24 hours post-dose. The results from this study were consistent with the previous two studies. The report noted that CVT-3759 was not found in plasma after administration of CVT-3758 and vice versa.

Assessing predictors of drug-induced torsade de pointes. Accepted in Trends in Pharmacological Sciences. Noted as scheduled for publication in December 2003.
This is a preprint of a manuscript authored by the sponsor the details the sponsor's criteria for deciding pro-arrhythmic risk from preclinical studies.

Attachment 5-2: In a telecom, this reviewer expressed a concern that none of the preclinical toxicology reports included an ophthalmologist's report, nor any indication that a board certified veterinary ophthalmologist had examined any of the animals in any studies. In fact, there are recent studies where it was specified that examinations were conducted by a staff veterinarian instead of an ophthalmologist. In response to that concern, the sponsor has sent the individual animal data for the ophthalmic exams for the following studies:

AT3465: RS-43285RHT: Three month oral toxicity study in rats
AT3935: RS43285 RJT: Six month oral toxicity study in rats with one month recovery period.
AT6544: RS-43285-RKT: One year rat toxicity study
AT3440: RS-43285DHT: Three month oral toxicity study in dogs
AT4050: RS-43285DJC:six month oral toxicity study in dogs
AT6971: RS-43285 DKC: One year oral toxicity study in dogs

While the individual animal sheets are signed, there is no indication that the person signing is a qualified veterinary ophthalmologist. There is no summary of the findings, and no statement from the person conducting the examinations as to a conclusion.

Attachment 5.3 Pathology data
In the above mentioned telecom, this reviewer also noted that there was no complete summary of histopathologic findings provided for any study and that the absence of data did not mean that one could assume that there was no problem. In this attachment, the sponsor has provided material for the following studies:
AT3465: RS-43285RHT: Three month oral toxicity study in rats
AT3935: RS43285 RJT: Six month oral toxicity study in rats with one month recovery period.
AT6544: RS-43285-RKT: One year rat toxicity study
AT3440: RS-43285DHT: Three month oral toxicity study in dogs
AT4050: RS-43285DJC:six month oral toxicity study in dogs
AT6971: RS-43285 DKC: One year oral toxicity study in dogs

The material submitted does not significantly contribute to elucidation of the histopathological questions.

Summary: The material submitted does not materially change the preclinical characterization of ranolazine.

Appears This Way
On Original

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Elizabeth Hausner
9/29/03 01:43:58 PM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
10/7/03 03:49:11 PM
PHARMACOLOGIST

Consult for NDA 21526

MEMORANDUM
DIVISION OF CARDIO-RENAL DRUG PRODUCTS INTERNAL CONSULTATION

To: Douglas Throckmorton, M.D., Division Director
HFD 110, Division of Cardio-Renal Drug Products

From: John Koerner, Ph.D., Pharmacologist
HFD 110, Division of Cardio-Renal Drug Products

Through: Charles Resnick, Ph.D., Supervisory Pharmacologist
HFD 110, Division of Cardio-Renal Drug Products

CC: Elizabeth Hausner, D.V.M., Pharmacologist
HFD 110, Division of Cardio-Renal Drug Products

Albert DeFelice, Ph.D., Supervisory Pharmacologist
HFD 110, Division of Cardio-Renal Drug Products

NDA: 21526
Sponsor: CV Therapeutics, Inc.
Drug: RANEXA, Ranolazine
Proposed Clinical Indication: Angina

Date Completed: 09/03/03

Re: Nonclinical Electrophysiological and Proarrhythmic Effects of Ranolazine

Executive Summary

Ranolazine's nonclinical electrophysiological effects are generally consistent with QT interval prolongation observed in clinical trials. However, the sponsor provided information arguing against proarrhythmic properties of ranolazine.

According to theory, torsade de pointes with QT prolonging drugs arises from increased transmural dispersion of repolarization and triggered activity (early afterdepolarizations) rather than from QT interval prolongation, per se. The elegant models of Antzelevitch argue that findings in isolated canine ventricular strips and wedge preparations, in particular action potential lengthening and early afterdepolarizations in M-cells, transmural dispersion of repolarization, and induction of torsade-like arrhythmias in vitro can predict proarrhythmic potential of a drug. Theory is supported in part utilizing data from positive control drugs such as amiodarone, cisapride, d-sotalol, erythromycin, quinidine and terfenadine.

While findings with ranolazine are primarily negative in the isolated canine ventricular wedge preparation, ranolazine increases M-cell action potential duration and transmural dispersion of repolarization under conditions of hypokalemia, consistent with its ability to inhibit repolarizing currents, I_{Kr} and I_{Ks}, at concentrations similar to those required for its proposed mechanism of action. Moreover, proarrhythmic potential was not adequately evaluated since ranolazine was not tested under conditions necessary to capture cisapride-induced arrhythmias in this model. Additionally, in vivo evaluation for proarrhythmia was not comprehensive.

Additional regulatory concerns reflect the complex nature of any proarrhythmia model, lack of evaluation of metabolites in this in vitro model, and adequacy of testing for proarrhythmia, utilizing known risk factors for clinical arrhythmias, e.g. female gender, different pacing modalities (pause, acceleration), adrenergic influences, and heart failure.

For these reasons, study results provided by the sponsor cannot preclude the risk of proarrhythmia with ranolazine.

Review and Evaluation

Ionic Currents

Ranolazine inhibited both repolarizing currents (I_{Kr} and I_{Ks}), depolarizing currents ($I_{Na\text{-}Late}$, and $I_{Ca\text{-}L}$), and I_{NaCa} in isolated canine ventricular myocytes.

Rank order for potency on native currents was $I_{Kr} = I_{Ks} > I_{Na\text{-}Late} > I_{NaCa} > I_{Ca\text{-}L}$.

Ionic Current (Canine Ventricular Myocytes)	IC50 (μM)
I_{Kr}	11.4
I_{Ks}	13.4
I_{K1}	> 100
$I_{Ca\text{-}L}$	296
$I_{Na\text{-}Late}$	21
I_{NaCa}	91

Ranolazine was also shown to weakly inhibit cloned human potassium channels (hERG and KvLQT1), with IC50s of 86 μM and 1.46 mM, respectively. While this data demonstrates the ability of ranolazine to block human repolarizing currents, it is not useful for potency estimation since studies were performed using a *Xenopus* oocyte expression system, which greatly underestimates potency. For example, IC50s for concurrent positive controls dofetilide and verapamil were approximately 20 -30 fold greater in oocytes than in mammalian expression systems (1,2). Additionally, KvLQT1 channels were not coexpressed with minK, which likely further underestimates ranolazine potency on this repolarizing channel (3,4).

Ionic Current (cloned human channel expressed in Xenopus oocytes)	IC50 (μM)	
	hERG	KvLQT1
Ranolazine	83	1460
Dofetilide*	0.3	-
Verapamil*	5.27	-

* IC50s for inhibition of hERG expressed in mammalian expression system: dofetilide, 0.015 μM; verapamil 0.143 μM (literature reference values (1,2).

The sponsor evaluated effects of several metabolites at a single concentration of 50 μM on I_{Kr} in canine ventricular myocytes. Several metabolites inhibited IKr by about 50% at this concentration. The sponsor did not evaluate effects of metabolites on other ionic currents, including I_{Ks}.

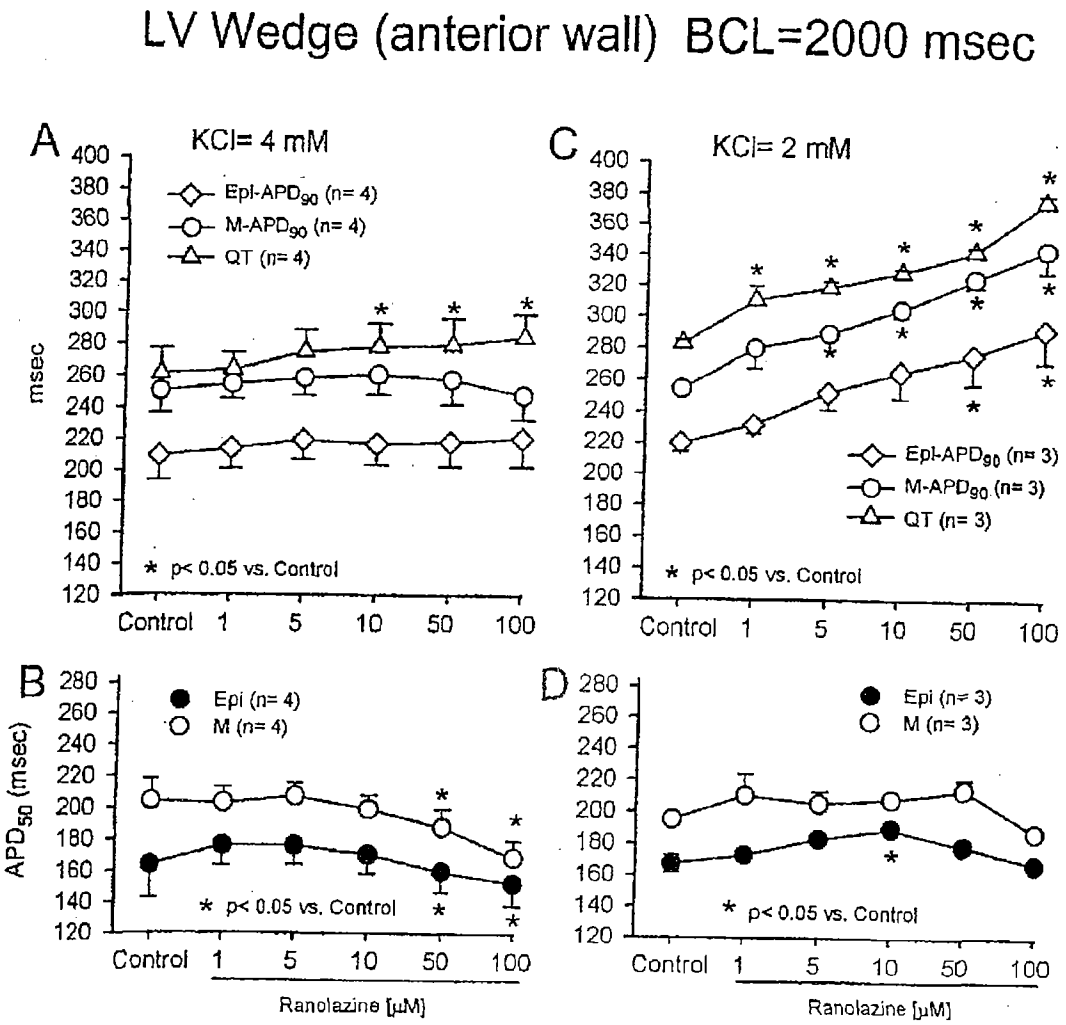
Appears This Way
On Original

Action Potential Duration

Ranolazine was evaluated for effects on action potential duration in several systems, including epicardium and M-cells from canine left ventricular strips. Concentrations evaluated ranged from 1 to 100 μM. In canine epicardial strips, ranolazine (≥ 5 μM) lengthened APD90 in a concentration dependent way. Effects were potassium and rate dependent, with lengthening slightly more pronounced with hypokalemia (2 vs 4 mM potassium) and a more rapid stimulation rate (500 ms vs 2000 ms basic cycle lengths; i.e., 120 vs 30 beats per minute). Ranolazine did not lengthen APD90 in M-cell strips.

Ranolazine was also evaluated for effects on action potential duration in isolated, buffer perfused canine ventricular wedge preparations. Ranolazine was evaluated in epicardium and M-cells at concentrations of 1- 100 μM, under conditions of normokalemia and hypokalemia (4 and 2 mM potassium) using basic cycle lengths of 500 and 2000 ms (stimulation frequencies of 120 and 30 beats per min, respectively).

In the presence of normokalemia, ranolazine did not lengthen APD90 in epicardium and M-cells. However, in the presence of hypokalemia, ranolazine lengthened APD90 in a concentration dependent way in both of these tissues.



Ranolazine also prolonged transmural QT interval, Tpeak to Tend, and transmural dispersion of repolarization, and altered T-wave morphology (wide, low, notched; see below). Ranolazine (10 and 100 μM) also lowered maximum upstroke velocity, indicating sodium channel blockade at concentrations evaluated. Maximum upstroke velocity was lowered by approximately 50% at 100 μM. Exposure duration and time course of effects were not provided.

Canine Left Ventricular Wedge: 4 mM $[KCl]_o$, BCL=2000

	Epicardium		M region				
Concentration	APD50 ± SE	APD90 ± SE	APD50 ± SE	APD90 ± SE	QT_{end}	$T_{peak} - T_{end}$	TDR
control	164 ± 21	209.3 ± 15.76	204.5 ± 13.9	250 ± 13.93	261.1 ± 15.76	34.25 ± 2.56	43 ± 6
1 μM	176.3 ± 12.25	213.8 ± 13.28	203.3 ± 9.621	254.3 ± 9.15	263.5 ± 10.56	34.5 ± 3.202	26.75 ± 8.045
5 μM	176.5 ± 11.85	219 ± 12.12	207.5 ± 8.627	258.3 ± 11.08	274.5 ± 13.73	37.75 ± 4.09	36 ± 2.449
10 μM	170.5 ± 12.03	216.5 ± 13.41	199 ± 9.083	260.3 ± 12.66	277.8 ± 14.99*	39.25 ± 5.54	30.75 ± 10.46
50 μM	159.5 ± 12.82*	218 ± 15.91	187.8 ± 11.21*	257.5 ± 15.47	279.3 ± 17.21*	41.25 ± 8.37	32.5 ± 6.278
100 μM	152.5 ± 14.44*	220.5 ± 18.26	169 ± 10.5*	247.8 ± 15.32	284.5 ± 14.39*	40.5 ± 4.94	23.75 ± 2.689

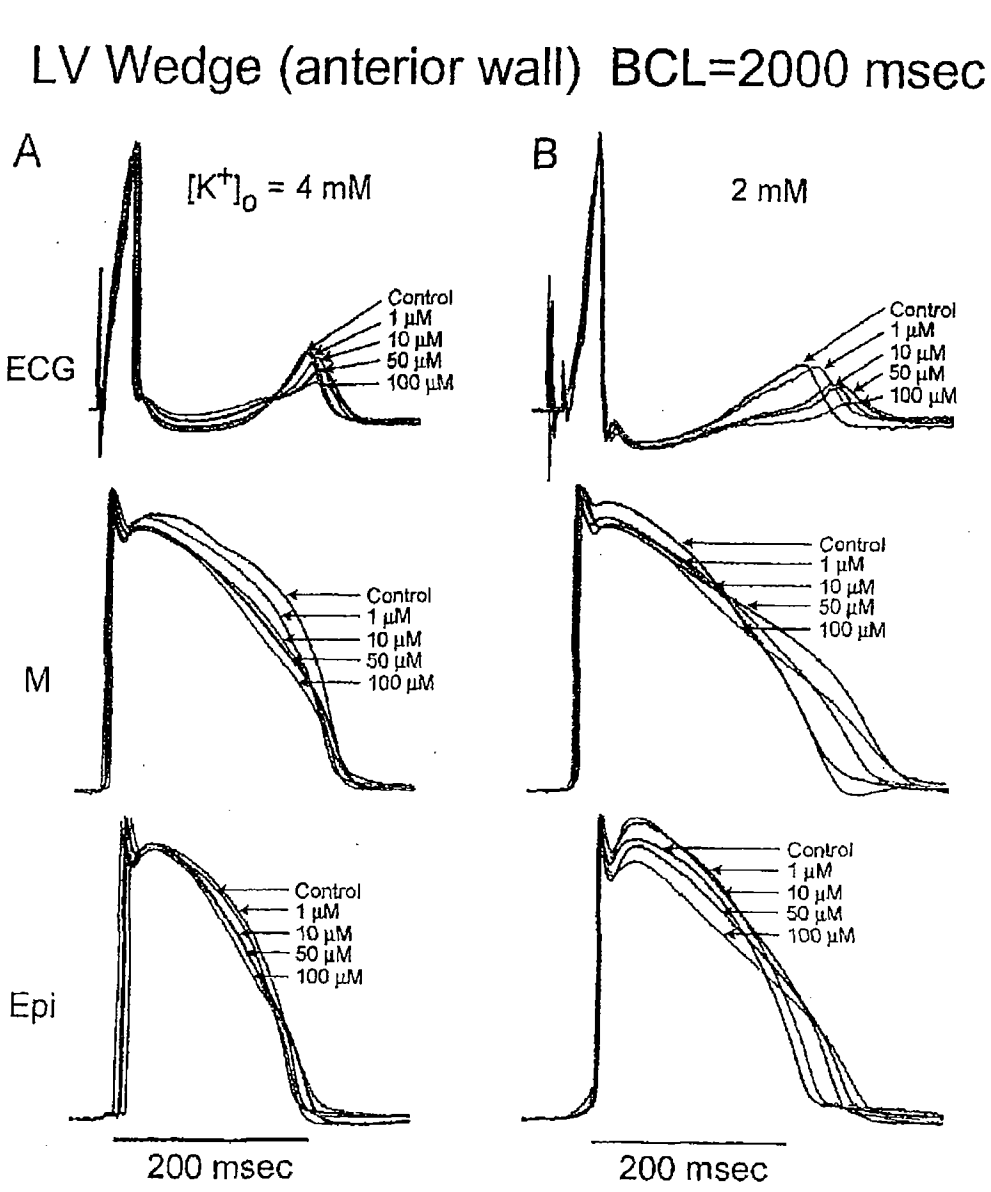
* $p<0.05$ vs. control $n\leq4$

Canine Left Ventricular Wedge: 2 mM $[KCl]_o$, BCL=2000

	Epicardium		M region				
Concentration	APD50 ± SE	APD90 ± SE	APD50 ± SE	APD90 ± SE	QT_{end}	$T_{peak} - T_{end}$	TDR
control	167.3 ± 5.548	220 ± 5.568	195.3 ± 3.283	254.3 ± 0.882	283 ± 2.08	24 ± 12.57	16 ± 9.238
1 μM	173 ± 2	232 ± 5.508	210.7 ± 13.53	280.3 ± 12.72	311 ± 9.5	35 ± 4.70	28.33 ± 11.46
5 μM	183.5 ± 1.5	252.5 ± 10.5	205.7 ± 7.881	289.7 ± 2.848*	319 ± 4.58	33 ± 1.33	15 ± 7
10 μM	190 ± 2*	265.5 ± 16.5	208.3 ± 3.48	305.3 ± 4.978*	329 ± 2.33	36 ± 4.09	23.5 ± 1.5
50 μM	179 ± 1	276.5 ± 18.5*	214.3 ± 6.333	325.5 ± 5.5*	343 ± 2.84	41 ± 6.35	35.5 ± 3.5
100 μM	167.5 ± 0.5	293.5 ± 21.5*	187.7 ± 4.978	345 ± 14.36*	376 ± 4.48	55 ± 1.00	35 ± 11

*$p<0.05$ vs. control $n\leq4$

Appears This Way
On Original


LV Wedge (anterior wall) BCL=2000 msec
A
$[K^+]_o$ = 4 mM
B
2 mM
ECG
M
Epi
Control
1 μM
10 μM
50 μM
100 μM
200 msec
200 msec

In comparison, most but not all positive control drugs showed pronounced M-cell APD90 lengthening and increased transmural dispersion of repolarization in canine ventricular strips or wedges (5-12).

Drug	Pronounced Effects	
	M-cell	Transmural Dispersion of Repolarization
Amiodarone (chronic)	no	no
Azimilide*	yes	yes
Cisapride*	yes	yes
Erythromycin	yes	yes
Quinidine	yes	yes
d-Sotalol	yes	yes
Terfenadine^	yes	yes

* Biphasic concentration – response relationship, with prolongation at low concentrations, and shortening or attenuation of effect at high concentrations.
^ Requires extended perfusion period (120 min) and hypokalemia (2 mM potassium) for effects.

In canine Purkinje fibers, ranolazine (1 to 100 μM) shortened APD90 and depressed maximal upstroke velocity. APD90 effects appeared independent of extracellular potassium (2 vs 4 mM) and basic cycle length (500 vs 2000 ms). Similar to findings in M-cells, ranolazine (10 and 100 μM) decreased maximum upstroke velocity, with approximately 50% inhibition at 100 μM. Ranolazine (5 and 10 μM) shortened d-sotalol induced APD lengthening and suppressed EADs in a concentration related way. Lack of action potential duration lengthening in canine Purkinje fibers with ranolazine is not impressive considering the poor sensitivity of this model to torsadogenic drugs such as terfenadine and terodiline (13, 14).

Proarrhythmia

Ranolazine (1- 100 μM) did not induce early afterdepolarizations (EADs) in canine ventricular M-cell strips and Purkinje fibers. Ranolazine (5 and 10 μM) reversed d,l-sotalol induced APD90 lengthening and prevented EADs in both canine M-cell strips and Purkinje fibers. Lower ranolazine concentrations were not evaluated for this property.

In comparison, the historical positive control erythromycin induced EADs in 2 of 10 M-cell strips at the highest concentration evaluated (8). Quinidine (3.3 μM) induced EADs in 3 of 5 M-cell strips and EAD-induced triggered activity in 2 of 5 M-cell strips with hypokalemia (2 mM potassium) but not with normokalemia (4 mM potassium) (9). M-cell strips from chronically amiodarone treated dogs did not show EADs or triggered activity (5). Additionally, chronic amiodarone attenuated d-sotalol induced APD90 lengthening and EADs.

The sponsor evaluated ranolazine for torsade-like polymorphic arrhythmias (TdP, spontaneous and programmed electrical stimulation (PES) inducible) in isolated canine ventricular wedge preparations. Ranolazine was evaluated in the presence and absence of hypokalemia (2 mM potassium). PES consisted of a single extrastimulus (S2) applied to the epicardial surface of the wedge preparation with the basic stimuli (S1) applied to the endocardium at basic cycle lengths of 500 and 2000 ms.

Ranolazine did not induce torsade like arrhythmias in these studies. However, the sample size was quite small (n = 4 for normokalemia, and n = 3 for hypokalemia), and positive historical control data from the Antzelevitch laboratory shows TdP incidences for d-sotalol, erythromycin and cisapride to be fairly low in this in vitro model (7, 8, 10, 11). Additionally, cisapride's proarrhythmic effects were limited to a single concentration, with negative proarrhythmia findings at both lower and higher concentrations, consistent with cisapride's biphasic effect on APD90 and transmural dispersion of repolarization. Most importantly, induction of TdP with cisapride required epicardial pacing (S1), which doubled baseline transmural dispersion of repolarization. As only endocardial pacing (S1) was utilized in the ranolazine experiments, cisapride's positive torsadogenic effect in this model is not applicable to the ranolazine experiments.

Indeed, the inability of cisapride to induce TdP in this model using endocardial pacing (S1), argues against the model's sensitivity to torsadogenic drugs when evaluated in this manner.

Drug	TdP Incidence	
	Spontaneous	PES-Inducible
Ranolazine (1-100 μM)	0 of 4	0 of 3
d-Sotalol (100 μM)	2 of 6 2 of 8	3 of 6 4 of 6
Erythromycin (100 μM)	no data	3 of 4
Cisapride (0.1-5 μM)	none	2 of 6*

* TdP inducible at 0.2 μM cisapride (only); required epicardial pacing (S1)

In Vivo Effects: QT Interval Prolongation and Proarrhythmia

Acute intravenous infusion of ranolazine was evaluated for effects on QT interval and proarrhythmia in anesthetized dogs with acute AV block. Ranolazine was administered at a dose of 0.5 mg/kg plus 1, 3 and 15 mg/kg/hr iv for 30 minutes/dose level (n=6). In one additional dog, ranolazine was administered at a dose of 1.5 mg/kg plus 15 and 30 mg/kg/min iv for 30 minutes per dose level. Sotalol (8 mg/kg followed by 4 mg/kg/hr iv for 20 minutes served as a positive control (n=5). Left ventricular effective refractory periods, QT interval, and QRS duration were determined at basic cycle lengths of 300, 400, 600 and 1000 ms following each dose of ranolazine or sotalol. All dogs then received bolus intravenous doses of phenylephrine (10, 20, 30, 40 and 50 μg/kg iv) in order to elicit arrhythmias.

In this study, ranolazine increased QT interval by approximately 10% (not statistically significant). The QT effect was biphasic, with the maximum increase observed at 3 mg/kg/hr. Ranolazine increased left ventricular refractoriness but only at the fastest stimulation rate (300 ms basic cycle length). Ranolazine increased QRS interval by 10% (statistically significant) at the next higher dose of 15 mg/kg/hr. Proarrhythmia was not observed with ranolazine in the presence of phenylephrine. The highest ranolazine dose of 30 mg/kg/hr induced acute heart failure and death.

In contrast to findings with ranolazine, sotalol induced a large increase in QT interval (33% increase at 1000 ms basic cycle length), and increased left ventricular refractoriness without altering QRS duration. Phenylephrine induced tachyarrhythmias, including TdP and ventricular fibrillation, in sotalol treated dogs.

Limitations of this study design include its acute nature, since chronic but not acute AV block has been shown to promote proarrhythmia by electrical remodeling and downregulation of ion channels, including I_{Kr} (15, 16). Additionally, ranolazine displaces radiolabeled ligand from α-1 adrenergic receptors, suggestive of potential functional antagonism (17). If ranolazine is indeed a functional α-1 adrenergic antagonist at doses administered above, then the in vivo canine model used for this study is inappropriate. Finally, positive control data is quite limited for this model, lessening the regulatory importance of negative findings.

References

1. Snyders DJ and Chaudhary A, High-affinity open-channel block by dofetilide of HERG expressed in a human cell line, *Mol Pharmacol* **49**: 949-955 (1996) [PMID: 8649354].

2. Zhang S, Zhou ZF, Gong QM, Makielski C, and January CT, Mechanism of block and identification of the verapamil binding domain to HERG potassium channels, *Circ Res* **84**: 989-998 (1999) [PMID: 10325236].

3. Yang T, Hideaki K, Roden D, Phosphorylation of the IKs channel complex inhibits drug block: Novel mechanisms underlying variable antiarrhythmic drug actions, *Circulation* **108**: 132-134 (2003) [PMID 12835205].

4. Busch A, Busch GL, Ford E, Suessbrich H, Lange HJ, etal, The role of IsK protein in the specific pharmacological properties of the IKs channel complex, *Br J Pharmacol* **122**: 187-189 (1997) [PMID 9313924].

5. Sicouri S, Moro S, Litovsky S, Elizari MV, Antzelevitch C, Chronic amiodarone reduces transmural dispersion of repolarization in the canine heart, *J Cardiovasc Electrophysiol* **8**:1269-79 (1997) [PMID: 9395170].

6. Burashnikov A, Antzelevitch C, A combination of IKr, IKs, and ICa or INa block produced a relatively homogeneous prolongation of repolarization of cells spanning the canine left ventricular wall, *PACE* **20**: II-1216, (1997).

7. DiDiego J, Belardinelli L, Antzelevitch C, Cisapride-induced transmural dispersion of repolarization and torsade de pointes in the canine left ventricular wedge preparation during epicardial stimulation, *Circulation*, **108**:1027-33 (2003) [PMID: 12912819].

8. Antzelevitch C, Sun Z, Zhang Z, Yan G, Cellular and ionic mechanisms underlying erythromycin-induced long QT intervals and torsade de pointes, *J Amer Coll Cardiol* **28**: 1836-1848 (1996) [PMID 8962574].

9. Sicouri S, Antzelevitch C, Drug-induced afterdepolarizations and triggered activity occur in a discrete subpopulation of ventricular muscle cells (M cells) in the canine heart: quinidine and digitalis, *J Cardiovasc Electrophysiol* **4**: 48-58, (1993) [PMID: 8287236].

10. Shimizu W, Antzelevitch C, Effects of a K(+) channel opener to reduce transmural dispersion of repolarization and prevent torsade de pointes in LQT1, LQT2, and LQT3 models of the long-QT syndrome, *Circulation*, **102**:706-12 (2000) [PMID: 10931813].

11. Shimizu W, Antzelevitch C, Differential effects of beta-adrenergic agonists and antagonists in LQT1, LQT2 and LQT3 models of the long QT syndrome, *J Am Coll Cardiol.* **35**:778-86, (2000) PMID: 10716483

12. Antzelevitch C, (personal communication).

13. Gintant GA, Limberis JT, McDermott JS, Wegner CD, Cox BF, The canine Purkinje fiber: an in vitro model system for acquired long QT syndrome and drug-induced arrhythmogenesis, *J Cardiovasc Pharmacol.* **37**:607-18 (2001) [PMID: 11336111].

14. Pressler ML, Warner MR, Rubart M, Rardon DP, Zipes DP, In vivo and in vitro electrophysiologic effects of terodiline on dog myocardium, *J Cardiovasc Electrophysiol* **6**: 443-54 (1995) [PMID: 7551314].

15. Vos MA, de Groot SH, Verduyn SC, van der Zande J, Leunissen HD, Cleutjens JP, van Bilsen M, Daemen MJ, Schreuder JJ, Allessie MA, Wellens HJ, Enhanced susceptibility for acquired torsade de pointes arrhythmias in the dog with chronic, complete AV block is related to cardiac hypertrophy and electrical remodeling, *Circulation*: **98**:1125-35 (1998) [PMID: 9736600].

16. Volders PG, Sipido KR, Vos MA, Spatjens RL, Leunissen JD, Carmeliet E, Wellens HJ, Downregulation of delayed rectifier K(+) currents in dogs with chronic complete atrioventricular block and acquired torsades de pointes, *Circulation* **100**:2455-61 (1999) [PMID: 10595960]

17. Hausner EA, Pharmacology Toxicology Review, NDA 21526.

Appears This Way
On Original

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

John Koerner
9/4/03 10:29:14 AM
PHARMACOLOGIST

review of nonclinical QT interval prolongation and proarrhythmia potentia

Charles Resnick
9/4/03 11:24:35 AM
PHARMACOLOGIST

Doug Throckmorton
9/4/03 11:37:51 AM
MEDICAL OFFICER

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 21-526

Date : 10/17/03

From : Albert DeFelice, Ph.D.,
Supervisory Pharmacologist, DCRDP, HFD-110

To : Douglas Throckmorton, MD
Director, Division of Cardiorenal Drug Products
OND/ODE-1/dcrdp(HFD-110)

Subject : Overview of Pre-clinical Reproductive Toxicology Studies of Ranolazine (NDA 21-526)

Sponsor : CV Therapeutics, Inc.

This is an overview of pre-clinical reproductive toxicity studies, and other relevant toxicity studies, performed by Syntex Research Instit. of Toxicol. Sci.. (Palo Alto, CA). They involve the rat and/or rabbit to evaluate effects of Ranolazine on fertility, organogenesis, and neonatal behavior. Contentious issues include impaired fertility (male/female rat), in-utero development tox (skeletal malformations in rat; embryotoxicity in rabbit); and neonatal developmental toxicity. It focuses on study design, especially systemic exposures relative to clinical drug levels; and on interpretation- interpretability of results *vis a vis* behavior in contemporary control cohorts. I also sought evidence of testis pathology in multiple standard sub-chronic/chronic rat toxicity tests to confirm that encountered at high dose (HD) of 300mg/Kg in the rat fertility test , and will convey at the outset that I could not find such – in either rat or multiple sub-chronic/chronic dog toxicity studies.
For a different perspective, I also sought to identify relative potency for targeted pharmacologic activity vs. depressed fertility toxicity i.e., a veterinary safety ratio in the rat. Such was indeterminite (ranolazine was not tested in a rat model of angina; and the pharmacodynamic activity underlying any anti-anginal activity is moot).
Pre-clinical reproductive and chronic toxicity studies have been comprehensively reviewed, and archived (9/2/2003), by Elizabeth Hausner, D.V.M.

Summary:

A. Male Rat Fertility:

[First off. it should be noted that in this fertility trial pregnancy rate in untreated females offered to untreated males was only 70% vs essentially 100% historical incidence, which immediately raises a questions regarding the status of breeders and/or the breeding conditions.].

Evidence of 30% depressed male fertility at high dose (HD) derives from a single study at 0, 5, 40, and 300 mg/Kg involving 20 males /40 females per breeding cell. At the HD,5 males and 4 females died prior to initial breeding (this drug is lethal in the rat even though it affords only 1-2 X the human AUC burden based on other rat PK studies. Pregnancy rates in the 30

control and the 25 HD females with evidence of mating was 98% and 64%, respectively. The surviving HD males were re-dosed at the HD in two successive follow-on trials (133 days; 156 days +32 days recovery: each involving untreated females) with the same results, namely 30% reduction in conception rate in the untreated females. So it is really three trials with the same male actors.

Regarding maternal reproductive performance as informed by mid-gestation caesarian: pregnancy rate in control and HD was 100 and 67%, respectively, confirming the impairment in survivors seen at term via natural delivery.

Testis histopath: Retrospectively, based on individual rat impregnating performance, four HD male rats with testicular lesions were identified which if sterile, would mathematically account for the same (30%) impairment in the initial trial and two follow-on trials. Accordingly. I searched for testis toxicity in other animal studies. Dr. Hausner reports that the "histopath for the standard 3-month rat study was incomplete with no incidence or summary tables; and that histopathology results for the 6 month rat study were not susceptible to easy interpretation. While the ability of those rat toxicology studies to confirm testicular histopathology is moot. , I do note that testis weight in those studies was unaffected, and that testis histology is evidently normal in a 1-year rat study.

B. Embryotoxicity and Teratology:

1.From Fertility study:

In the fertility study above, incidence of pregnancy with at least 1 resorption was 75% in all treated dams, but fully 50% in control. However, there was no drug-associated reduction in mean litter size, mean no.of total resorptions, or mean no.of implantations. at Caesarian,

II. Segment II teratology studies : These were performed in rats and rabbits at up to maternotoxic high dosages of 400 and 150 mg/Kg, respectively, administered during the critical period of fetal embryogenesis followed by Caesarian at end of term. The HD is an $LD_{35\%}$ and $LD_{25\%}$, in rats and rabbits, respectively. In my judgement, these tests were still informative since there were 15 litters available for analysis in rat and 13-15 in rabbit – even though 16 to 20 is recommended by ICH). I see no evidence of selective fetal toxicity in these studies, indeed there is absence of terata even at the egregiously toxic HD:

Rat: Fully 24-52% of litters from controls had delayed or reduced ossification (in this case, pelvic, cranial and possibly sternebral) and mis-shapen sternebrae vs 33-80% of litters from HD dams. Such "lesions" are relatively common .and not considered to be manifestations of teratogenesis. In my opinion, excesses in these "lesions" are a non-issue. Moreover, all other bones were evidently normally ossified. Whether this is or is not an important lesion, it is noted that this ossification "anomaly" occured at probably approx. 1.5X the human AUC. Minimum live litter size was decreased at MD and HD, but this could reflect drug-unrelated decreased ovulation at these dosages (min, corpora lutea way down at MD and HD).
Fetal weight was significantly decreased, and incidence of pregnant dams with 100% still-born fetuses egregiously increased, at HD. However, the HD approaches an $LD_{50\%}$, with cyanosis and convulsions, in this study – hardly the profile of selective embryocidal toxicity. One may even have expected to see frank terata at such a systemically toxic dosage. There were none.

Rabbit: First off, as noted, I consider 13-14 litters in all cohorts to be evaluable, and not egregiously below the suggested limit. The **implantation index** [(implantations/corpora lutea) x100] was indeed significantly reduced at the MD(45 mg/Kg) and the HD (150 mg/kg), but this

is related in part to, strangely enough, a "dose -related" increase in number of corpora lutea (this cannot be drug-related as they are treated after ovulation). Furthermore, there was no impairment of gestation survival index [(live fetuses/total fetuses)X100] , or resorption index [(total resorptions/implantations) X100], or live litter size. Fetal weight was unaffected, even at the lethal HD.Reduced ossification of sternebrae is again a non-issue - it occured in 54% of control litters and 77% of HD litters. These results do not reveal any selective fetal toxicity in my opinion.

C. Neo-natal development: In the above fertility trial summarized above , delayed neonatal development per standard landmarks (e.g., eye-opening) was clearly evident . Development did "catch-up" to control by the last monitoring period. Mean pup weight was non-dose-relatedly reduced, but only by 5% on day 4 and 3% on day 21 (HD vs control). Reduced neonatal survival was statistically significant and dose-related, but not appreciable: mean survival index being 95 % in treated groups vs. 100% in control up through weaning, attributable to excess neonatal mortality only in the post-natal days 1-4 interval.

In a Segment III trial ,which are typically specifically designed to look at neonatal behavior, there were, inexplicably, no developmental landmarks monitored. However, survival and mean body weight of pups from dams treated at up to 200 mg/Kg were indistinguishable from control. (Recall that the 300 mg/kg dosage is lethal in females and males).

A. Fertility (study 116-R-86-43285-PO-RMF)

[This study informs rat fertility and neonatal development. It is the only trial of fertility, as rabbits are not used for such testing]

Pregnancy rates: Fertility study 116-R-86-43285-PO-RMF involved 20 males offered to 40 females per mating cohort; 0, 5, 40, or 300 mg/Kg rats total per cohort; and with both males and females treated. It provided persuasive *prima facie* evidence of appreciably (30%) impaired capacity of males to impregnate at the convulsant lethal high dose (HD) of 300 mg/Kg, (i.e., impregnation was 64 % and 93% of HD and control females, respectively ($P<0.05$.) who presented with evidence of having mated. [I note that only 75% of control co-habited females had evidence of mating, which is appreciably lower than the ca. 100% incidence usually encountered in such studies.] The dose-response possibly begins at 40 mg/Kg,(approx.10% impairment), the next lowest dose tested. This is based on a reduction in gravidity of female consorts both at Caesarean sacrifice(where, from each cohort, the first 12-15 dams with evidence of mating were sacrificed at mid-gestation) , and as evident from pregnancy status in the surviving females allowed to litter at term.

Sponsor asserts that "the pregnancy rate in treated rats given 300 mg/kg/day was within the range seen in primiparous control rats". Documentation of that assertion.was not provided.

Because of the impaired fertility, surviving HD sires (P1) were not sacrificed, but, rather, were re-tested twice in two consecutive longer term trials, each of which revealed 30% impaired ability to impregnate untreated females after 133 days of re-instated high-dosing (study 176-R-87) , and, again, after 156 days high-dosing followed by a 32-day recovery (study 195 -R-87). In both follow-up studies , 100% of control males impregnated females vs. 69% of HD males, establishing -and confirming - that it was male fertility which was evidently compromised at the HD.

Regarding effects on female fertility, there was no cohort of treated females exposed to untreated males. Accordingly, I do not know whether an increase in incidence of gravid females with resorptions noted at Caesarian of all treated dams, (about 75% with at least one resorbed fetus vs. 50% of controls) reflects impaired maternal reproductive performance , or, conceivably, an effect on sperm to reduce embryo viability. Dr. Hausner did not note that this "impairment" is mitigated by essentially unchanged Resorption Index [(total resorptions/implantations)X100] and Implantation Index [(implantations/corpora lutea) X100]. Accordingly, there was no significant decrease in litter size.

Neonatal development: In the initial trial, development delays – eye opening; vaginal opening; negative geotaxis and reduced survival – were seen in all drug-treated groups. However, I note that neonatal development informed by such landmarks had "caught-up" to controls by the last monitoring period. The reduced neonatal survival was statistically significantly, but not appreciably, impaired : mean survival index being 95 % at in treated groups vs. 100% in control up through weaning, attributable to excess neonatal mortality in the post-natal days 1-4, and no exacerbation subsequently through weaning. Dr. Hausner notes, correctly, that "Mean pup weights in the HD group were decreased compared to control at all points of determination and reporting"; but the decrement was only 5% on day 4 and 3% on day 21. No neonatal monitoring was performed in the second or third follow-up trials to confirm these unimpressive and/or reversible changes in behavior of neonates exposed to ranolazine *in utero* and *via* lactation.

Parental toxicity: The HD was clearly toxic, convulsant, and evidently lethal: 7 males and 4 females died prior to or subsequent to mating due, according to Sponsor, " principally to aspiration and/or malintubation , possibly related to excess salivation and convulsions". The HD of 300mg/Kg is close to 400 mg/Kg which in teratology study AT3758 (See below)is also convulsant and an $LD_{35\%}$.

Histopathology of treated males: It was determined retrospectively from individual fertility data, that five repeatedly used HD males contributed to the reduced fertility in the main study and its two "extensions". Autopsy performed on 4 of these 5 rats at the end of study three revealed atrophied testes and/or epididymides. Three of these HD males (all of which, by then, had been exposed to ranolazine for approx. 1 year followed by a 1-month drug-free recovery), presented with epididymal atrophy and virtual aspermia (2 rats)or hypospermia(1 rat). Four of these 13 HD males surving the 1 year HD regimen had atrophied seminiferous tubules vs. 2 of 20 control males.

Dr. Hausner did the math and confirms that sterility in the 4 identified rats could account for the 31% decrease in fertility in the last two consecutive re-dosing trials

It is indeterminate when in the course of the three consecutive trials these lesions in 4 rats occurred, as there were no biopsies, and no HD sacrifices until after the end of the third re-trial followed by a month recovery. If these four damaged rats account for the HD impairment of fertility, then the lesions were present by the end of the initial 80 day day dosing which preceded the first testing of fertility, and neither abated - nor were additional rats affected - over a subsequent 9 months of re-challenge (i.e., two follow-on trials) in view of the identical 30% impairment in all three trials.

Testis weight/histology in other rat and dog tox. studies:

I looked for evidence of testicular injury in standard chronic rat (and dog) toxicity studies where testis weight (both absolute and as %body wt) and histology are routinely monitored.

Rat: Dr. Hausner's review does not identify the testis as an affected organ in 3, 6, or 12 month rat toxicity studies done at up to 200 or 500 mg/Kg, except that in the 3 month study mean testis weight was unchanged at 250 mg/Kg and actually slightly increased (11%) at an egregiously systemically toxic 500 mg/kg. dosage. Regarding the veracity of these "non-findings", Dr. Hausner reports that the "histopath for the 3-month study was incomplete with no incidence or summary tables. Histopathology results for the 6 month rat study were not susceptible to easy interpretation". However, at least testis weights were given, and data revealed no atophy as I just noted. Clearly, histopath. was provided for the 1 year rat study at up to 200 mg/Kg, where clear drug-associated changes were noted in adrenals, pituitary and lungs, but no mention of excess testicular pathology.

Dog: :It is also evident from Dr. Hausner's review of chronic conventional toxicity studies that the dog testis is not consistently affected vis a vis weight or histology, in 3, 6, or 12 month toxicity studies also done at up through systemically toxic dosages (up to 80 mg/Kg). At 60 mg/Kg, testis weight was slightly increased in a 3-month study, decreased 25% in a 6 mo. study, and unchanged in a 12-mo. study. Her review is silent on any testis histopathology, which presumably was absent.]

B. Teratogenicity/embryotoxicity:

As noted in the summary,Rat and a Rabbit Segment II teratogenict y studies were performed at up to maternocidal dosages. I believe that sufficient number of litters were available (13-15), even at the HDs, to be revealing, and saw no evidence of any teratogenic activity or selective embryocidal activity of veterinary importance to project any clinical concern. This drug is toxic in both rats and rabbits at AUC exposures (at least in the rat, and probably rabbit as well) approximating clinical (we saw the same thing with ACE inhibitors in the rabbit tests of their teratogenic potential.). To get a handle on their inherent reproductive liability, a veterinary safety ratio of , say, an Fertility.$_{30\%}$/ $ED_{30\%}$ might be revealing. I could not develop such surrogates. Dr. Hausner just received reports CVT303.064-P and CVT303.062-P on anti- beta and anti-alpha adrenergic activities , respectively, of ranolazine in conscious rats. Perhaps data theirein could afford such safety ratios.

Drug exposures and projected safety multiples: In the fertility study, No blood levels were provided or referenced to compare to AUC of 33,700 ng.hr/ml in humans who received 1000 mg of ranolazine t.i.d. for 5 days. However, in a 6 month rat toxicity study, dosages of 50 or 150 mg/Kg /day for 3 months afforded AUCs of approx 14,000 and 64,000 ng.hr/ml., respectively, in the males. Accordingly, if we assume that the threshold level for impairing male fertility was 40 mg/Kg (not enough dosages were tested to firmly establish such) then rats may begin to reveal impaired fertility at approx 0.5X the human AUC. What can more confidently be said is that important impairment of male rat fertility was clearly observed at 300 mg/Kg which affords approx. twice the human AUC – not a very re-assuring safety multiple.

[It should be noted, at least in passing, that impairment of fertility was not progressive. Impairment was still approx. 30% after about 1 year HD (300 mg/Kg)exposure when , based on other rat studies, the $AUC_{0\text{-}24\ hr}$ is expected to be approx 300,000 ng.hr/ml or 9X the human exposure (200 mg/Kg afforded 200,000 ng.hr/ml in the 1 year standard tox. study)]

Therapeutic ratio : I could not calculate a toxic dose/pharmacodynamic dosage in the rat because the drug was not tested in an in vivo rat model of angina pectoris. Furthermore, as the pharmacodynamic basis underlying therapeutic effect is moot, I could not identify a surrogate marker for anti-anginal activity.

CONCLUSIONS:

In a study of male rat fertility, ranolaziner impaired fertility at the lethal HD, which likely reflected no or reduced sperm counts in 4 rats. No other chronic rat or dog toxicity study identified any testicular toxicity as informed by organ weight, although Dr Hausner notes that histopath tables were not forthcoming in all potentially relevant toxicity studies. Regarding teratogenicity, there was none, even at maternocidal dosages. Regarding embryotoxicity, there were reductions in impantation indices but not, as far as I can determine, when corrected for increases in ovulation . There were decrements in fetal weight and neonatal developmental delays, especially at the HD.

The evaluation of any reproductive toxicity in the context of maternal and paternal toxicity, and at or within a few-fold of human AUC exposures, The extent to which such reproductive toxicity is selective is indeterminate, in my judgement, due to an excessive interval between mid and high dosages which precluding determination of toxicity thresholds.

Appears This Way
On Original

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Albert Defelice
10/17/03 01:36:45 PM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY COVER SHEET

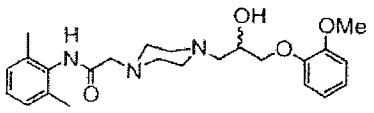
NDA number:21-526
Review number: 1
Sequence number/date/type of submission: NDA
Information to sponsor: Yes ( ) No ( )
Sponsor and/or agent: CVT Therapeutics
Manufacturer for drug substance :

Reviewer name: Elizabeth A. Hausner, D.V.M.
Division name: Division of Cardio-Renal Drug Products
HFD #: 110
Review completion date: August 27, 2003
Drug:

Trade name: Ranexa
Generic name (list alphabetically): ranolazine
Code name: RS-43285-193, RS-43285-003, CVT-303, RAN D, Ran4
Chemical name:

IUPAC:	N-(2,6-dimethylphenyl)-2-{4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]piperazinyl}acetamide
CAS[1]:	1-piperazineacetamide, N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-
Other:	(±)-4-[2-hydroxy-3-(o-methoxyphenoxy)propyl]-1-piperazineaceto-2',6'-xylidide

CAS registry number: 95635-55-5
Mole file number:
Molecular formula/molecular weight: $C_{24}H_{33}N_3O_4$/427.54
Structure:



Ranolazine

Relevant INDs/NDAs/DMFs: IND 43,735
Drug class: anti-anginal
Indication: angina
Clinical formulation: sustained release tablets of 375 and 500 mg. Tablet: methacrylic acid copolymer, microcrystalline cellulose, hydroxypropyl methylcellulose, sodium hydroxide, magnesium stearate. Coatings: titanium dioxide, polyethylene glycol, FD&C blue # 2 lake or FD&C yellow #6 lake, polysorbate 80, carnauba wax. Maximum recommended dose of 1000 mg b.i.d.
Route of administration: oral
Proposed use: angina for those patients in whom all other anti-anginals are inadequate or not tolerated
Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

I. Recommendations

A. Recommendation on Approvability: Preclinically ranolazine has been shown to interact with cardiac ion channels. Approximately 7 of the known major metabolites have also been shown to interact with cardiac ion channels including Ikr. There is no projected margin of safety between plasma levels in animals where various adverse effects have appeared and the plasma levels measured in humans. Approvability depends upon the clinically demonstrated risk:benefit ratio.

B. Recommendation for Nonclinical Studies: 1)Exploration of the long-term effects of ranolazine upon the pigmented structures of the eye. 2) Clarification of the apparent reproductive liability of the drug.

C. Recommendations on Labeling: Under "Description": There is insufficient information to warrant the statement that ranolazine '[redacted]

The "Mechanism of Action" section should be restated to reflect the uncertainty of the mechanism of action. The paragraph regarding [redacted] should be removed.

"Carcinogenesis, Mutagenesis, Impairment of Fertility": The sponsor's statement should be changed to read [redacted]

"Pregnancy—Category C" The sponsor's first two sentences in this section should be removed and replaced with [redacted]

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings: Safety pharmacology was presented for cardiovascular, gastrointestinal, nervous and pulmonary systems. The cardiovascular safety study showed that cumulatively increasing doses of intravenous ranolazine caused a deterioration in cardiac function manifested as decreased cardiac output, decreased contractile force and decreased left ventricular systolic pressure which could reflect direct cardiac toxicity or increased afterload. Left ventricular minute work was also decreased while total peripheral resistance was increased, suggesting that afterload indirectly hurt cardiac performance. No ECG data was provided. The sponsor was asked by telephone to provide the ECG data. July 2, 2003 the sponsor

replied verbally to this request and said that the ECG data was unavailable and could not be produced. The sponsor's estimate of total cumulative exposure for the 2 doses where these effects were most prominent showed that the plasma concentration was within the range seen clinically.

In a July 31, 2003 telecon with representatives from CV Therapeutics, Drs Koerner and Hausner requested the ECG data for the dog toxicology studies. August 18, 2003 in a telephone call, Maragaret Dillon of CV Therapeutics informed this reviewer that while the ECG data had been located, it had not been analyzed. She estimated that it would take some 6 months of work to quantitate the various ECG intervals. After consultation with Drs Koerner and Gordon, it was decided to forgo review of the dog ECG data. This was communicated back to the sponsor later on 8/18/03.

A variety of in vitro studies indicate that ranolazine has the ability to interact with cardiac ion channels. Lacking informative ECG analyses, this creates a concern for the drug's potential to alter repolarization.

Central sedation was noted in several of the CNS assessments. Neurologic deficits were identified at doses where sedation was not apparent. Ranolazine treatment also contributed to stress-induced hyperphagia, indicating an effect on the hypothalamic-pituitary-adrenal axis. This was reinforced in the general toxicology studies and several special toxicology studies that specifically examined the effect of ranolazine on the adrenal gland (see below).

The gastrointestinal safety study lacked a positive control but was suggestive of a mild delay in GI transit.

Consistent across toxicology studies and species were clinical signs of sedation and salivation. Signs reported primarily in rodents included hunching, piloerection, dyspnea or tachypnea, ataxia, tremors and convulsions. Dogs also showed ataxia, respiratory signs, tremors, thrashing and convulsions. There seemed to be a decreased incidence of signs with prolonged dosing. The sponsor suggested hypotension to explain the signs but it is unclear that hypotension could produce all of the signs reported. The safety pharmacology studies also reported neurologic effects in rodent species at doses for which we do not have information regarding blood pressure. Doses used in the nonclinical studies were limited by the neurologic signs. The highest doses used produced only low multiples of human exposure. Adverse effects in animals were found at plasma drug levels at or below those achieved in humans.

The adrenal gland was identified as a target organ. Special studies showed both acute and chronic effects of ranolazine on the HPA axis. Two oral doses of ranolazine depressed both basal and post-stress ACTH levels as well as corticosterone in plasma and the adrenal gland. All these parameters increased in response to stressors but not to the extent of the vehicle-treated animals. There were some inconsistencies between studies. However, dose-related increases in adrenal weight were seen in both dogs and rats. Histological changes such as vacuolation of the zona fasiculata repeated across studies. While the neurologic effects and to some extent the dermal effects reported in one dog

study are relatively non-specific, opioids have been shown to affect the HPA axis both acutely and chronically. This fact and receptor binding studies suggest that either parent compound or one of the metabolites may have opioid receptor activity. Or, this may be indirect support of the sponsor's proposed mechanism of action (See Summary of Special Toxicology Studies).

Reproductive and developmental toxicology studies indicated adverse effects upon fertility in both sexes. Furthermore, general toxicology studies in both rodents and dogs showed 1) dose-related increases in pituitary size in female rats (1 year study) 2) dose related decrease in absolute and normalized uterine weight in dogs in both 3-month and 12-month studies 3)in males, an increase in absolute and normalized testicular weight was seen in 3 and 12-month studies 4) in the 1 year study, the prostate weight was increased at all doses while testicular weight was decreased at LD and MD and increased at the HD. Alterations in adrenal function may also underlie, in part, the observed effects upon fertility.

Increased developmental variations were seen in both rats and rabbits. In the data as presented, the skeletal system was affected, primarily at doses causing maternal toxicity.

Two developmental toxicology studies were conducted. The earlier study used insensitive detection methods yet still showed delays in eye opening and negative geotaxis. The second study did not present any data.

Genotoxicity assays included the Ames assay, in vivo mouse micronucleus test and in vitro cytogenetics using Chinese hamster ovary cells. An increase in aberrations per cell and the percent cells with aberrations was reported for one concentration (576μg/ml) +S9 activation given 10 hours of incubation. This effect was not seen in the 400 and 800 μg/ml concentrations incubated for 20 hours.

A radiolabeled tissue distribution study indicated that presence of drug-derived radioactivity in the retinal pigment epithelium of pigmented animals. The elimination half-life of radioactivity from the RPE after a single dose of drug was 23 days. While melanin binding is not unusual, three concerns are presented:

1) There is no indication in the study reports that a qualified veterinary ophthalmologist has examined any of the animals used in the toxicology studies.
2) There is no characterization of the effect of repeated dosing upon the pigmented structures of the eye or pigmented skin (e.g., is there a potential for phototoxicity?)
3) The mechanism of action of the drug is proposed to be an alteration of mitochondrial fatty acid metabolism. The highly metabolically active RPE is rich in mitochondria. If the drug truly alters (down-regulates) mitochondrial fatty acid oxidation, what effect will this have on the RPE?

The drug is highly metabolized with over 100 metabolites reported. Several of these (approximately 8) are present in multiple species, including humans, in significant amounts. Three study reports from July 2002 report the identification of CVT4786, a

major new metabolite in rats, mice and dogs. In humans, CVT4786 has been found in amounts up to 30-40% of the parent drug. No pharmacologic/toxicologic characterization of that major metabolite has been located in the submission. Overall, there is a lack of systematic characterization of the pharmacology/toxicology of the major metabolites.

B. Pharmacologic Activity: In anesthetized dogs, ranolazine caused an increase in coronary artery blood flow and a decrease in systolic arterial pressure.There is data to show that ranolazine may in fact decrease fatty acid oxidation. There is data to show that ranolazine also binds to a number of receptors which may in turn secondarily affect cardiac energy metabolism.

C. Nonclinical Safety Issues Relevant to Clinical Use: see above. The ability of the drug and metabolites to interact with cardiac ion channels creates the potential for effects upon cardiac repolarization. Based upon the cardiovascular safety study, there is a concern that use of this drug in patients with congestive heart failure could precipitate a marked decrease in contractility and left ventricular function and increased afterload (TPVR). The neurologic effects seen in animals create concern for potential neurologic effects in humans.

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

C. cc: list:

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

Appears This Way
On Original

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Primary pharmacodynamics: unclear, possibly reducing cardiac oxygen demand at a given workload.

Mechanism of action: unclear. Possibly calcium channel blockade

Drug activity related to proposed indication: unclear. Possibly decreasing cardiac oxygen demand.

Secondary pharmacodynamics: cardiovascular deterioration (loss of contractility and increased afterload (TPVR)), central, multifocal neurologic effects and some effect on the HPA axis.

[ ]' *Pharmacology Report for Ranolazine free Base August 11, 1997.* [ ] *138249.* The compound dissolved in 0.5% DMSO was tested in radioligand binding assays for maximum total binding and nonspecific binding against a number of different receptors. Nonspecific binding was defined as the proportion of total binding not displaced by unlabeled ligand. The summary of results meeting significance criteria is shown below.

It may be seen that the free base interacts with a number of receptors. Some interactions do not quite achieve significance but are close, such as Ca^{2+} channel binding.

Significant responses (meeting significance criteria) were noted in the assays listed below:

CAT.#	PRIMARY ASSAY NAME	CONC.	% INH.	IC_{50}	K_i	nH
20210	Adrenergic α_{1A}	10 µM	83			
20320	Adrenergic α_{1B}	10 µM	55			
20352	Adrenergic α_{2A}	10 µM	57			
20411	Adrenergic β_1	10 µM	58			
27111	Serotonin 5-HT$_{1A}$	10 µM	53			

CAT.#	SECONDARY ASSAY NAME	DOSE/CONC.	CRITERIA	RESULT	ACTIVITY
40501	Adrenergic α_1 Antagonism	10 µM	≥ 50%	61	Y
45900	Serotonin 5-HT$_{1A}$ Agonist	10 µM	≥ 50%	97	Y

PHARMACOLOGY REPORT
EXPERIMENTAL RESULTS
BIOCHEMICAL ASSAYS

PT#: 138249
CODE: CV-10
E4-NE-002

August 11, 1997

M.W.: 500 Solvent: 0.5% DMSO

CAT.#	TARGET	CONC.		PERCENT INHIBITION %	IC50	Ki	nH
20050	Adenosine A_1	10.0	µM	-5			
20060	Adenosine A_{2A}	10.0	µM	-7			
20070	Adenosine A_3	10.0	µM	17			
◆ 20310	Adrenergic α_{1A}	10.0	µM	57			
◆		10.0	µM	53			
		1.0	µM	22			
		0.1	µM	24			
		0.01	µM	4			
◆ 20320	Adrenergic α_{1B}	10.0	µM	50			
		10.0	µM	43			
		1.0	µM	10			
		0.1	µM	-4			
		0.01	µM	-1			
◆ 20362	Adrenergic α_{2A}	10.0	µM	59			
◆		10.0	µM	57			
		1.0	µM	14			
		0.1	µM	18			
		0.01	µM	4			
20370	Adrenergic α_{2B}	10.0	µM	36			
20401	Adrenergic β_1	10.0	µM	43			
◆ 20411	Adrenergic β_2	10.0	µM	65			
◆		10.0	µM	58			
		1.0	µM	11			
		0.1	µM	19			
		0.01	µM	-5			
20420	Adrenergic β_3	10.0	µM	31			
21000	Angiotensin AT_1	10.0	µM	-8			
21010	Angiotensin AT_2	10.0	µM	-14			
21150	Bombesin	10.0	µM	27			
21250	Bradykinin B_1	10.0	µM	6			
21260	Bradykinin B_2	10.0	µM	7			
21450	Ca^{2+} Ch. (L), Benzothiazepine	10.0	µM	23			
21460	Ca^{2+} Ch. (L), Dihydropyridine	10.0	µM	17			
21500	Ca^{2+} Ch. (L), Phenylalkylamine	10.0	µM	-17			
21600	Ca^{2+} Channel (N)	10.0	µM	-1			
21701	Cannabinoid CB_1	10.0	µM	-6			
21710	Cannabinoid CB_2	10.0	µM	-8			
21801	Cholecystokinin CCK_A	10.0	µM	21			
21811	Cholecystokinin CCK_B	10.0	µM	4			
21950	Dopamine D_1	10.0	µM	8			

Results with ≥50% stimulation or inhibition are boldfaced. (Negative values correspond to stimulation of binding or enzyme activity)

138249BP.CV

NDA 21-526

PHARMACOLOGY REPORT
EXPERIMENTAL RESULTS
BIOCHEMICAL ASSAYS

PT#: 138249
CODE: CV-10
E4-NE-002

August 11, 1997

M.W.: 500 Solvent: 0.5% DMSO

	TARGET			PERCENT INHIBITION	IC_{50}	K_i	nH
				-100 -50 0 50 100			
21960	Dopamine D_{2L}	10.0	µM	8			
21980	Dopamine D_3	10.0	µM	12			
21990	Dopamine $D_{4.2}$	10.0	µM	13			
22020	Dopamine D_5	10.0	µM	22			
22400	Endothelin ET_A	10.0	µM	-5			
22410	Endothelin ET_B	10.0	µM	1			
22600	Estrogen	10.0	µM	1			
22650	$GABA_A$, Agonist Site	10.0	µM	5			
22660	$GABA_A$, Bezodiazepine, Cen.	10.0	µM	16			
22680	$GABA_A$, Chloride Channel	10.0	µM	18			
23200	Glucocorticoid	10.0	µM	8			
23270	Glutamate, Kainate	10.0	µM	-3			
23280	Glutamate, NMDA, Agonist	10.0	µM	-7			
23900	Glycine, Strychnine-Sens.	10.0	µM	-3			
23950	Histamine H_1, Central	10.0	µM	16			
23970	Histamine H_2	10.0	µM	15			
23980	Histamine H_3	10.0	µM	14			
24350	Interleukin-1α	10.0	µM	-7			
25060	Leukotriene D_4	10.0	µM	5			
25260	Muscarinic M_1	10.0	µM	-17			
25270	Muscarinic M_2	10.0	µM	-3			
25280	Muscarinic M_3	10.0	µM	21			
25290	Muscarinic M_4	10.0	µM	-15			
25300	Muscarinic M_5	10.0	µM	-4			
25551	Neurokinin NK_1	10.0	µM	19			
25560	Neurokinin NK_2	10.0	µM	5			
25700	Neuropeptide Y_1	10.0	µM	-6			
25710	Neuropeptide Y_2	10.0	µM	31			
25850	Nicotinic Acetylcholine, Cen.	10.0	µM	5			
26010	Opiate-δ	10.0	µM	17			
26020	Opiate-κ	10.0	µM	-17			
26040	Opiate-µ	10.0	µM	17			
26560	K^+ Channel [K_{ATP}]	10.0	µM	6			
26570	K^+ Channel [K_V]	10.0	µM	7			
26580	K^+ Channel [SK_{Ca}]	10.0	µM	-4			
◆ 27111	Serotonin 5-HT_{1A}	10.0	µM	65			
◆		10.0	µM	51			
		1.0	µM	30			
		0.1	µM	23			
		0.01	µM	21			

Results with ≥50% stimulation or inhibition are **boldfaced**. (Negative values correspond to stimulation of binding or enzyme activity)

PHARMACOLOGY REPORT
EXPERIMENTAL RESULTS
BIOCHEMICAL ASSAYS

PT#: 138249
CODE: CV-10
E4-NE-002

August 11, 1997
M.W.: 500 Solvent: 0.5% DMSO PAGE 6

	TARGET			PERCENT INHIBITION	IC_{50}	K_i	nH
27160	Serotonin 5-HT_2	10.0	µM	23			
27180	Serotonin 5-HT_3	10.0	µM	-10			
27200	Serotonin 5-HT_4	10.0	µM	12			
27810	Sigma, σ_1	10.0	µM	25			
27820	Sigma, σ_2	10.0	µM	9			
28500	Testosterone	10.0	µM	8			
28550	Thromboxane A_2	10.0	µM	-2			
28650	TNF-α	10.0	µM	-5			
28701	Vasoactive Intest. Pep. - VIP_1	10.0	µM	-5			
28750	Vasopressin V_1	10.0	µM	16			

The screening lab came to the conclusion that displacement of radioligand from adrenergic α1A and adrenergic α1B binding sites was related to functional receptor antagonism. Displacement of radioligand from serotonin 5-HT1A binding sites appeared to be related to functional receptor agonism. Displacement of radioligand from adrenergic α2A and adrenergic β2 were concluded to be unrelated to functional receptor agonism or antagonism.

pharmacology report for the S-enantiomer of ranolazine free base. August 11, 1997. 138250

The S-enantiomer of ranolazine was dissolved in 0.5% DMSO and tested against the same panoply of receptors. The summary of significant responses is shown below.

PT#: 138250
CODE: CV-8 M.W.: 500 Solvent: 0.5% DMSO PAGE 3

Biochemical assay results are presented as the percent inhibition of specific binding or activity throughout the report and unless noted are the average of duplicate tubes tested at each concentration.

- *For primary assays, only the lowest concentration with a significant response is shown in this summary. Quantitative data (K_i, IC_{50}, nH) is shown only where applicable to client request.*
- *Where applicable, all secondary assay results meeting significance criteria are shown in this summary.*
- *Please see Experimental Results section for details of all responses.*

Significant responses (meeting significance criteria) were noted in the assays listed below:

CAT#	PRIMARY ASSAY NAME	CONC.	% INH.	IC_{50}	K_i	nH
20050	Adenosine A_1	10 µM	80			
20310	Adrenergic α_{1A}	10 µM	59			
20383	Adrenergic α_{1B}	10 µM	52			
20411	Adrenergic β_2	10 µM	51			
27111	Serotonin 5-HT_{1A}	10 µM	63			

CAT#	SECONDARY ASSAY NAME	DOSE/CONC.	CRITERIA	RESULT	ACTIVITY
40801	Adrenergic α_1 Antagonism	10 µM	≥ 50%	65	Y
46950	Serotonin 5-HT_{1A} Agonist	10 µM	≥ 50%	86	Y

The S-enantiomer, like the free base, showed activity for the adrenergic receptors as well as the calcium channel and opiate receptors. The sponsor also came to the same conclusion that displacement of radioligand from adrenergic α1A and adrenergic α1B binding sites was related to functional receptor antagonism. Displacement of radioligand from serotonin 5-HT1A binding

sites appeared to be related to functional receptor agonism. Displacement of radioligand from adrenergic α2A and adrenergic β2 were concluded to be unrelated to functional receptor agonism or antagonism.

[ ] *pharmacology report for the R-enantiomer of ranolazine free base. August 11, 1997.* [ ] *138251.*

The R-enantiomer was dissolved in 0.5% DMSO and tested against the various receptors as was done with the free base and S-enantiomers. The lab reported that no significant responses were observed in any primary assay. It may still be seen that there was substantial binding activity for the adrenergic receptors, serotonin 5HT1A, calcium channels and opiates.

PT#: 138251
CODE: CV-9
PA-18877-70

EXPERIMENTAL RESULTS
BIOCHEMICAL ASSAYS
August 11, 1997
M.W.: 500 Solvent: 0.5% DMSO
PAGE 4

CAT.#	TARGET	CONC.		PERCENT INHIBITION %	IC_{50}	K_i	nH
20050	Adenosine A_1	10.0	µM	-5			
20060	Adenosine A_{2A}	10.0	µM	8			
20070	Adenosine A_3	10.0	µM	-15			
20310	Adrenergic α_{1A}	10.0	µM	45			
20320	Adrenergic α_{1B}	10.0	µM	15			
20362	Adrenergic α_{2A}	10.0	µM	35			
20370	Adrenergic α_{2B}	10.0	µM	36			
20401	Adrenergic β_1	10.0	µM	-1			
20411	Adrenergic β_2	10.0	µM	11			
20420	Adrenergic β_3	10.0	µM	10			
21000	Angiotensin AT_1	10.0	µM	11			
21010	Angiotensin AT_2	10.0	µM	14			
21150	Bombesin	10.0	µM	-8			
21250	Bradykinin B_1	10.0	µM	15			
21260	Bradykinin B_2	10.0	µM	6			
21450	Ca^{2+} Ch. (L), Benzothiazopine	10.0	µM	18			
21460	Ca^{2+} Ch. (L), Dihydropyridine	10.0	µM	15			
21500	Ca^{2+} Ch. (L), Phenylalkylamine	10.0	µM	-16			
21600	Ca^{2+} Channel (N)	10.0	µM	5			
21701	Cannabinoid CB_1	10.0	µM	11			
21710	Cannabinoid CB_2	10.0	µM	15			
21801	Cholecystokinin CCK_A	10.0	µM	28			
21811	Cholecystokinin CCK_B	10.0	µM	23			
21950	Dopamine D_1	10.0	µM	2			
21960	Dopamine D_2	10.0	µM	23			
21980	Dopamine D_3	10.0	µM	1			
21990	Dopamine $D_{4.2}$	10.0	µM	-2			
22020	Dopamine D_5	10.0	µM	19			
22400	Endothelin ET_A	10.0	µM	-7			
22410	Endothelin ET_B	10.0	µM	-3			
22600	Estrogen	10.0	µM	8			
22650	$GABA_A$, Agonist Site	10.0	µM	-13			
22660	$GABA_A$, Benzodiazepine, Cen.	10.0	µM	6			
22680	$GABA_A$, Chloride Channel	10.0	µM	5			
23200	Glucocorticoid	10.0	µM	9			
23270	Glutamate, Kainate	10.0	µM	12			
23280	Glutamate, NMDA, Agonist	10.0	µM	-15			
23300	Glycine, Strychnine-Sens.	10.0	µM	20			
23950	Histamine H_1, Central	10.0	µM	12			
23970	Histamine H_2	10.0	µM	20			

Results with ≥50% stimulation or inhibition are boldfaced. (Negative values correspond to stimulation of binding or enzyme activity)
138251BP.CV [ ]

NDA 21-526

PHARMACOLOGY REPORT
EXPERIMENTAL RESULTS
BIOCHEMICAL ASSAYS

PT#: 138251
CODE: CV-9
PA-18877-70

August 11, 1997

M.W.: 500 Solvent: 0.5% DMSO

	TARGET			PERCENT INHIBITION	IC50	Ki	nH
				-100 -50 0 50 100			
23980	Histamine H_3	10.0	µM	12			
24350	Interleukin-1α	10.0	µM	2			
25060	Leukotriene D_4	10.0	µM	7			
25260	Muscarinic M_1	10.0	µM	18			
25270	Muscarinic M_2	10.0	µM	16			
25280	Muscarinic M_3	10.0	µM	24			
25290	Muscarinic M_4	10.0	µM	13			
25300	Muscarinic M_5	10.0	µM	-4			
25551	Neurokinin NK_1	10.0	µM	-12			
25560	Neurokinin NK_2	10.0	µM	3			
25700	Neuropeptide Y_1	10.0	µM	-7			
25710	Neuropeptide Y_2	10.0	µM	4			
25860	Nicotinic Acetylcholine, Cen.	10.0	µM	21			
26010	Opiate-δ	10.0	µM	20			
26020	Opiate-κ	10.0	µM	-11			
26040	Opiate-µ	10.0	µM	4			
26560	K^+ Channel [K_{ATP}]	10.0	µM	10			
26570	K^+ Channel [K_V]	10.0	µM	6			
26580	K^+ Channel [SK_{Ca}]	10.0	µM	-4			
27111	Serotonin 5-HT_{1A}	10.0	µM	7			
27160	Serotonin 5-HT_2	10.0	µM	21			
27190	Serotonin 5-HT_3	10.0	µM	-11			
27200	Serotonin 5-HT_4	10.0	µM	17			
27810	Sigma, σ_1	10.0	µM	33			
27820	Sigma, σ_2	10.0	µM	33			
28500	Testosterone	10.0	µM	5			
28550	Thromboxane A_2	10.0	µM	7			
28650	TNF-α	10.0	µM	0			
28701	Vasoactive Intest. Pep. - VIP_1	10.0	µM	-3			
28750	Vasopressin V_1	10.0	µM	10			

Results with ≥50% stimulation or inhibition are **boldfaced**. (Negative values correspond to stimulation of binding or enzyme activity)

AT4927 The effect of RS-43285-193 on opioid receptor binding in Guinea Pig brain. November 1988. RS-43285-193 was tested for the ability to compete for mu, kappa and delta opioid receptor binding in guinea pig brain homogenates. Concentrations used were 0.1 nM - 100μM. Tritiated etorphine (kappa binding ligand), penicillamine-enkephalin (delta binding) and glyol (mu binding) were used as competitors. The opioid antagonist naloxone was used at several concentrations that included 15 nM, 21 nM and 146 nM.

Ranolazine was reported to be inactive at all concentrations tested for delta binding but showed activity at concentrations ≥ 10μM for mu and kappa binding. The only data presented was the textual comment that at 100μM, ranolazine caused 27% and 24% inhibition of mu and kappa receptor binding respectively. Naloxone was reported to give 50% inhibtion in mu, kappa and delta binding assays at 15nM, 21nM and 146 nM respectively. By comparison, ranolazine showed lower affinity for the opioid receptors.

AT4707 The effects of RS-43285-197 in K^+ depolarised smooth muscle; interactions with Ca^{2+} and Ca^{2+} channel activators. January 1986.

Taenia were isolated/prepared from the cecums of female guinea pigs and established in organ baths with a calcium free K^+-Tyrode solution. Cumulative calcium response curves for Ca^{2+} (0.01, 0.03, 0.1, 0.3, 1,3 mmol/l) were obtained by increasing calcium at 3 minute intervals. In some experiments, preparations were incubated with RS-43285 for 30 minutes prior to being half-maximally contracted with Ca^{2+} (0.3×10^{-3} mol.l^{-1}). After the contractions had stabilized, a concentration-response curve was obtained to the Ca^{2+}channel activators Bay K 8644 (10^{-9} to 3×10^{-6} mol.l^{-1}) or palmitoyl carnitine (10^{-5} to 3×10^{-4} mol.l^{-1}).

Results: Concentrations of RS-43285 from 10^{-5} to 10^{-3} mol.l^{-1} antagonized responses to Ca^{2+} in the K^+ depolarized taenia preparations. The sponsor reported that the combination of ranolazine with either Bay K 8644 or palmitoyl carnitine did not affect the augmentation of calcium-induced contractions by the latter two compounds.

The sponsor concluded that although ranolazine's inhibition of K+-depolarized smooth muscle was indicative of calcium channel blockade, "the concentration of RS-43285 necessary to achieve this effect is so high,... that RS-43285 must act as an anti-anginal agent by some other mechanism. This suggestion is further confirmed by the lack of effect with the Ca2+ channel activators Bay K 8644 and palmitoyl carnitine."

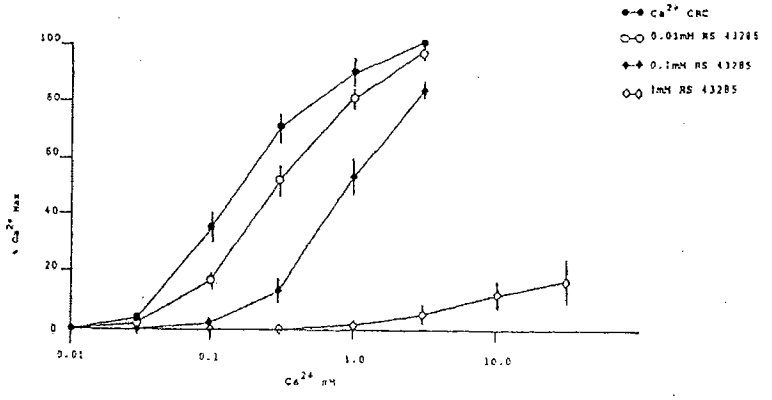


Figure 1: The effect of RS-43285 on K^+-depolarised guinea-pig taenia smooth muscle preparations.

AT5425 Effects of RS-43285-193 on guinea pig cardiac ventricular action potentials in vitro under normal and ischemic conditions. Dec. 1990
Female Duncan-Hartley guinea pigs were euthanized and the hearts collected in ice cold physiologic "salt" solution. Free running papillary muscles, 1 mm diameter were dissected from the right ventricle and transferred to a perfusion chamber. The muscle preparations were perfused with a physiologic salt solution (PSS). Solutions were preheated and oxygenated. The preparation was stimulated at a basal frequency of 1 Hz and supramaximal intensities. Resting membrane potentials and action potentials were recorded from an intracellular microelectrode. The effects of each concentration of ranolazine were monitored for over 30 minutes. At the end of each 30 minute period the stimulation frequency was increased to 3.3 Hz to investigate use-dependent blockade of sodium channels.

In a separate set of tissues, the effects of ranolazine on ischemia-induced changes in electrical and mechanical activity were examined. After electrode placement, the tissue was exposed to physiologic salt solution in which the glucose had been replaced by mannitol and which was vigorously gassed with N_2. Changes in APD, contractility and tension were monitored every 5 minutes for 30 minutes. The tissue was then superfused with standard physiologic salt solution for 40 minutes. Drug or vehicle was then added to the superfusing solution and equilibration allowed for 20 minutes. In the continuing presence of drug or vehicle the tissue was then exposed to glucose-free PSS(gassed with N2) for a further 30 minute period.

Results:
Over the concentration range of 0.1, 1, 10 and 30 μM, ranolazine had no effect on maximum diastolic potential. At concentrations of 10^{-6}, 10^{-5} and $3x10^{-5}$, a dose-dependent prolongation of action potential duration was apparent. The maximum rate of depolarisation at both 1.0 and 3.3 Hz was decreased compared to control at these concentrations. However, the only significant change was at the highest concentration at a frequency of 3.3 Hz. The sponsor's results are shown below. Data are mean ±sem from 4 separate experiments.

Effects of RS-43285-193 on maximum diastolic (resting) potential, maximum rate of depolarisation (V_{max}) and action potential duration

Concentration (M)	Membrane Potential (mV)	Maximum rate of Depolarisation 1.0 Hz (V/s)	3.3 Hz	Action Potential Duration (ms)
0	-82.4 ± 2.46	209.3 ± 15.00	202.9 ± 13.76	194.0 ± 2.37
10^{-7}	-83.8 ± 1.51	202.5 ± 8.82	195.4 ± 8.77	193.0 ± 4.08
10^{-6}	-82.7 ± 1.70	203.6 ± 9.28	194.3 ± 7.66	195.1 ± 2.77
10^{-5}	-82.8 ± 2.27	200.4 ± 8.50	171.1 ± 6.44	211.8 ± 2.06**
$3x10^{-5}$	-83.5 ± 2.05	191.8 ± 6.57	134.3 ± 8.76**	224.4 ± 4.11**

** indicates a value significantly different from control ($p < 0.01$, Analysis of Variance and Dunnett's t-test).

'e mear s.e. of mean from 4 separate experiments.

Appears This Way
On Original

Ischemia-induced increases in tension were larger in the presence of drug than in control tissues . The sponsor conludes that the increase in APD was either due to calcium or potassium channel effects. The conclusion goes on to state that as ranolazine has no positive inotropic effects at the concentrations used, the effect is probably due to blockade of cardiac potassium channels. Given the effects in this study on Vmax and APD, the sponsor summarizes that in this model system, the drug has effects similar to Class I and Class III anti-arrythmic drugs. The study would be stronger for the inclusion of positive controls, either historical or concurrent.

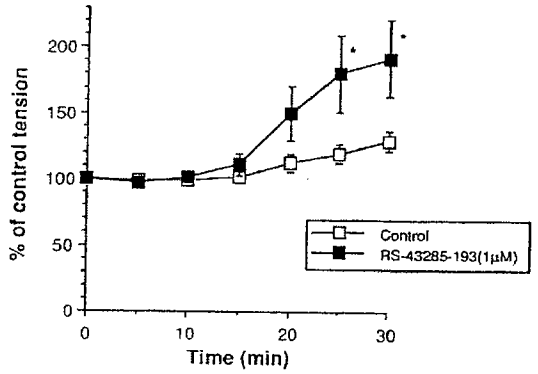


Figure 6.
Effects of RS-43285-193 on ischaemia-induced changes in tension.

Data are means ± s.e. mean (n=4-5 preparations). * indicates a significant difference relative to vehicle control tissues ($p<0.05$; Analysis of Variance and Bonferroni modified t-test).

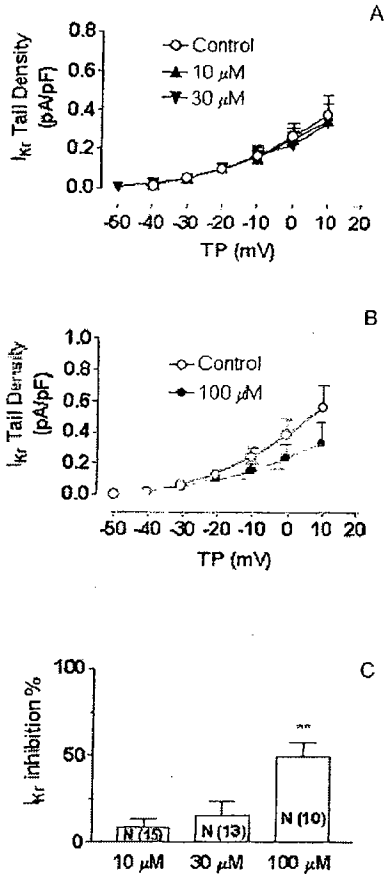
CVT303.041P Effects of ranolazine on I_k , I_{Na} and I_{Ca} in canine atrial myocytes. September 2002.

Adult mongrels were anesthetized and the hearts collected and immersed in Tyrode solution. Left atrial myocytes were isolated via collagenase perfusion and kept in high K+ storage. Nimodipine (1μM), dofetilide(1μM) and atropine(1μM) were used to block I_{Ca}, I_{kr} and acetylcholine-dependent K+ current respectively. General voltage clamp techniques were used.

Total I_k currents were elicited by 3-s depolarizing pulses from –50 to +70 mV followed by 2-s repolarizations to –40 mV to observe tail currents. I_{ks} was studied in the presence of E-4301 (5μM) to inhibit I_{kr}. I_{kr} recordings were obtained with 200 ms pulses from –60 mV to +10mV followed by 2 s repolarizations to –40 mV to observe tail currents.

I_{Ca} was recorded on 240 ms depolarizing pulses from –50mV to voltages ranging from –40mV to +60 mV.

Figure 2 Effect of Ranolazine on I_{Kr} Based on Tail Current Measurements



Panel A: Effect of 10 and 30 μM ranolazine on I_{Kr} tail currents (in pA/pF). Panel B: Effect of 100 μM ranolazine on I_{Kr} tail currents (in pA/pF). *P<.05; **P<.01 vs control. Panel C: Percent reduction in I_{Kr} by ranolazine concentrations indicated, based on tail currents following an activating step to 0 mV.

100% inhibition of the calcium channels was achieved.

I_{Na} was recorded during 40 ms depolarizations applied at 1 and 2 Hz from a holding potential of –140 to –40 mV.

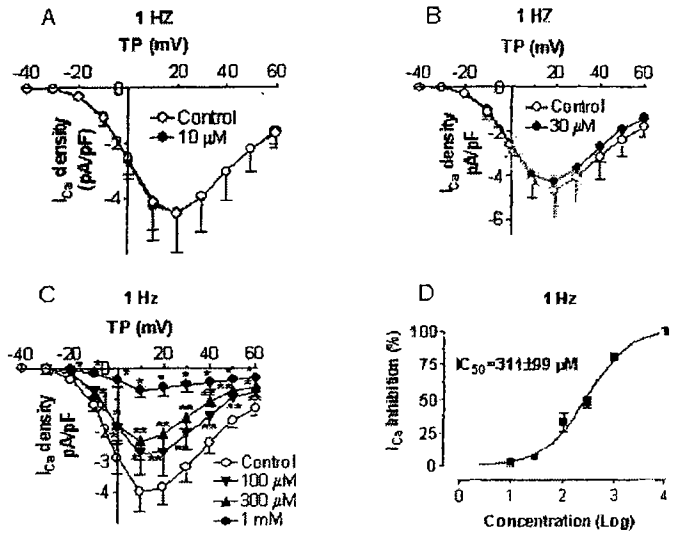
Recordings were performed before drug appliction as the control, after 10 minutes of superfusion with ranolazine and after washout (time undefined) of drug.

Results:
Note: The numbers given below were taken from the text of the results section.
Ranolazine caused a dose-dependent decrease in I_{kr} tail density: 8.2%(10μM), 15.2%(30μM) and 49.3%(100μM) compared to control conditions.

Ranolazine decreased I_{ks} density by 16%(100μM), 17% (300μM) and 27%(1000μM).

Ranolazine also had an affect on I_{Ca} at concentrations ≥100 μM. Based upon the graphical results,

Figure 4 Inhibition of I_{Ca} by Ranolazine During Depolarizing Pulses at a Frequency of 1 Hz



Panels A, B, and C: Current density-voltage relations for I_{Ca} under control conditions and in the presence of 10 (n = 6), 30 (n = 6), 100 (n = 8), 300 (n = 7), and 1000 μM (n = 5) ranolazine. Panel D: Concentration-response curve for ranolazine inhibition of I_{Ca} (at +10 mV, n = 8). Results are mean ± SEM. *P < 0.05 and **P < 0.01 vs control.

In canine atrial myocytes under the conditions used in this study, ranolazine inhibits I_{kr}, I_{ks} and I_{Ca} with a potency order of $I_{Kr}>I_{Ca}>I_{Ks}$ but an efficacy order of $I_{Ca}>I_{Kr}>I_{ks}$. The study would be stronger for the inclusion of positive controls or comparator compounds.

CVT303.048-P Electrophysiological effects of ranolazine in the guinea pig heart in vivo. March 2002.

The report describes this as a novel in vivo guinea pig model. Anesthetized guinea pigs were intubated and ventilated with room air. The right carotid artery was cannulated for direct blood pressure measurements. A custom made catheter was introduced through the left carotid artery into the left ventricle to record endocardial monophasic action potentials (MAP). Another electrode catheter was positioned in the right atrium and ventricle for atrial and ventricular pacing.

The treatment groups included:

Group I	Control
Group II	Vehicle control
Group III	Ran 2.5 mg/kg bolus + 90µg/kg/min iv (LD Ran)
Group IV	Quinidine 10 mg/kg bolus + 50µg/kg/min, iv
Group V	Quinidine 10 mg/kg bolus + 50µg/kg/min, iv + Ran 2.5 mg/kg bolus + 90µg/kg/min iv
Group VI	Ran 5 mg/kg bolus + 180 µg/kg/min, iv (HD Ran)
Group VII	Quinidine 10 mg/kg bolus + 50µg/kg/min, iv + HD Ran

For all groups, electrophysiological parameters were recorded and determined following instrumentation, a 20 minute stabilization period and a 15 minute saline infusion, vehicle infusion,quinidine, low and high doses of Ranolazine and in the presence of quinidine. Blood samples for plasma level determination of drug were collected at 15 minutes following the administration of Ran.

Results: We do not know the stability of this model over the duration of time that it took to collect the data. Ranolazine caused a dose-related increase in $MAPD_{100}$. The effect was additive with quinidine. The sponsor's results are shown below.

Table 1: Sinus cycle length (SCL), systemic blood pressure (BP), and left ventricular monophasic action potential duration at 100% repolarization ($vMAPD_{100}$) before (Baseline) and 15 min after commencement of various treatments (Treatment).

Group	Baseline			Treatment (15 min)		
	SCL (ms)	BP (mm Hg)	$vMAPD_{100}$ (ms)	SCL (ms)	BP (mm Hg)	$vMAPD_{100}$ (ms)
Control	241±8	51±2	123±9	248±11	51±3	123±9
Carrier	232±7	49±2	129±2	246±14	49±4	126±3
Quinidine	220±7	53±2	120±3	248±8*	54±3	134±5*
Low-dose Ranolazine	256±20	58±3	121±2	278±12	56±3	133±4
Low-dose Ranolazine + Quinidine	221±16	56±2	128±4	292±8*	54±2	152±6*
High-dose Ranolazine	215±6	49±5	118±4	260±10*	50±4	144±5*
High-dose Ranolazine + Quinidine	218±6	46±2	119±4	306±8*	46±3	166±5*

Table 2: Values of electrophysiological parameters measured before (control) and after treatment with Ranolazine (RAN), alone and in combination with Quinidine (Q). Vehicle (carrier), low dose RAN (2.5 mg/kg + 90 µg/kg/min, IV) and high-dose RAN (5 mg/kg + 180 µg/kg/min, IV), Quinidine (10 mg/kg + 50 µg/kg/min, IV), alone and in the presence of low and high doses of RAN.
Values are mean ± SEM.

Parameter (msec)	Control (n=8)	Carrier (n=8)	Low RAN (n=8)	Q (n=8)	Low RAN + Q (n=8)	High RAN (n=8)	High RAN + Q (n=8)
	After 15 min infusion						
VRP_{90}	96±2	95±2	107±4	107±3	124±2*	127±5†	143±5††
VRP_{80}	94±3	92±2	106±4	106±3	120±3*	124±5†	136±6††
ARP_{90}	67±3	67±4	75±2	82±4	91±2*	93±6†	113±8††
ARP_{80}	67±3	66±4	76±3	81±4	90±2*	92±6†	111±8††
$SNRT_{90}$	290±18	266±10	336±24*	253±6	284±10	325±13*	326±13*
$SNRT_{80}$	296±18	269±13	348±29*	265±7	293±8	326±16*	335±15*
AVB_W	151±4	148±6	165±12	144±3	165±11	162±6	189±10*

$VRP_{90,80}$, $ARP_{90,80}$ and $SNRT_{90,80}$ are the ventricular and atrial refractory periods and sinus node recovery times, respectively, measured at 80% and 90% of SCL. AVB_W is the AV nodal Wenckebach point defined as the longest atrial cycle length at which second-degree AV block occurs. * P<0.05 vs. control, carrier, Quinidine alone; † P<0.05 vs. control, carrier, L_RAN; †† vs. all other groups.

Best Possible Copy

ECGs were monitored but ECG intervals including QT, QRS, PR etc were not provided.

From the sponsor's data above it may be seen that repolarization periods of both the atria and ventricles increased with increasing dose of ranolazine. Both doses of ranolazine when combined with quinidine caused a greater effect than either drug alone.

CVT303.012N: Effects of ranolazine on membrane potentials and currents of guinea pig isolated ventricular myocytes .January 2002.
Single ventricular myocytes were isolated from the hearts of adult male guinea pigs (breed unspecified). Myocytes were placed in a recording chamber and superfused with Tyrode solution at 35°C. Transmembrane voltages were measured with glass electrodes containing appropriate solutions. Initial experiments showed that ranolazine increased the action potential duration and $I_{Ca(L)}$.Therefore additional studies were conducted with nitrendipine for comparison.
The effect of ranolazine on membrane potentials: action potentials were induced by 5 ms-depolarizing pulses applied at a frequency of 0.5 Hz. The duration of the action potential was measured at APD_{50} and APD_{90}. Ranolazine was applied at concentrations of 1,3,10, 30 and 100 µmol/l. The effect at each concentration was determined on 5 myocytes.

Resting membrane potential (RMP) was determined as the stable diastolic potential. The effect of ranolazine on the RMP was determined after the cells were treated with 10, 30 or 100 µmol/l ranolazine.

Effect on I_{k1}: Ik1 was elicited by a 4.8 second ramp voltage clamp pulse from –130-+50 mV at a frequency of 0.16 Hz. The amplitude of the current of 4 myocytes ± ranolazine (1-30 µmol/l) was determined.

Effect on $I_{Ca(L)}$: A 150-200 ms voltage clamp pulse from –40mV to 0mV was applied at a frequency of 0.5Hz and K+ in both Tyrode and pipette solutions was replaced by equimolar Cs+. The amplitude of the $I_{Ca(L)}$ was determined as the maximal inward current. Ranolazine was applied at concentrations of 3 and 30 µmol/l. Each concentration was tested on 5-6 myocytes.

Effect on I_k: IK was elicited by a 1-s depolarizing pulse from –40mV to +30mV at a frequency of 0.16 Hz. The amplitude of the tail current was measured to determine the amplitude of Ik. 30µmol/l Ran was tested on 6 myocytes.

Nitrendipine: The inhibitory effects of nitrendipine on $I_{Ca(L)}$ at concentrations of 0.1-1 µmol/l were tested. It was found that nitrendipine at 0.1 µmol/l mimicked the effect of ranolazine on $I_{Ca(L)}$. Further experiments were therefore performed to assess the affect of this concentration of nitrendipine on the APD.

Results: The sponsor's results are shown below. Ranolazine at concentrations of 1-100µM shortened APD. When expressed as a percentage of the control: 7±2 %(1 µM,APD_{50}), 18±2% (100 µm, APD_{50}) and 5±2%(1 µM, APD_{90}), 12±1% (100 µM, APD_{90}).
No effect on Ik1 was shown.
Ranolazine caused a decreased amplitude of $I_{Ca(L)}$ which was only partly reversible after washout of the drug. Nitrendipine at 0.1 µmol/l mimicked the effect of ranolazine on the calcium channel.
Reviewer's Summary of Results

	control	Ranolazine (µM)				
		1	3	10	30	100
APD_{50}(ms)	238± 20(5)	222± 20*		208±15		
	246± 17(5)		221± 15*		206± 14*	
	249± 23(5)					203± 15*
APD_{90}(ms)	272± 21(5)	257± 18		246± 15*		
	279± 18 (5)		254± 15*		248± 16*	
	284± 24 (5)					249± 18*
RMP (mV)	-80.9±1.3(14)				-80.8± 1.3	
$I_{Ca(L)}$(nA)	1.30±0.25 (5)		1.21± 0.24*			
	1.13±0.25 (6)				0.970.20*	
$I_{k(tail)}$ (pA)	155± 16 (6)				107±28*	

The sponsor was specifically asked (February 25, 2003) to identify the studies that were crucial to support the proposed mechanism of action and electrophysiological claims. The following are summary reviews of those studies.

Mechanism of Action

AT6055:The effect of ranolazine enantiomers, RS-43285-197 (s-isomer) and RS-43285-198 (r-isomer), compared to ranolazine racemate (RS-43285-193) on ischemic ECG changes and hemodynamic function in the dog model of transient myocardial ischemia. August 1988

Adult Beagles of both sexes were anesthetized and subjected to cardiac pacing at 50-80 beats per minute above resting heart rate for 1 minute prior to LAD occlusion. Pacing plus occlusion was carried out for another 2 minutes. Ten minutes of rest was followed by another pacing event. Episodes of pacing and occlusion were repeated at least four times or until 2 consecutive challenges produced the same degree of S-T segment elevation with a return to baseline between challenges. ST segment measurements were made at 45, 60, 75, 90 and 105 seconds into the occlusion period and at 2 second intervals (10 values) after termination of occlusion and pacing. Two pre-drug conditioning S-T segment elevations for each electrode were averaged and this value taken as 100%. Each test event thereafter was expressed as a percentage of this baseline value. The dose range covered cumulative iv doses of 5, 15-20, 50, 200, 500 and 1500 μg/kg with each successive dose increment being injected 5 minutes into the reperfusion phase following each ischemic challenge. The treatment group sizes were: control (n=9), racemate (n=13), R isomer (n=7), S isomer (n=8).

Results: Repeated ischemic episodes produced S-T elevation. The time-related rise after injury was described as small compared to that apparent upon reperfusion. Some 77% of the dogs (n=35) showed a range of mean ±se percentage increase of S-T elevation of 180%-400% (259±10%, n=27). There was a wide range of baseline S-T segment elevation in response to ischemia. Results were presented as percentage change of the baseline. The use of percentages makes one wonder what effect would have been observed had the ranges of absolute response been shown. Note also that the acute nature of this model means that there is no time for electrical remodeling or alterations to the myocardium. This is essentially a healthy heart and conduction system. Blood pressure changes were shown graphically, with minor changes apparent but unquantifiable given the presentation. There appeared to be a dose-related increase in diastolic blood pressure with the R-isomer. Heart rate changes were apparent in the graphical presentations. Based on the results presented, the enantiomers and the racemate appear to decrease ST segment elevation as a percentage of baseline in this model.

Appears This Way
On Original

Figure 3a

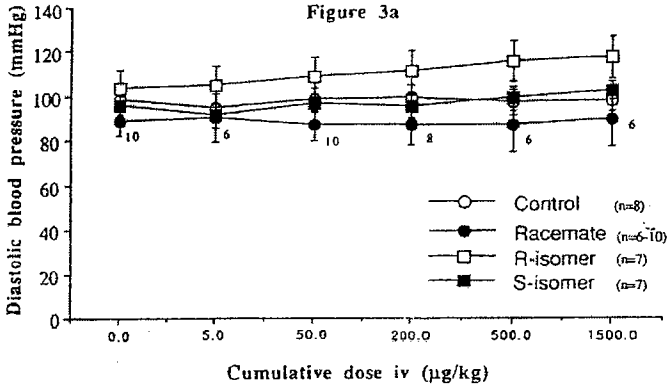


TABLE 1

The effect of ranolazine racemate and its R- and S- isomers on S-T segment changes in epicardial electrocardiograms from the dog ischaemic myocardium stressed by electrical pacing

Insult phase

Dose mcg/kg	Untreated (n=9)	S-isomer (n=8)	R-isomer (n=7)	Racemate (n=6-13)
Control	100%	100%	100%	100%
5	101.0 ± 2.6	91.7 ± 5.1	98.3 ± 4.6	87.8 ± 3.8(7)**
20	103.8 ± 3.2	87.8 ± 3.3***	89.8 ± 2.7***	89.2 ± 2.4(6)***
50	102.1 ± 3.5	90.5 ± 8.1	89.1 ± 4.1*	76.6 ± 5.6(12)**
200	95.8 ± 3.0	82.5 ± 4.0**	87.9 ± 2.5+	70.8 ± 5.4(13)****
500	95.7 ± 2.8	76.5 ± 5.7***	77.4 ± 4.4***	75.0 ± 7.6(8)*
1500	93.5 ± 2.1	73.1 ± 4.2****	73.9 ± 2.8****	67.3 ± 6.5(8)***

Recovery phase

Dose mcg/kg	Untreated (n=8-9)	S-isomer (n=8)	R-isomer (n=7)	Racemate (n=7-11)
Control	100%	100%	100%	100%
5	101.4 ± 3.1	87.4 ± 4.4**	102.2 ± 7.2	98.3 ± 5.6(7)
20	99.5 ± 3.5	84.9 ± 4.3**	88.3 ± 4.3	90.2 ± 8.4(7)
50	102.8 ± 4.4	84.9 ± 5.9*	93.5 ± 5.4	87.3 ± 4.6(9)*
200	98.2 ± 2.7	82.6 ± 5.1**	89.0 ± 2.7	80.9 ± 3.9(11)****
500	98.4 ± 2.9	79.6 ± 5.1***	82.7 ± 4.1	81.1 ± 5.4(7)**
1500	98.4 ± 4.1(8)	75.1 ± 4.3***	74.9 ± 4.1***	76.7 ± 6.6(7)**

Data are means ± s.e.m. Numbers in parenthesis denote number of animals.
Statistical significances are shown: *P<0.05, **P<0.02, ***P<0.01, ****P<0.001 versus the corresponding untreated value by the Student's t-test.
Corresponding Bonferonni adjustment for multiple comparisons: *P<0.075(NS), **p<0.03, ***p<0.015, ****p<0.0015.
+p<0.03 for the effect of R-isomer versus Racemate; no other isomer to racemate or isomer to isomer comparisons were found to be statistically significantly different from one another.

AT3378: The effects of RS-43285 on the biochemical consequences of transient myocardial ischemia in the dog.

Three anesthetized dogs were paced at 50 – 80 bpm above resting heart rate for 1 minute before occlusion of the LAD. Pacing and occlusion lasted for 2 minutes during which time blood was collected from the coronary vein. The next cycle was begun after a 10 minute rest period. Five control challenges were performed followed by 3 post-drug challenges. RS-43285 was given iv for cumulative doses of 50, 200 and 500 µg/kg. Blood gases, electrolytes, glucose, lactate and ffa were measured.

"Production" and "uptake" parameters were calculated based on arterio-venous differences:

(arterio-coronary venous difference)/arterial x 100%

"consumption" = (arterio-venous difference)x regional myocardial blood flow ($ml.100g^{-1}.min^{-1}$) divided by (weight myocardial "ischemic bed"(g) x 100)

It was said that limitations of coronary blood flow data made calculation of the 500 µg/kg dose level impossible.

Results:
There was no table of values. Data was given graphically. Numbers were found in the textual description of results. The ischemic insult produced a 24% increase in FFA uptake from myocardial venous blood. Ranolazine caused a decrease in FFA uptake in the myocardium. At all 3 doses RS-43285 caused an increase in the disappearance of NEFAs from femoral arterial blood. The ischemic insult caused an increase in glucose extraction from 4.3% to 21.5%. Ranolazine decreased myocardial glucose extraction approximately 59% at all doses. The ischemic insult produced an 8% rise in myocardial glucose consumption. This was reduced to 2.9% and 4.6% by 50 and 200 µg/kg RS-43285 respectively (47%-66% fall from the basal value). Myocardial glucose consumption was also slightly decreased by 2.9% and 4.6% at 50 and 200 µg/kg respectively. It was stated that ischemia caused a decrease in oxygen consumption and that ranolazine further decreased the O_2 consumption. There was insufficient information to quantify this difference.

Between untreated controls and all drug-treated groups there was a decrease in myocardial venous lactic acid production expressed as % of baseline. There was no dose-response apparent. There were no differences between treated and untreated groups in cephalic venous or femoral arterial blood. K+ efflux during electrical stress pacing was decreased in blood samples collected from the 3 different blood vessels in treated animals compared to controls. NEFA uptake was decreased in all treated myocardial samples. NEFA uptake in cephalic venous samples were decreased at LD and increased at MD and HD compared to control. The percent glucose extraction decreased relative to control in all treated samples. Myocardial glucose consumption increased in control and treated groups. The increase in control was ~9% compared

to 3 and 5% in the treated groups. Myocardial O_2 extraction expressed as a percent increase from baseline was the same in control and LD (~5.5%), MD was at 3% and the HD was at 4.5%. It's not clear if these differences are variability of the system or real. There was no effect on myocardial pH values. Some of the differences between the groups, shown as percentage of baseline values, are very small (e.g. half a percentage) and one may wonder if a number of the differences are real.Use of a comparator compound might increase confidence that the model can be used to detect treatment differences.

Appears This Way On Original

CVT303.024-P Effects of acute intravenous ranolazine on left ventricular function in dogs with chronic heart failure.Oct. 1998-Aug 1999. Reported Nov 11, 1999

LV dysfunction and failure (LVEF 27±2%) was produced in 7 dogs by multiple sequential intracoronary microembolizations over a period of 5-15 weeks. Following a 2-3 week recovery period, hemodynamic and angiographic measurements were made before and 40 minutes after iv administration of ranolazine (0.5 mg/kg bolus and a 40 minute continuous iv infusion of 1.0 mg/kg/hour). Each animal served as its own control.

Results: There were no differences in arterial-coronary sinus samples for glucose, lactate or ffa after ranolazine. There were no significant effects on HR, systolic pressure, diastolic pressure, mean aortic pressure, LVEDP, peak dP/dt or several other parameters. There were non-significant increases in systolic aortic pressure, diastolic aortic pressure, mean aortic pressure, peak dP/dt and peak – dP/dt. Ejection fraction (%) increased from 27±5 to 35±5 ($p<0.001$) and stroke volume (SV, ml) went from 20±4 to 27±4 ml ($p<0.001$). The author of the report offered the opinion that the assays were insufficiently sensitive to show differences in the measured biochemical parameters.

CVT303.035-P Effects of acute intravenous ranolazine in cardiac function and mechanical efficiency in dogs with heart failure. September, 2000.

Eight anesthetized, healthy dogs were measured before and after dobutamine administration for LVEF, SV, coronary blood, MVO2 and the arterial-coronary sinus difference for glucose, lactate and ffa. Subsequent LV dysfunction and heart failure was produced by multiple sequential intracoronary microembolizations. Measurements were then repeated ± ranolazine or dobutamine.

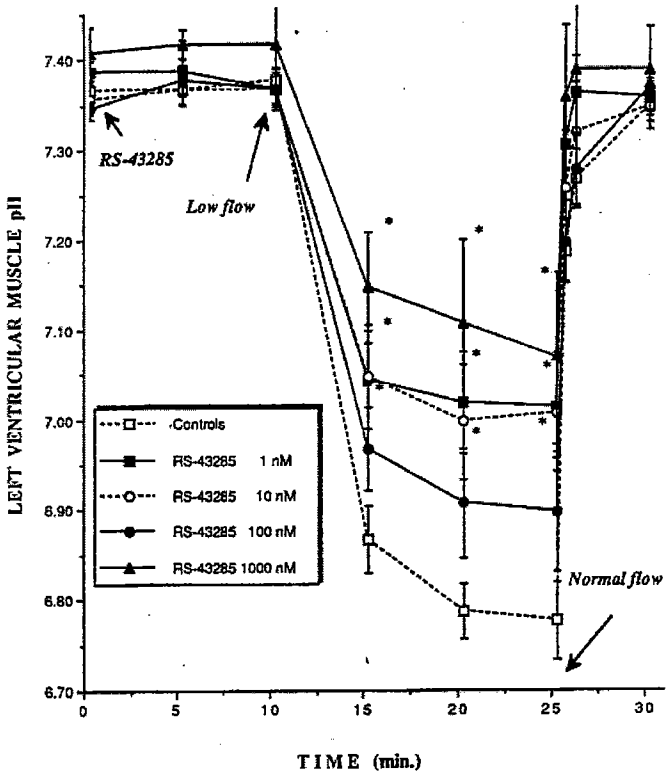
Results: In dogs with heart failure, dobutamine caused an increase in FFA uptake compared to the pretreatment levels ($p<0.01$). There was also a significant decrease in arterial glucose concentration ($p<0.02$). Ranolazine had very little effect on cardiac function in healthy dogs. It did increase glucose and ffa uptake in healthy dogs and decreased ffa and glucose uptake in heart failure dogs: There was no increase in MVO2 with ranolazine treatment although the LV efficiency increased.

AT4537: Anti-ischemic effects of ranolazine (RS-43285) in isolated rat hearts subjected to low perfusion flow. November 1988

Hearts were collected from male Sprague-Dawley rats then perfused with Kreb's solution. A microelectrode was implanted into the ventricular wall. Hearts were perfused at 14 ml/min to obtain a stable baseline ventricular pH. By adjusting the pump speed the flow was decreased to 1 ml/min for 15 minutes. Flow was restored to the initial rate for 15 minutes. Measurements of coronary flow were made at 30 seconds, 1 minute and 5 minutes and 5 minute intervals thereafter. Infusions of ranolazine (1 nM, 10 nM, 100 nM and 1000 nM) were started 10 minutes prior to reducing the flow rate and were continued for the rest of the experiment.

Fig. 1

EFFECT OF RANOLAZINE ON THE VALUES OF LEFT VENTRICULAR MUSCLE pH BEFORE, DURING AND AFTER LOW FLOW GLOBAL ISCHAEMIA IN ISOLATED PERFUSED RAT HEARTS.
n= 7-10/GROUP



* Significantly different from controls. ($p<0.05$ Unpaired Student's t-test)

Results: Left ventricular muscle pH was decreased by 5 minutes after the start of low flow and returned to normal with restoration of flow. The pH of the ventricular muscle was on average lowest in the controls. The pH for the treated animals did not go as low as the controls. There was no difference in effect between the 1 nM and 10 nM concentrations and the 100 nM concentration had no apparent effect. Under the conditions of the assay, 1000 nM ranolazine prevented the ventricular pH from decreasing to the extent shown by the control samples. Use of a comparator compound such as a calcium channel blocker or opioid would strengthen the evidence that the effect is unique to ranolazine.

AT5697 In vitro effects of ranolazine (RS-43285-193) on K+ and lactate release during 15 minutes of 96% low flow global ischemia of rat hearts. April 1991.

Ranolazine was administered at concentrations of $1x10^{-6}$ and $1x10^{-5}$M to isolated paced Langendorff perfused rat hearts subjected to low flow global ischemia to study the possible effect on K+ and lactate release, considered indicators of ischemia. A 5 minute stabilization period was followed by 15 minutes of low flow. Coronary perfusate was collected just before start of low flow and at 3 minute periods throughout the low flow.

Results: Lactate efflux was decreased with the addition of ranolazine. K+ efflux was increased early on and decreased later. When the data was averaged into cumulative values, the ranolazine seemed to decrease K+ efflux.

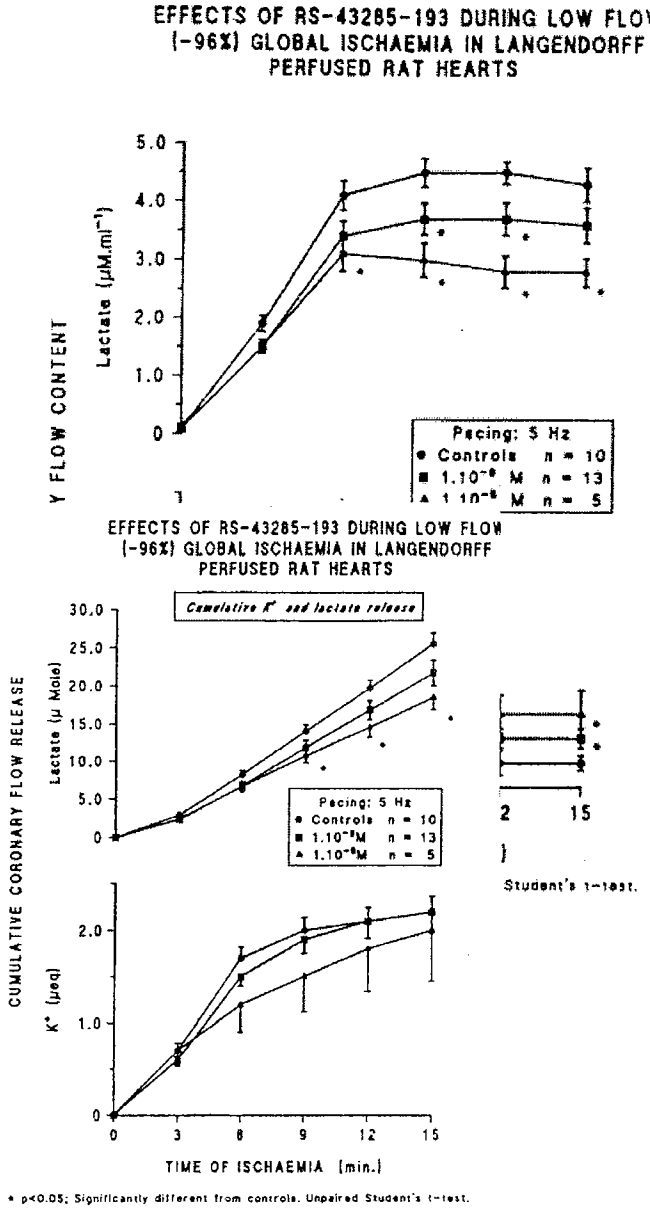


Figure 2

Appears This Way On Original

Under the conditions of the assay, the concentrations of ranolazine tested decreased lactate release. The addition of positive controls is desirable as is the addition of comparator compounds.

Appears This Way On Original

AT7009 Effects of ranolazine on high energy phosphate content and mitochondrial Complex I activity after global ischemia in isolated rat hearts. June 1995

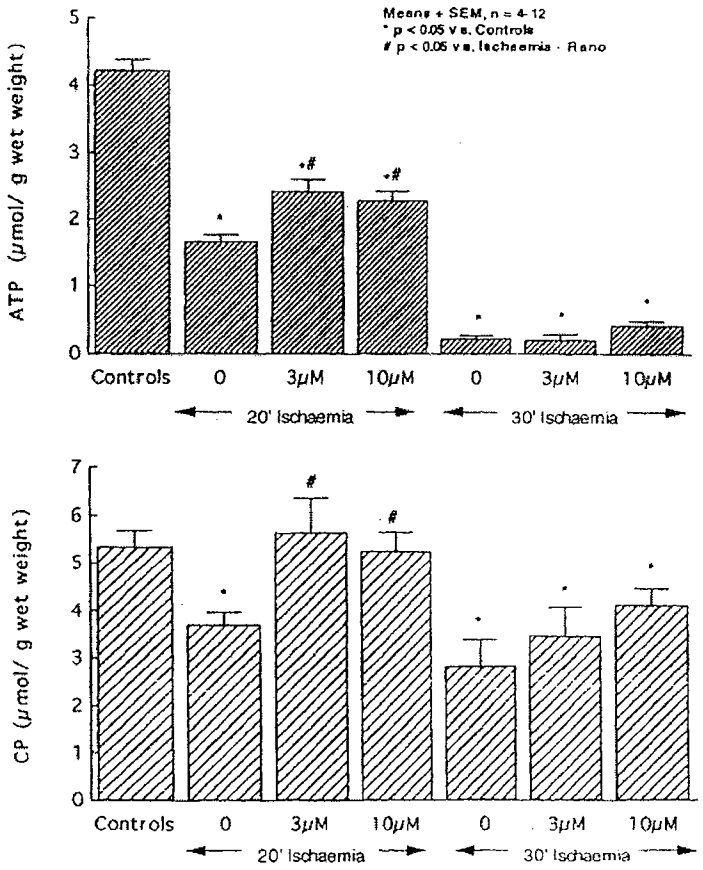
Ranolazine was tested at 3μM and 10 μM in Langendorff-perfused rat hearts subjected to 20 or 30 minutes of global ischemia and 5 minutes of reperfusion. At the end of each experiment the hearts were freeze-clamped between pre-cooled aluminum plates and assayed for ATP and creatinine phosphate concentrations. In a separate series of perfusions the hearts were used to make mitochondria as described in another report.

Appears This Way On Original

Results: A table of results was presented pertaining to mitochondria. It is incompletely explained and subject to misinterpretation.The "effects of ranolazine on functional parameters and O2 consumption in normoxic working hearts" shows no difference between treated and control hearts. No positive controls were included. The graph of ATP content shows minimal differences between the doses at either time point. The graph of CP content showed a dose response at 30 minutes of ischemia. Use of standard deviations instead of standard error of the mean would increase confidence in the results.

13

Figure 3 Effects of Ranolazine on the loss of high energy phosphates following global ischaemia in Langendorff-perfused rat hearts



AT6734 Cardioprotective effects of ranolazine (RS-43285) in the rabbit isolated perfused heart. June 1994

Hearts from male NZW rabbits were perfused via a Langendorff preparation at a constant flow. After equilibration, hearts were treated with either 10 or 20 µM ranolazine or vehicle for 10 minutes before exposure to a 30 minute period of global ischemia and 60 minutes of reperfusion. A normoxic control group was included. Aliquots of coronary effluent were collected from treated hearts at baseline, 15, 30, 45 and 60 minutes of reperfusion and analyzed for creatine kinase concentrations and K+ efflux. At the end of the experimental period hearts were analyzed for myocardial calcium and tissue ATP content.

Results: The presentation of results indicates that during the reperfusion, ranolazine-treated hearts 1)showed a dose-related decrease in EDP that progressed over time 2)showed a dose-related increase in LVD(developed)P vs control animals.

Creatine kinase increased in all groups, but to a lesser extent in the ranolazine animals. K+ increased very slightly in the control animals and stayed apparently constant in the treated animals. Tissue calcium increased over baseline in all groups, but to a lesser extent in the treated groups. Tissue ATP reached slightly greater (but statistically significant) levels compared to vehicle samples.

TEM was used to examine specimens from each group. Control hearts showed blurring of the myofibrillar z-bands, disrupted cristae, electron dense bodies that the sponsor said were suggestive of irreversible damage. Lanthanum chloride, used as an indicator of blood vessel integrity was scarce on the luminal surface of the vessels. The ranolazine-treated samples were reported to show few pathological changes. We are not told how samples were taken to ensure that they were representative.

The sponsor states that the mechanism by which ranolazine caused the reported changes is not known, but postulates that the results are consistent with what might be expected if the drug modulated anaerobic glycolysis.

Under the conditions of the assay, adding ranolazine to the isolated perfused hearts of healthy rabbits mitigated the effects of the low flow conditions.

AT7002 Protective effects of ranolazine (RS-43285) in isolated ginea[sic] pig hearts and their association with increases in active pyruvate dehydrogenase. Started January 1992- ended Jan. 1995

Perfused isolated hearts from female Duncan-Hartley guinea pigs were paced at 25% above the spontaneous rate (321±6 beats per minute) and electrical impulses delivered to the ventricles by implanted electrodes. A 30 minute equilibration period was followed by a 20 minute pre-treatment period ±ranolazine. The hearts were then perfused for another 30 minutes under low flow(0.7 ml/min) ischemic conditions ± ranolazine. Time-matched non-ischemic controls were used for comparison. Frozen hearts (not specified if they were from the first part of the study) were assayed for active, non-phosphorylated, pyruvate dehydrogenase (PDHa) and total PDH activity. Glycogen content and short-chain acylCoAs were also measured.

Results: PDHa was decreased compared to baseline both ± 10 mcM ranolazine but was

Table 1
Effects of ranolazine on tissue contents of short chain CoA esters and CoASH in low-flow ischaemic guinea pig hearts

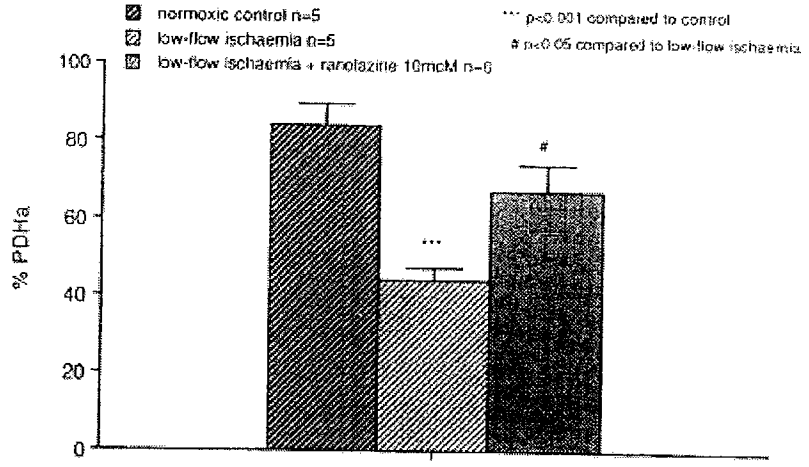
Condition	Malonyl CoA	Acetyl CoA	Succinyl CoA	Glutathione CoA	CoASH	Acetyl CoA/ CoASH
Normoxic control	0.26 ± 0.03	1.35 ± 0.36	4.72 ± 0.52	1.69 ± 0.15	5.75 ± 0.50	0.27 ± 0.05
LFI control	0.47 ± 0.01	4.10 ± 0.09	2.49 ± 0.12	1.50 ± 0.17	5.04 ± 0.29	0.97 ± 0.04
LFI + 1 μM ranolazine	0.52 ± 0.03	4.05 ± 0.29	2.63 ± 0.38	1.35 ± 0.05	5.63 ± 0.54	0.86 ± 0.03
LFI + 10 μM ranolazine	0.41 ± 0.04	4.76 ± 0.62	3.18 ± 0.43	2.55 ± 0.42	5.73 ± 0.38	0.98 ± 0.09

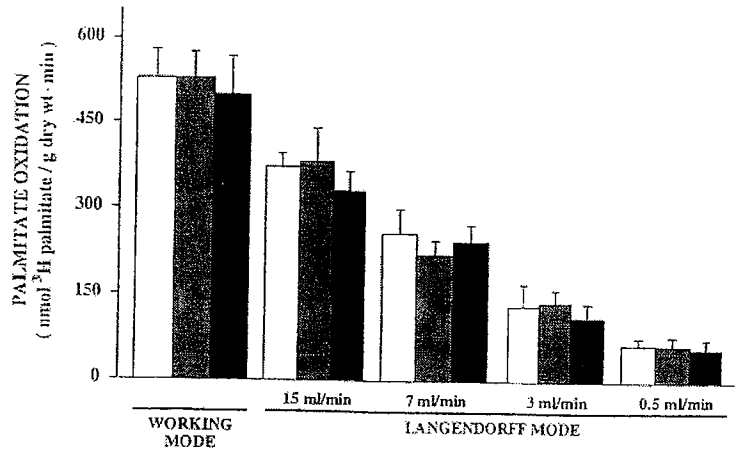
Values are nmol/g wet weight (or a ratio) and are given as mean ± s.e.m. for 5 (control, LFI control, LFI + 1 μM ranolazine) or 6 (LFI + 10 μM ranolazine) hearts. See text for statistical analyses.

Appears This Way On Original

decreased less in the presence of this one concentration of ranolazine. Lactate release did not differ between the controls and 1 μM ranolazine but was somewhat decreased with the 10 μM ranolazine. Lactate dehydrogenase (mIU.min.gm dry weight) did not differ between the two reported concentrations of ranolazine but both were lower values than the controls. CPK levels were highest for the untreated controls, followed by the 10 μM ranolazine and the lowest values shown by the 1 μM ranolazine samples. There does not appear to be any effect on short chain acyl CoAs.

Figure 1
The effect of 10 mcM ranolazine on tissue levels of activated pyruvate dehydrogenase (PDHa) after 30 mins low-flow ischaemia





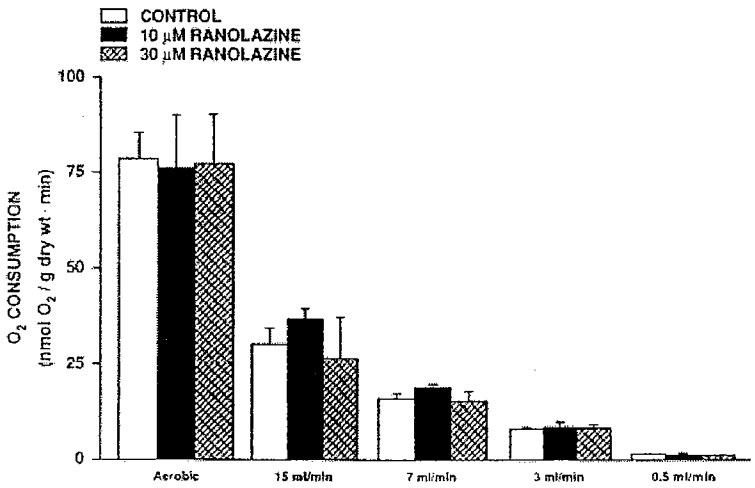
Summary: The concentrations tested were not specified in the methods. Only 2 concentrations were presented, and in one assay critical to supporting the hypothesis only 1 concentration was presented (shown alongside). This study is not as convincing as it might be if more concentrations had been tested.

AT7004 Effects of ranolazine on substrate oxidation in isolated rat hearts. April 1993- January 1995.

Isolated rat hearts were perfused with Krebs-Henseleit buffer +3% albumin under conditions of normoxia, and on reperfusion after 30 minutes of no-flow ischemia and under conditions designed to give either low or high Ca (1.25 mM or 2.5mM),low or high FA (0.4 or 1.2 mM palmitate) ±

insulin. Hearts were either paced at 280 bpm or unpaced.

Glycolysis and glucose oxidation were measured by perfusing hearts with [5-^{3}H/U-^{14}C]glucose. Fatty acid oxidation was measured in separate experiments using either [1-^{14}C]palmitate or [9,10-^{3}H]palmitate. For low flow ischemia studies ranolazine was added to the perfusate at 1,10 or 30 µM. Steady state rates of glycolysis were determined by measuring tritiated water production as released at the enolase step after its separation from radiolabelled glucose.



Results: Palmitate oxidation was decreased in all groups over time. There was no apparent effect on palmitate oxidation with ranolazine treatment. There was no apparent difference between control and treated in terms of oxygen consumption.

The rate of glucose oxidation with 10 µM ranolazine was increased under most conditions. There was an apparent dose response in increased glucose oxidation with increasing concentration of ranolazine except under the lowest flow condition.

Isolated rat hearts, under some of the conditions studied, when treated with 1, 10 and 30 µM ranolazine showed a tendency to a dose-related increase in glucose oxidation. Glycolysis and palmitate oxidation were either unaffected or inconsistently affected.

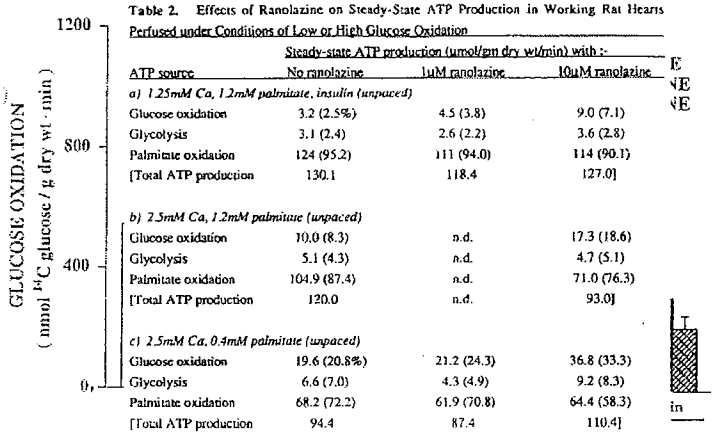


Table 2. Effects of Ranolazine on Steady-State ATP Production in Working Rat Hearts Perfused under Conditions of Low or High Glucose Oxidation

ATP source	Steady-state ATP production (µmol/gm dry wt/min) with :- No ranolazine	1µM ranolazine	10µM ranolazine
a) 1.25mM Ca, 1.2mM palmitate, insulin (unpaced)			
Glucose oxidation	3.2 (2.5%)	4.5 (3.8)	9.0 (7.1)
Glycolysis	3.1 (2.4)	2.6 (2.2)	3.6 (2.8)
Palmitate oxidation	124 (95.2)	111 (94.0)	114 (90.1)
[Total ATP production	130.1	118.4	127.0]
b) 2.5mM Ca, 1.2mM palmitate (unpaced)			
Glucose oxidation	10.0 (8.3)	n.d.	17.3 (18.6)
Glycolysis	5.1 (4.3)	n.d.	4.7 (5.1)
Palmitate oxidation	104.9 (87.4)	n.d.	71.0 (76.3)
[Total ATP production	120.0	n.d.	93.0]
c) 2.5mM Ca, 0.4mM palmitate (unpaced)			
Glucose oxidation	19.6 (20.8%)	21.2 (24.3)	36.8 (33.3)
Glycolysis	6.6 (7.0)	4.3 (4.9)	9.2 (8.3)
Palmitate oxidation	68.2 (72.2)	61.9 (70.8)	64.4 (58.3)
[Total ATP production	94.4	87.4	110.4]
d) 2.5mM Ca, 0.4mM palmitate, paced (280bpm)			
Glucose oxidation	51.2 (44.0%)	57.3 (44.2)	100.0 (64.8)
Glycolysis	10.3 (8.8)	10.9 (8.4)	11.7 (7.6)
Palmitate oxidation	54.9 (47.2)	61.3 (47.3)	42.7 (27.7)
[Total ATP production	116.4	129.5	154.4]

Data are calculated from the steady-state rates given in Table 1, from where n values and indication of error values can be obtained; n.d., not determined. Values in parentheses are calculated percentages from the µmol/gdw data shown; it should be noted that this only uses the measured parameters and does not include oxidation of endogenous substrates (for instance endogenous fat oxidation) [31], so that the total ATP production values given are therefore only derived from the exogenous sources measured.

Best Possible Copy

Appears This Way
On Original

In the tabular comparison of the high vs low calcium and palmitate, ±insulin and ± pacing, all of the many possible permutations of conditions were not examined. Also, discernible patterns of dose-response are not always apparent in the numbers. Another concentration of ranolazine would have been useful in this respect. There is no apparent effect on glycolysis or palmitate oxidation. There does appear to be an increase in glucose oxidation at the highest concentration of ranolazine. Table 4 shows the effects of 1 concentration of ranolazine added at reperfusion to hearts previously subjected to an ischemic period.

Table 4. Effects of ranolazine (10μM), added at reperfusion to hearts previously subjected to a 30min period of ischemia, on steady-state rates of glucose oxidation, glycolysis, and palmitate oxidation.

	Pre-ischemic	hearts (n) reperfused with :-	
Parameter	Control	No ranolazine	10μM ranolazine
glucose oxidation	337±36	377±66(8)	801±123(10)*@
glycolysis	3110±190	3570±440(8)	4440±380(10)@
palmitate oxidation	722±64	968±279(7)	566±108(8)

Hearts were perfused and rates determined as described in the legends to Figures 5 and 6. Rates shown are derived from the data shown in Figure 6 which has been averaged the last 30min period. *Indicates a significant effect of ranolazine compared to the appropriate "No ranolazine" value (unpaired t-test) and @indicates a significant difference from the appropriate pre-ischemic control value (paired t-test); the summed value for this latter value (i.e. all hearts) is shown in each instance.

Under the conditions of the study, ranolazine produced an increase in glucose oxidation.

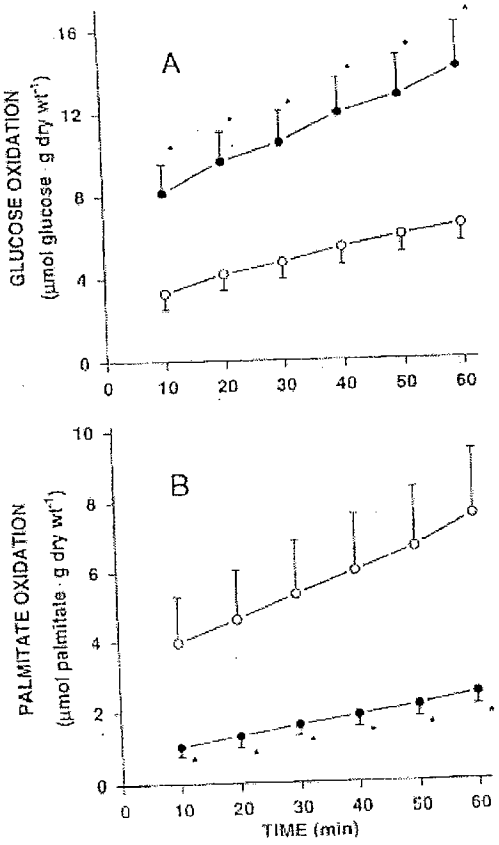
Effects on glycolysis and palmitate oxidation were inconsistent. The sponsor notes on p.283-284 of vol. 6 that calcium channel antagonists protect the heart by decreasing workload and metabolic energy demand.

Appears This Way On Original

AT7005 Effects of ranolazine on energy utilisation in skeletal muscle. Sept. 1994-February 1995

The "epitrochlearis" muscle was isolated from rats and superfused with oxygenated Krebs buffer containing 3% albumin + 0.4 mM palmitate, 5.5 mM glucose, 0.5 mM lactate + a "physiologic" amino acid mixture and either [5-^{3}H/U-^{14}C]glucose to measure glucose oxidation and glycolysis or [9,10-^{3}H]palmitate and [U-^{14}C]lactate to measure palmitate and lactate oxidation ±10μM ranolazine for up to 1 hour following a 5-10 minute pre-equilibration period. Contralateral muscles from the same animal were used.

Oxygen consumption was monitored under several flow conditions. Samples were analyzed for distribution of acetyl CoA carboxylase isoforms. Short chain CoA esters were extracted and analyzed by HPLC.



Results: 10μM ranolazine-treated muscle preps showed somewhat greater rates of glucose oxidation and glycolysis than the untreated preps. Lactate oxidation was unchanged and palmitate oxidation decreased (closed circles in the graph are + ranolazine). There was no significant difference between control and treated regarding tissue contents of CoA, acetyl CoA, succinyl CoA and malonyl CoA.

Under the conditions of the study, the one concentration of ranolazine tested on isolated rat skeletal muscle decreased palmitate oxidation and increased glucose oxidation. Oxygen consumption was slightly (NS) decreased relative to the controls. A dose-response effect would strengthen the study as would the use of comparator compounds.

CL7069 Ranolazine effects on pyruvate dehydrogenase activity in perfused normoxic rat hearts: evidence for enzyme activation by an indirect mechanism. Jan 1994- February 1995 Reported June 1995

Hearts were collected from male Wistar rats and perfused with Krebs-bicarbonate buffer. Hearts were perfused with Krebs-bicarbonate buffer and one of the following concentrations of palmitate (0,0.4, 0.8 or 1.2 mM). In some cases the palmitate was replaced with either octanoate or acetate (±albumin). Hearts were pre-perfused for 10 minutes without palmitate, octanoate or acetate then for 30 minutes with palmitate (0, 0.4, 0.8 or 1.2 mM) ±20 μM ranolazine and/or 1mM dichloroacetate (DCA, a pharmacological analogue of pyruvate).In some cases the palmitate was replaced with either octanoate or acetate ±albumin. Cardiac function and heart rate were continuously monitored by an isometric tension device. At the end of the perfusion period the hearts were freeze clamped and stored in liquid nitrogen until analysis.

Cardiac mitochondria were prepared from freshly euthanized rats. Mitochondria were incubated in buffer with respiratory substrates (10mM 2-oxoglutarate, 0.5mM malate), ± ranolazine, for 4 minutes. Uncoupled mitochondria were not given the respiratory substrates; 1μM FCCP, Mg.ATP and oligomycin were added instead.
Initial pyruvate dehydrogenase activity (PDHa) was assayed spectrophotometrically from disrupted mitochondrial pellets. Total PDH was assayed after conversion of all the enzyme into the active form using pig heart phosphate phosphatase. PDHa kinase was assayed either by incorporation of ^{32}P from [γ-^{32}P]-ATP into purified bovine or human PDH. Glutamate dehydrogenase was assayed as an alternative mitochondrial marker and PDHa expressed over this activity. PDHa kinase was assayed either by following incorporation of 32P from [γ-32P]ATP into purified bovine or human PDH or by following rate of inactivation of pig heart PDH. Unspecified powdered tissue was analyzed for CoA esters and free CoA.

Results:
Ranolazine inconsistently affected PDHa content in the presence of varying concentrations of palmitate. Ranolazine + DCA (a known PDHa stimulator) produced results different from DCA or ranolazine alone.

Appears This Way
On Original

PDHa/GDH content of hearts perfused with :-

[palmitate]	No additions	20μM ranolazine	1mM DCA	ranolazine + DCA
0mM	0.21 ± 0.03	0.19 ± 0.03	0.42 ± 0.03*	0.52 ± 0.02*
0.4mM	0.08 ± 0.01	0.18 ± 0.02*	0.25 ± 0.02*	N.D.
0.8mM	0.09 ± 0.03	0.18 ± 0.02*	0.32 ± 0.02*	0.44 ± 0.01*†
1.2mM	0.08 ± 0.03	0.08 ± 0.02	0.13 ± 0.02	0.33 ± 0.03*†

Hearts were perfused (plus 3% albumin) for 30min under the conditions shown (see Methods for full details). PDHa is expressed as a ratio of the glutamate dehydrogenase (GDH) activity; this was not altered by any of the conditions tested and was in the range 6-10units/g wet weight. Similar results were obtained if PDHa was expressed over total PDH activity (not shown); this was also not altered by any of the conditions tested and was in the range 3-6units/g wet weight. The average PDH(total)/GDH ratio was 0.62 ± 0.05 from all hearts. N.D., not determined. Results are means ± s.e.m. for at least 4 hearts. *Significantly different from no addition; †significantly different from ranolazine or DCA alone.

Table 3. Effects of ranolazine and other agents on the steady-state PDHa content of isolated rat heart mitochondria

Other conditions	steady-state PDHa/GDH content of mitochondria incubated with :- no ranolazine	100μM ranolazine
Control, coupled	0.03 ± 0.01	0.03 ± 0.01
1mM DCA	0.15 ± 0.03*	0.16 ± 0.04*
0.2mM pyruvate	0.18 ± 0.04*	0.17 ± 0.03*
50nM free Ca^{2+}	0.16 ± 0.02*	0.18 ± 0.02*
50nM Ca^{2+} + 1mM DCA	0.30 ± 0.03*†	0.31 ± 0.04*†
10μM palmitoyl carnitine	0.01 ± 0.003	0.01 ± 0.003
palmitoyl carnitine + 1mM DCA	0.11 ± 0.02*†	0.12 ± 0.03*†
10mM L-carnitine	0.20 ± 0.04*	0.21 ± 0.05*
100μM DCA	0.08 ± 0.01*	0.08 ± 0.01*
1mM L-carnitine	0.07 ± 0.01*	0.07 ± 0.01*
100μM DCA + 1mM carnitine	0.14 ± 0.01*†	0.14 ± 0.02*†
Control, uncoupled	0.15 ± 0.04	0.15 ± 0.03
1mM DCA	0.28 ± 0.04*	0.30 ± 0.04*

See Methods for full details of incubations which were for 4min, but essentially similar results were obtained after 8min; in incubations up to 30min ranolazine did not give values any different from controls. GDH activity was not altered by any conditions tested and averaged 153 ± 12 mU/mg protein. Total PDH activity (not shown) was not always measured but also did not change and averaged 109 ± 9 mU/mg protein. Results are means ± s.e.m. of values obtained from at least 3 different preparations. *Significantly different from appropriate control; †significantly different from either agent alone (i.e. effect of DCA). Ranolazine had no significant effects at this concentration or at any other over the range 0.0001-1mM; in contrast, DCA, pyruvate, carnitine and Ca^{2+} showed concentration-dependent effects (not shown).

Best Possible Copy

Appears This Way
On Original

Table 2. Effects of pyruvate, DCA and ranolazine on PDHa kinase activity.

	Kinase activity (% control) in the presence of :-	
Conditions	No ADP	0.5mM ADP
Control	(100)	94
1mM ranolazine	103	99
5mM DCA	71	42
20mM DCA	56	35
4mM pyruvate	90	35

Activity was measured by following the incorporation of ^{32}P from [γ-^{32}P]-ATP into PDH (see Methods); essentially similar results were obtained when activity was measured by following changes in amounts of PDHa (see Methods). A typical experiment is shown; this was repeated on 3 separate occasions. Ranolazine had no effects over the range 0.001-1mM. The enhancement of the effects of DCA and pyruvate by ADP is consistent with the results of Pratt and Roche (1979).

Appears This Way
On Original

Lack of effect of ranolazine on PDH or its interconverting enzymes

To bring about increases in PDHa within the tissue, either PDH phosphate phosphatase must be activated and/or PDHa kinase must be inhibited. We examined the effects of ranolazine on these two enzymes using purified preparations (see Methods), and in comparison to effects of known regulators (see Introduction). For completeness, we also studied the catalytic activity of PDH. In all these experiments no evidence for any direct effect of ranolazine on the PDH

Appears This Way
On Original

system was found. A typical example is shown in Table 2 for PDHa kinase. Here it is seen (see also legend) that the inhibitory effects of pyruvate and DCA (and ADP) on this enzyme are all readily evident, whereas ranolazine, up to 1mM, had no effects at all. In the case of the phosphatase, the activatory effects of Mg^{2+}, Ca^{2+} and spermine (see Introduction) were all readily evident but again no effects of ranolazine (0.001 - 1mM) were observed (not shown). There were also no effects of ranolazine (up to 1mM) on the catalytic activity of PDH itself; this was the case in terms of the enzyme's Vmax or Km for any of its substrates (pyruvate, CoA or NAD^+), and for the Ki of its end-product inhibitors NADH and acetyl CoA (not shown).

There remained the possibility that some other mitochondrial co-factor was required for ranolazine to have an effect on an enzyme of the PDH system, and therefore an extensive series of experiments was conducted with isolated rat heart mitochondria which contain all of these enzymes. However, the results in Table 3 (see also legend) show that ranolazine had no effect on the steady-state PDHa content of rat heart mitochondria incubated under a variety of conditions. In contrast, known regulators of the kinase (DCA, pyruvate) and phosphatase (Ca^{2+}) again had the expected effects (Table 3) (see McCormack *et al.*, 1982). Ranolazine also had no effect in the presence of these other effectors, even though additive effects of e.g. DCA and Ca^{2+} (as an effector of the kinase plus an effector of the phosphatase) were readily evident. This suggests that ranolazine does not affect either PDH kinase or phosphatase directly to bring about its effects on PDHa. However, PDHa can be altered indirectly by events leading to changes in the matrix content of effectors of these enzymes. This is shown in Table 3 in the case of carnitine which has no direct effects on the enzymes, but causes a reduction in the matrix acetyl CoA/CoA ratio (Lysiak *et al.*, 1988) and in this way leads to kinase inhibition.

Appears This Way
On Original

The sponsor postulates that while ranolazine has no direct effect on PDH kinase or phosphatase, the drug may cause a decrease in mitochondrial acetyl CoA content which will lead to a decrease in kinase activity. A decrease in Acetyl CoA will also decrease end-product inhibition of the catalytic activity of PDH.

CVT303.021-N: Attempt at optimization of the energy balance of the cardiac myocyte by the synergistic action of a dietary fatty acid and a cytoprotective pharmaceutical agent.1998

This report is marked as a "training thesis" and is not completely translated from the original French. Cultured neonatal rat cardiomyocytes were used to assay $^{14}CO_2$ production from 3 substrates: palmitate, glucose and octanoate. Cellular contraction was also monitored and quantitated. The method used contains various original components such as an electronic collimator and original software for processing the signal to establish contraction rhythm. This equipment was used to evaluate the contraction rhythm before the study to select cultures with a spontaneous rhythm of 90-150 contractions per minute. The set-up was also used to evaluate the influence of treatments on the cellular rhythms. Unfortunately, no form of validation or standardization data was presented for this apparatus.

Results: Addition of either trimetazidine or ranolazine decreased the contraction rate of the myocardial cells. (p.26/318). Both drugs seemed to decrease $^{14}CO_2$ production from palmitate to the same extent. Neither seemed to have a significant effect when the substrate was octanoate. There were slight increases in CO2 production compared to "temoin" from both drugs, a greater increase from ranolazine, when the substrate was glucose. These differences are marked as statistically significant. Under the conditions of the study, the addition of ranolazine to the cultures decreased CO2 production when compared to the control cultures with a substrate of palmitate, increased CO2 production when the substrate was glucose and was no different than control when the substrate was octanoate.

AT5450 The binding of [^{3}H]-RS-43285-193 to rat cardiac mitochondria. February 1987-August 1989.

Hearts were collected from rats and mitochondria prepared. The mitochondria were incubated with tritiated ranolazine. In competitive binding experiments compounds were present over the concentration range of $10^{-4} - 10^{-10}$M. Incubations were carried out at 25°C for 45 minutes. The inhibition of specific binding of [^{3}H]-RS-43285-193 was determined in the presence of 10μMRS-87505 or 10 μMRS-88216. These compounds were undefined.

Results: The sponsor reported that radiolabelled Ranolazine associated to rat mitochondria with a $t_{1/2}$ for association of 2.5 minutes. Binding reached equilibrium within 10 minutes and was stable for another 40 minutes. The addition of unlabelled RS-43285 caused a rapid dissociation

of the labelled material. The addition of KCN decreased the binding/association of ranolazine with the mitochondria to background levels. The process was inhibited by KCN (suggesting energy dependence).

Under the conditions of the study, [^{3}H]-RS-43285-193 rapidly associated and dissociated from isolated rat cardiac mitochondria. The binding was non-saturable, low affinity and unlikely to be a classical receptor. The isomers were reported to be equipotent at binding in this system, indicating a lack of stereospecificity.

AT6052 Studies of ^{3}H-ranolazine (RS-43285-193) uptake by isolated rat hearts during normoxic perfusion according to Langendorff. October 1990-December 1990.

Tritiated ranolazine at concentrations from 0.814 x 10^{-9}M (18.7μCi.l^{-1}) to 16.34 x 10^{-9}M (374 μCi.l^{-1})was infused into isolated rat hearts perfused under normoxic conditions according to the methods of Langedorff. The 25 minute perfusion followed a 5 minute stabilization period. After the perfusion, mitochondria were isolated and the radioactivity measured.

Results: The amount of radioactivity administered vs the amount of radioactivity found in the mitochondria and/or in the whole heart was linear. The sponsor proposed that this indicated a lack of mitochondrial accumulation. The conclusion is thus that there is a linear relationship between amount of radioactivity perfused through the hearts and that recovered from the tissue.

CVT303.007-N Effect of ranolazine on fatty acid β oxidation in rat heart mitochondria, part I. Sept 1996- March 1997.

Rat heart mitochondria were prepared from male Sprague-Dawley rats. The fatty acid oxidation of [1-^{14}C]palmitoylcarnitine ± ranolazine was assayed by liquid scintillation counting. Oxygen uptake by the rat heart mitochondria ± inhibitor ± palmitoyl carnitine or acyl CoA was measured by an oxygen electrode.
Ranolazine was added to the incubation mixtures as an ethanolic solution. The sponsor states that the amount of ethanol used did not affect the rate of fatty acid oxidation but does not present data to support this.
The effect of ranolazine on fatty acid oxidation was studied with purified enzymes of β-oxidation. Ten β-oxidation enzymes catalyzing 4 types of reactions were assayed ± 30 or 100 μM ranolazine.
Results: It was unknown how rapidly ranolazine was taken into mitochondria so in a preliminary experiment, rates of β-oxidation were determined after preincubating mitochondria with ranolazine for different periods of time. Inhibition was optimal without first incubating the mitochondria with the drug. The β-oxidation capacity of the mitochondria decreased significantly during the preincubation time, therefore, mitochondria were not preincubated with ranolazine in subsequent experiments.

Table3
Effect of Ranolazine on the Rates of Myocardial Respiration Supported by Palmitoyl-L-carnitine, Palmitoyl-CoA, Linoleoyl-CoA, or Pyruvate

Substrate	Ranolazine (μM)	Respiration Rate (nmol O/min/mg)	Inhibition (%)
Palmitoylcarnitine	0	196 ± 4.9 (3)	0
	30	173 (1)	12
	100	132 ± 1.4 (3)	33
Palmitoylcarnitine	0	191 ± 1.2 (3)	0
	30	168 ± 1.1 (4)	12
	100	134 ± 4.2 (3)	30
Palmitoyl-CoA	0	96 ± 1.1 (3)	0
	30	66 ± 0.5 (3)	31
	100	47 ± 0.7 (4)	52
Linoleoyl-CoA	0	100 ± 1.1 (3)	0
	30	89 ± 1.2 (3)	12
	100	62 ± 1.2 (3)	38
Pyruvate	0	193 ± 1.2 (3)	0
	30	189 ± 2.2 (3)	2
	100	184 ± 2.4 (3)	5

The data is presented as "Inhibition of myocardial β-oxidation by ranolazine as a function of the preincubation time." Mean without SD was presented.

Ranolazine was tested in a concentration range from 1-100 μM. The acid soluble products (nmol/min/mg protein) decreased from the control value of 1.99± 0.04 to 1.43±0.05 at 100μM ranolazine (28% inhibition) . A repeat experiment produced a control value of 2.74±0.06 and the high dose drug concentration gave a value of 1.64±0.05 (40% inhibition).

Two reported concentrations of ranolazine caused some inhibition of respiration with different substrates.

Ranolazine had essentially no effect on the mitochondrial enzymes of β-oxidation. There was a slight effect on long-chain enoyl-CoA hydratase and an even smaller effect on enoyl-CoA hydratase (crotonase). The sponsor stated that inhibition of crotonase was likely to be the cause of impaired fatty acid oxidation in the presence of ranolazine and therefore was further investigated. It was their interpretation of further studies that the inhibition was non-competitive with a Ki of 180 μM.

Effect of Ranolazine on the Activities of Mitochondrial Enzymes of the β-Oxidation Spiral

Enzyme	Ranolazine (μM)	Activity (Units/mg)	Inhibition (%)
Short-chain acyl-CoA dehydrogenase	0	0.79 ± 0.03 (6)	0
	30	0.78 ± 0.02 (6)	0
	100	0.77 ± 0.01 (7)	0
Medium-chain acyl-CoA dehydrogenase	0	2.2 ± 0.02 (6)	0
	30	2.2 ± 0.02 (7)	0
	100	2.2 ± 0.06 (6)	0
Long-chain acyl-CoA dehydrogenase	0	2.1 ± 0.01 (6)	0
	30	2.1 ± 0.03 (7)	0
	100	2.1 ± 0.04 (6)	0
Very long-chain acyl-CoA dehydrogenase	0	0.74 ± 0.01 (6)	0
	30	0.79 ± 0.02 (6)	0
	100	0.77 ± 0.03 (6)	0
Enoyl-CoA hydratase (crotonase)	0	2920 ± 70 (6)	0
	30	2550 ± 50 (6)	13
	100	2120 ± 60 (6)	27
Long-chain enoyl-CoA hydratase	0	10.9 ± 0.2 (4)[1]	0
	30	9.5 ± 0.3 (7)[1]	13
	100	6.9 ± 0.3 (5)[1]	37
3-Hydroxyacyl-CoA dehydrogenase	0	323 ± 4 (6)	0
	30	340 ± 5 (6)	0
	100	350 ± 6 (6)	0
Long-chain 3-hydroxyacyl-CoA dehydrogenase	0	12.2 ± 0.1 (7)[1]	0
	30	12.7 ± 0.4 (6)[1]	0
	100	13 ± 0.2 (6)[1]	0

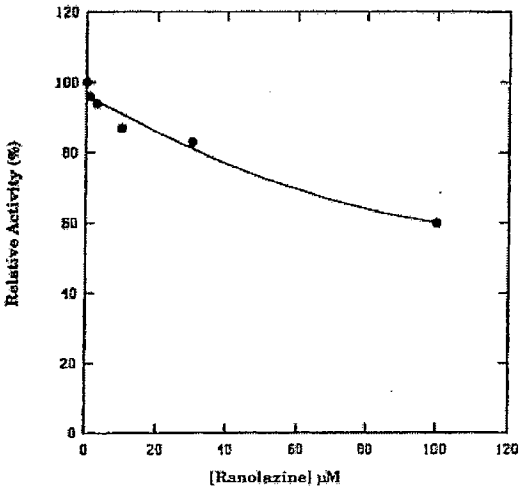


Fig. 1. Inhibition of β-oxidation by ranolazine in isolated rat heart mitochondria. [1-^{14}C]palmitoyl-L-carnitine served as the substrate.

Appears This Way On Original

CVT303.008-N Effect of ranolazine on fatty acid β-oxidation in rat heart mitochondria, part 2. September 1997-December 1997, Reported Jan. 1998.

Mitochondria were isolated from male rat hearts and loaded with carnitine by incubation. The assay mixture contained buffer, rotenone, antimycin A and the carnitine-loaded mitochondria. In control experiments, mersalyl acid was added to inactivate the translocase. The uptake of carnitine was initiated by the addition of 0.5mM [^{3}H-methyl]-L-carnitine to the assay mixture. The reaction was stopped by acidification and centrifugation. Enoyl CoA hydratases were assayed for activity as was carnitine palmitoyltransferase (CPT) and carnitine:acylcarnitine translocase(CAT).

Results: There was no drug effect on CPTII. The Triton X-100 used in the assay inactivates CPT I so it was assumed that measured activity was for CPTII. There was a dose-related decrease in CAT activity.

Table 2
Effect of Ranolazine on the Activity of Carnitine: Acylcarnitine Translocase

Experiment	Ranolazine (μM)	Activity (dpm/10 min)	Inhibition (%)
I	0	1,460 ± 50 (5)	0
	30	1,140 ± 40 (5)	22
	100	920 ± 70 (5)	37
	300	430 ± 70 (5)	71
II	0	1,400 ± 50 (5)	0
	30	1,130 ± 20 (5)	19
	100	750 ± 50 (4)	46
	300	530 ± 70 (5)	62

There was a dose-related decrease in mitochondrial respiration when the substrates provided were either palmitoylcarnitine (15% and 35% less than 0 mM ranolazine) or octanoate (28% and 39% less than 0 mM ranolazine).

It was mentioned in the results that R- and S-ranolazine produced essentially identical results to the racemate regarding the inhibition of long chain enoyl CoA hydratase (Ki values of 0.195mM, 0.19 mM and 0.19mM respectively). Non-competitive inhibition was again reported.

CVT303.013-N Effect of ranolazine on fatty acid β-oxidation in rat heart mitochondria, Part 3. December 1998- December 1999.

Carnitine:acylcarnitine translocase was assayed by measuring uptake of radioactive L- carnitine in isolated rat liver mitochondria preloaded with L-carnitine. (R-) and (S-) ranolazine were both evaluated for effects on β-oxidation of palmitoyl-L-carnitine in coupled rat heart mitochondria. Results: Both enantiomers inhibited β-oxidation. At concentrations of 0.1mM and 0.3 mM, (R)-ranolazine was twice as effective as the (S-) isomer while at the lowest concentrations there was no apparent difference.

differences were detected. Inhibitions of 28% and 40% previously observed with 0.1 mM racemic ranolazine (1) are similar to the level of inhibition caused by the S-isomer.

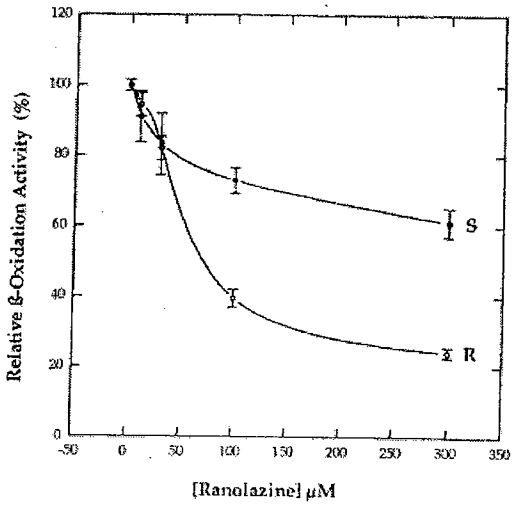


Fig. 1. Effects of (R)- and (S)-ranolazine on the ß-oxidation of[1-^{14}C]palmitoyl-L-carnitine in rat heart mitochondria. For further details see Attachment 1.

Neither enantiomer nor the racemic mixture showed any effect in this system on the β-oxidation of octanoate. Both isomers showed an inhibition of carnitine:acylcarnitine translocase

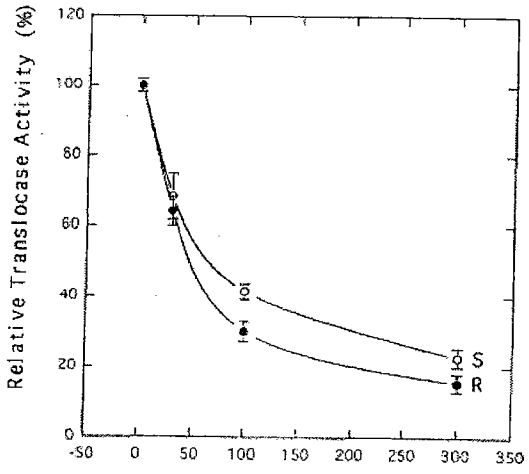


Fig. 2. Effects of (R)- and (S)-ranolazine on the activity of carnitine:acylcarnitine translocase in rat liver mitochondria. For detailed data see Attachment 2.

Effect of Ranolazine on Carnitine:Acylcarnitine Translocase of Rat Liver Mitochondria

Mersalyl (μM)	Ranolazine S-, or R-Isomer (μM)	Carnitine:Acylcarnitine Translocase Activity (dpm/5 min) orig. count	(count-backgrd[1])	Rel.Act. (%)	Inhibition (%)
None	None	2100 ± 40 (5)	1170	100 ± 2	0
4	None	940 ± 45 (5)			
None	S- 30	1740 ± 140 (4)	800 §	68 ± 6	32
None	S- 100	1430 ± 46 (5)	490 §	42 ± 2 *	58
None	S- 300	1210 ± 57 (5)	270 §	23 ± 3 *	77
None	R- 30	1700 ± 86 (4)	750 §	64 ± 4	36
None	R- 100	1290 ± 60 (5)	350 §	30 ± 3 *	70
None	R- 300	1130 ± 55 (4)	190 §	16 ± 3 *	84

1 Counts corrected for non-specific uptake determined in the presence of the translocase inhibitor mersalyl.
§ Rate of translocation is different from the rate observed in the absence of mersalyl at $P < 0.001$.
* Inhibition of translocation by S-isomer is different from the inhibition by the R-isomer at $P < 0.01$

Appears This Way On Original

When comparing the two enantiomers of ranolazine, the (R) isomer is the more effective inhibitor of both ß-oxidation and translocase. However, the patterns of inhibition obtained with (S)-ranolazine for ß-oxidation and translocase differ significantly from each other, whereas the responses of ß-oxidation and translocase to the (R) isomer are very similar. This imperfect relationship between the inhibitions of ß-oxidation and translocase may be an artifact attributable to the translocase assay. Use of an assay method that yields initial velocities may result in a better correlation and improve the confidence about the cause-effect relationship between the ranolazine-induced inhibitions of translocase and ß-oxidation.

Effect of Ranolazine on the ß-Oxidation of [1-^{14}C]-Palmitoyl-L-carnitine by Rat Heart Mitochondria

Ranolazine Isomer	Concn. (μM)	Acid Soluble Products (nmol/min/mg)	Rel. Activity (%)	Inhibition (%)
R-isomer	None	1.78 ± 0.03 (13)	100.0 ± 1.6	0
	10	1.68 ± 0.06 (5) §	94.4 ± 3.3	5.6
	30	1.46 ± 0.06 (6) ‡	82.0 ± 3.3	18.0
	100	0.70 ± 0.05 (8) ‡	39.5 ± 2.6 *	60.5
	300	0.43 ± 0.03 (9) ‡	24.2 ± 1.5 *	75.8
S-isomer	None	1.78 ± 0.03 (13)	100.0 ± 1.6	0
	10	1.62 ± 0.13 (8) §	91.0 ± 7.3	9.0
	30	1.48 ± 0.16 (6) ‡	83.2 ± 8.7	16.8
	100	1.30 ± 0.06 (7) ‡	72.9 ± 3.6 *	27.1
	300	1.09 ± 0.07 (12) ‡	61.1 ± 4.1 *	38.9

* Inhibition of ß-oxidation by the R-isomer is different from the inhibition by the S-isomer at $P < 0.001$.

Rate of ß-oxidation is different from the rate observed in the absence of the inhibitor at $P < 0.005$ (§); at $p < 0.001$ (‡).

AT5184 Effects of ranolazine on mitochondrial function: Respiration, calcium uptake and release and carnitine palmitoyl transferase activity. April 1989 – March 1990.

Cardiac mitochondria were isolated from male Sprague-Dawley rats. Mitochondrial NADH-Cytochrome c reductase activity was determined by spectrophotometric methods. Mitochondrial OGDH (oxoglutarate dehydrogenase)activity was determined by fluorimetry. To determine the rate of efflux of calcium from the mitochondria, 200 nM free calcium was added to the mitochondrial mix until formation of NADPH reached equilibrium. Efflux of calcium was initiated by the addition of 10 nM NaCl and the decay in the absorbance signal (340 nM) followed for 5 minutes.

CPT1 activity was measured in mitochondria isolated from Sprague-Dawley rats by measuring the formation of palmitoyl [^{3}H]-carnitine from palmitoyl-CoA and L-[^{3}H]-carnitine.

Results:
In the textual summary of results, it was reported that tight coupling of the respiration and oxidative phosphorylation within the mitochondria were evidenced by the need to add 180mcM ADP to the malate/glutamate containing preps. The respiratory control ratio (state 3/state 4 respiration) was decreased in the presence of $1x10^{-3}$ and $1x10^{-4}$M ranolazine but not with $1x10^{-5}$ M ranolazine. Ranolazine inhibited oxidation of NADH with a lower affinity than that of rotenone (pki 4.24±0.09 vs pki 8.31±0.10 for rotenone). It was also stated that mitochondrial CPT1 was inhibited by ranolazine but only at high concentrations.
A table labelled "Effect of ranolazine on malate/glutamate stimulated respiration" is without legend, concentrations without units are given and as such the whole is uninterpretable. Table 2 lists 3 compounds and gives a pKi value, presumably for Cytochrome c reductase activity. All tables are without legends. We don't know how many concentrations of ranolazine were tested or what those concentrations were. There is a mention of diltiazem being used to inhibit the calcium dependent stimulation of NADH-OGDH as a comparator compound. Again, the number of concentrations tested and what those concentrations were could not be located in the report. The report does not provide enough material for the reviewer to come to an independent conclusion.

AT7037 Effects of ranolazine on the mitochondrial beta-oxidation pathway in vitro. January 1995- October 1995.

Mitochondria were prepared from rat heart and skeletal muscle. Radiolabelled palmitate was added and terminated at 0, 4 or 10 minutes for samples for the measurement of acyl carnitine intermediates. Beta-oxidation flux was determined in a separate experiment by measurement of the acid-soluble radioactivity generated from the labelled palmitate. Ranolazine was tested at 30μM and 100 μM.

Effects of ranolazine on flux through the beta-oxidation pathway of rat skeletal muscle mitochondria

Condition	Rate of fatty acid oxidation
H_2O control	30.7
Placebo control	30.6
30 μM ranolazine	18.5
100 μM ranolazine	16.5

Rates are given as nmol C_2 units/min/ml and have been calculated over the 12 min period of incubation (see Methods). Rates obtained were linear (see below). (Samples were collected at 0, 4, 8 and 12 min).

Results: It was reported that the isolated rat heart mitochondria had too high activities of both ATPase and acyl CoA hydrolase to "make the assay feasible." It was therefore decided to assess the actions of the drug using rat skeletal muscle mitochondria.
Ranolazine decreased the flux through the β-oxidation pathway. There was little difference in effect between the two concentrations.

Ranolazine had some effect on the C2 and C4 acyl carnitine intermediates after 4 minutes of incubation. A difference was also noted at 10 minutes. The sponsor's comment with the 10-minute table is noteworthy.

Table 3

Effects of ranolazine on acyl carnitine intermediates after 4 min incubation of rat skeletal muscle mitochondria

Acyl Cn	Content of acyl Cn (nmol/ml) in samples treated with:-			
	Placebo	Blank	30 µM ranolazine	100 µM ranolazine
C2	204.3	226.2	132.1	144.7
C4	1.7	1.9	1.2	0.9
C6	0.9	1.2	0.8	0.6
C8	0.3	0.4	0.5	0.5
C10	0.3	0.5	0.4	0.3
C12	0.6	0.7	0.7	0.4
C14	0.4	0.4	0.5	0.3
C16	5.9	8.0	9.0	8.0
C16:1	0.21	0.35	0.31	0.45
C16:OH	0.14	0.16	0.13	0.16

* These samples have been ignored for the analyses of the results. They are inconsistent with the results in Table 3 (as more C2 build-up is expected with time) and with the flux results (Table 1). The reason why these samples gave erroneous results was not found out.

Effects of ranolazine on acyl carnitine intermediates after 10 min incubation of rat skeletal muscle mitochondria

Acyl Cn	Content of acyl Cn (nmol/ml) in samples treated with:-			
	Placebo*	Blank	30 µM ranolazine	100 µM ranolazine
C2	145*	313	233	215
C4	0.8*	2.5	1.4	1.6
C6	0.6*	1.1	0.9	1.0
C8	0.19*	0.36	0.27	0.19
C10	0.4*	0.7	0.6	0.7
C12	1.0	0.8	0.7	0.7
C14	0.7	0.7	0.6	0.7
C16	9.0	6.8	7.6	8.0
C16:1	0.9	0.7	0.7	0.7
C16:OH	0.34	0.43	0.46	0.35

In the results, the sponsor states that:

> determine the site(s) in fatty acid oxidation affected by ranolazine. However, one possible and already known site of action of the drug could be involved. It is a weak inhibitor of respiratory Complex I (NADH-CoQ oxidoreductase) (SS/023/95), although interestingly, it is more potent in this effect in broken or uncoupled than in coupled mitochondria (which is apparently quite unique (SS/023/95)). Thus, there is potential for its causing a build-up of NADH (which could easily be assayed under the present conditions) and thus inhibiting fatty acid oxidation at the 3-hydroxyacyl CoA dehydrogenase step. However, in the present studies no particular evidence for build-up of 3-hydroxyacyl intermediates was obtained. In a previous study on a patient with <5% normal Complex I activity, a similar degree of fatty acid oxidation flux and reduction in acetyl CoA was observed as at present, but in this case hydroxyacyl intermediates were seen to build up (Watmaugh *et al.*, 1990). The build-up of acyl carnitine intermediates perhaps suggests that the acyl-CoA dehydrogenase (which uses FAD) is a more likely site. The incubation conditions used are likely to cause most of the mitochondria to be coupled, and in this case the effect of ranolazine on Complex I is very weak (k_i >300 µM) (SS/023/95). NADH build-up would also lead to inhibition of flux through pyruvate dehydrogenase and glucose oxidation, yet these are promoted by ranolazine (SS/016/95; SS/018/95; SS/019/95; SS/021/95; SS/022/95). Also, the

Electrophysiology
N.B.- The following are studies specifically identified by the sponsor as crucial to their claims.

CVT303.034-P Electrophysiologic effects of ranolazine in isolated myocytes, tissues and arterially perfused wedge preparations from the canine left ventricle. Oct. 2000-July 2001. Report date July, 2001.

Isolated left ventricular cells: Whole cell currents were recorded from isolated canine left midmyocardial and epicardial cells at 37°C using conventional whole cell patch clamp techniques. I_{k1}, I_{Ks}, and I_{Kr} were recorded at 37°C using whole cell voltage clamp techniques. I_{K1} was measured using an external solution containing oubain and nifedipine to block the sodium-potassium current and L-type calcium current (I_{Ca}, L) respectively. I_{Ks} was measured in the presence of E-4031 and nifedipine to block I_{Kr} and I_{Ca}. Ranolazine was applied to the cells at concentrations of 0.1, 0.5, 1.0, 5.0, 10 and 100 µM. I_{Ks} was elicited by depolarization to 40 mM for 3 sec from a holding potential of -50 mV followed by a repolarization step to 0 mV (4.5 sec). The time-dependent tail current elicited by the repolarization was termed I_{Ks}. I_{Kr} was measured as the time-dependent tail current elicited at a potential of -40 mV following a short depolarizing pulse to 30 mV. I_{K1} was recorded during 900 msec of 10 mV voltage steps applied from a holding potential of -40 mV to test potentials ranging from -100 mV to 0 mV, and was characterized as the 5 msec average of the steady state current at the end of the test pulse.

Action potentials were recorded from epicardial and M cell preparations. The effects of ranolazine were determined at concentrations of 1,5,10, 50 and 100 μM, with recordings started 30 minutes after the addition of each concentration of drug. Rate-dependence of ranolazine's effects were determined by recording transmembrane action potentials at BCL of 300, 500, 800, 1000, 2000 and 5000 msec. The data recorded at BCLs of 500 and 2000 msec are presented in the report. Vmax was recorded ±10 and 100 μM of ranolazine at a BCL of 500 msec. Two separate experiments were performed, one using standard $[K+]_0$ of 4 mM and the other with low $[K+]_0$ of 2 mM.

Tissue slices from ventricular epicardial and M region: Tissue slices were isolated from left ventricular epicardial and M regions and allowed to equilibrate in a tissue bath for 4-6 hours while superfused with Tyrode's solution and paced at a basic cycle length (BCL) of 2 Hz using field stimulation.

Left ventricular wedges were placed in Tyrode's solution of either standard $[K+]_0$ of 4 mM or low $[K+]_0$ of 2 mM. Transmembrane action potentials were recorded from epicardial and subendocardial (M) regions using floating microelectrodes. A transmural pseudoECG was recorded along the same axis as the transmembrane recordings. The wedges were allowed to equilibrate for 2 hours while paced at basic cycle lengths of 2000 msec. A constant flow rate was set before ischemia to reach a perfusion pressure of 40-50 mm Hg.

Results:

Ventricular tissue slices:
The prolongation of APD was hypokalemia-dependent in both the epicardial and M-cells. Prolongation was greater at faster rates for the epicardial preparations (use dependent). Biphasic effects were apparent in some M cell preparations, with APD prolonged at low concentrations and shortened at high concentrations.

Wedge Preparations:
At low K+ concentration, ranolazine produced a dose-related increase in QT interval, T_{peak}-T_{end}, action potential duration and transmural dispersion of repolarization. This is shown in the sponsor's table below.

Appears This Way
On Original

Table 3. Canine Left Ventricular Wedge: 4 mM $[KCl]_o$, BCL=2000

	Epicardium		M region				
Concentration	APD50 ± SE	APD90 ± SE	APD50 ± SE	APD90 ± SE	QT_{end}	$T_{peak} - T_{end}$	TDR
control	164 ± 21	209.3 ± 15.76	204.5 ± 13.9	250 ± 13.93	261.1 ± 15.76	34.25 ± 2.56	43 ± 6
1 μM	176.3 ± 12.25	213.8 ± 13.28	203.3 ± 9.621	254.3 ± 9.15	263.5 ± 10.56	34.5 ± 3.202	26.75 ± 8.045
5 μM	176.5 ± 11.85	219 ± 12.12	207.5 ± 8.627	258.3 ± 11.08	274.5 ± 13.73	37.75 ± 4.09	36 ± 2.449
10 μM	170.5 ± 12.03	216.5 ± 13.41	199 ± 9.083	260.3 ± 12.66	277.8 ± 14.99*	39.25 ± 5.54	30.75 ± 10.46
50 μM	159.5 ± 12.82*	218 ± 15.91	187.8 ± 11.21*	257.5 ± 15.47	279.3 ± 17.21*	41.25 ± 8.37	32.5 ± 6.278
100 μM	152.5 ± 14.44*	220.5 ± 18.26	169 ± 10.5*	247.8 ± 15.32	284.5 ± 14.39*	40.5 ± 4.94	23.75 ± 2.689

* $p<0.05$ vs. control $n\leq4$

Table 4.
Canine Left Ventricular Wedge: 2 mM $[KCl]_o$, BCL=2000

	Epicardium		M region				
Concentration	APD50 ± SE	APD90 ± SE	APD50 ± SE	APD90 ± SE	QT_{end}	$T_{peak} - T_{end}$	TDR
control	167.3 ± 5.548	220 ± 5.568	195.3 ± 3.283	254.3 ± 0.882	283 ± 2.08	24 ± 12.57	16 ± 9.238
1 μM	173 ± 2	232 ± 5.508	210.7 ± 13.53	280.3 ± 12.72	311 ± 9.5	35 ± 4.70	28.33 ± 11.46
5 μM	183.5 ± 1.5	252.5 ± 10.5	205.7 ± 7.881	289.7 ± 2.848*	319 ± 4.58	33 ± 1.33	15 ± 7
10 μM	190 ± 2*	265.5 ± 16.5	208.3 ± 3.48	305.3 ± 4.978*	329 ± 2.33	36 ± 4.09	23.5 ± 1.5
50 μM	179 ± 1	276.5 ± 18.5*	214.3 ± 6.333	325.5 ± 5.5*	343 ± 2.84	41 ± 6.35	35.5 ± 3.5
100 μM	167.5 ± 0.5	293.5 ± 21.5*	187.7 ± 4.978	345 ± 14.36*	376 ± 4.48	55 ± 1.00	35 ± 11

*$p<0.05$ vs. control $n\leq4$

The effects in the wedge preparations were hypokalemia-dependent. As only 2000 ms BCL was evaluated, use-dependence cannot be assessed.

The effect on I_{Na} was measured by rate of rise of the upstroke of the action potential. Both concentrations of ranolazine that were tested decreased Vmax.

Ranolazine inhibited I_{Kr} (IC_{50} 11.5μM) and I_{Ks} (IC_{50} 13.4μM) in a concentration dependent manner but did not appear to alter I_{K1}.

Torsade de Pointes were not observed to develop spontaneously. Programmed electrical stimulation did not produce TdP under any of the test protocols.

T-wave morphological changes were noted in both tissue slices and wedges: widened, low and notched, especially at low K+.
There was triangulation of the AP and decreased plateau height in both the epicardial and M cells of the tissue slices.

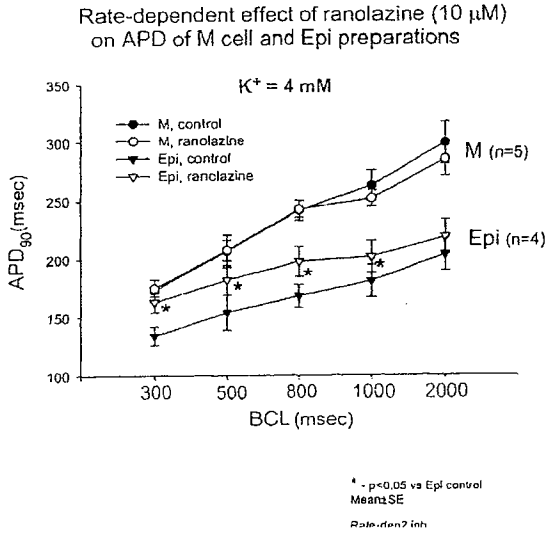
Individual cells, tissue slices and tissue wedges were used in this study. It was not always clear as to which was represented in the results as shown and the reporting was incomplete. Another point that required clarification was the stability of the various test systems over time. This is relevant given the length of the equilibration periods. The duration of exposure to the drugs was not specified for the wedge preparations. It should be noted that evaluations in the presence of

catecholamines have not been made. It should also be noted that ranolazine behaved differently in the tissue slices versus the wedge preps. This is summarized in the reviewer's table below.

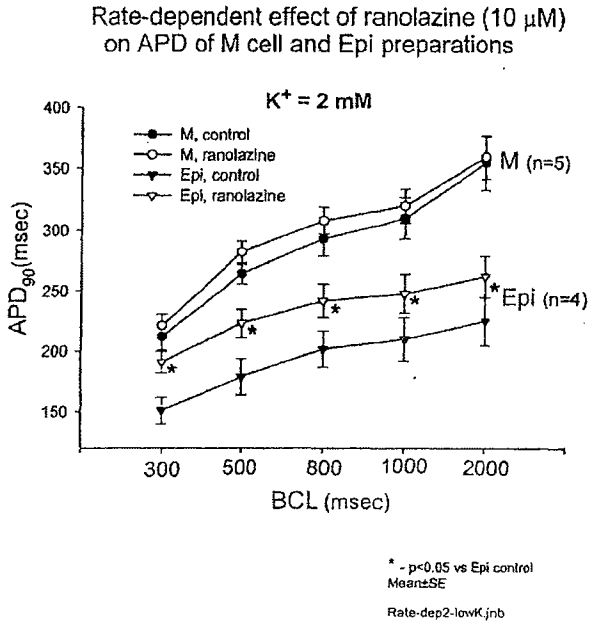
preparation	APD50	APD90
Tissue slice	4mM K+: concentration-dependent shortening	4mM K+: concentration and use-dependent shortening
Wedge	4mM K+: decreased (50, 100 μM) 2mMK+: no change	4mM K+: no change More effect on epicardium Than M cells 2mM: prolonged (5-100 μM) more effect on M cells than on epicardium

Electrophysiologic effects of ranolazine in isolated myocytes, tissues and arterially perfused wedge preparations from the canine left ventricle. Amendment to CVT 303.034P , August 2002.

Electrophysiologic effects of ranolazine in isolated myocytes, tissues and arterially perfused wedge preparations from the canine left ventricle were given further elaboration in this report, in fact to add the data from isolated myocytes, tissue sections and wedge preparations that were not included in the main report. Ranolazine at 10 μM was tested for its effect on the APD90 at different basic pacing cycle lengths (BCL) of 300, 500, 800, 1000 and 2000 msec in tissue slices of epicardial and M cells. E-4301, an I_{kr} blocker was used for comparison. The superfusing Tyrode solution contained either 2 mM K+ or 4mM K+. At 4 mM K+, and at pacing rates below 1000 msec, there was prolongation of APD90 in the epi cells but not the M cells. In the M cells, at BCL of 1000 and 2000, there was a slight shortening of the APD90. The effect in the epi cells appeared to be greater at lower BCL. The E-4301 caused a marked prolongation of APD90 in the M cells and a minimal change in the epi cells. At 2 mM K+, ranolazine produced a prolongation of APD in both M cells and epicardial cells. The sponsor's graphs are shown below.



Best Possible Copy



Appears This Way On Original

Ranolazine's effect in this system appeared to be greater at longer cycle lengths and at low potassium concentrations.

CVT303.036-P Electrophysiologic effects of ranolazine on late I_{Na}, I_{Ca}, I_{to} and $I_{Na\text{-}Ca}$ in isolated canine left ventricular myocytes. July 2001-January 2002. Reported January 2002.

Whole cell currents were recorded from isolated canine left ventricular midmyocardial and epicardial cells at 37°C using conventional patch clamp or perforated patch clamp (I_{Na}) to determine concentration-response relationships for the transient outward current (I_{to}), late sodium (late I_{Na}), calcium (I_{Ca}) and sodium-calcium exchange ($I_{Na\text{-}Ca}$) currents. The single

myocytes were obtained following enzymatic dissociation. The effects of ranolazine were determined over the concentration range of 0.5 – 100 μM. Tetrodotoxin was used to block steady state of the late I_{Na} channels. $CdCl_2$ was used to block I_{Ca}. For each ion channel, the sponsor states in the methods that a series of pulses was applied and then the effects measured in the final pulse of the sequence or 4 minutes after addition of each drug concentration. This procedure was repeated for each concentration tested. Data was not provided as to the stability of the test system during this interval.

Results: Ranolazine had minimal effect on $I_{to.}$ 100 μM reduced I_{to} by 16±3% and 17±4% at potentials of 0 and 10 mV respectively ($p<0.001$). Late I_{Na}, I_{Ca} and $I_{Na\text{-}Ca}$ were significantly inhibited by ranolazine with IC_{50} values of 21, 296 and 91 μM respectively.
The sponsor compares the effects with those of amiodarone (reduced I_{kr}, I_{ks}, late I_{Na} and I_{Ca}) and proposes this to explain why ranolazine like amiodarone prolongs the QT interval but may not cause torsades de pointes (TdP). However amiodarone does cause TdP in a small percentage of patients. The effect on late I_{Na} is postulated as to perhaps reduce transmural dispersion of repolarization and suppress EAD activity.

CVT303.050-P Use- and voltage-dependent effects of ranolazine on late I_{Na} during action potential voltage clamp.March 2002-August 2002. Reported August 9, 2002
Whole cell currents were recorded from isolated canine left ventricular midmyocardial cells obtained by enzymatic dissociation using the action potential voltage clamp technique. Late I_{Na} was recorded at 37°C. Action potentials elicited at basic cycle lengths of 300 and 2000 ms were used as command waveforms for the voltage clamp. Drug effects during a train of 30 pulses at repetition rates of 300 and 2000 ms were determined over a concentration range of 1 – 50 μM. Late I_{NA} was defined as the tetrodotoxin sensitive current.

Results: Late I_{Na} was evaluated during both the plateau and final repolarization of the action potential clamp. Increasing concentrations of ranolazine caused a decrease in late I_{Na} normalized current with an IC_{50} value of 20.75μM for a BCL of 2000ms, strikingly close to the IC_{50} of 21μM reported in a previous study (CVT303-036P). The IC_{50} was reported as 11.53 μM for a BCL of 300mS which the sponsor proposes as suggestive of use dependence. Another interpretation is that the numbers are the same within normal experimental variability.
According to the sponsor, potency of the block was greatest at plateau potentials and during rapid stimulation. At BCL of 2000 msec, IC_{50} was 20.75 μM with a plateau voltage of –28 mV and 5.86 μM with a plateau voltage of 20 mV. At a 300 ms BCL, half-inhibition of late INa was 5.04 μM during the plateau at a voltage of 13 mV and 11.5 μM during the final repolarization at a voltage of –28 mV. The sponsor's conclusion was a voltage and use dependent inhibition of late I_{Na} by ranolazine. The study would be stronger for the inclusion of positive controls or comparator compounds.

CVT303.042-P Electrophysiological effects of ranolazine in isolated canine purkinje fibers. September 2001- November 2001. Reported January 2002.

Standard microelectrode techniques were used to record transmembrane action potentials from free-running Purkinje fibers isolated from canine right and left ventricles. The preparations were

placed in tissue baths and allowed to equilibrate for ≥30 minutes while superfused with oxygenated Tyrodes solution and paced at a a basic cycle length of 1 Hz. The tissues were exposed to gradually increasing concentrations of ranolazine (1, 5, 10, 50 and 100 μM) at 20-30 minute intervals. The preparations were stimulated at basic cycle lengths of 300, 500, 800, 1000, 2000 and 5000 msec. Data from only the BCLs of 500 and 2000 msec were presented as representative of the relative pacing rates. Two separate sets of experiments were performed using extracellular K+ concentrations ($[K+]_o$) of 3 and 4 mM. The sponsor does not present data indicating the stability of the preparation over time.

Results

At a $[K+]_o$ of 4 mM, ranolazine altered resting membrane potential at concentrations of 50 and 100 μM. At these same concentrations, Overshoot and phase 0 amplitude of the action potential were decreased. This is shown in the reviewer's version of the sponsor's table.

Effects at [K+] =4.0 mM, BCL = 500 msec

	control	Ranolazine in μM				
		1	5	10	50	100
Amplitude	122±5	120±9	124±3	122±7	117±7	106±12*
RMP	-91±1	-90±2	-90±2	-90±3	-89±3	-87±3*
overshoot	32±4	32±7	34±7	32±6	28±7	19±11*

Values are mean ± SD, n=7, * $p<0.05$ compared to control

Lowering $[K+]_o$ to 3 mM did not substantially modify the effects of ranolazine on electrophysiological parameters of Purkinje fibers. Early afterdepolarizations were not reported for any conditions. Graphs of APD versus concentrations of ranolazine show a downward drift with increased concentration. Is this truly a specific effect or due to deterioration of the test system? Stability data or a reference system would be helpful to answer this question. EAD induced by d-sotalol (100 μM) was suppressed by ranolazine at concentrations down to 5 μM.

The next 3 study reports were not identified by the sponsor as critical to the mechanistic argument. However, these reports help to characterize the metabolites.

CVT303.037-P Effects of ranolazine and ranolazine metabolites on the duration of action potential of guinea pig ventricular myocytes. Conducted Nov-Dec 2000, April 2001 and reported March 2002.

Whole-cell patch electrode technique was performed on guinea pig ventricular myocytes. Action potentials were induced by 5-ms depolarizing pulses applied at frequencies of 0.5, 1 or 2 Hz. Values of action potential duration at APD50 and APD90 were measured ±ranolazine (3,10 and 30 μmol/l). Action potentials for ranolazine (10μmol) were also determined in the presence of 5μmol/l quinidine at a frequency of 0.25Hz.

To determine the effect of the selected metabolites, ventricular myocytes were paced at a frequency of 1 Hz. The action potential duration was measured ± one of three metabolites: RAN-2 (RS-94287)(3,10 and 30 μmol/l), CVT2512(RS-88640)(10 μmol/l) and CVT2514(RS-88390)(10μmol/l).

Results: Independent of the pacing frequency, ranolazine caused a dose-related decrease in both APD50 and APD90. The shortening was partially reversible after washout of the drug. At 10 μmol/l ranolazine attenuated the effect of quinidine. The 3 metabolites that were tested had no effect on the duration of action potential elicited at a frequency of 1Hz.

The study would be stronger with the inclusion of positive controls and if ranolazine and the metabolites had been tested according to the same parameters (concentrations and pacing frequencies). If there is a rate-dependent prolongation of action potential duration, the conditions of the present study would not necessarily show the phenomenon. That is, a faster stimulation frequency may be needed. The sponsor does make the point that the decrease in action potential observed in the presence of ranolazine may be attributed to the fact that ranolazine inhibits $I_{Ca(L)}$.

CVT303.040-P Effects of ranolazine, ranolazine enantiomers SAR-103143/SAR-85179, metabolites RS-88390, RS-88640 and RS-94287 and comparators dofetilide and verapamil on HERG and IsK. September 2002.

Concentration-response relationships for dofetilide (1, 10, 100 nM, 1 and 10 μM) and verapamil (1, 3, 10, 100 μM and 1 mM) to block HERG currents were obtained. After baseline I_{HERG} measurements were obtained in the absence of drugs (control), then progressively higher concentrations of dofetilide or verapamil were added to the superfusion solution until complete block was achieved. Steady state responses to both drugs were recorded. Currents from Xenopus oocytes expressing HERG were also recorded under control conditions and in the presence of 10, 30, 100 μM and 1 mM ranolazine. The sponsor did not specify steady state conditions nor was data presented to indicate this. The effects of the S-enantiomer(SAR-103143) and R-enantiomer (SAR-85179) on HERG and IKs was tested under control (no drug) conditions or in the presence of 10μM and 100 μM S- and R-enantiomer. The effects of the named metabolites were determined at concentrations of 0, 10μM and 100μM.

Results: Dofetilide and verapamil inhibited HERG in a concentration-dependent manner with no voltage dependence observed. Ranolazine significantly inhibited HERG currents in a dose and voltage dependent manner. At a concentration of 100μM, ranolazine inhibited HERG by ~50%. In the graphical presentation of results, it was shown that sufficiently high concentrations of ranolazine caused complete I_{HERG} block. Voltage dependence of the block was also observed. The reviewer has summarized this in the table below.

Reviewer's summary of results presented in report text

Ranolazine conc	Isk voltage	Significance
100μM	0-40mV	P<0.05
300μM	-20-40mV	P<0.01,p<0.001
1mM	-30-40mV	P<0.05
3mM	-30-40mV	P<0.01, p<0.001

R- and S- ranolazine also inhibited HERG in a concentration and voltage dependent fashion.

Reviewer's summary of results presented in report text

Ranolazine conc	Isk voltage	Significance

S-ran 10 μM	30, 40 mV	P<0.05
R-ran 10 μM	30, 40mV	Qualitatively but not statistically significant
S-ran 100 μM	-40, -20mV	P<0.05
	-30, 20-40mV	P<0.01
R-ran 100 μM	-20 and 20 mV	P<0.05
	30 and 40 mV	P<0.01

Neither enantiomer had an effect on Isk at the concentrations tested. But overall, 100μM concentrations of either enantiomer inhibited I_{HERG} by 50%, similar to the potency of ranolazine.

Of the metabolites tested, RS-88390 inhibited HERG with approximately the same potency of the parent compound. No effect of the metabolites on Iks was detected.

CVT303.043-P Electrophysiological effects of ranolazine metabolites in myocytes isolated from the canine left ventricle. Conducted July 2001- February 2002. Reported February 2002.

Cells from the epicardial and midmyocardial regions of the left ventricle were used following enzymatic dissociation from the LV free wall. Rapid delayed rectifier and slow delayed rectifier potassium currents were recorded at 37°C using whole cell voltage clamp techniques. I_{kr} was measured as the time-dependent tail current elicited at a potential of –30mVfollowing a 250 ms depolarizing pulse to 30 mV from a holding potential of –50 mV. I_{ks} was measured as the time-dependent tail current elicited at a potential of –30 mV following a 2 sec depolarizing pulse to 40 mV from a holding potential of –50 mV. Metabolites were tested a final concentration of 50 μM. The voltage clamp protocol was repeated 4 times before drug, 4 times during drug (beginning 4 minutes after exposure of the cell to drug) and 4 times after washout of the drug (beginning 4 minutes after initiation of washout). The data for each of 4 runs was averaged. The stability of the preparation over time was not discussed in the report. For I_{kr} experiments, chromanol, a blocker of I_{ks} was added to the recording solutions. For I_{ks} studies, the I_{kr} blocker E-3401 was added to the recording solutions just before the experiment.

Results: Reduction of the I_{kr} tail current was produced with metabolites RS-94287, RS-88390, RS-89961, RS-88681, RS-89983, RS-88772 and RS-88597. Maximum I_{kr} inhibition (51%) was produced by RS-88390. Metabolites CVT-2543 acid (CVT4786?)and RS-89289 produced significant reduction of I_{ks} (39%). The variability of the I_{kr} measurements for CVT2534 acid were disproportionately large compared to the other measurements in this segment of the study: Mean I_{kr} (pA) of 145.3± 61.6 vs RS-88772, 37.8±6.3 or CVT2738 101.5±14.4. The study would be stronger for the inclusion of positive controls.

CVT303.038-P Effects of ranolazine on QT prolongation and arrhythmia induction in anesthetized dog: comparison with sotalol Sept.2002

AV block was induced in mongrel dogs by radio-frequency ablation. Racemic sotalol (n=5)was given iv at a loading dose of 8 mg/kg and a maintenance infusion of 4 mg/kg/hr. Ranolazine (n=5) was given as 0.5 mg/kg intravenous loading dose followed by a first, second and third infusion of 1.0, 3.0 and 15 mg/kg/hr. One dog received ranolazine as a 1.5 mg/kg loading dose followed by infusions of 15 and 30 mg/kg/hr. Twenty minutes (sotalol) or 30 minutes (ranolazine) after starting the maintenance infusion, electrophysiological measurements of right and left ventricular effective refractory period (ERP), QT and QRS were made at basic cycle lengths of 300, 400, 600 and 1000 ms. After the measurements, bolus phenylephrine challenges (10, 20, 30, 40 and 50 μg/kg) were given intravenously and arrhythmias monitored.

Results: All 5 dogs treated with sotalol died from Torsade de Pointes (TdP). The effective refractory period was reported to be prolonged in a reverse use-dependent manner.

One of the 5 dogs treated with ranolazine died during the 30 mg/kg/hr infusion with no electrophysiological measurements made. The sponsor lists the cause of death as pump failure with no further explanation. Ranolazine increased QT at each of the cycle lengths used, but not to the extent of sotalol. TdP was not observed in any of the ranolazine dogs. Runs of ventricular tachycardia were reported, qualified as that which would be expected from phenylephrine alone.

At a basic cycle length of 1000 ms, sotalol increased QT interval from 333±27 to 441±14ms (32% increase). At this cycle length, the maximum increase reported for ranolazine was 348±9 to 384±14 ms (36 msec, 10% increase). The maximum prolongation of QT interval was seen at 3 mg/kg/hr and declined slightly at the higher dose of 15 mg/kg/hr (although still above control levels). Effects on QT are summarized in the reviewer's table below. There is substantially more variability in the controls for the sotalol arm of the study compared to the controls for the ranolazine group. QTc values were not presented.

Summary of Effects of ranolazine and sotalol on QT interval (ms)

Basic cycle length	Mean QT± SE					
	sotalol		ranolazine			
	control	Sot 8+4	control	Ran0.5+1	Ran 3	Ran 15
1000	332.7± 77.00	440.93± 76.93**	348.40± 9.07	352.52± 9.05	384.02± 13.9	369.80± 11.6
600	309.85± 73.60	354.67± 74.73**	318.20± 8.58	323.50± 7.74	345.00± 10.04	336.34± 11.43
400	262.73± 74.53	299.14± 73.53**	285.40± 6.02	286.50± 5.76	306.46± 10.38	302.18± 9.33
300	238.40± 74.07	266.40± 74.07*	263.60± 6.61	266.16± 6.36	272.72± 6.09	274.82± 6.48

The drugs both increased the effective refractory period. The baseline between the two groups of dogs differed slightly. This is summarized in the reviewer's table below.

Summary of Effects of ranolazine and sotalol on RV ERP (ms)

Basic cycle length	Mean ERP RV					
	sotalol		ranolazine			
	control	Sot 8+4	control	Ran 0.5+1	Ran 3	Ran 15
1000	206.00±8.86	255.50± 9.56**	240.20±9.9	254.00± 9.31*	249.50±6.19	253.16±7.77
600	191.00±7.1	223.50± 9.07**	218.50±8.93	227.50±8.87	224.50±4.83	229.50±6.19
400	174.00±7.85	195.67± 7.53**	194.00±6.83	201.50±6.45	199.66±3.75	206.50±5.79
300	162.00±6.82	181.33± 8.21**	175.00±5.25	182.84±6.67	181.00±2.32	185.00±5.76

Values are Mean ±SE

Summary of Effects of ranolazine and sotalol on LV ERP (ms)

Basic cycle length	Mean ERP LV					
	sotalol		ranolazine			
	control	Sot 8+4	control	Ran 0.5+1	Ran 3	Ran 15
1000	252.50±17.5	286.25± 16.25*	252.16± 14.13	259.38± 18.18	265.43± 19.42	260.43± 19.32
600	227.50±12.5	262.50± 27.5*	226.16± 11.29	233.13± 12.43	238.13± 13.25	237.50± 14.11
400	202.50±15	226.25± 21.25	198.50± 9.7	204.38± 11.01	211.45± 9.2	215.00± 10.05
300	182.50±10	201.25± 18.75	180.50± 7.18	185.00± 8.1	189.38± 8.32	196.88± 17.53*

Values are Mean ±SE

Under the conditions of this study, it appears that ranolazine can prolong QT interval and extend the effective refractory period of both ventricles although not to the extent of sotalol. We do not know the blood levels of ranolazine achieved in this study.

Pharmacology summary: The sponsor claims in Item 5, volume 1, p.36, that ranolazine increases pyruvate dehydrogenase activity (PDHa) and inhibits the long-chain and short-chain enoyl-CoA hydratase and carnitine:acylcarnitine translocase. However, there were multiple studies to show that ranolazine binds to several non-target receptors. For example, both enantiomers and the racemate interact with α and β adrenergic receptors as well as 5HT1 receptors. This is summarized in the most recent study, a standard radioligand binding assay. There are further studies showing interaction with opioid receptors and some cardiac calcium channel blocking ability. The parent drug has also been shown to block the I_{kr} channel and to delay repolarization. The properties of the metabolites are incompletely described. For example, receptor binding profiles for the

major metabolites have not been provided and the electrophysiological characterization was minimal. Clinically, the drug has also been shown to prolong the QT interval. The sponsor submitted electrophysiology data that they felt supported their position that ranolazine would not contribute to repolarization abnormalities despite the increased QT interval.

Pharmacology Summary: Electrophysiology

The sponsor presents a series of in vitro studies from the laboratory of Charles Antzelevitch, Ph.D., F.A.C.C., one of the leading experts in cardiac electrophysiology. The studies indicate the ability of ranolazine to interact with cardiac ion channels. As the studies would be stronger for the inclusion of positive control data, either historical or concurrent, to indicate the sensitivity of the models, the Division made specific requests for this material which the sponsor provided in the form of published material and a pre-print of studies conducted in the wedge model using other drugs. While ranolazine did not produce early after depolarizations (EAD) in this model, it should be noted that known arrhythmogenic agents such as erythromycin, d-sotalol and cisapride do not produce EADs or torsade de pointes routinely. The incidence reported for spontaneous occurrence in this model was in the order of 20-30% (Antzelevitch, Sun, Zhang and Yan. J Am Coll Cardiol 1996: 28:1836-48; Diego, Belardinelli and Antzelevitch. Unpublished manuscript "Cisapride-induced transmural dispersion of repolarization and torsade de pointes in the canine left ventricular wedge preparation during epicardial stimulation."Shimizu and Antzelevitch. J Am Coll Cardiol 2000; 35: 778- 786). The series of provided references also suggests that testing compounds in the presence of beta adrenergic stimulation is necessary for complete characterization (J Am Coll Cardiol 2000; 35:778-786). In the wedge studies provided, ranolazine was not tested with catecholamines or some other form of beta adrenergic stimulation. It should be noted that several studies showed that approximately 7 of the major, identified, metabolites have the ability to interact with cardiac ion channels. Several of these metabolites show in vitro potency comparable to the parent drug. More detailed commentary on the electrophysiology in general and the wedge model in particular will be provided in a separate document by John Koerner, Ph.D. In this reviewer's opinion, while the electrophysiology data may potentially be useful as an in vitro elucidation of mechanistic possibilities, it is not possible to extrapolate the information to indicate potential human safety.

Pharmacology Summary: Mechanism of Action

The sponsor presents a series of studies indicating the ability of ranolazine to modulate cardiac energy metabolism. The systems used were isolated mitochondria, various in vitro tissue preparations, perfused hearts and several dog studies. While the data suggest the possibility of direct or indirect effects upon cardiac energy metabolism, the sponsor fails to show how all other potential mechanisms of action were excluded. Should the proposed shift in metabolism from fatty acid oxidation to glucose metabolism actually be the primary mechanism by which ranolazine exerts pharmacologic effects, rather than secondary to some other mechanism, such as calcium channel blockade for example, there are serious concerns surrounding this metabolic shift.

More detailed discussion of proposed mechanism

The sponsor's proposed mechanism of ranolazine's action is a shift from fatty acid oxidation to greater reliance on glucose, primarily through inhibition of carnitine-acylcarnitine translocase and enoyl CoA hydratase.

Ischemia corresponds to partial or total decrease in blood flow to a tissue or organ with loss of oxygen supply to the cells. The hypoxia inhibits oxidative phosphorylation and so ATP decreases while ADP transiently accumulates and is then degraded with an accumulation of phosphate. Anaerobic glycolysis temporarily compensates for the decrease in oxidative phosphorylation but with bi-products of lactate and decreased tissue pH. Persistent ischemia and decreased ATP will lead to failure of the Na+/K+ ATPase which will eventually lead to an increase in Ca^{2+} (Morin et. al. Adv Drug Deliv Rev. 49(2001) 151-174).

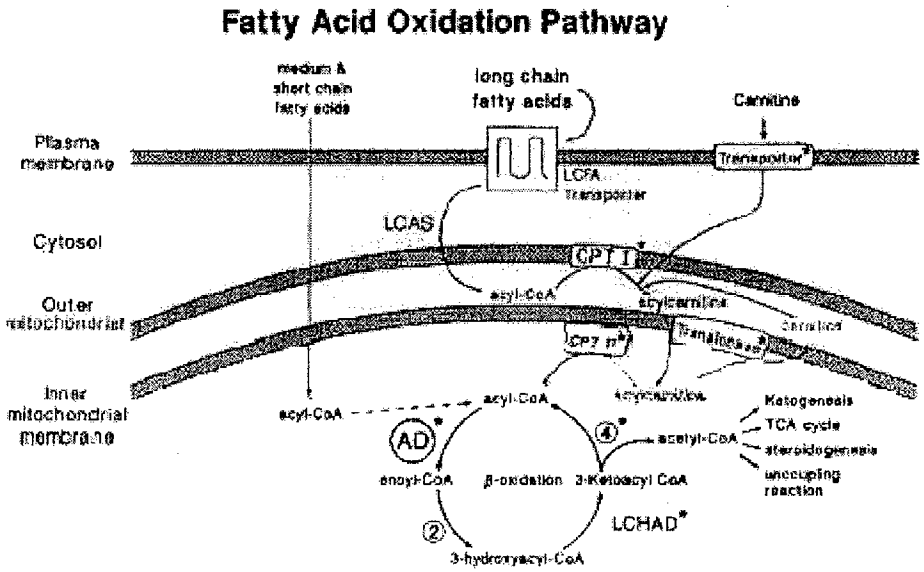
After birth, mitochondrial fatty acid β-oxidation becomes the major source of myocardial energy. It has been demonstrated by a number of investigators that defects in the transport, mitochondrial uptake and β-oxidation of long-chain fatty acids causes cardiomyopathy(CM) in infants and children. (Mathur, A. et al. Circulation 1999; 99:1337-1343).

The glucose transporters GLUT1 and GLUT4 have been identified in the myocardium. Insulin and ischemia cause a translocation of these transporters from the intracellular space into the plasma membrane, resulting in an increased capacity for glucose transport. The interstitial glucose concentration is a function of the arterial glucose concentration and blood flow, thus interstitial glucose levels and the transmembrane glucose gradient are decreased during ischemia and increased by hyperglycemia (Stanley, W.C. Cardiovascular Research 33(1997):243-257.). It would thus seem paradoxical that during angina, when glucose is in decreased supply, to shift metabolism to utilize more glucose.

It has already been demonstrated that myocardial ischemia or increased cardiac work will cause a fall in glycogen concentration. Myocardial substrate metabolism during ischemia is dependent upon the severity of ischemia. A decrease in flow of 20-60% causes a decreased myocardial oxygen consumption (~10-50%), a transient increased dependence on anaerobic glycolysis, reduced rate of FFA oxidation and somewhat more severe contractile dysfunction (ibid.). However, the primary oxidative fuel during mild to moderate ischemia is fatty acids. More severe reductions in flow (>70%) result in greater rates of lactate accumulation and glycogen breakdown. When there is complete elimination of flow, there is total dependence on anaerobic metabolism with glycogen as the sole glycolytic substrate as there is no blood flow to deliver glucose to the tissues.

Carnitine-acylcarnitine translocase(CT) is an inner mitochondrial membrane protein that allows transfer of carnitine and its esters across the inner mitochondrial membrane. CT deficiency is characterized by 2 syndromes, the milder of which includes muscle weakness and hypoketotic hypoglycemia. The more severe syndrome includes development of cardiomyopathy. (Antozzi,

C. and Zeviani, M. Cardiovascular Research, 35 (1997): 184-199). Arrhythmias also seem to be associated with inhibition of carnitine-acylcarnitine shuttling to the mitochondria. (Bonnet D., et al. Circulation. 1999;100:2248-2253.)



Best Possible Copy

Figure 1. The ß-oxidation pathway of fatty acids and specific defects causing cardiomyopathy. The pathways of fatty acid oxidation and cellular carnitine metabolism are shown. Defects known to cause cardiomyopathy (indicated by the asterisks) include abnormalities in carnitine and acylcarnitine transport-carnitine transport defect, carnitine palmitoyltransferase II (CPT II) deficiency, and carnitine-acylcarnitine translocase (translocase) deficiency - and errors in steps 1 and 3 of mitochondrial ß-oxidation, long-chain and medium-chain acyl-coenzyme A dehydrogenase (AD) deficiencies and long-chain 3-hydroxyacyl-coenzyme A dehydrogenase (LCHAD) deficiency, respectively. LCFA, long-chain fatty acid; LCAS, long-chain acyl-CoA synthetase.

Thus, the proposed mechanism of action for ranolazine appears to involve shifting to glucose metabolism at a time when glucose is in short supply and also to accumulate the cardiotoxic intermediates of fatty acid metabolism.

The apparent paradox in the sponsor's proposed mechanism of action is succinctly stated in the work by V.G. Davila-Roman et al "Altered myocardial fatty acid and glucose metabolism in idiopathic cardiomyopathy." JACC 2002; 40:271-277. This is shown below in Davila-Roman's own words.

Alterations in myocardial substrate metabolism have been implicated in the pathogenesis of contractile dysfunction and heart failure (HF) (1–3). Animal models of HF have shown that in the progression from cardiac hypertrophy to ventricular dysfunction, the expression of genes encoding for mitochondrial fatty acid beta-oxidation (FAO) enzymes is coordinately decreased, resulting in a shift in myocardial metabolism that recapitulates the fetal heart gene program, with glucose instead of fatty acids becoming the primary energy substrate (4–8).

The reactivation of the metabolic fetal gene program may have detrimental consequences on myocardial contractile function. The downregulation of mitochondrial FAO enzymes is associated with increased myocardial utilization of oxygen-sparing glycolytic pathways for the production of high-energy phosphates (4). Although this allows for reduced oxygen demands in the hypertrophied and failing heart, the reliance of the myocardium on glucose may produce a relatively energy-deficient state that over a long time may result in decreased contractile performance (1–3). Alternatively, the inability to metabolize fatty acids in the presence of excess availability may be associated with accumulation of nonoxidized toxic fatty acid derivatives, resulting in lipotoxicity and HF (9). This hypothesis is supported by the development of myocardial hypertrophy, HF and sudden cardiac death in children with genetic defects in myocardial FAO enzymes (10–12). Furthermore, myocardial FAO enzyme expression is downregulated in humans with dilated cardiomyopathy, suggesting that a gene regulatory program is responsible for the alterations in myocardial energy substrate utilization (13).

Animal studies have provided significant insight into the metabolic alterations that occur in HF; however, studies in

It may be argued that in the naturally occurring deficiencies in fatty acid oxidizing enzymes the degree of deficiency is greater than what would occur in a pharmacologically-induced situation. However, some other considerations remain:

1. Those affected by CAT deficiency show signs generally within the first day or two of life. How long will it take for a lesser degree of pharmacologically-induced deficiency to become apparent? That is, to produce signs of arrhythmia from the build-up of arrhythmogenic intermediates or from heart failure?
2. Why is a pharmacologically induced deficiency less deleterious and more beneficial than the natural deficiency?

The pharmacology studies presented to support the proposed mechanism of action made little use of comparator compounds to get some idea of the sensitivity of the assay systems. For example, Morin et al (Ibid.) reviewed drugs that have been shown to modulate mitochondrial metabolism. Drugs such as diazoxide, amiodarone, carvedilol, gingko biloba, propofol, cyclosporin A, clonazepam and diltiazem can all be shown to influence different enzymes of fatty acid oxidation. However, it can be argued that this is not the accepted therapeutic mechanism of action for any of these drugs.

The sponsor has not presented studies to exclude contribution of other mechanisms such as binding to opioid receptors, calcium channel effects or alpha and beta adrenergic effects. Both opioids and calcium channel blockers may decrease cardiac oxygen consumption, left ventricular end diastolic pressure and cardiac work. While the parent drug shows low to moderate binding to calcium channels, opioid receptors and alpha and beta adrenergic receptors, there is no information apparent as to the affinity of any of the major metabolites for these receptors and channels.

There is an assay that is used clinically to identify those who have an inherited or congenital deficiency in the carnitine:acylcarnitine translocase (Brivet et al. "Rapid diagnosis of long chain and medium chain fatty acid oxidation disorders using lymphocytes." Ann Clin Biochem 1995;32:154-159; Saudubra et al. "Recognition and management of fatty acid oxidation defects: A series of 107 patients." J. Inher.Metab Dis 22(1999)488-502.; Brivet et al. "Defects in activation and transport of fatty acids." J. Inher. Metab Dis.22 (1999)428-441).Why did the sponsor not use this assay to generate data from the species of interest, the human, or at least, human tissue samples, to support the proposed mechanism?

Pharmacology conclusions: The sponsor was given a specific request by telephone to identify the studies that support the proposed mechanism of action and to show how other possible mechanisms were discounted. The sponsor presents numerous studies indicating that ranolazine may exert pharmacological effects upon the metabolism of isolated mitochondria, various organ preparations and some dog models. No evidence is presented to show how other possible mechanisms of action (the "non-target" receptors to which ranolazine and the major metabolites bind) were eliminated from consideration. The electrophysiology data indicates the potential for ranolazine to interact with cardiac ion channels and may be useful in the in vitro characterization of mechanistic questions. A number of the major metabolites were also shown to interact with cardiac ion channels

equipotent to parent drug. No extrapolations to human safety may be made from this electrophysiology data.

References

Antozzi, C. and M. Zeviani.Cardiomyopathies in disorders of oxidative metabolism. Card. Res.(1997)184-199.

Barron, B. Opioid peptides and the heart. Card. Res 43 (1999) 13-16.

Bonnefont, J-P, et. al. Carnitine palmitoyltransferase deficiencies. Mol.Gen. Metab. 68, 424-440 (1999).

Bonnet, D., et. al. Arrhythmias and conduction defects as presenting symptoms of fatty acid oxidation disorders in children. Circulation. 1999;100:2248-2253.

Brivet, M. et al. Impaired mitochondrial pyruvate importation in a patient and a fetus at risk. Mol. Gen. Metab. 78 (2003) 186-192.

Brivet, M. et. al. Diagnosis of carnitine acylcarnitine translocase deficiency by complementation analysis. J. Inherit. Metab. Dis 17 (1994) 271-274.

Brivet, M. et.al. Defects in activation and transport of fatty acids. J. Inher. Metab. Dis. 22 (1999) 428-441.

Brivet, M. et. al. Rapid diagnosis of long chain and medium chain fatty acid oxidation disorders using lymphocytes. Ann Clin. Biochem. (1995); 32; 154-159.

Davila-Roman, V.G., et. al. Altered myocardial fatty acid glucose metabolism in idiopathic dilated cardiomyopathy. JACC (2002); 40(2): 271-277.

Demmelmair, H., et.al. New insights into lipid and fatty acid metabolism via stable isotopes. Eur J Pediatr (1997) 156 [Suppl 1]: S70-S74.

Drolet, G., et. al. Role of endogenous opioid system in the regulation of the stress response. Prog. Neuro-Psychopharmacol & Biol Psychiat. 2001 Vol 25, 729-741.

Jakobs, C. et. al. In vivo stable isotope studies in three patients affected with mitochondrial fatty acid oxidation disorders: limited diagnostic use of 1-^{13}C fatty acid breath test using bolus technique. Eur J Pediatr (1997) 156[Suppl 1]: S78-S82.

Kantor, P. et. al. The antianginal drug trimetazidine shifts cardiac energy metabolism from fatty acid oxidation to glucose oxidation by inhibiting mitochondrial long-chain 3-ketoacyl coenzyme A thiolase. Circ. Res. 2000; 86: 580-588.

Kelly, D. and A. Strauss. Inherited cardiomyopathies. NEJM. 1994 vol 330, 913-919.

Mathur, A. et. al. Molecular heterogeneity in very-long-chain acyl-CoA dehydrogenase deficiency causing pediatric cardiomyopathy and sudden death. Circulation. 1999; 99: 1337-1343.

Millington, W. et. al. Localization of pro-opiomelanocortin mRNA transcripts and peptide immunoreactivity in rat heart. Card. Res. 43 (1999) 107-116.

Morin, D. et. al. Mitochondria as target for antiischemic drugs. Advanced Drug Delivery Reviews. 49(2001)151-174.

Morris, A. et. al. A patient with carnitine-acylcarnitine translocase deficiency with a mild phenotype. J. Pediatr1998; 132:514-6.

Nuoffer, J-M, et.al. Familial neonatal SIDS revealing carnitine-acylcarnitine translocase deficiency. Eur J Pediatr (2000) 159:82-85.

Oliver, M.F. and L.H. Opie. Effects of glucose and fatty acids on myocardial ischemia. Lancet (1994) 343:155-158.

Pande, S. and M. Murthy. Carnitine-acylcarnitine translocase deficiency: implications in human pathology. Biochim Biophys Acta (1994)269-276.

Pande, S. et. al. Carnitine-acylcarnitine translocase deficiency with severe hypoglycemia and auriculo ventricular block. J Clin Invest (1993)91:1247-1252.

Pepe, S. et. al. 'Cross talk" between opioid peptide and adrenergic receptor signaling in isolated rat heart. Circulation (1997) 95:2122-2129.

Prasad, C. et. al. Hepatic carnitine palmitoyl transferase 1 (CPT1 A) deficiency in North American Hutterites (Canadian and American): evidence for a founder effect and results of a pilot study on a DNA-based newborn screening program. Mol. Gen. Metab. (2001) 73:55-63.

Roe, D., et. al. Evidence for a short-chain carnitine-acylcarnitine translocase in mitochondria specifically related to the metabolism of branched chain amino acids. Mol. Gen. Metab. (2000)69, 69-75.

Saudubray, J., et. al. Recognition and management of fatty acid oxidation defects: A series of 107 patients. J. Inher. Metab. Dis. 22 (1999) 488-502.

Sack, M. and D. Kelly. The energy substrate switch during development of heart failure: Gene regulatory mechanisms (Review). Int. J. Mol. Med. (1998) 1: 17-24.

Slama, A. et. al. Complementation analysis of carnitine palmitoyltransferase I and II defects. Ped Res. (1996);40(4): 542-546.

Stanley, W., et. al. Regulation of energy substrate metabolism in the diabetic heart. Card. Res. (1997);34:25-33.

Stanley, C., et. al. Brief report: A deficiency of carnitine-acylcarnitine translocase in the inner mitochondrial membrane. NEJM (1992); 327: 19-23.

Strauss, A., et. al., Molecular basis of human mitochondrial very-long-chain acyl CoA dehydrogenase deficiency causing cardiomyopathy and sudden death in childhood. Proc Natl Acad Sci USA (1995); 92:10496-10500.

Yang, B-Z., et. al. Carnitine/acylcarnitine translocase deficiency (neonatal phenotype): successful prenatal and postmortem diagnosis associated with a novel mutation in a single family. Mol.Gen.Metab. (2001);73:64-70.

Appears This Way
On Original

II. SAFETY PHARMACOLOGY:

Neurological effects:

The effect of RS-43285 on motor coordination in the rat as determined using the accelerating rotarod AT3377, Conducted June 1984, reported April 1985. vol. 4, p. 76

Sprague-Dawley rats [ ] of unspecified sex were used. The methods are not entirely clear, but it appears that rats received a single oral dose of 10 mg/kg of RS-43285 and one hour later were observed on an accelerating rotarod apparatus. Length of time each rat remained on the rod was determined. Eight rats per group were used. Clonidine and vehicle controls were used for comparison. Clonidine-treated rats stayed on the rod for approximately 25 seconds on average compared to 75 seconds on average for the vehicle controls and approximately 70 seconds for the ranolazine-treated rats. The study is inadequate in that only one dose of drug was tested. There is no way of assessing the existence or absence of a dose-response effect. In light of what has been observed in essentially every toxicology study in each species and has also been reported in various other pharmacokinetic and pharmacology studies, the reviewer disagrees with the sponsor's conclusion that ranolazine "is unlikely to produce overt CNS sedation or stimulation"

The effect of RS-43285 on clonidine-induced hypoactivity in rats. AT3248, April –May 1983. Reported December 1984.

Ligand binding studies had shown that ranolazine possesses some affinity for the α2-adrenoceptor in rat cerebral cortex. This study examined the effect of single oral doses of 0

(water), 10, 30 or 100 mg/kg ranolazine and possible central α2-adrenoceptor antagonist activity in conscious rats as demonstrated by a reversal of the effect of α2-adrenoceptor agonist clonidine (0.3 mg/kg) on locomotor activity. Group size was not disclosed.

Results were expressed in activity units without explanation of how activity was assessed.

treatment	Activity units
Control (untreated)	1000
clonidine	200-300
Ranolazine + clonidine	
ranolazine	447 (n=1)

There is a problem with the results. In the text, the sponsor states that the control animals showed 1000 units of activity. However, in the graphical presentation of the data, the control animals show much less (≤300 units of activity). If one gives any weight to the results, it must be considered that ranolazine itself appears to have a sedative effect in this model.

Neuropharmacological Activity AT3829,vol 4, p152.
Overt Behavior: Groups of 3 male Sim:(ICR)fBR were given intraperitoneal doses from 10-300 mg/kg of ranolazine. Exact doses were not specified. The sponsor reports normal behavior at 10 mg/kg. Crouching posture was noted at 30 mg/kg in one (1/3 or 30%) animal. At 100 mg/kg, signs reported include decreased spontaneous locomotion, wobbly gait, decreased induced activity, decreased grip strength, loss of orientation, ataxia, loss of righting reflex, decreased muscle tone, decreased muscle temperature and mydriasis. Acute mortality occurred at 300 mg/kg for 3/3 mice. Cause was not determined. The sponsor states that 100 mg/kg i.p. "...was a central nervous system depressant."

Induced aroused and unaroused loss of the righting reflex in the mouse: Five groups of ten male Sim:(ICR)fBR mice were given intraperitoneal doses of 100, 110, 120, 160 and 250 mg/kg. At periodic intervals after dosing the mice were placed on their backs. If mice were unable to right within 30 seconds they were regarded as having lost the righting ability while in the unaroused state (ULRR). The mice were then aroused by vigorously rolling in a person's hands for 10 seconds and again placed on their backs. If they were now unable to right, they were considered to have lost their righting ability in the aroused state (ALRR) The ratio of ED_{50} (mg/kg, ip) for unaroused and aroused loss of righting was calculated and compared to data for known central nervous system depressants. This is summarized in the reviewer's table below.

Compound	ED_{50} (mg/kg, i.p.) and 95% confidence limits		AED_{50}/UED_{50}
	Unaroused	aroused	
RS-43285	120 (112-128)	174 (160-190)	1.45
phenobarbital	112(99-121)	142(136-150)	1.3
glutethimide	105(91-115)	115(109-122)	1.1
promazine	65(55-73)	91(81-103)	1.4

The sponsor notes that the ratio of aroused:unaroused ED50s was a value comparable to that of phenobarbital and meprobamate, suggesting general CNS depressant activity.

Induction of neurological deficiency (Neurological and skeletal muscle coordination and function): Groups of 10 male Hla: (ICR)BR mice were dosed intraperitoneally with either water or doses of ranolazine from 10-100 mg/kg. Fifteen minutes after dosing, the mice were observed for their ability to remain on a suspended wire for 10 seconds. Immediately after this the mice were subjected to an electroshock test. The drug was effective in inducing a neurologic deficit with an ED_{50} of 64 (53-70) mg/kg. Sedation was reported for higher dose levels. Mean time on the wire was decreased compared to the controls at all doses and showed a dose-related response. Therefore, neurologic effects were apparent even at the lowest dose of 10 mg/kg. Sponsor's results are shown below.

Summary of Test for Induction of Neurological Deficit

compound	Dose mg/kg	#unimpaired/#tested	Mean time on wire sec±SEM
water		10/10	30±0
RS-43285	10	9/10	27.5±2.5
	30	10/10	21.2±2.4
	60	6/10	11.5±1.4
	80	2/10	4.9±1.3
	90	0/10	1.8±0.8
	100	0/10	2.2±1.1

No NOEL was found for the induction of a neurological deficit.

Effect on Hexobarbital-induced sleep time: Four groups of 10 male Sim: (ICR)fBR mice were used. The mice were treated with water or an aqueous solution of ranolazine at doses of 1, 10 and 50 mg/kg i.p. Fifteen minutes after dosing, 100 mg/kg hexobarbital was given intraperitoneally. Mice were observed for onset and duration of loss of the righting reflex (sleep). Onset of sleep was not reported. Duration of hexobarbital-induced sleep was significantly ($p = 0.03$) increased at the highest dose of 50 mg/kg: 112±11 vs 74± 12 min for the control.

Effect on the electroencephalogram, reticular formation multiple unit activity and sleep of cats: The three cats used in the study were from a colony of cats having electrodes implanted at various specified loci in the brain. Ranolazine was given at 10 or 30 mg/kg. Each cat received both doses with at least 2 weeks between doses. Baseline recordings of EEG, RFMUA(a signal characteristic of the sleep stages in the cat) and EMG were obtained for 48 hours prior to dosing. No observations were made for the first 24 hours to eliminate the first night effect. EEG and behavior were observed for 1 hour. Each cat receiving the HD showed signs of salivation, retching and emesis. The sponsor reported that there were no effects on the parameters measured. However, there was a dose-related increase in REM sleep and REM latency as shown in the reviewer's summary of the sponsor's data below:

Summary of effects on Cat REM sleep (N=3)

	Mean percent ±SE of 24 hour			
	Day -1	Day 1	Day 2	Rem latency min± S.E.
Historical control	14.1±0.3	12.2±0.8	12.9±1.1	90±33

Ran 10 mg/kg	13.8±2.3	14.3±1.4	13.6±1.3	92±21
Ran 30 mg/kg	16.5,17.4*	12.5±1.7	14.3±1.2	107±10

*data on one cat was lost due to recorder failure

While the differences are not significant, there does appear to be a mild dose-related effect upon the duration of REM sleep.

Hot Plate Analgesia Effects: Groups of ten male Hla: (ICR) BR mice were tested 3X at 30 minute intervals prior to dosing to establish a baseline. Water or ranolazine at doses within the range of 1 – 100 mg/kg was administered intraperitoneally. The mice were then tested three more times at 30, 60 and 90 minutes after receiving the drug. Drug efficacy was defined as doubling of each individual mouse's average control response latency. Ptosis and sedation were reported for the HD. Latency relative to the control was not apparently affected. There was therefore no apparent effect on analgesia.

Stress-Induced Hyperphagia in rats: Groups of eight male Hla: (SD)BR rats received either water or ranolazine at 1,10 or 100 mg/kg p.o. Thirty minutes later, the rats were restrained and allowed access to sweetened condensed milk. The volume of milk consumed was measured. There was a dose-related increase in the amount of milk consumed in the treated animals as shown in the reviewer's table below.

Summary of milk volume consumed

treatment	Volume of milk consumed ml±sem
Water	4.7±1.0
Ranolazine 1mg/kg	5.0±1.0
10	5.3±1.1
100	5.9±1.2

If the premise of the study is that stress causes an increase in milk consumption, then it would appear that increased doses of drug caused increased stress.

Anti-convulsant effect using electrically-induced seizures: Groups of 10 Hla: (ICR) BR mice received either water or drug intraperitoneally. Doses used were 10, 30, 60, 80, 90 and 100 mg/kg. At 10 and 30 mg/kg there was increased mortality (80% and 90% respectively compared to 40% for the control group) as a result of the seizures. At 80 and 90 mg/kg the tonic extensor seizures were incomplete. The animals were also observed to be sedated at these doses. At 100 mg/kg the seizures were absent (0/10). The animals also appeared to be sedated.
Low doses of ranolazine appeared to increase the lethality of seizures. The high doses that caused signs of sedation decreased the incidence of induced seizures.

Antagonism of apomorphine-induced climbing: Two studies were conducted using groups of 8 male Sim: (ICR)fBR mice. The mice were treated intraperitoneally with vehicle or ranolazine 15 minutes prior to dosing with 3 mg/kg apomorphine subcutaneously. Six minutes later and every

30 seconds for 15 minutes, the mice were observed for climbing behavior. The initial study used doses of 0.3-30 mg/kg. The LD-treated mice showed significantly less climbing behavior than the controls. Therefore the doses were decreased in the subsequent study to 0.03- 1.0 mg/kg. There were no significant differences in the second study.

Oxotremorine-induced tremors (central anticholinergic activity): Four groups of 10 male Sim: (ICR)fBR mice were treated with water or ranolazine at 1, 10 or 50 mg/kg i.p. Fifteen minutes later, oxotremorine 2 mg/kg was given intraperitoneally. The mice were observed for tremors at 5, 10,15 and 30 minutes after oxotremorine administration. There was no apparent effect upon numbers of tremors and no other signs were reported.

Effects of ranolazine in in vitro neurotoxicity models. AT7011 July 1994-April 1995. Reported June 1995.

Ranolazine(1-100μM) was tested using cultured rat hippocampal neurones against 1) glutamate-induced cell death and 2) glutamate-induced intracellular Ca^{2+} transients. All further experimental details were referenced to 2 papers.
Results: There was a statement that ranolazine did not alter, prevent or protect cultured rat hipocampal cells from 10μM glutamate. No data was presented.

Effects of RS-43285 on the electroretinogram of guinea-pig retina. AT4944 Jan 1989- Feb 1989. Reported August 1989.

Female guinea pigs of unspecified breed (albino Duncan Hartleys?) were euthanized, the eyes collected and the retina isolated. After a 1 hour equilibration period control ERG's were recorded and the tissue then made anoxic by bubbling the superfusing solution with 100% nitrogen. ERG's were again recorded 2 and 4 minutes after beginning anoxic conditions. After 4 minutes of anoxia, the superfusing solution was bubbled with 100% oxygen and a recovery ERG recorded. The protocol was repeated after 30 minutes to obtain a second control. Fifteen minutes later the anoxia procedure was repeated. When recovery from anoxia was observed, the ranolazine was removed from the superfusing solution and "30 minutes left before obtaining a final control response from the retina to anoxia." Some additional control preparations were treated as described above with the exception that the RS-43285 was not added to the perfusing solution on the third anoxic challenge.

Results: There were no significant differences reported between the anoxia-induced reduction in ERG amplitude ± ranolazine (10^{-5}M) at either time point. Given that there was no comparator compound and only one concentration of ranolazine was tested, limited information is derived from the study.

Cardiovascular effects:

Hemodynamic effects of intravenous infusions of RS-43285 in pentobarbitone-anesthetised dogs. AT3300, vol 3, p.325. June 1984-Sept 1984. Reported January 1985.

Mongrel dogs were infused with cumulatively increasing doses of 1, 10, 100 and 1000µg/kg/min with each dose infused for 15 minutes. The results were compared to those obtained in control animals given saline (1 ml/min for 1 hour). Flow probes were installed in the LAD and aortic root. Pressure transducers were used for pulmonary arterial flow and left ventricular pressure. A lead II ECG was recorded.

Results: The dogs infused with saline showed no apparent changes in cardiovascular function. The dogs receiving the 2 lowest doses of ranolazine showed approximately 22% increase in coronary blood flow. LVEDP was approximately doubled at these two doses also. Systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and mean arterial pressure (MAP) showed dose-related decreases. The sponsor's results are shown here.

There were dose-related decreases in ABF (aortic mean blood flow), SV, MAP, SAP, DAP, HR,

HEMODYNAMIC EFFECTS OF RS-43285 IN PENTOBARBITONE-ANESTHETISED DOGS (N = 4)

Parameter	Initial Baseline value	Values during RS-43285 infusion at various doses for 15 min µg/kg/min i.v.			
		1	10	100	1000
CBF	45.0 ± 4.2 ml/min	54.5 ± 8.0	56.0 ± 13.0	47.0 ± 3.0	42.5 ± 8.0
CVR	2.14± 0.5 mmHg/ml/min	1.6 ± 0.2	1.9 ± 0.4	1.5 ± 0.2	1.3 ± 0.3
ABF	1.3 ± 0.2 l/min	1.2 ± 0.2	1.3 ± 0.2	0.7 ± 0.1*	0.5 ± 0.1*
TPR	77.7 ± 13.0 mmHg/l/min	82.0 ± 9.0	74.0 ± 15.0	103.0 ± 6.0	122.0 ± 19.0
SV	8.2 ± 1.8 ml/beat	7.5 ± 2.0	8.3 ± 2.0	4.6 ± 0.5	3.2 ± 0.6*
MAP	94.9 ± 2.6 mmHg	95.6 ± 4.0	88.0 ± 7.0	71.0 ± 14.0	51.0 ± 13.0*
SAP	115.0 ± 2.8 mmHg	112.0 ± 5.0	107.0 ± 9.0	86.0 ± 17.0	66.0 ± 14.0*
DAP	83.6 ± 2.0 mmHg	86.0 ± 5.0	77.0 ± 6.0	62.0 ± 13.0	42.0 ± 12.0*
HR	167.0 ± 12.0 beat/min	170.0 ± 11.0	168.0 ± 12.0	147.0 ± 22.0	134.0 ± 14.0*
LVSP	125.0 ± 18.0 mmHg	127.0 ± 15.0	124.0 ± 17.0	93.0 ± 35.0	77.0 ± 26.0*
LVEDP	4.3 ± 2.4 mmHg	7.0 ± 2.8	8.0 ± 3.0	3.5 ± 2.0	8.0 ± 4.0
+dp/dt	3893.0 ± 241.0 mmHg/sec	3962.0 ± 219.0	3928.0 ± 157.0	2313.0 ± 793.0	1672.0 ± 525.0*
-dp/dt	5381.0 ± 772.0 mmHg/sec	5451.0 ± 699.0	4831.0 ± 570.0	3871.0 ±1449.0	2572.0 ± 946.0*
dp/dt/P	33.75± 2.6 sec^{-1}	34.5 ± 2.0	31.0 ± 4.0	21.4 ± 7.0	21.0 ± 7.0
LVAW	2.2 ± 0.2 kg.m/min	2.2 ± 0.3	2.3 ± 0.3	1.2 ± 0.6*	0.3 ± 0.6*
PAP	10.5 ± 1.5 mmHg	11.0 ± 1.7	10.1 ± 1.3	9.6 ± 1.2	10.0 ± 2.0

* denotes difference with respect to initial value significant $p < 0.05$ by analysis of variance or t-test.

LVSP, +dp/dt(left ventricular contractility), dp/dt/p (left ventricular contractility) and LVMW (LV minute work). No ECG data was presented.

Increasing doses of ranolazine caused loss of cardiovascular function manifested as decreased cardiac output, contractile force and left ventricular systolic pressure. Left ventricular minute work was decreased while total peripheral resistance was increased. The sponsor notes that the 2 higher doses were equivalent to total doses of 25 mg and 250 mg for a 15 kg dog.

25 mg/15kg= 1.67 mg/kg ÷ 1.8 = 0.93 mg/kg Human equivalent dose (HED)
250 mg/15 kg = 16.67 mg/kg ÷1.8= 9.26 mg/kg HED

Best Possible Copy

Human Cmax: 5290 ng/ml

A 70 kg human receiving the maximum dose of 1000 mg ranolazine receives a dose of 14.28mg/kg. Therefore, the dogs showing depression of cardiac function in the above study are not even receiving exposure equivalent to the human therapeutic levels.

Effect on norbormide-induced sudden death in rats: Norbormide at 50-200µg/kg produces general vasoconstriction, coronary vasoconstriction, arrythmias, respiratory depression, hypotension and cardiac arrest in rats. An unspecified number of male Sim: (SD)fBR rats per group were anesthetized, vessels cannulated and maintained on artificial respiration. The rats received drug intravenously 30 seconds – 2 minutes before 125µg/rat of norbormide was given i.v. Ranolazine had no effect on survival at doses of 1,3,5 or 20 mg/kg. At 10 mg/kg 3 animals survived (0 survived in all other groups including the controls). There was no dose-related effect. The results are most likely accidental or spurious.

Hemodynamics in the conscious dog: Two instrumented female dogs trained to slings were used. Baseline readings were made then isoproterenol was continuously infused in step-wise fashion using 3 doses (0.02, 0.07 and 0.21 µg/kg/min) for 5 minutes at each dose. Monitoring was done at the end of each 5 minutes. The animal was allowed to recover to pre-isoproterenol levels. Ranolazine or propylene glycol was given in a gelatin capsule. Each dog was observed for 3 hours post-dose and readings made at 15 minute intervals. Doses of ranolazine used were 200 µg/kg, 1 mg/kg and 5 mg/kg. Each dose was given to a single dog on a separate day. The HD was studied twice while the LD and MD were studied only once. The results, for n=1, do indicate drug effects upon cardiovascular parameters. However, the study is underpowered and inadequate. In the multi-study summary that accompanies the set of studies in which this one was included, the sponsor mentioned that the positive inotropic and chronotropic effects of challenges with epinephrine, norepinephrine and isoproterenol were mildly inhibited, but not the blood pressure decrease elicited by isproterenol, suggesting β-adrenergic blocking properties (p.303). The results were determined in a single dog (n=1, p.329).

Hemodynamics in an anesthetized dog: One dog was anesthetized, a midsternal thoracotomy performed and strain gauges used to determine contractile force. Bilateral vagotomy was also performed. Ranolazine was given intravenously at doses of 0.316, 1.0 and 3.16 mg/kg at intervals of approximately 1 hour. Challenge drugs were administered at 10-15 minute intervals before and after each dose of RS-43285. The drugs used were epinephrine, norepinephrine, isoproterenol, angiotensin, acetylcholine, histamine and bilateral carotid occlusion. The sponsor notes that due to time constraints these were not given after each dose of ranolazine. The only numerical data presented was 2 tables, titled "Percent inhibition of heart rate responses following intravenous administration of RS-43285 to an anesthetized dog" and "Percent inhibition of myocardial contractile force responses following intravenous administration of RS-43285 to an anesthetized dog". Only epinephrine, norepinephrine and isoproterenol results were shown. The sponsor states that ranolazine exhibited some "mild cardiac β-adrenergic blocking properties against autonomic nervous system changes." Given the n of 1 and the lack of reported results, the study is inconclusive.

Pulmonary effects:

Respiration in the anaesthetized dog AT3386, vol 4, p.113

Female beagles [redacted] were anesthetized with sodium pentobarbitone and maintained with this. The dogs were intubated and body temperature maintained. Continuous recording of respiratory rate, tidal volume and flow rate was made. Blood gases were monitored as well as mean arterial blood pressure. The test article was dissolved in saline and administered intravenously giving cumulative doses of 15, 50, 200, 500, 1500, 3000, 7000 and 15000 µg/kg. Blood samples were taken 10 minutes after each dose then measurements recorded for 2 minutes. A dosing-recording cycle of 13 minutes was thus established. The sponsor stated that it was not necessary to show standard error bars as there were no significant differences and thus the error bars were not shown in any of the figures. No positive control data was shown. Results are summarized in the reviewer's table below.

Summary of respiratory parameters (N=3): values approximate

parameter	control	Drug-treated
Femoral venous pH	7.34	50-1500 µg/kg: 7.36-7.37; 3000-15000µg/kg:7.34-7.32
Femoral arterial pH	7.34	All drug-treated groups: 7.36-7.38
Femoral arterial pO_2 (no units)	~72	≥50 µg/kg : 75-80
Femoral venous pO_2 (no units)	50	50-7000 µg/kg: 50-46
Femoral arterial pCO_2(no units)	40	All drug-treated groups: 36-32
Femoral venous pCO_2(no units)	41	All drug-treated groups: 39-33
Expiratory flow rate (ml/sec)	110	15-200µg/kg: 11-115; 500-15000µg/kg: 105-95
Tidal volume(ml)	109	All drug-treated groups: 115-120
Respiratory rate(breaths /min)	15	No apparent effect
Minute flow (ml/min)	1540	200-3000µg/kg: 1700-1800 ml/min
Mean arterial blood pressure(mm Hg)	127	Began falling >500 g/kg. At 15000 µg/kg was 116 mm Hg.

The values were obtained from graphical presentations of the data and are therefore approximate.

The system shows a great deal of variability. A positive control, either historical or concurrent, would strengthen the study.

Renal effects: no studies found

Gastrointestinal effects:

The effect of RS-43285 on gastric secretion in the isolated perfused stomach of the mouse. AT3364 Vol 4, p.60.

Male CD-1 mice were killed by cervical dislocation, the stomachs isolated, cannulated, washed and placed in an organ bath. RS-43285 was added to the serosal solution in a cumulative fashion, with a 15 minute equilibration allowed at each concentration. The pH of the perfusate was determined at the end of each 15 minute period. The concentrations of RS-43285 used were

from $1x10^{-8}$ to $1x\ 10^{-4}$ M. It does not appear that control samples were included in the study design. No experimental results were shown. The sponsor stated that the concentrations tested were without effect on the pH of the perfusate. The study is inconclusive.

The effects of RS-43285 on the intestinal transit of radiochromium (^{51}Cr) marker in vivo AT3365 Vol 4., p68. Male CD-1 mice were orally treated with either RS-43285 (80 mg/kg) or saline vehicle. Thirty minutes later 0.2 ml of radiochromium (0.5μCi $Na^{51}CrO_4$ in saline) was orally instilled into the stomach. Thirty minutes after chromium administration the animals were killed by cervical dislocation. The small intestine was removed and divided into 10 equal segments. The radioactivity in each segment was assessed by gamma counting. Differences in gastrointestinal transit were assessed by calculation of the geometric center (GC) using the equation GC= sum of (fraction of ^{51}Cr per segment x segment number). No results were presented except for the sponsor's statement that GC for the vehicle was 6.38±0.34 and for the drug was 6.05±0.66. Use of positive controls, either historical or concurrent would strengthen the study.

Abuse liability: no studies found

Other:

Safety pharmacology summary: Safety pharmacology data was presented for the cardiovascular, gastrointestinal, neurological and pulmonary systems.
The cardiovascular safety study showed that cumulatively increasing intravenous doses of 1, 10, 100 and 1000 μg/kg/min , with each dose infused over 15 minutes, caused a profound deterioration in cardiovascular function at 100 and 1000 μg/kg/min manifested as decreased cardiac output, contractile force and left ventricular systolic pressure. Left ventricular minute work was decreased while total peripheral resistance was increased. There were dose-related decreases in ABF (aortic mean blood flow), SV, MAP, SAP, DAP, HR, LVSP, +dp/dt(left ventricular contractility), dp/dt/p (left ventricular contractility) and LVMW (LV minute work). No ECG data was presented. The sponsor was contacted by telephone 5/16/03 and asked to provide the ECG data.

There was no plasma drug concentration data. However, the sponsor notes that the 2 higher doses were equivalent to total doses of 25 mg and 250 mg for a 15 kg dog.

250 mg/750 ml = 0.33 mg/ml
human Cmax 5290 ng/ml

25 mg/15kg= 1.67 mg/kg ÷ 1.8 = 0.93 mg/kg Human equivalent dose (HED)
250 mg/15 kg = 16.67 mg/kg ÷1.8= 9.26 mg/kg HED

A 70 kg human receiving the maximum dose of 1000 mg ranolazine receives a dose of 14.28mg/kg. Therefore, the dogs showing depression of cardiac function in the above study are not even receiving exposure equivalent to the human therapeutic levels.

The neurologic effects evaluation included: motor coordination (accelerating rotarod), overt signs, induced aroused and unaroused loss of righting reflex, induction of neurologic deficiency, effect on hexobarbital sleep time, effect on the electroencephalogram and reticular formation multiple unit activity and sleep in cats, hot plate analgesia effect, stress-induced hyperphagia, anti-convulsant effects, antagonism of apomorphine-induced climbing and oxotremorine-induced tremors.

A point that emerged in the overt behavior, induced aroused and unaroused loss of righting reflex, induction of neurologic deficits, hot plate analgesia effects and anti-convulsant effects was that of sedation. The sponsor noted in several of these studies that central sedation appeared to be a characteristic of ranolazine. The sponsor compared the AED_{50}/UED_{50} (aroused/ unaroused) for ranolazine to that of phenobarbital, glutethimide and promazine. Mydriasis was reported in the CNS overt signs study at a dose of 100 mg/kg in addition to decreased activity, ataxia, decreased grip strength, loss of orientation, loss of righting reflex and decreased temperature and muscle tone. In the neurologic deficits study, deficits were elicited at every dose tested (no NOEL identified) with an ED_{50} of 64 mg/kg (range of 53-70 mg/kg). Sedation was reported for doses ≥80 mg/kg. This is supported by the hexobarbital sleep study in which 50 mg/kg, the highest dose tested, significantly (p=0.03) prolonged the sleeping time (a dose not associated with sedation in these studies). Although sedation was noted in the hot plate analgesia test, there was no apparent effect on analgesia.

Stress-induced hyperphagia yielded interesting results. Treatment with ranolazine produced dose-related increases in the volume of milk consumed after restraint-stress.

The antagonism of apomorphine-induced climbing produced effects where ranolazine-treated mice showed significantly less climbing behavior than the controls. When the doses were decreased in a subsequent study to 0.03 –1 mg/kg (clinically irrelevant exposure levels) there were no significant differences. Note: apomorphine is a potent dopaminergic stimulant in mice. The results showing antagonism of this effect may have been due to dopaminergic antagonism or general sedation (although that was not reported). The study should have been repeated at the original doses to confirm by repetition the original results.

The anti-convulsant study showed that doses of 10 and 30 mg/kg ranolazine increased the lethality of seizures(80% and 90% respectively compared to 40% for the control group) At 80 and 90 mg/kg the tonic extensor seizures were incomplete. The animals were also observed to be sedated at these doses. At 100 mg/kg the seizures were absent (0/10). Low doses of ranolazine appeared to increase the lethality of seizures while doses that caused signs of sedation decreased the incidence of induced seizures.
There was no apparent effect upon oxotremorine-induced tremors.

Gastrointestinal transit time data was presented in a somewhat undetailed report that makes it difficult to evaluate the data. No positive controls were presented and no results except for the sponsor's statement that the geometric center for the controls was 6.38±0.34 while the GC for the drug-treated group was 6.05±0.66. This result is difficult to interpret with no comparator compounds.

Pulmonary effects were examined over 15-15000 μg/kg. No positive control data was presented, no error bars were present on the graphs and no tabular data was presented, only graphical. The system showed a great deal of variability which confounds interpretation. The sponsor concluded that there were no significant differences from control. Given the cardiovascular effects that have been demonstrated to occur within the dose range used, secondary pulmonary effects might reasonably be expected.

No renal pharmacology data was presented.

No abuse potential data was presented.

Safety pharmacology conclusions: As tested and reported in the studies provided, ranolazine appears to have:

1) **significant cardiovascular liability**
2) **several central nervous system effects that include: a central sedative effect, the ability to induce neurologic deficits in the absence of sedation, increasing the lethality of induced seizures at low doses and some effect on the HPA axis that is manifested as an exaggerated response to stress.**

The negative inotropic effects seen in the cardiovascular safety study are comparable to calcium channel blocking effects. This raises a concern for the clinical use of this drug in patients with congestive heart failure where one might expect to see a marked decrease in contractility and left ventricular function (Goodman and Gilman's 10th edition).

We know nothing about the contribution of the many metabolites to these results. There is no margin of safety between the plasma levels at which these effects occurred in the animals and the therapeutic levels achieved in humans.

Appears This Way
On Original

III. PHARMACOKINETICS/TOXICOKINETICS:

*The data for the human plasma levels was obtained from Peter Hinderling, M.D., the biopharmaceutics reviewer for this NDA.

PK parameters:

Absorption:

Absorption and excretion studies in dog following oral and intravenous administration of ^{14}C-RS 43285 at 5 mg/kg AT3407/SS/038/85 Sept 1984.

Male Beagles were given either an oral or an intravenous dose of ^{14}C-RS-43285-193 at 5 mg/kg. Blood, urine and feces were collected at unspecified times and radioactivity determined by tlc, HPLC and liquid scintillation counting.

Results: Peak plasma levels of radioactivity were reached within 30 minutes in 3 dogs and by 90 minutes in the 4th dog. Mean urinary excretion was 41% and 37% following oral and intravenous dosing respectively. Mean fecal excretion was 56% and 59% following oral and intravenous administration respectively. Mean total excretion of dosed radioactivity in urine and feces of 97% and 96% were obtained following oral and intravenous administration respectively. The ratio of oral to intravenous AUC values show mean bioavailability of >80% for the radioactivity and 17% for RS43285.

		Radioactivity			RS 43285		
Dog No.	Route of Administration	C_{max} (ng equiv.ml^{-1})	t_{max} (h)	AUC (0-infinity) (ng equiv.h.ml^{-1})	C_{max} (ng.ml^{-1})	t_{max} (h)	AUC (0-12 h) (ng.h.ml^{-1})
M4CA1	oral	3551	0.5	11451	146	0.5	99
M4CA2	oral	1854	1.5	9605	163	0.5	326
M4CA4	oral	3060	0.5	14831	372	0.25	520
M4CA5	oral	2788	0.5	9094	380	0.25	439
M4CA1	intravenous	-	-	12690	-	-	1373
M4CA2	intravenous	-	-	10824	-	-	2146
M4CA4	intravenous	-	-	11194	-	-	1995
M4CA5	intravenous	-	-	8066	-	-	1512

Summary: Consistent with other studies, ranolazine appears to be rapidly absorbed and excreted. The difference in bioavailability of the radioactivity and the drug is not clear, but contribution of metabolites may be involved.

^{14}C-RS 43285: Oral and intravenous absorption and excretion studies in the rat at 5 and 250 mg/kg AT3413/SS/020/85 July 1985

Male and female Sprague-Dawley rats were given 5 mg/kg oral and intravenous doses of radio-labeled ranolazine. Male rats also received 250 mg/kg oral doses of radio-labeled ranolazine. Blood, urine and feces were collected. Quantitation of radioactivity was by HPLC with a radioactivity monitor and an integration program.

Results: Atypical plasma profiles from 1 male and 1 female were omitted. Partial subcutaneous administration was suspected. After 5 mg/kg, Cmax was achieved within 30-60 minutes of oral

dosing. Mean urinary excretion accounted for 56 and 57% of dosed radioactivity after oral and intravenous administration respectively in male rats and 47 and 54% respectively in female rats. Rapid absorption was reported after the 250 mg/kg dose with a plateau in plasma levels from 1 to 6 hours. Urinary excretion accounted for 54% of dosed radioactivity. The sponsor's PK data is shown below.

Summary of PK data

Sex + N	Target dose Mg/kg	Route of Administra	Mean $AUC_{0-\infty}$ equiv.h.ml^{-1}	Mean Cmax (g equiv.g^{-1})	Median t_{max} (Hour)
M , 4	5	Oral	7.3	1630	0.50
F,4	5	Oral	5.6	1209	0.97
M,4	250	Oral	450.9	39468	3.85
M,3	5	IV	17.2		
F,3	5	IV	12.6		

SUMMARY OF PLASMA LEVELS (mcg/ml) IN MALE AND FEMALE RATS 1 HOUR POST-DOSE AFTER 1, 3 AND 6 MONTHS ADMINISTRATION OF RANOLAZINE AT 5, 50 AND 200 mg/kg

	Dose (mg/kg/day)		
Time Point	5	50	200
Males			
1 Month	0.803 ± 0.208 (0.559-1.34)	6.48 ± 2.24 (3.39-9.76)	18.4 ± 4.62 (12.1-25.9)
3 Months	0.724 ± 0.176 (0.352-0.896)	9.39 ± 3.12 (2.67-13.0)	22.2 ± 6.57* (15.4-35.8)
6 Months	1.02 ± 0.388 (0.721-1.92)	8.48 ± 2.32 (4.59-11.9)	36.7 ± 9.25* (23.6-48.4)
Females			
1 Month	1.39 ± 0.30 (0.945-1.89)	8.24 ± 2.42 (5.82-13.6)	24.4 ± 7.74 (12.7-33.5)
3 Months	0.998 ± 0.234 (0.690-1.33)	10.6 ± 3.41 (6.21-16.9)	32.4 ± 9.63 (20.2-46.6)
6 Months	2.91 ± 0.655* (2.16-4.29)	15.2 ± 4.59 (10.1-25.3)	43.7 ± 11.0 (33.8-66.3)

Values represent means ± S.D (Range)
n=10 except * where n=9

plasma exposure in rats orally dosed with ranolazine.

Evidence of absorption of ranolazine in the six month oral toxicity study in rats. AT5878, December 1991

Ranolazine in solution was given orally to male and female Sprague-Dawley rats at doses of 0, 5, 50 and 200 mg/kg/day for six months. Blood samples were collected at one month, three months and six months approximately 1 hour post-dose. Plasma levels of ranolazine were determined by HPLC.

Results: Plasma levels increased with increasing dose. Plasma levels increased in a relatively linear manner in males with the exception of the 3 month time point. At that time, the increase from 50 to 200 mg/kg was less than proportional. In the females, at all time points, the increase in plasma level from 50 to 200 mg/kg was less than proportional. Results are summarized in the sponsor's table below.

Since this is the comparison of plasma levels at one time point only, rather than a comparison of AUC values, all that can be said is that there was evidence of

Evidence of absorption of ranolazine in the six month oral toxicity study in dogs AT5879, December 1991

Ranolazine formulated in gelatin capsules was given to male and female Beagles at doses of 0, 5, 25 and 60 mg/kg/day once a day for 6 months. Blood samples were collected from all dogs days 43-46, days 106-109 and days 182-185. The collection times were before dosing, 30 minutes, 1, 2, 4 and 8 hours after dosing. Determination of plasma drug concentration was by HPLC methodology.

Results:

In both sexes, the increase in AUC with increasing dose was greater than proportional.

Reveiwer's Summary: Female PK Parameters

	Ranolazine Cmax (ng/ml)					
	5 mg/kg/day ***		25 mg/kg/day ***		60 mg/kg/day ***	
1 month	484±254		3580±1561		8858±1909	
3 months	710±124		4348±1078		10855±2713	
6 months	705±471		3958±895		9918±5096	
	AUC (ng.hr/ml)					
1 month	866±625	0.026x	7104±2081	0.211x	27430±8437	0.814x
3 months	1291±561	0.038x	8989±669	0.267x	27443±7945	0.814x
6 months	1399±702	0.042x	7361±1874	0.218x	27817±10337	0.825x

***multiple of human exposure

Reveiwer's Summary: Male PK Parameters

	Ranolazine Cmax (ng/ml)					
	5 mg/kg/day	***	25 mg/kg/day	***	60 mg/kg/day	***
1 month	712±246		3600±2423		8960±1060	
3 months	867±189		5170±1119		11895±2566	
6 months	697±217		3678±1185		9235±2040	
	AUC (ng.hr/ml)					
1 month	1343±361	0.040x	12318±2706	0.366x	28126±4265	0.835x
3 months	1720±202	0.051x	14364±4720	0.426x	37740±8458	1.120x
6 months	1655±388	0.049x	10319±3221	0.306x	29610±7995	0.879x

*** multiple of human exposure

Distribution:

^{14}C-RS-43285: Tissue distribution studies by whole body autoradiography in albino and pigmented rats RS-43285-193AT3356 April 1985

Albino Sprague-Dawley rats and Long Evans pigmented rats received a single oral dose of ^{14}C-RS-53285 dihydrochloride at 50 mg/kg. The albino rats were euthanized at 1,6 and 24 hours and the pigmented rats at 24 hours. Sagittal whole body sections were obtained at several levels throughout the carcass, exposed to radiograph film and contact prints developed to show distribution of radioactivity. It appears that there was one albino rat per time point and 1 pigmented rat at 24 hours.

Results: A visual discernment of distribution of radioactivity was made. At 1 hour, the highest apparent concentrations of radioactivity were in the GI tract, adrenals, kidney, lacrimal gland, liver, preputial gland and pituitary. The lowest levels were reported for the muscle, bone marrow, lung and testes. The sponsor states that no evidence of radioactivity was discerned in the brain or spinal cord. In the pigmented rat, levels of radioactivity in the eye were much greater than in the albino rat, possibly due to melanin binding. After 6 hours, levels of radioactivity in most tissues were apparently lower. By 24 hours post-dose, the highest levels of radioactivity were present in the contents of the stomach and large and small intestines. Levels of radioactivity were much greater in the eye of the pigmented rat than the albino rat. Because so few animals were studied, the report cannot be given too much weight.

^{14}C-RS-43285: Tissue distribution studies in the rat. RS-43285 AT3357, April 1985

Parallel studies with albino Sprague-Dawley rats and pigmented Long-Evans rats, n=4 per time point, were given a single oral dose of ^{14}C-RS-43285, 50 mg/kg. Rats were euthanized (AT3413) in groups of 4 at 1, 6, 24 and 72 hours after dosing. After euthanasia, thyroid, heart, lungs, testes, bone marrow, kidneys, liver, spleen, arterial wall, GI tract, xiphoid cartilage, fat, adrenals and brain were collected and weighed. Urine and fecal samples were collected daily from the group of rats euthanized at 72 hours.

Ocular studies: following a single oral dose of ^{14}C-RS-43285 at 5 mg/kg, Long Evans rats in groups of 2 were euthanized at 1, 2, 3, 7, 10, 14, 21 and 28 days post-dose. Control albino rats were euthanized at 1 and 2 days post-dose.

Quantitation of radioactivity was done by scintillation counting.

Results: Levels of radioactivity were reported to be highest at 1 hour and declined rapidly thereafter. In descending order, the principal tissue levels of radioactivity were found in the GI tract, liver, adrenals, kidney, thyroid, arterial wall, bone marrow, heart and brain. At 72 hours post-dose the radioactivity was principally associated with liver, kidney, GI tract and thyroid, although detectable levels were reported for all the original tissues.

Pigmented eyes at 1 hour contained 21.47 % dose x 10^{-3} vs 0.35% dose x 10^{-3} in the albino eyes. There was no detectable radioactivity in the albino eyes after day 2 while the pigmented eyes showed levels of 5.62 % dose x10^{-3}. The terminal half-life of elimination of radioactivity from the pigmented rat eye was ~ 23 days.

Mean urinary and fecal excretion accounted for 53% and 43% respectively of the dosed radioactivity.

Summary: Drug-derived radioactivity was found primarily in liver, adrenals, kidney thyroid and gastrointestinal tract. Drug-associated radioactivity was cleared rapidly from tissues with the exception of the pigmented eye. Skin was not discussed.

The tissue distribution of total radioactivity in the albino and pigmented rat following oral administration of [^{14}C]-ranolazine. December 1999-October 2001 CVT303.006R

Twenty nine male albino rats each received a single oral dose of [^{14}C]-ranolazine at a target dose level of 50 mg/kg. At each time point of 0.5, 1, 2, 4, 6, 8, 24 and 72 hours post-dose, 3 rats were euthanized and a number of tissues and/or blood collected. Urine, feces and cage wash were collected for the 5 remaining animals, housed in metabolism cages. The intervals for urine collection were: predose, 0-6, 6-24, 24-48, 48-72, 72-96, 96-120 hours. The intervals for fecal collection were: 0-24, 24-48, 48-72, 72-96 and 96-120 hours. The following organs were collected from the animals euthanized at 1, 6, 24, 72 and 120 hours post-dose: adrenals, bile duct, blood, bone mineral and marrow, brain, eyes, fat, harderian gland, heart, kidneys, large intestine, liver, lungs, muscle, pancreas, plasma, carcass, salivary gland, skin, small intestine, spleen, stomach, testes, thyroid, urinary bladder. Blood only was collected from rats in the 0.5, 2, 4 and 8 hour groups. Levels of total radioactivity were determined for each tissue and sample of excreta by liquid scintillation counting and combustion analysis.

Another 6 male pigmented rats each received a single oral dose of [^{14}C]-ranolazine at 50 mg/kg. One rat was euthanized at each of 6 timepoints: 24, 72, 168, 336, 504 and 672 hours post-dose. Levels of radioactivity were determined in one of the eyes and a blood sample by combustion analysis and liquid scintillation counting. Quantitative whole body autoradiography was also performed.

Results:
Albino rats: Mean total recovery of radioactivity over 120 hours was 98% of administered dose. Elimination was primarily through the feces with a mean of 53% (range 47 –62%). Elimination via the urine was on average 39%(range 30-43%). Cage washings accounted for 3-7% of radioactivity. 87% of the radioactivity was recovered within the first 24 hours after dosing.

The radioactivity was quickly distributed to every organ sampled. There were detectable levels of radioactivity in the adrenals, brain, eyes, heart and kidneys (and other organs) to 120 hours after dosing. The quantitative whole body autoradiography was not as sensitive. However, there were detectable levels of radioactivity in testes, adrenals and eyes.

Appears This Way
On Original

Table 2 ***Mean Concentration of Total Radioactivity in Tissues Following Single Oral Administration of [^{14}C]-Ranolazine to Male Albino Rats. Target Dose Level: 50 mg.kg^{-1} Results expressed as µg equiv.g^{-1}***

Sample	Timepoint									
	1 h Mean	1 h SD	6 h Mean	6 h SD	24 h Mean	24 h SD	72 h Mean	72 h SD	120 h Mean	120 h SD
Adrenals	46.70	5.10	6.75	2.59	1.52	0.22	0.61	0.02	*0.37	*0.12
Bladder	43.03	32.11	13.85	8.17	7.25	10.34	0.24	0.04	*0.14	*0.09
Bone Marrow	39.39	16.66	6.31	3.57	1.09	0.57	*0.09	*0.12	*0.11	*0.08
Bone Mineral	10.24	1.849	2.50	0.76	*0.42	*0.03	*0.09	*0.09	*0.13	*0.05
Brain	5.26	0.94	1.54	0.21	0.28	0.11	*0.05	*0.04	*0.06	*0.03
Eyes	9.80	1.90	2.10	0.25	0.22	0.06	*0.06	*0.01	*0.04	*0.03
Fat-Subcutaneous	12.36	0.83	1.50	0.28	0.69	0.45	0.25	0.03	0.20	0.05
Harderian Gland	111.64	36.68	16.96	6.40	2.19	0.60	0.39	0.05	*0.16	*0.07
Heart	22.49	2.91	2.68	0.27	*0.37	*0.09	0.16	0.01	0.12	0.03
Kidneys	60.80	8.36	11.98	0.73	2.58	0.73	0.91	0.03	0.57	0.17
Liver	138.62	24.60	22.76	7.75	4.36	1.30	1.51	0.20	0.83	0.22
Lungs	45.78	10.63	3.73	0.60	0.79	0.38	*0.17	*0.15	0.17	0.05
Muscle	14.72	1.80	2.25	0.31	0.36	0.14	0.13	0.01	0.13	0.01
Pancreas	36.05	15.17	4.01	0.34	0.57	0.18	0.25	0.03	0.17	0.04
Salivary Gland	44.41	1.57	7.36	1.71	0.72	0.28	0.20	0.03	0.17	0.02
Skin	17.62	1.22	4.24	0.44	0.64	0.07	0.40	0.08	0.27	0.01
Spleen	41.76	8.87	3.68	0.71	0.60	0.13	0.29	0.02	0.24	0.02
Testes	9.38	1.85	2.67	0.15	0.84	0.32	0.11	0.03	0.09	0.01
Thyroid	33.42	2.76	3.46	0.71	2.18	0.40	0.47	0.05	*0.63	*0.35
Bile duct	36.52	50.57	13.15	8.34	4.35	N.A.	*0.29	*0.25	*0.21	*0.20
Stomach	335.40	208.23	13.61	7.46	33.81	44.67	1.86	2.26	0.12	0.00
Stomach Contents	2181.50	785.83	14.88	6.33	78.52	109.50	4.76	4.23	*0.02	*0.02
Small Intestine	261.47	27.16	57.00	5.63	15.93	9.16	0.76	0.47	0.12	0.02
Small Intestine Contents	580.04	158.86	72.84	8.77	30.28	19.38	1.22	0.96	0.07	0.02
Large Intestine	35.49	9.49	341.52	67.94	38.61	11.34	1.90	1.07	0.14	0.03
Large Intestine Contents	20.01	3.13	391.47	53.49	50.02	22.23	2.24	1.22	0.09	0.03
Carcass	11.51	2.70	2.84	0.96	0.60	0.29	0.40	0.28	0.33	0.18
Whole Blood	14.31	2.31	2.92	0.38	0.55	0.17	0.18	0.01	0.12	0.02
Plasma	17.91	2.06	3.37	0.46	0.58	0.19	0.43	0.33	0.07	0.01

Values are mean and SD of data from 3 (1,6,24 and 72 h timepoints) or 5 (120 h timepoint) rats.
N.A.= Not available/analysed
*=Mean includes results calculated from data less than 30 d p m above background

Table 4 ***Concentration of Total Radioactivity in Tissues (QWBA) Following Single Oral Administration of [^{14}C]-Ranolazine to Pigmented Rats. Target Dose Level: 50 mg.kg^{-1}***

Results expressed as µg equiv.g^{-1}

Tissue	001M	002M	003M	004M	005M	006M
	24 h	72 h	168 h	336 h	504 h	672 h
Adrenal Gland	2.1	1.1	NM	NM	NM	NM
Bladder	NM	NM	NM	NM	NM	NP
Blood	NM	NM	NM	NM	NM	NM
Bone Marrow	1.6	NM	NM	NM	NM	NM
Brain	NM	NM	NM	NM	NM	NM
Brown Fat	1.9	NM	NM	NM	NM	NM
Epididymis	NM	NM	NM	NM	NM	NM
Eye	102.7	53.9	37.4	9.5	16.3	6.1
Harderian Gland	6.1	NM	NM	NM	NM	NM
Heart	NM	NM	NM	NM	NM	NM
Kidney	4.0	2.5	1.1	0.2	NM	NM
Lachrymal Gland	NM	NM	NM	NM	NM	NM
Large Intestine Wall	NM	NM	NM	NM	NM	NM
Liver	6.8	2.4	0.8	0.1	NM	NM
Lung	NM	NM	NM	NM	NM	NM
Lymph Node	NM	NM	NM	NM	NM	NM
Pancreas	1.3	NM	NM	NM	NM	NM
Pituitary Gland	NM	NM	NM	NM	NM	NM
Preputial Gland	2.5	1.1	NM	NM	NM	NM
Prostate	NM	NM	NM	NM	NM	NM
Rectum	NM	NM	NM	NM	NM	NM
Salivary Gland	1.6	NM	NM	NM	NM	NM
Seminal Vesicles	NM	NM	NM	NM	NM	NM
Skeletal Muscle	0.7	NM	NM	NM	NM	NM
Skin (Albino area)	0.8	0.7	NM	NM	NM	NM
Skin (Pigmented area)	15.0	7.5	NM	NM	NM	NM
Small Intestine Wall	NM	NM	NM	NM	NM	NM
Spinal Cord	NM	NM	NM	NM	NM	NM
Spleen	1.4	0.9	NM	NM	NM	NM
Stomach Wall	NM	NM	NM	NM	NM	NM
Testis	0.9	0.3	NM	NM	NM	NM
Thymus	NM	NM	NM	NM	NM	NM
Thyroid Gland	NM	NM	NM	NM	NM	NM
White Fat	0.2	NM	NM	NM	NM	NM
Limit of Reliable Measurement	0.3	0.3	0.3	0.3	0.2	0.3
Eye*	47.4	36.3	16.5	9.5	7.2	4.6
Blood*	0.5	0.2	0.1	0.1	0.1	0.1

* = Measured by combustion and LSC
NM = Not measurable
NP = Not present

Pigmented rats: The eye contained the highest concentrations of total radioactivity. The rate of elimination from both skin and eyes appeared to be slower than other tissues. The concentration in the eye at 28 days after dosing was ~6% of that measured at 24 hours post-dose (p.225, vol 42).

Concentrations in the eye were reported to diminish with a half life of ~ 8 days. Although it was a very small sampling of pigmented rats, the results are suggestive of melanin binding. This has not been confirmed with in vitro studies nor

Best Possible Copy

described to any greater extent. Neither potential toxic effects upon the visual system nor potential for phototoxicity have been explored.

Metabolism:

Pharmacokinetics of ranolazine in male guinea pigs after a single intravenous dose of ranolazine at 6 or 30 mg/kgCVT303.008-R August-October, 2000. Reported: July 17, 2002

This is an amendment to the original report. Male guinea pigs, surgically cannulated, were given single intravenous doses of either 6, 30 or 60 mg ranolazine dissolved in saline. Blood was collected via the canulas at 2, 5, 15, 30 minutes and 1, 2, 4, 6, 8 and 24 hours after dosing. Plasma concentrations of ranolazine and 3 metabolites found in humans (RS-88390 (CVT-2514); RS-88640(CVT-2512); RS-94287(CVT-2738)) were determined by LC/MS/MS. The range of quantification was listed as 50 – 10,000 ng/ml for ranolazine and 10 – 10,000 ng/ml for the 3 metabolites. The method was referenced to a paper regarding the use of human plasma. There is no indication that this assay was validated for guinea pig plasma.

Results: No adverse effects were reported for the 6 mg/kg dose. At 30 mg/kg, signs included hyperactivity, hyperventilation, tremors and prostration. These were reported to resolve by 1 hour. The 60 mg/kg dose was lethal. No further details were given regarding the HD effects. The pharmacokinetic parameters that were determined are shown in the sponsor's table below.

Summary of PK values

Dose(mg/kg)	6	30
Number of animals	2[a]	3[b]
$AUC_{(0-\infty)}$(ng.hr/ml)[c]	1089	6340±2010
CLp (ml/min/kg)	90.7	72.2±23.2
Vdβ(l/kg)	6.36	5.61±2.11
Elimination $t_{1/2}$	nd[d]	0.89±0.05[e]

[a] mean of 2 animals [b]Values are mean and SD. [C]- values were $AUC_{(0-t)}$ for the 6 mg/kg dose. [D]nd=not determined

[e]-half life determined on plasma concentrations between 1 and 4 hours.

The 3 specified metabolites were present in guinea pig plasma. These metabolites result from N-dealkylation at the piperazine ring, O-demethylation of the methoxyphenyl group and O-dearylation of the methoxyphenyl group. It is not specified in the report if the sponsor looked for other metabolites also.

Summary of metabolite data: AUC ng.hr/ml

metabolite	6 mg/kg	30 mg/kg
RS-88390	161	275
RS-94287	57.8	847
RS-88640	33.7	417

Given the low numbers of animals used for this study, limited conclusions may be drawn. It may be said that following single intravenous doses of ranolazine, the above 3 metabolites were found in guinea pig plasma.

Effect of RS43285 on hepatic drug metabolising activity in the rat and mouse. AT3397/SS/011/85, July 1985.

Several approaches were taken:

3. Male CD-1 mice received oral doses of phenobarbitone 250 mg/kg or ranolazine 250 mg/kg once a day for 5 days.
There were 10 mice per group (control, phenobarbitone and RS-43285). Day 6, the mice were given an intraperitoneal injection of pentobarbitone(70 mg/kg). Induction time was described as time from injection to the loss of righting reflex. Sleeping time was the time between loss and regaining the righting reflex.
4. Male Sprague Dawley rats (n=3 per group) were given oral doses of 250 mg/kg/day ranolazine or a saline vehicle once a day for 5 days. The rats were euthanized 24 hours after the last dose. Livers were collected and microsomal protein, microsomal CYP450 content and flavoprotein reductase content determined.
5. A single oral dose of saline, 25 mg/kg or 100 mg/kg of RS43285 was given to male rats one hour prior to an intraperitoneal dose of pentobarbitone (65 mg/kg). One hour later, the rats were given an intraperitoneal injection of pentobarbitone (65 mg/kg). Induction time and sleeping time were measured as above.
6. Control male rat livers were used for in vitro evaluation of potential inhibitory activity of RS43285 (0, 10, 50, 100 and 500 μM) on aminopyrine N-demethylase, biphenyl 4-hydroxylase and ethoxyresorufin O-deethylase.
7. NADPH-cytochrome c reductase activity was measured over a microsomal protein concentration range of 0.5-2 mg/ml.

Results: There was no significant difference between control and ranolazine for induction and pentobarbitone-induced sleeping time in mice. Liver weight and microsomal protein content were significantly increased in ranolazine-treated rats.

Appears This Way On Original

Effect of Chronic Oral Administration of RS 43285 to Male Rats on Some Indices of Hepatic Phase I Drug Metabolising Activity (DMAE 373)

Dosed Group	Final Body Weight (g)	Final Liver Weight (g)	Microsomal Protein Content[1]	Specific Microsomal Cytochrome P-450 Content[2] (uncomplexed)	Total Cytochrome P-450 Content[2] (complexed plus uncomplexed)	Microsomal NADPH-Cytochrome c Reductase Activity[3]
Control	221.7 ± 3.8	9.1 ± 0.7	11.7 ± 0.1	0.60 ± 0.02	ND	45.7 ± 3.8
RS 43285	228.3 ± 8.7	10.5 ± 0.6*	13.1 ± 0.2*	0.54 ± 0.02	0.55 ± 0.00	51.2 ± 6.7

RS 43285 was dosed orally at 250 $mg.kg^{-1}.day^{-1}$ for 5 consecutive days.

Results are given as the mean ± S.D. for three determinations. The statistical difference between the means was determined by the Student's t-test.

* Values statistically different from control ($P < 0.05$).

1 mg protein.g liver^{-1}
2 nmol.mg protein^{-1}
3 nmol.mg protein^{-1}.min^{-1}
ND = not determined

Best Possible Copy

There was significant inhibition of ethoxyresorufin O-deethylase activity and biphenyl 4-hydroxylase (BPH) at 100 and 500 µM. The inhibition of BPH was 35 and 48% mean inhibition at those 2 concentrations, respectively.

Ranolazine produced a slight increase in sleeping time acutely at 100 mg/kg. Chronic ranolazine administration caused a slight decrease in sleeping time.

Pentobarbitone induction and sleeping times

	ranolazine: acute administration		ranolazine:chronic administra	
	Induction time (minutes)	Sleeping time (minutes)	Induction time (minutes)	Sleeping time (minutes)
Control	2.6±0.5 (n=6)	135±18	2.7±0.6	56.2±14.5
Ranolazine 25 mg/kg	3.2±1.2 (n=5)	129±15	2.8±0.6	45.7±11.9
Ranolazine 100 mg/kg	2.2±0.3 (n=6)	144±18	6.0±2.8*	19.5±7.3#

*statistically different from control $p<0.05$; # $p<0.001$

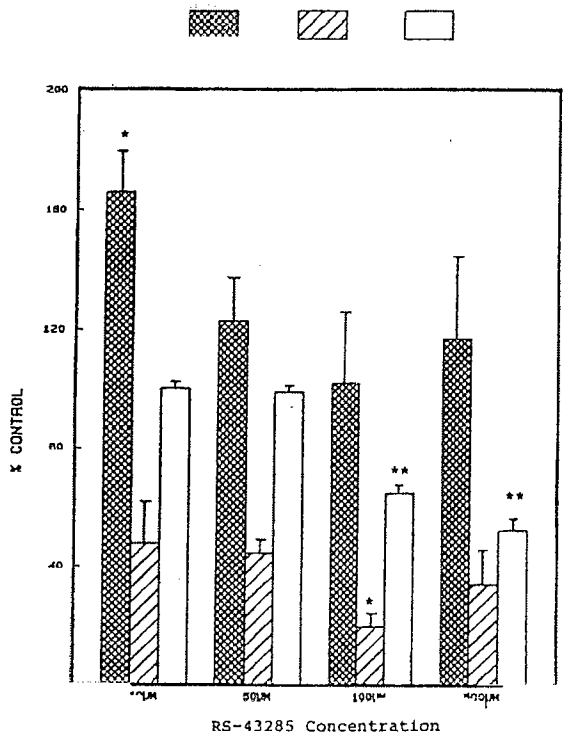


Figure 2 Effect of RS-43285 Added In Vitro on the Activity of Three Microsomal Drug Mono-oxygenases in Rat Liver.
APD- Aminopyrine N-Demethylase; EROD- Ethoxyresorufin O-Deethylase; BPH- Biphenyl 4-Hydroxylase
* $P<0.05$; ** $P<0.01$.

The hepatic rate of RS43285 metabolism: an in vitro assessment. AT3474. September 1985. The racemate RS 43285-193 (lot 125SS0584), the R enantiomer RS 43285-198 (lot number 5282-27) and the S enantiomer RS 43285-197 (lot number 5325-32) were compared against nicardipine hydrochloride. Male Sprague-Dawley rats and male CD-1 mice (both species from [redacted], and male Beagles [redacted], were used. After euthanasia, livers were collected from each animal and post mitochondrial supernatants prepared. Triplicate incubations for each condition were prepared. Samples were taken from each reaction mixture at time intervals up to 2 hours then processed for and analyzed by HPLC for parent compound levels. Unfortunately, the report does not specify how many animals per species were used nor the concentrations tested. One statement made in the results section suggests that only 10 µM and 100 µM concentrations were tested for one of the 3 compounds under consideration. We do not know about the usage of nicardipine.

Results The results are presented as 1)a table of rate constants for 2 concentrations of RS43285 with no indication as to whether this was the racemic mix or one of the enantiomers; 2) "An interspecies comparison of the relative in vitro rates of metabolism of nicardipine and RS43285 by liver post-mitochondrial supernatant" presents rate constants for 100 µM nicardipine and 100 µM RS43285 for the rat and dog; 3) a third table gives rate constants for 10 µM of the

enantiomers in rat liver(R: 1.20±0.27 h^{-1}; S: 1.08±0.18h^{-1}; racemate: 0.60 h^{-1}) 4) the 4th table gives reaction velocity (µg RS43285.g liver-1.min-1) for 0.103, 1.03 and 10.3 mM RS43285. The lack of data for multiple concentrations, lack of data for the enantiomers versus the racemic mix and the different concentrations reported for different species make it very difficult to extract useful information from this report. The study is inconclusive.

A further investigation of the potential inhibitory effect of RS 43285 on hepatic drug metabolising activity in the rat. Addendum to SRS Report No. SS/011/85, AT3619 June 1986

Male Sprague-Dawley rats ( ) were used as a source of hepatic microsomes. Microsomal ethoxyresorufin O-deethylase (EROD) activity was measured. Apparent Km and Vmax were determined for RS43285 concentrations of 0, 0.01, 0.05 and 0.10 mM using 7 substrate concentrations somewhere over the range 0.01-5 µM. Biphenyl 4-hydroxylase (BP4H) activity was also determined with apparent Km and Vmax determined for 4 concentrations of RS 43285 (0, 0.01, 0.05 and 0.10 mM) using 5 substrate concentrations over the range 0.01- 2 mM biphenyl. For unexplained reasons, control rat liver microsomes were used at 0.34 mg protein/incubation for the EROD assays and 2.0 mg protein. m^{-1}/incubation for the BP4H assays. The difference in designations of concentrations are the sponsor's.

Results: Vmax and Km increased slightly from 0 - 0.05 mM RS43285 for ethoxyresorufin O-deethylase. Vmax decreased slightly for biphenyl 4-hydroxylase with increasing concentration of RS43285. This is summarized in the reviewer's table below.

RS43285 conc (mM)	Ethoxyresorufin O-deethylase		Biphenyl 4-hydroxylase	
	Vm#	K_M µM	Vm*	K_m mM
0	128(91.7-213)	0.26(0.14-0.50)	2.63(1.33-8.33)	0.47(0.16-2.00)
0.01	167(122-270)	0.21(0.12-0.41)	3.03(1.33-33.33)	1.00(0.33-10.00)
0.05	196(130-385)	0.45(0.25-1.11)	1.26(0.90-2.85)	0.19(0.06-0.58)
0.10	169(115-333)	0.43(0.23-1.00)	0.95(0.53-2.22)	0.20(0.07-0.62)

pmol.min^{-1}.mg $protein^{-1}$ *nmol.min^{-1}. mg $protein^{-1}$ Values in parentheses are sponsor's 95% confidence intervals

CYP1A1 and CYP1A2 both metabolize 7-ethoxyresorufin. The specificity of biphenyl 4-hydroxylase is not mentioned in the report.

Summary: From the given data, it appears that under the conditions of the assay there was minimal effect of RS43285 on activity of either ethoxyresorufin O-deethylase or biphenyl 4-hydroxylase.

An in vitro assessment of the sites of metabolism of RS 43285 in the rat. AT3867 April 1987
Male Sprague-Dawley rats ( ) were euthanised and the liver, lungs, kidneys and an unspecified portion of the small intestine were collected. Post-mitochondrial supernatants were prepared from each tissue. Samples were incubated for intervals up to 1 hour. Concentrations and possible variations on chemicals used were not specified. After the metabolic reaction, protein was precipitated and the supernatant analyzed by HPLC for parent compound. The sponsor presents a single table of data, with each value from a single rat, showing rate constants

for RS43285 metabolism. Data is shown only for the liver and 1 value for the kidney with the note that no metabolism was detected in any other sample. As presented, the reviewer cannot come to an independent interpretation of the data.

The isolation and characterization of the metabolites of ranolazine in the rat. AT5956/ SS/020/89 February, 1992.
Male Sprague-Dawley CD(SD)BR rats ( ) were orally gavaged with 250 mg/kg of ranolazine and placed in metabolism cages for separate collection of urine and feces. Urine collected was pooled to give a 0-24 hour sample.

Other rats were anesthetized and the bile ducts cannulated. After a recovery period the rats were orally dosed with 200 mg/kg. Bile was collected for 24 hours post-dose.

Semi-preparative and analytical HPLC were performed.

In vitro metabolism studies were also performed using ^{14}C-ranolazine in a buffered incubation system containing an S9 fraction prepared from rat livers. The samples were analyzed for metabolites by HPLC. Fractions from the semi-preparative hplc of the urine and bile fractions were hydrolyzed with glusulase. Mass spectrometry was performed on all prepared samples.

Results: There was no tabular summary of distribution of the metabolites only a textual presentation of the results. It is not clear from the report how many animals were used to generate the data. One comment in the report states that the bile data came from 1 rat. It was also stated that overall recovery of radioactivity was >70%. The sponsor proposed that the 31% of the total radioactivity remaining after extraction of the urine sample was conjugated material. The report also states that the hplc systems used failed to fully separate the monohydroxylated metabolites RS-88597, RS-88772/88835 and RS-89961 as well as the positional isomers RS-89664, RS-88597, RS-89356 and RS-89649 (3,4,5 and 6–monohydroxylated respectively in the methoxyphenyl ring). HPLC evidence suggested that only RS88597 was formed in vivo.
The most abundant metabolite in rat urine was RS-89289, accounting for ~9% of the dose. RS-94287 and a glucuronide conjugate of RS-88390were next most predominant at 6% and 5% of the dose respectively. All other components were reported to be present at ≤ 4%.

Metabolites in the bile of the one rat were reported to be exclusively conjugated species including the glucuronide conjugate of RS-88390 (16%) and sulfate conjugates of RS-88597 and RS-88640. Quantitation was confounded by co-chromatography.

The in vitro sample (n=1, p.164) showed ranolazine and 5 major metabolites as well as a number of minor metabolites. Potential stereoselectivity in the metabolism of ranolazine was not investigated in the present study. Metabolites were tentatively identified as RS-88250 and/or RS88755, RS88640, RS94287 and RS88597.

There is insufficient data presented to allow for an independent review of the material. A reasonable interpretation of the data is that certain metabolites were identified in the urine and bile of an unspecified number of male rats.

The identification of the metabolites of ranolazine in the rat. AT7014,May –Sept, 1994. Reported: January, 1995

THE ABUNDANCE OF RANOLAZINE METABOLITES IN 0-24 h MALE RAT URINE

Metabolite	Abundance *
RS-89289	9%
RS-89983	3%
RS-94287	6%
RS-89961	2%
ranolazine	4%
RS-88250/88755 - conjugate	3%
RS-89983 - sulphate	3%
RS-91347 - conjugate	2%
RS-88390 - glucuronide	5%

* Expressed as a percentage of the dose

On average 43% of dosed material was excreted within 24 h of dose.

Al other metabolites present at 1% or less of dose

Thirty male and 30 female rats (:CD(SD)BR, [redacted]) were given oral doses of 150 mg/kg. A group of 9 male and 9 female rats received a single dose of non-radiolabelled ranolazine Day 1 and another group (21 male, 21 female) received a single daily dose of non-radiolabelled ranolazine for 7 days with a further single dose containing ^{14}C-RS-43285 (15-20 µCi/animal) on Day 8. Blood samples were collected day 1 at 0.5, 2.5 and 5 hours after dosing. Day 8, blood samples were collected at 0.5, 1.0, 2.5, 5, 10 and 24 hours post-dose. Urine and fecal samples were collected from 3 male and 3 female rats placed in metabolism cages. The excreta samples were removed at 6, 24 and 48 hours after administration of the radiolabelled dose. Pooled samples of urine, feces and plasma were prepared for analysis by liquid scintillation counting, LC, NMR and MS.

Results

Plasma: Nine compound-related components were found in male rat plasma. Of these, 3 were each present at >10%.The significant metabolites were RS-91437 +RS-89983 (28% total), RS-88390 glucuronide (13%) and ranolazine (40%).

Six radiochemical components were found in female rat plasma of which 2 were considered significant. These were the glucuronide conjugate of RS-88390 (12%) and ranolazine (66%).

Urine: Eighteen compound –related components were reported for male urine samples. Three peaks were present at >10%: possibly RS-89289(20%), RS-91437(14%) and glucuronide conjugate of RS-88390(19%). Ranolazine was present at 5% of the urinary radioactivity.

Female urine contained 17 compound-related species. The greatest peaks were RS-91437(28%), RS-88390 glucuronide (21%) and ranolazine (23%).

Feces: Male feces contained 13 components with 3 significant: RS-88755(11%), RS-88597 (12%) and RS-88390 (29%). Ranolazine was present at 9%.

Female feces contained 25 peaks with 3 significant: RS-88250(17%), RS-88390 glucuronide (14%) and RS-88390 (20%). Ranolazine was present at 3% of total radioactivity in the fecal sample.

The study indicates sex-related differences in metabolism. The sponsor did not tabulate the relative abundance of the various metabolites in the different matrices but discussed selected metabolites in the text of the report. In the female rats, 5 hours post-dose, ranolazine accounted for ~2/3 of total circulating radioactivity compared to ~ 40% for male rats. Unchanged ranolazine was present in 0-24 hour female urine as ~23% of radioactivity compared to 5% in male urine. In male plasma, the major metabolite was RS-91347, with RS-89983 and the glucuronide of RS-88390 of approximately equal significance (to each other or to RS-91347 is not clear). In female rats, RS-91437 was the principal metabolite followed by RS-88390 glucuronide and RS-88597. RS-89289 was not identified in the female rat plasma.

The metabolism in both sexes appeared to be due predominantly to hydrolysis (RS-91437), O-demethylation (RS-88390) followed by glucuronidation and then N-dealkylation (RS-89983) which is further metabolised to RS-89289.

Effect of RS43285 on hepatic microsomal levels of cytochrome P-450 and protein in dogs following oral administration for six months. AT4031 March, 1988.

RS 43285 (lot 171SS0986), formulated with lactose and magnesium stearate in gelatin capsules was orally dosed to male and female Beagles ( ) at doses of 0, 5, 25 and 60 mg/kg/day for 6 months. After euthanasia, liver samples were collected from the control and 25 mg/kg animals. Microsomes were prepared and the microsomal protein content and CYP450 concentrations determined.
Results: There were no apparent differences in any of the parameters measured in either sex. The study would be more convincing if all dose groups had been examined or if at least the HD group had been examined also.

Appears This Way On Original

Effect of RS43285 on hepatic microsomal levels of cytochrome P-450 and protein in rats following oral administration for six months. AT4032, April 1988.

RS 43285-193(lot 153SS7085) was given orally to male and female Sprague-Dawley rats [redacted] at doses of 0, 5, 50 and 200 mg/kg, once a day for six months. After euthanasia, liver samples were collected from the control group and MD group. Microsomes were prepared and microsomal protein and cytochrome P-450 content determined.

Results: There was a 25% increase in microsomal CYP450 content in the males and a 26% decrease in the same parameter in females. The report would be more convincing if all dose groups had been sampled or at least the HD group had been included in the analysis.

The isolation and characterisation of the metabolites of ranolazine in mouse urine. AT6580, August 1989, Reported: February 1994

Six male mice in the three month oral dose ranging study were housed in metabolism cages and given a single oral dose of ^{14}C-ranolazine at 35 mg/kg. The urine was pooled by percentage volume (110 ml, 0-24 hours). Analysis of samples was by hplc and MS. Relative amounts of the metabolites were determined by liquid scintillation counting. For calculation purposes, the sponsor assumed that the total of the fractions isolated from mouse urine was equivalent to the mean percentage of the dose excreted in that sample:
[(radioactivity in isolated fraction)/(sum of radioactivity in all isolated fractions) x percentage of dose excreted in sample] = relative abundance

Standards used for metabolite identification were RS-88390, RS-88640, RS-89537, RS-89681, RS-89289, RS-89983, RS-89961, RS-88772, RS-88835, RS-88755, RS-88597, RS-89664, RS-88250, RS-91437, RS-89356, RS-89649 and RS-94287.

THE ABUNDANCE OF MAJOR RANOLAZINE METABOLITES ISOLATED FROM 0-24 h MOUSE URINE

Metabolite	Abundance in Urine % of dose	Fraction
RS-89983 Glucuronide	9%	Fr 2
RS-89289	25%	Fr 3*
Unidentified	4%	Fr 4
RS-94287	18%	Fr 5
RS-88390 Glucuronide	3%	Fr 6

Mean levels of 61.1% of the dose were excreted in urine in 0-24 h. Abundances are expressed as a percentage of the total radiochemical dose. All other components, including ranolazine, were present at less than 1% of the dose.

* multi-component fraction.

Results: An average of 61% of the radiochemical dose was eliminated in the urine in 24 hours, somewhat lower than the rat which averages ~90% in the first 24 hours. The major component identified in the urine was RS-89289. The sponsor's results are shown here.
Ranolazine was reportedly excreted as a minor component (<1% of dose, p 276).

It was proposed that the in vivo metabolism of ranolazine in the mouse was by one of two oxidative pathways: N-dealkylation or O-demethylation, sometimes followed by glucuronic acid conjugation. Hydroxylation did not appear to be a significant means of elimination of ranolazine in the mouse.

The metabolite profiles of ranolazine in rat, mouse, hamster and dog. AT6360, Conducted Feb.1987-Feb-1988. Reported: May 1993.

The report compares the metabolite profiles of ranolazine in various animal species as determined by hplc with radiochemical detection. Radioactive components were tentatively identified solely on the basis of chromatographic retention relative to ranolazine and using unlabelled standards of known molecular structure. The samples used for analysis were generated in studies reported elsewhere. The identification of those studies was:

DMAE 510/513 male and female rat plasma after single oral administration of ^{14}C-ranolazine.
Dose used 100mg/kg, n= 6 per sex

DMAE 517- male rat plasma after single oral administration of ^{14}C-ranolazine
Dose used 25 mg/kg, n=8

DMAE 467, 468, 472 male rat bile samples after single oral administration of ^{14}C-ranolazine
Dose used 5 mg/kg, n=4

The characterization of the dog metabolites was referenced to a report in preparation. The references for the rat studies are different than the designations that have been used in this NDA to identify the reports.

The standards used for identification of the metabolites were RS-89289, RS-89983, RS-88597, RS-88640, RS-94287, RS-88755, RS-88772, RS-88835, RS89664 and RS-88390.

Results: The sponsor notes that metabolite fractions do not necessarily represent discrete metabolite species but may represent heterogeneous mixtures of co-eluting components. The data is presented as representative chromatograms for the different species and matrices.

Reviewer's summary of textual description of results

Metabolites	20 minutes post-IV	6 hours post IV	Males		Females	
			1hr, po	6 hr, po	1hr,po	6hr,po
Ranolazine	61% total radioactivity		44%		58%	"similar"
RS89289	8		9%			
RS-89983	11	"almost solely"	27%	73%		
RS-88640	10		11%		36%??	"similar"
RS-88597						
RS-94287						
RS-88755						
RS-88772						
RS-88835						
RS-89664						
RS-88390						
RS-43285						

The sponsor states that there was no dependence on route of administration or dose in the observed metabolite profiles. There was insufficient data presented in the report to allow the reviewer to evaluate the statement. Ranolazine predominated at 1 hour post-dose as greatest percentage of total radioactivity (inconsistent with other reports that show a rapid disappearance of ranolazine). The major metabolites in males were the N-dealkylation products RS-89289, RS-88640 and RS-88640 (O-dealkylation). In female rats the sole entity other than ranolazine that was reported was RS-88640. The biliary results suggest " conjugated metabolite species."

Comparison of the in vitro metabolism of ranolazine in mouse, rat, dog and human liver microsomes. CVT303.005-MET, Jan-Feb 2002. Reported: July, 2002.

The sponsor states that both liver S9 and microsomes were used for the comparison of metabolism but only the microsomal data was reported. The rationale was that the data from the S9 and the microsomes was 'essentially similar."

Liver microsomes from male CD-1 mice, Sprague-Dawley rats, Beagles and humans were purchased from a commercial supplier. The protein content, total P450 concentrations and the activities of the major CYP450 isozymes were characterized by the vendor and not subsequently confirmed before use. Microsomal protein at presumably a final concentration of 0.20mg/l was used with ranolazine at a final concentration of 20 µM. A two minute preincubation was followed by the addition of NADPH to initiate the reaction. Incubation times were 5, 10,20, 30 and 60 minutes. LC/MS/MS analysis was used to assess disappearance of ranolazine and formation of metabolites. Quantification was by use of an internal standardization method. The sponsor defined "rate of formation" of metabolites as the maximal rate of appearance of individual metabolites between two subsequent time points.

Table 1 Comparison of Metabolites Formed in Liver Microsomes Prepared from CD-1 Mice, Sprague Dawley Rats, Beagle Dogs and Humans

Route/Metabolites	Maximal Rates of Metabolism of Ranolazine and Formation of Metabolites (pmole/min/mg microsomal protein) [a]			
	Mouse	Rat	Dog	Human
Total metabolism				
Ranolazine	894	753	491	589
N-dealkylation at the N4 piperazine nitrogen				
RS-94287	**145**[b]	**102**	**60.6**	**160**
CVT-2534	**65.8**	**78.6**	31.9	**99.5**
CVT-4786	10.5	4.43	7.46	**33.7**
N-dealkylation at the N1 piperazine nitrogen				
RS-88681	**441**	**150**	**81.7**	17.3
RS-89983	59.3	29.7	11.7	5.4
RS-89289	5.14	3.41	3.24	6.91
Hydroxylation at the dimethylphenyl ring				
RS-89961	**88.5**	30.0	21.5	17.1
RS-88772	12.6	3.71	7.58	8.67
RS-88835	48.6	23.2	**85.4**	7.00
Hydroxylation at the methoxyphenyl ring				
RS-88597	17.6	25.3	**128**	18.6
RS-89664	2.10	1.47	ND[c]	1.58
RS-89356	10.9	16.2	53.7	9.57
O-Demethylation				
RS-88390	19.1	**39.7**	50.6	**110**
O-Dearylation				
RS-88640	1.31	2.43	2.70	5.05
Amide bond cleavage				
RS-91347	2.04	2.14	1.47	1.09
Downstream metabolites				
Desmethyl RS-88681	6.35	28.4	0.794	1.17
RS-88755	2.00	13.2	0.447	0.886
RS-101647	2.04	1.53	0.307	0.882

[a] Values represent the overall metabolism (disappearance of ranolazine in incubate) and formation (appearance) of various metabolites.

[b] Values in bold represent the top five metabolites for each species.

[c] ND denotes not detected

Results: Except for RS-89664, not found in the dog microsomes, the 18 metabolites for which the sponsor analyzed were present in all 4 species. There were quantitative differences. These 18 Phase I metabolites are products of 11 metabolic routes. These 18 metabolites accounted for 86%, 90%, 92% and 79% of the total radioactivity in the mouse, rat, dog and human liver microsomes respectively.

For most of the primary metabolites, maximal rates of formation occurred between 0-5 minutes. Some metabolites exhibited higher rates of formation at later time points. One may ask whether this was real or an artifact of in vitro methods (e.g. such as the degree and uniformity of mixing).

Summary: Overall, there was a qualitative similarity between the in vitro results obtained between the 4 species examined in this study. A weakness of the study is that: 1) the sponsor did not confirm the total protein and microsomal information of the purchased materials. 2) the results presented contained no error bars on the graphs. The tabular results did not contain a ±SD. Were replicate samples processed or were results based upon N=1 per time point per species?

The isolation and characterisation of the metabolites of ranolazine in the dog. AT6377, Conducted January 1985-November 1992. Reported: May 1993.
The metabolism of ranolazine in the dog was investigated in vitro using S9 liver preparations and in vivo using urine and bile samples from dogs dosed orally with ranolazine 60 mg/kg. Metabolites were isolated by solid and liquid phase extraction followed by semi-preparative hplc. Mass spec methods were used on the radiolabeled fractions. Liquid scintillation counting was used for detection of radioactivity. Standards used for identification were RS-89289, RS-89983, RS-94287, RS-88640, RS-88250, RS-88755, RS-88835, RS-88772, RS-89961, RS-88681, RS-89537, RS-88597, RS-89664, RS-89649, RS-89356, RS-91347 and RS-88390.

Results: The sponsor's results are summarized in the table below:
Reviewer's condensation of sponsor's table summarizing abundance of major metabolites in dog urine/bile (pp. 178-179)

metabolite	Abundance in urine	Abundance in bile
RS-94287	3%	
RS-88640	6%	
RS-88597	1%	
RS-88835/88772 glucuronide	2%	
RS-88597 glucuronide*,**		
RS-88597 glucuronide*	1%	
RS-88390 conjugate	2%	
RS-88597 glucuronide	3%	
RS-88835/88772 conjugate	4%	
RS-88597 conjugate		
Ranolazine glucuronide	3%	
RS-88835/88772 sulphate		3%

* RS-88597 has the potential to form two glucuronide conjugates differing only in the position of conjugation. **The sponsor speculated that this peak may have been due to cross contamination from another peak.

The in vitro liver assay was textually described as producing 9 fractions, 4 of which were characterised against standards. The named metabolites, RS-94287, RS-88597, RS-88835/RS88772 and RS-88390 were reported to have been identified in rat in vitro studies.

Previous studies have indicated that approximately 30-32% of administered ranolazine is excreted in the urine and the remainder is excreted via the feces.

The identification of the metabolites of ranolazine in Baboon plasma AT6812,Conducted Oct.1993-Dec 1993. Reported: November 1994.

This report does not specify the number, age, sex or origin of the baboons used nor the rationale for the use of baboons. The in life methods were described as "sample origin". The present report states that "Full details of animals, dose preparation and administration and of samples collected are contained in the report pertaining to that study.[1]" The footnote was found to be listed as "Alps et al., (1991) SRS report no. SS/082/91. Reduction of myocardial enzyme release by RS-43285 (ranolazine) in a subhuman primate model of ischemia with reperfusion: post-reperfusion treatment with RS-43285-193 (racemate) and its enantiomers RS-43285-197 (S-isomer) and RS-43285-198 (R-isomer). The introduction states that the baboons had either undergone a 12 hour continuous iv infusion with either RS-43285-197 or RS-43285-198 at a target dose level of 50 μg/kg/hr following a loading dose of 500 μg/kg. Blood samples were "removed" 12 hours post-infusion and one sample analysed per animal as part of the metabolite characterisation.

Analysis of the one sample collected per animal was analysed by HPLC, MS and LC/MS. Control plasma samples were spiked with known amounts of standards for semi-quantitation of the metabolites found.

The protocol also states that liver microsomal and in vitro hepatocyte metabolism experiments will also be included in this study, possibly using ^{14}C-ranolazine. The protocol also states that blood samples will be taken at sequential times or at a single timepoint. Urine, feces and bile were also supposed to be collected. There appears to be some inconsistency between the written protocol and the data reported.

Results
Based upon the presentation of results, it appears that the baboons received either the racemic mix or one of the enantiomers. The baboon receiving the R-enantiomer showed at least 22 possibly drug-related components in the LC-MS analysis. Shoulders on a variety of peaks indicated the potential for other metabolites. Ten metabolites were positively identified. The analyses of plasma extracts from 3 baboons dosed with the S-isomer showed a similar wide range of metabolites. There were no significant qualitative differences apparent although there was some variation in absolute and relative peak intensities (p. 20).

The major plasma metabolite was RS-94287 (up to 29% of parent compound). Four other compounds at concentrations >10% of parent compound concentration were RS-88390 (16%), RS-89983(up to 13%), RS-88640(up to 12%) and RS-89961 (up to 11%).

The interanimal inconsistency of finding RS-89289 and RS-91347 was addressed by the sponsor as possibly due to the analytical method being suboptimal for these particular compounds. Given the uncertainty of the methodology, one may summarize this study by saying that single timepoint plasma samples from baboons given intravenous ranolazine show evidence of extensive metabolism. Both Phase I and Phase II metabolism are suggested. One may ask

whether a single sample taken 12 hours after the end of an intravenous infusion was the optimum methodology for examining the metabolic profile.

Excretion:

The disposition of ranolazine following oral administration of 50 mg.kg^{-1} [^{14}C]-ranolazine in the mouse CVT303.009-R May 17, 2002

A single oral dose of 50 mg/kg [^{14}C]-ranolazine (specific activity 925MBq/mmol) was given to 6 male CD-1 mice housed singly in metabolism cages. Urine was collected at 0-6, 6-24, 24-48, 48-72, 72-96 and 96-120 hours post-dose. Feces was collected 0-24, 24-48, 48-72, 72-96 and 96-120 hours post-dose. Twenty-four male CD-1 mice received single oral doses (as described above) for plasma level determination of drug. Terminal blood samples were collected at 0.5, 1, 2, 4, 6, 8, 24 and 48 hours post-dose. Levels of total radioactivity were determined for the plasma. RBCs were discarded. Liquid scintillation counting was used to quantify the radioactivity.

Results: Following oral administration the total radioactivity showed a peak mean plasma level at the first sampling time of 0.5 hours. The concentration of total radioactivity decreased to ~60% of peak levels by 1 hour. Total plasma radioactivity continued to fall as summarized in the reviewer's table below.

Reviewer's summary of total plasma radioactivity for mice given a single oral dose of [^{14}C]-ranolazine

Mean plasma total radioactivity (µg base equiv.ml^{-1})	Post-dose timepoint			
	0.5hours	1hour	24hours	48hours
	24.461	15.042	0.239	0.077

From vol 43, p.117

Approximately 30% of the total radioactivity was excreted in the urine in the first six hours. Urinary excretion accounted for 48.4±3.7% of the administered dose (drug or radioactivity not specified) and feces accounted for 45.8±2.7% over the collection period. Including gastrointestinal contents and carcass, 94% (range 91 - 97%) of the administered dose was recovered.

Summary: Peak plasma levels of radioactivity were achieved by the first sampling point of 0.5 hour. Excretion was approximately equally divided between urine (if cage wash was included) and feces and was essentially complete by 120 hours post-dose.

An investigation of the pharmacokinetics of ranolazine in the male rat following oral dosing for 7 days. AT6152, August, 1992.

Thirty male Sprague-Dawley rats were orally dosed at 50 mg/kg/day and blood samples from 2 rats per timepoint were collected day 7 at 10 min, 20 min, 30 min, 40 min, 1 hr, 1.5 hr, 2, 2.5, 3, 4, 6, 8, 12 and 24 hours. Plasma drug levels were determined by HPLC. This is a rare study where a calibration range ([illegible] ng) for ranolazine was mentioned.

The sponsor states that the purpose of the current study was to determine pk data after once daily dosing to steady state.

Sponsor's summary of PK parameters in male rats after 7 days of dosing.

Parameter	Male rat value
Cmax (ng/ml)	4130
Tmax(hour)	0.50
AUC_{0-24}(ng.h/ml)	18300
Clpo (ml/min/kg)	49.9

The sponsor does not present data to support that these are steady state values.
The sponsor cites AUC_{0-24} values for doses of 240, 320 and 400 mg given t.i.d to steady state (1992 SS/022/92 Safety and tolerability of multiple oral doses of ranolazine in healthy young male volunteers). This data was used to calculate the dose exaggeration in the animals and is summarized in the reviewer's table.

Reviewer's Summary of Relative Dose Exposure

Human dose (mg, tid)	Human AUC_{0-24}	Rat multiple of the human exposure
240	13200	1.4
320	19100	0.96
400	23100	0.79
1000	33700	0.54

The pharmacokinetics of ranolazine in male and female rats following single intravenous and single oral administration. AT6154,Conducted October 1987-March 1988. Reported: August 1992

Thirty male and 30 female rats were dosed intravenously at 25 mg/kg and 24 male and 24 female rats were dosed orally at 100 mg/kg. All doses were given as aqueous solutions (5%dextrose for iv, water for oral). Blood samples were taken from 3 animals/sex/timepoint at 5 min, 10 min, 20 min, 40 min, 1 hour, 2,3,4,6, and 8 hours after intravenous dosing and at 20 minutes, 40 minutes, 1 hour, 2,3,4,6 and 8 hours after oral dosing.

Results: Sex-related differences were apparent in the clearance values and subsequent exposure as shown by AUC. The females showed lower clearance after IV dosing but had lower AUC values. The sponsor's values are shown below.

Appears This Way
On Original

PHARMACOKINETIC PARAMETERS OF RANOLAZINE FOLLOWING SINGLE INTRAVENOUS AND SINGLE ORAL ADMINISTRATION TO RAT

INTRAVENOUS			ORAL		
Parameters	Male	Female	Parameters	Male	Female
C_0 (ng/ml)	16700	25200	C_{max} (ng/ml)	10800	19500
			t_{max} (h)	0.333	0.667
AUC_{0-inf} (ng.h/ml)	10700	30100	AUC_{0-inf} (ng.h/ml)	30400	76500
$t_{1/2}$ (h)	1.05	2.42	$t_{1/2}$ (h)	6.20	3.65
Systemic Cl (ml/min/kg)	38.9	13.8	Oral Cl (ml/min/kg)	54.8	21.8
Vd (L/kg)	3.54	2.89			
			ioavailability (%)	71.0	63.3

Appears This Way On Original

A study to investigate the pharmacokinetics of ranolazine in male and female rats following once daily oral dosing at 2,5, 50 and 150 mg/kg for 6 months. AT6811,Conducted Feb 1992. Reported: November 1994

Sixty-five male and 65 female Sprague-Dawley rats — CD(SD)BR, [illegible] were dosed once a day by oral gavage for 6 months at the above-mentioned doses. Doses were based on daily weight for the first 10 weeks of the study and weekly weight thereafter. There were a number of rats dosed by oral gavage only on day 1, and at 1, 3 and 6 months, equivalent to 4 single oral doses. Blood samples were taken on day 1, and at 1,3 and 6 months at 0.5, 1.5, 3,6 and 24 hours after dosing. It is not clear from the report how many animals were sampled per time point. The summary indicates that blood was collected from 2 rats per time point. Plasma levels were determined by hplc methodology. The LOQ for the assay was [illegible] ng/ml.

Results: For both sexes, at all doses (both the multiple and single dose protocols), Cmax and AUC $_{0\text{-}24}$ increased over time. The only exceptions to this were the AUC values for the single dose males. The increases in AUC with increasing dose were greater than proportional in most cases. Results are summarized in the sponsor's table below. The apparent increase in exposure in the multiple dose rats may be due to accumulation, an age-related phenomenon or due to variability resulting from the small sample size.

Table 7
RANOLAZINE C_{max} AND AUC VALUES FOLLOWING MULTIPLE ORAL ADMINISTRATION AT 2, 5, 50 AND 150 mg/kg/day FOR SIX MONTHS AND SINGLE ORAL ADMINISTRATION AT 5 and 150 mg/kg ON DAYS 1, 32, 92 AND 183

Parameter	Dose	Females				Males			
		Day 1	Day 32	Day 92	Day 183	Day 1	Day 32	Day 92	Day 183
C_{max} (ng/ml) Multiple Dose	2 mg/kg/day	197	380	420	403	90.6	160	284	139
	5 mg/kg/day	548	974	983	1720	438	454	953	690
	50 mg/kg/day	4380	4960	5450	7020	2740	3920	4140	5160
	150 mg/kg/day	10000	13800	21200	15600	9840	13200	12200	19200
C_{max} (ng/ml) Single Dose	5 mg/kg	857	777	1230	1750	344	402	247	361
	150 mg/kg	15600	16700	11200	21200	14200	7980	8460	17000
$AUC_{0\text{-}24h}$ (ng.h/ml) Multiple Dose	2 mg/kg/day	464	840	943	1020	165	238	339	227
	5 mg/kg/day	1480	2220	2300	5000	660	936	1530	1260
	50 mg/kg/day	29000	36400	38900	50300	7210	12200	14100	22800
	150 mg/kg/day	99800	169000	126000	167000	59500	118000	63600	117000
$AUC_{0\text{-}\infty}$* (ng.h/ml) Single Dose	5 mg/kg	1360	1610	1830	3600	617	734	454	736
	150 mg/kg	136000	161000	114000	197000	77900	76700	54900	89100

* $AUC_{0\text{-}\infty}$ = $AUC_{0\text{-}24h}$ at 5 mg/kg and $AUC_{0\text{-}24h}$ at 150 mg/kg

Metabolic profiles of ranolazine following oral administration of a single 50 mg/kg dose of [^{14}C]ranolazine to male albino rats.CVT303.003-MET Amendment August, 2002

The stated reason for the amendment is to add metabolites that were either not detected or were below the quantification limit of the assay in the summary tables and footnotes.
Male Sprague-Dawley rats were given single oral doses of 50 mg/kg of [^{14}C]-ranolazine. Concentrations of total radioactivity in plasma and recoveries of the radioactive dose in urine and feces up to 5 days were determined. Details of the timepoints of collection of samples were not provided. Samples of plasma and urine were subjected to LC/MS/MS quantification for 18 metabolites. The details of the samples analyzed (time points) were not provided. These samples were also hydrolyzed with β-glucuronidase/sulfatase for estimation of conjugated metabolites.

Results: Peak concentrations of ranolazine and metabolites were reported to be reached within 1 hour. Peak concentrations for total radioactivity and ranolazine were reached 0.5 hour after dosing (p.257, vol 40). At Cmax, ranolazine accounted for 25% of total radioactivity in plasma, decreasing to 1% at 8 hours post-dose. Based on AUC_{0-24} ranolazine accounted for 13% of total radioactivity in plasma.

At least 40 metabolites were found in plasma and more than 80 were found in the urine. Of the plasma metabolites, 20 had AUC values >1% that of ranolazine. Approximately 9 had AUC values >10% that of ranolazine: RS-89983(113%), RS-91347 (46.3%), CVT-4786 (31.6%), RS-89289 (18.8%), RS-94287(15.3%), RS-89961(13.6%), RS-88390(16.3%) and ranolazine glucuronide conjugates(17.7%) and RS-88597 unspecified conjugate(10.6%) had AUC values > 10% that of ranolazine (p.258, vol 40).

Following oral administration an average of 39% and 53% of the radioactive dose was recovered in 5 days in urine and feces respectively. The 0-24 hour urine pool contained 96% of the radioactivity recovered in the urine. This pool was used for the metabolic profiling (a point mentioned in the results, not the methods section).

Ranolazine and the metabolites quantified accounted for 78.5% of the total radioactivity in urine. Of that , only 4% of the dose was recovered in urine as unchanged parent compound. The metabolites not quantified and unknown metabolites combined to account for the remaining 21.5% of total radioactivity in the urine.

CVT-4786 was listed as a major new metabolite identified in plasma and urine in this study. This was also mentioned in a similar dog metabolism study. This metabolite accounted for 9.4% of total radioactivity in urine.
Two N-oxides at the N-1 and N-4 piperazine positions were noted as identified for the first time in rats also in this study.

Concentrations of Total Radioactivity, Ranolazine and Metabolites in Pooled Plasma Following Oral Administration of a Single 50-mg/kg Dose of [^{14}C]-Ranolazine to Male Rats

(Concentrations of Ranolazine and Metabolites Determined by ( ) Method)

Time Point	Concentration in Plasma (µg Ranolazine Equivalents/mL for Total Radioactivity or µg/mL for Ranolazine and Metabolites)													
(hr)	Total ^{14}C	Ranolazine (% of Total ^{14}C)		RS-88390	RS-88640	RS-88681	RS-94287	RS-89983	CVT-2534	CVT-4786	Des-methyl RS-88681	RS-88755	RS-101647	RS-91347
0	BQL	BQL	-	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
0.5	20.8	5.23	(25.1)	0.172	0.043	0.269	0.730	1.42	0.437	1.64	0.474	0.072	0.070	1.88
1	17.9	3.01	(16.8)	0.107	0.040	0.208	0.631	1.69	0.317	1.38	0.364	0.069	0.071	1.48
2	11.9	2.21	(18.5)	0.097	0.034	0.084	0.328	1.41	0.127	0.505	0.158	0.047	0.032	1.13
4	6.04	1.29	(21.4)	0.063	0.020	0.027	0.167	0.853	0.045	0.275	0.049	0.042	0.012	0.666
6	3.37	0.112	(3.3)	0.010	BQL	BQL	0.030	0.729	0.010	0.084	0.012	0.010	BQL	0.064
8	2.33	0.027	(1.2)	BQL	BQL	BQL	BQL	0.511	BQL	0.025	BQL	BQL	BQL	BQL
24	0.582	BQL	(0.0)	BQL	BQL	BQL	BQL	0.102	BQL	BQL	BQL	BQL	BQL	BQL
72	0.433	BQL	(0.0)	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
120	0.069	NA		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pharmacokinetic Parameter														
Cmax (µg/mL or µgEquiv/mL)	20.8	5.23		0.172	0.043	0.269	0.730	1.69	0.437	1.64	0.474	0.072	0.071	1.88
% Cmax for Ranolazine		100		3.29	0.82	5.14	13.9	32.3	8.35	31.4	9.06	1.38	1.36	36.0
Tmax (hr)	0.5	0.5		0.5	0.5	0.5	0.5	1.0	0.5	0.5	0.5	0.5	1.0	0.5
AUC$_{(0\text{-}t\ hr)}$	62.9	11.0		0.45	0.12	0.44	1.69	7.77	0.75	3.36	0.86	0.25	0.15	5.14
AUC$_{(0\text{-}24\ hr)}$	86.2	11.2	(13.0)	0.46	0.14	0.47	1.72	12.7	0.76	3.56	0.87	0.26	0.16	5.20
T1/2 (hr)*	31.2	0.7		3.8	2.9	1.0	1.2	6.5	1.1	1.2	1.1	1.8	1.2	1.0
AUC$_{(0-\infty)}$	126	11.0		0.50	0.21	0.48	1.74	13.6	0.76	3.40	0.88	0.36	0.15	6.06
% AUC$_{(0\text{-}24\ hr)}$ for Ranolazine		100		4.07	1.27	4.19	15.3	113	6.73	31.6	7.71	2.2	1.43	46.3

Units for AUC are µg *hr/mL or µgEquiv *hr/mL
BQL: Below the quantification limit of the assay
NA: Not analyzed.
*Half-life estimated from last three time points that have measurable levels.

Appears This Way On Original

The sponsor states the major pathways of metabolism in the rat as N-dealkylation at both nitrogens of piperazine, amide hydrolysis and O-demethylation of the methoxy group at the methoxyphenyl ring.

Determination of routes and rate of excretion and metabolic profiles of [^{14}C]-ranolazine following oral administration to Beagle dogsCVT303.007-R November16, 2000

Group I consisted of 4 male Beagles and Group II consisted of 3 bile-duct cannulated male Beagles. Dogs were drug-free for at least 3 weeks prior to start of the study. Each dog received a single oral dose of 25 mg/kg aqueous [^{14}C]-ranolazine (~6.25µCi/kg). Blood for determination of plasma radioactivity was collected from all animals pre-treatment, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 hours post-dose.
Urine was collected: 0-4, 4-8,8-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168. Feces were collected pre-dose, 0-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hours post-dose. Bile was collected from Group II animals pre-dose, 0-1,1-2, 2-4, 4-6,6-8, 8-10, 10-24, 24-48, 48-72, 72-96, 96-120 hours post dose.
Analysis was by tissue combustion and liquid scintillation counting.

Results: All animals appeared lethargic at 4 hours post-dose but returned to normal appearance by 6 hours. One dog continued to be lethargic at 8 hours after dosing. Maximum concentrations of plasma radioactivity were reported to occur for individuals of both groups from 0.5 – 1.0 hour after dosing. Maximum mean concentrations were observed at 0.5 hours. Mean concentrations decreased to non-detectable levels 4 hours post-dose for Group II and 12 hours post-dose for Group I (vol 43, p.26).

Urinary excretion of radioactivity through 168 hours was 49.29±4.47% of administered dose for Group I animals and 30.28±3.6% for the bile duct cannulated dogs. The majority of radioactivity was excreted within the first 48 hours post-dose for both groups.

Fecal excretion of radioactivity was 42.84±2.13% for the intact dogs and 3.61±0.90% for the cannulated dogs. The majority of excretion was in the first 48 hours. The total amount of radioactivity excreted in the bile (group II) through 120 hours was 59.89±2.70% of the administered dose of radioactivity. Approximately 50% of the administered dose was excreted via the bile within the first 6 hours.
Summary: Consistent with the mouse results, the absorption following oral dosing was rapid with rapid excretion via the urine and bile/feces. Approximately 90% of the dose was excreted within 48 hours for both groups. Greater than 95% was recovered in excreta within 168 hours. Urinary excretion reached 49% in intact animals compared to 30% for the bile duct cannulated animals. This suggests that re-absorbed biliary material contributed to systemic levels in the intact animals. The onset of signs (lethargy) at 4 hours, after the Cmax, may be due either to an active metabolite, delayed distribution of drug to a specific target site or secondary to some pharmacologic process that requires several hours for full effect. See the next report for characterization of metabolites.

Appears This Way On Original

Metabolic profiles of ranolazine following oral administration of a single 25 mg/kg dose of [^{14}C]-ranolazine to Beagle dogs.CVT303.002-MET. Amendment to CVT303.007-R August 2002.

The stated purpose of the amendment was to add metabolites that were either not detected or were at concentration that were below the quantification limit of the assay (BQL) in the summary table and footnotes of table 4-21. The various biological matrices that were collected following single oral doses to intact or bile duct cannulated dogs were assessed for metabolites by LC/MS/MS and radiochromatography. Concentrations of 18 metabolites in plasma, urine and bile were quantified by LC/MS/MS. The samples were also subjected to β-glucuronidase/sulfatase hydrolysis to estimate levels of conjugated metabolites.

Results: in the seven days of the study, approximately 49% and 43% of the administered radioactivity was recovered in the urine and feces respectively. At Tmax (0.5 hours), unchanged ranolazine accounted for 44% of the total radioactivity in plasma and decreased to 12% at 8 hours post-dose. Values of plasma AUC_{0-24} of ranolazine accounted for an average of 26% of that of total radioactivity in plasma (p.20, vol 40).

At least 80 metabolites were found in plasma and over 100 were detected in urine. Due to the large numbers of metabolites, complete chromatographic resolution could not be achieved. Of the metabolites that were quantified in plasma, 12 phase I metabolites showed AUC values ≥ 1% of that of ranolazine. The sponsor's findings are shown here.

Metabolites of Ranolazine	Plasma		Urine		Bile
	$AUC_{(0\text{-}24\ hr)}$ (μg .hr/mL) (%Ranolazine AUC)[a]		% Dose		% Dose
Phase I Metabolites	Intact Dogs (n=4)	BDC Dogs (n=3)	Intact Dogs (n=4)	BDC Dogs (n=3)	BDC Dogs (n=3)
Ranolazine (RS-43285, CVT-303)	13.5 (100)	21.1 (100)	2.38	3.55	0.07
RS-88597 (CVT-5030)	6.28 (46.5)	2.30 (10.9)	5.47	2.03	0.00
RS-88640 (CVT-2512)	2.78 (20.6)	0.51 (2.4)	3.91	0.65	0.37
RS-94287 (CVT-2738, Ran 2)	2.79 (20.6)	2.88 (13.6)	2.64	3.04	0.00
CVT-4786	1.92 (14.2)	1.84 (8.7)	3.12	2.69	0.00
RS-89961 (CVT-2551)	1.70 (12.6)	1.80 (8.5)	1.04	0.99	0.02
RS-88681 (CVT-2513)	1.52 (11.2)	1.59 (7.5)	1.52	1.77	0.00
RS-88835 (CVT-5028)	1.00 (7.4)	0.32 (1.5)	0.16	0.00	0.00
RS-88390 (CVT-2514)	0.97 (7.2)	1.00 (4.7)	0.12	0.11	0.00
RS-89289 (CVT-2537)	0.79 (5.8)	0.70 (3.3)	1.83	1.65	0.00
RS-89356 (CVT-5031)	0.60 (4.4)	0.41 (1.9)	0.34	0.19	0.00
RS-88772 (CVT-3388)	0.22 (1.7)	0.16 (0.8)	0.57	0.27	0.00
CVT-2534	0.14 (1.0)	0.20 (0.9)	0.00	0.00	0.00
RS-89983 (CVT-2535)	0.12 (0.9)	0.16 (0.7)	0.00	0.00	0.00
RS-101647 (CVT-3248)	0.0 (0.0)	0.0 (0.0)	0.10	0.10	0.00

Appears This Way On Original

Some 10 metabolites had AUC values > 10% that of ranolazine. The sponsor describes these textually as:

Reveiwer's summary of sponsor's textual description of plasma metabolites AUC as % of ranolazine

metabolite	RS-88597	RS-94287	RS-88640	CVT-4786	RS-89961	RS-88681
AUC rel to ranolazine	46.5	20.6	20.6	14.2	12.6	11.2
metabolite	RS88390	RS-88835	RS-89289			
AUC rel to ranolazine	7.2	7.4	5.8			

Data from vol 40., p.20

Pharmacokinetics of Total Radioactivity, Ranolazine, and Metabolites in Intact Dogs Following a Single 25-mg/kg Oral Dose of [^{14}C]-Ranolazine

(Primedica Study BCAZ-101)

(Mean ± SD, N = 4)

Analyte ID	Cmax (μgEquiv/mL or μg/mL)	Tmax (hr)	$AUC_{(0\text{-}24\,hr)}$ (μgEquiv·hr/mL or μg·hr/mL) (% AUC for Ranolazine)	$AUC_{(0\text{-}last)}$ (μgEquiv·hr/mL or μg·hr/mL) (% AUC for Ranolazine)	$T_{1/2}$ (hr)
Phase I Metabolites					
Total ^{14}C	16.9 ± 0.89	0.75 ± 0.29	51.2 ± 11.0	51.3 ± 9.73	2.77 ± 0.58
Ranolazine	6.96 ± 1.69	0.63 ± 0.25	13.5 ± 4.91 (100)	13.6 ± 4.99 (100)	4.92 ± 1.54
RS-88597	1.25 ± 0.13	0.75 ± 0.29	6.28 ± 1.58 (46.5)	7.05 ± 2.38 (51.7)	7.49 ± 2.71
RS-94287	0.310 ± 0.170	0.63 ± 0.25	2.79 ± 1.48 (20.6)	3.02 ± 1.43 (22.2)	6.69 ± 2.47
RS-88640	0.179 ± 0.059	1.25 ± 0.50	2.78 ± 0.67 (20.6)	3.90 ± 0.72 (28.6)	13.9 ± 7.02
CVT-3786	1.04 ± 0.63	0.63 ± 0.25	1.92 ± 0.93 (14.2)	1.97 ± 0.87 (14.4)	3.48 ± 1.87
RS-89961	0.363 ± 0.044	0.75 ± 0.29	1.70 ± 0.20 (12.6)	1.70 ± 0.22 (12.4)	3.65 ± 0.97
RS-88681	0.263 ± 0.113	0.63 ± 0.25	1.52 ± 0.70 (11.2)	1.57 ± 0.69 (11.5)	5.25 ± 0.75
RS-88390	0.280 ± 0.035	0.75 ± 0.29	0.97 ± 0.24 (7.2)	1.00 ± 0.30 (7.3)	4.49 ± 1.72
RS-88835	0.324 ± 0.034	0.63 ± 0.25	1.00 ± 0.14 (7.4)	1.17 ± 0.21 (8.6)	9.41 ± 3.74
RS-89289	0.419 ± 0.254	0.63 ± 0.25	0.79 ± 0.47 (5.8)	0.82 ± 0.47 (6.0)	3.69 ± 2.33
RS-89356	0.245 ± 0.039	0.63 ± 0.25	0.60 ± 0.17 (4.4)	0.63 ± 0.18 (4.6)	4.87 ± 2.19
RS-88772	0.074 ± 0.011	0.75 ± 0.29	0.22 ± 0.05 (1.7)	0.25 ± 0.05 (1.8)	2.64 ± 0.80
CVT-2534	0.101 ± 0.074	0.63 ± 0.25	0.14 ± 0.10 (1.0)	0.15 ± 0.10 (1.1)	0.69 ± 0.18
RS-89983	0.087 ± 0.049	0.63 ± 0.25	0.12 ± 0.07 (0.9)	0.12 ± 0.07 (0.9)	0.73 ± 0.14
Phase II Metabolites[a]					
RS-88597 Conjugate[b]	1.29 ± 0.24	1.50 ± 0.58	8.70 ± 2.32 (64.4)	10.1 ± 2.99 (73.7)	8.53 ± 2.51
Ranolazine Glucuronide	2.65 ± 1.00	1.00 ± 0.00	8.13 ± 2.97 (60.1)	8.11 ± 2.92 (59.4)	3.78 ± 0.83
RS-88835 Conjugate	1.04 ± 0.289	0.75 ± 0.29	4.96 ± 1.50 (36.7)	5.99 ± 1.87 (43.9)	10.0 ± 4.1
RS-89356 Conjugate	0.435 ± 0.095	1.25 ± 0.50	2.81 ± 0.77 (20.8)	3.19 ± 0.86 (23.4)	7.81 ± 2.72
RS-88390 Conjugate	0.272 ± 0.073	1.13 ± 0.63	2.16 ± 0.39 (16.0)	2.72 ± 0.73 (19.9)	10.4 ± 6.8
RS-88640 Conjugate	0.076 ± 0.025	1.75 ± 0.50	0.46 ± 0.18 (3.4)	0.63 ± 0.39 (4.6)	9.93 ± 10.9
RS-89983 Conjugate	0.059 ± 0.023	1.33 ± 0.58 (n=3)	0.15 ± 0.11 (1.1)(n=3)	1.52 (n=2)	

[a] Conjugates of RS-89664, RS-89961, RS-88772, CVT-2534, desmethyl RS-88681, and RS-88755 were either not detected or levels were BQL.

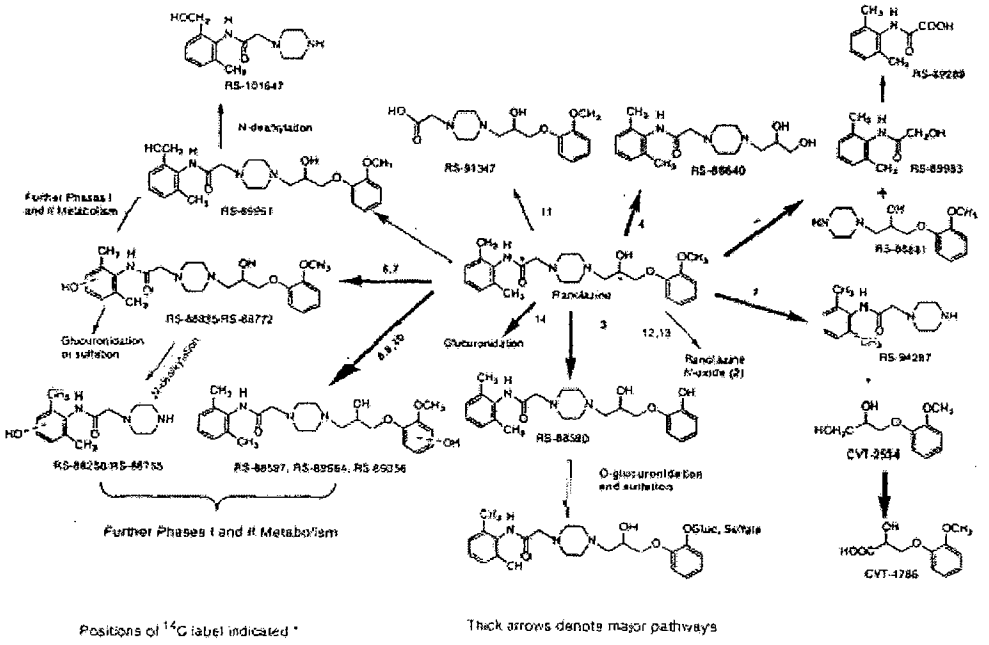
[b] Concentrations of conjugates were estimated from the differences before and after β-glucuronidase hydrolysis.

The hydrolying enzyme used contained both β-glucuronidase and sulfatase.

Appears This Way On Original

Metabolites of Ranolazine	Plasma		Urine		Bile
	$AUC_{(0\text{-}24\ hr)}$ (μg .hr/mL) (%Ranolazine AUC)[a]		% Dose		% Dose
Phase II Metabolite[b]	Intact Dogs (n=4)	BDC Dogs[c] (n=3)	Intact Dogs (n=4)	BDC Dogs (n=3)	BDC Dogs (n=3)
RS-88597 (CVT-5030) Conjugate	8.70 (64.4)	3.05 (14.4)	4.01	1.48	6.29
Ranolazine Glucuronide	8.13 (60.1)	6.12 (29.0)	4.32	3.71	33.41
RS-88835 (CVT-5028) Conjugate	4.96 (36.7)	1.16 (5.5)	1.62	0.22	4.67
RS-89356 (CVT-5031) Conjugate	2.81 (20.8)	0.79 (3.7)	2.75	1.04	5.03
RS-88390 (CVT-2514) Conjugate	2.16 (16.0)	1.02 (0.48)	0.90	0.35	3.48
RS-88640 (CVT-2512) Glucuronide	0.46 (3.4)	0.47 (2.2)	0.57	0.20	0.48
RS-89983 (CVT-2535) Conjugate	0.15 (1.1)	0.00 (0.0)	0.15	0.11	0.00
RS-89961 (CVT-2551) Conjugate	0.00 (0.0)	0.00 (0.0)	0.33	0.27	0.57
RS-88772 (CVT-3388) Conjugate	0.00 (0.0)	0.00 (0.0)	0.10	0.09	0.15
CVT-2534 Conjugate	0.00 (0.0)	0.00 (0.0)	0.28	0.24	0.00

[a]Values represent % AUC of ranolazine based on μg.hr/mL. Ranolazine plasma AUC was set at 100%.
[b]Units for conjugates are expressed as μg of the corresponding Phase I metabolite equivalent.hr/mL..
The following metabolites were either not detected or at levels that were below the quantification limit of the assay: RS-91347 (CVT-3369) and conjugates of RS-89664 (CVT-5029), desmethyl RS-88681 (CVT-3247) and RS-88755 (CVT-3389).



The sponsor reports that the metabolic profile of ranolazine in dogs was qualitatively similar to humans. Quantitative differences were noted. For example, direct glucuronidation of ranolazine and hydroxylation of the methoxyphenyl and dimethylphenyl rings were major routes of metabolism in the dog and minor in humans. O-demethylation followed by sulfation and glucuronidation were major in humans but minor in dogs.

A major new metabolite identified in the current study was CVT4786, the carboxylic acid derivative of CVT-2534. Other newly identified metabolites were two N-oxides at the N1 and N4 piperazine positions.
Other metabolites that the sponsor felt worthy of special mention were:
N-dealkylation (at the N1 position of piperazine ring):
RS-88681 and RS-89983 (further oxidized to RS-89289)
O-dearylation- RS-88640
O-demethylation- RS-88390
Amide hydrolysis- RS-91347 and

Glucuronidation, N-dealkylation, O-demethylation and hydroxylation appear to be main metabolic routes in the dog.

The sponsor reports that there were no metabolites unique to plasma. However, endogenous compounds may mask the identification of products of drug metabolism. The reviewer is unaware of any studies examining the presence of interfering endogenous substances for any of the preclinical species.

Excretion studies in mice in support of a three month carcinogenicity dose ranging study with ranolazine (RS-43285 VHT(2)) AT4945, October 1989
Satellite groups of mice were used for this study. Male and female mice in the satellite groups received the LD (5 mg/kg/day) and HD (35 mg/kg/day) from the 3 month study. A second satellite group was used for a single dose excretion study. These male mice received a single oral administration of 5 mg/kg ^{14}C-ranolazine (labelled on the carbonyl carbon of the acetamide group). Urine and feces were collected daily. Metabolism cages were washed at the end of the observation period to account for all possible radioactivity. Radioactivity was analyzed by liquid scintillation counting.

Results: There were essentially no differences between the single dose results and those who received multiple doses. The results are summarized in the reviewer's table below.

Reviewer's Summary of Mean Excretion of Radioactivity (p. 288)

Parameter	5 mg/kg ^{14}C-ranolazine		
	Single dose males (n=6)	Multi-dose males (n=6)	Multi-dose females (n=6)
Urinary excretion	51±4	55±7	54±7
Fecal excretion	41±4	39±6	40±9
Total excretion	91±6	94±3	95±6
	35 mg/kg ^{14}C-ranolazine		
Urinary excretion		61±7	60±5
Fecal excretion	Not reported	35±6	36±4
Total excretion	Not reported	96±2	96±2

Values are mean % dose recovered ±SD

Approximately 90% of dosed radioactivity was recovered within 24 hours.
There was minimal recovery of radioactivity from the carcass and GI contents under all conditions. A slight increase in urinary pH was reported for the multiple dose animals of both sexes compared to the single dose animals where there was no reported change in urinary pH. The LD animals went from pH 7.1-pH 8.0. The HD mice went from pH 7.1 to pH 7.9. The significance of this is not clear.

Plasma level and excretion studies in mouse following multiple oral administration of ranolazine in support of the two year carcinogenicity study (RS-43285 VMT) AT6153, January 1989-January 1991. Report: July 1992.

A satellite group of thirty male and 30 female VAF CD1 mice were assigned to each of two dose groups. Ranolazine was given at dose levels of 5 or 50 mg/kg/day for the duration of the study. Blood samples were collected at 30 minutes post-dose from up to 10 animals per sex group on day 1 and at 3,6,12,18 and 24 months of the study. Plasma was examined for ranolazine concentration. On selected days at approximately 3 and 9 months, the usual daily dose was replaced by a ^{14}C-ranolazine dose. On these occasions, male and female mice (n=4 or 6 from each dose group) were housed in polycarbonate metabolism cages until 96 or 120 hours post-dose and excreta collected daily. Urine and feces were assayed for radioactivity at the 3 month timepoint. Urine only was assayed for radioactivity at the 9 month timepoint. HPLC detection was conducted.

Results: Ranolazine was found in the plasma at each sampling period for both doses. There was insufficient data generated for AUC determination. The differences between the plasma levels at single timepoint determinations really can't be given the same level of comparison as AUC data.

For all groups, excretion of radioactivity was primarily through the urine. The percent dose recovered was from 47%-58% for both sexes, all dose groups at both 3 and 9 months. "Representative" urinary metabolite profiles were presented as chromatograms with minimal information.

A preliminary investigation of the pharmacokinetics of ranolazine in mouse following single oral and chronic oral administration AT6291, January 1993.
In the preliminary study, male mice were given single oral doses of ^{14}C-ranolazine at 15 and 35 mg/kg. Terminal blood samples from 3 animals per time point (20 min., 40 min., 1, 1.5, 2,4,6,8 and 24 hours post-dose) were analyzed for ranolazine and radioactivity. An additional study was undertaken in 3 mice to examine earlier timepoints of 5,10,15,20, 25 and 30 minutes post-dose. In the carcinogenicity dose ranging study, there were two active satellite treatment groups with 20 male and 20 female mice in each dose group. The dose levels were 5 and 35 mg/kg/day. Blood samples were taken from each mouse at 30 minutes post-dose on Days 1, 8, 28 and 84 of the study. Plasma samples were analyzed for ranolazine.

Results: In the preliminary study, exposure increased more than proportionally with increasing dose as seen in both Cmax and AUC. This non-proportional increase was seen in both ranolazine and drug-derived radioactivity. This is summarized in the sponsor's table below. It is not clear if the extra mice used for the early timepoints were included in these calculations.

Table 4
MEAN C_{max}, t_{max} and AUC_{0-} VALUES FOLLOWING SINGLE ORAL ADMINISTRATION OF ^{14}C-RANOLAZINE AT 15 AND 35 mg/kg TO MALE MICE

	Dose	
	15 mg/kg	35 mg/kg
Ranolazine		
C_{max} (ng/ml)	1480	4670
t_{max} (h)	0.333	0.333
AUC_{0-} (ng.h/ml)	2920	9100
Radioactivity		
C_{max} (ng/ml)	4790	11200
t_{max} (h)	1.00	0.333
AUC_{0-} (ng.h/ml)	13200	34100

In the second study, where a single sample was taken at 30 minutes after dosing, The mean plasma level of ranolazine remained relatively constant over the duration of the study. This is summarized in the reviewer's table below.

Reviewer's summary of mean plasma ranolazine at 30 minutes post dose (units not given in report pp. 84-87)

	Day 1	Day8	Day 28	Day 84

Females 5 mg/kg/day	253±89.7	189±56.8	201±59.1	176±54.4
Males 5 mg/kg/day	224±79.8	244±151	263±87.5	242±99.5
Females 35 mg/kg/day	2350±909	2300±775	2900±860	2990±1530
Males 35 mg/kg/day	2960±808	3230±928	2620±691	3500±1480

Metabolic profiles of ranolazine following oral administration of a single 50-mg/kg dose of [^{14}C]-ranolazine to male mice. CVT303.006-MET, July 30, 2002

Male CD-1 mice received single oral doses of [^{14}C]-ranolazine at 50 mg/kg. Concentrations of total radioactivity in plasma and recoveries of the radioactive dose in urine and feces up to 5 days post-dose were determined. Plasma and urine samples were analyzed by LC/MS/MS. The samples were also subjected to β-glucuronidase/sulfatase hydrolysis to estimate levels of conjugated metabolites. The report does not state the number of mice used, how many were sampled per time point and the times at which plasma samples were collected. Urine samples were apparently collected over 5 days with 0-48 hour samples pooled "proportionally to the total volume to make one urine pool for each mouse."

Results
Urine: Nine major metabolites were detected by radiochromatography of the 0-48 hour pooled sample. Fifty-nine additional minor metabolites were also detected by both radiochemical detection and MS.

Plasma: the sponsor states that most urinary metabolites were also detected in plasma with no metabolites that were unique to plasma. However, the sponsor adds a caveat that the presence of major novel metabolites may have been masked by significant chemical noise from endogenous compounds in the total ion chromatogram generated by LC/MS. It is stated in the report that concentrations of 18 Phase I and 13 Phase II metabolites were determined. It does not state how many metabolites overall were identified or counted. Apparently there were sufficient numbers of metabolites that complete resolution could not be achieved. The greatest exposure based on AUC was to ranolazine (18% of total ^{14}C) followed by RS-94287 (6%), RS-88390 conjugate (7%), RS-88681 conjugate (5%), RS88597(5%) and RS-89983(4%).

The sponsor's summary is shown below.

Metabolites of Ranolazine RS Number (CVT Number)	Plasma $AUC_{0\text{-}24\ hr}$ (µg x hr/mL) (% AUC for Ranolazine)	Urine % Urinary Total Radioactivity (% of Administered Dose)
Phase I Metabolites		
Ranolazine (RS-43285, CVT-303)	8.88 (100)	2.44 (1.18))
RS-94287 (CVT-2738, Ran 2)	2.88 (32.4)	13.7 (6.61)
RS-88597 (CVT-5030)	2.25 (25.3)	1.16 (0.56)
RS-89983 (CVT-2535)	2.04 (23.0)	0.88 (0.43)
CVT-4786 (Acid of CVT-2534)	1.31 (14.7)	18.7 (9.05)
RS-88681 (CVT-2513)	1.22 (13.7)	10.5 (5.07)
RS-89289 (CVT-2537)	0.93 (10.5)	13.9 (6.73)
RS-89961 (CVT-2551)	0.88 (9.90)	2.22 (1.07)
RS-91347 (CVT-3369)	0.77 (8.69)	0.98 (0.47)
CVT-2534	0.23 (2.56)	0.00 (0.00)
RS-101647 (CVT-3248)	0.14 (1.55)	0.94 (0.46)
RS-88390 (CVT-2514)	0.12 (1.37)	0.00 (0.00)
RS-88755 (CVT-3389)	0.12 (1.30)	0.67 (0.33)
RS-89356 (CVT-5031)	0.10 (1.15)	0.00 (0.00)
Desmethyl-RS-88681 (CVT-3247)	0.06 (0.64)	0.74 (0.36)
RS-88250	0.03 (0.32)	0.55 (0.27)
RS-88640 (CVT-2512)	0.011 (0.12)	0.97 (0.47)
RS-88772 (CVT-3388)	0.012 (0.14)	0.00 (0.00)
RS-88835 (CVT-5028)	0.008 (0.08)	0.00 (0.00)
Phase II Metabolite[b]		
RS-88390 (CVT-2514) Conjugate	3.16 (35.6)	5.37 (2.62)
Desmethyl-RS-88681 (CVT-3247) Conjugate	2.54 (28.6)	3.30 (1.59)
RS-89983 (CVT-2535) Conjugate	2.06 (23.2)	6.12 (2.95)
RS-88597 (CVT-5030) Conjugate	1.98 (22.3)	3.68 (1.79)
Ranolazine (RS-43285 or CVT-303) Glucuronide	1.32 (14.9)	0.71 (0.35)
RS-88835 (CVT-5028) Conjugate	0.42 (4.76)	0.95 (0.46)
RS-88250 Conjugate	0.40 (4.53)	0.30 (0.15)
RS-89356 (CVT-5031) Conjugate	0.36 (4.10)	1.19 (0.58)
RS-88772 (CVT-3388) Conjugate	0.30 (3.42)	0.61 (0.30)
RS-88755 (CVT-3389) Conjugate	0.28 (3.15)	0.37 (0.18)
RS-88640 (CVT-2512) Conjugate	0.22 (2.52)	0.00 (0.00)
RS-89961 (CVT-2551) Conjugate	0.18 (2.00)	0.00 (0.00)
CVT-2534 Conjugate	0.11 (1.21)	0.65 (0.31)

[a]Values represent % AUC of ranolazine. Ranolazine plasma AUC was set at 100%.
[b]Units for conjugates are expressed as µg of the corresponding Phase I metabolite equivalent.hr/mL.

Results of this study are consistent with previous reports that ranolazine is extensively metabolized following oral administration in the mouse. In addition to the metabolites already identified in previous studies, several new metabolites were found. These included CVT-4786, which was the oxidative product of CVT-2534, the complimentary half of RS-94287. N-oxides of ranolazine, several di- and tri-hydroxylated ranolazine and RS-88390 derivatives and their methylated metabolites.

It may be concluded that ranolazine is extensively metabolized in male mice. Several previously unreported metabolites were identified. These included CVT-4786 and 2 N-oxides at the N1 and N4 piperazine positions. There were other new, methylated metabolites derived from dihydroxylated ranolazine. One metabolite found in human plasma but not in the mouse is a conjugate of RS-89664, a hydroxylated metabolite at the 3-position of the methoxyphenyl ring.

Best Possible Copy

There are quantitative differences in the human vs mouse metabolite profiles. N1-dealkylation was a major route of metabolism in mice but was minor in humans. O-demethylation and O-dearylation were significant metabolic routes in humans but not in mice.

Plasma level and excretion studies in the male dog following single oral administration of ^{14}C-ranolazine at 60 mg/kg AT5957 Conducted Oct. 1986, Reported: January 1992.

Ranolazine (lot E6-ML-001) and ranolazine labelled with ^{14}C on the carbonyl carbon atom of the thioacetamide group (specific activity 0.74 MBq/mg) was used. Three male Beagles (
) were given aqueous solutions of drug in a single oral dose of 60 mg/kg. The dose level used in this study matched the dose used in the 6-month dog oral toxicity study. Urine and fecal samples were collected each day for 7 days.
Plasma levels of drug were determined by HPLC methods. The report does not specify when the blood samples were collected, but a page in the results section suggests that the times were 0.25, 0.5, 0.75, 1 1.5, 2, 3, 4, 6, 8, 12, 24 ,30, 48 and 72 hours after dosing.

Results: Drug derived radioactivity persisted in the plasma for a longer time than did the parent drug. Bioavailability of the parent drug was high, on average 65±15%. The sponsor's results are shown at right. The mean tmax values were 1.9 and 2.5 hours for ranolazine and radioactivity respectively. Both Cmax and AUC for radioactivity were higher than the respective values for ranolazine.

Pharmacokinetics of Ranolazine and [illegible] activity in the Male Dog Following Single Oral Administration of ^{14}C-Ranolazine (60 mg/kg)

Parameter	Dog No. M4CA1	M4CA2	M4CA5	Mean ± SD
Radioactivity				
C_{max} (ng equiv/ml)	12800	8140	7650	9530 ± 2840
t_{max} (h)	2.0	1.5	4.0	2.5 ± 1.3
AUC(0-infinity) (ng equiv.h/ml)	142000	102000	85200	110000 ± 29200
Terminal $t_{1/2}$ (h)	17.4	20.0	14.6	17.3 ± 2.7
Ranolazine				
C_{max} (ng/ml)	2140	2310	2150	2200 ± 95.4
t_{max} (h)	0.8	1.0	4.0	1.9 ± 1.8
AUC(0-infinity) (ng.h/ml)	11900	12100	11500	11800 ± 306
Terminal $t_{1/2}$ (h)	5.60	4.10	10.9	6.87 ± 3.57
Oral Clearance (ml/min/kg)	84.0	82.6	87.0	84.5 ± 2.25
Bioavailability** (%)	78.7	49.3	67.9	65.3 ± 14.9

^{14}C AUC (po, 5 mg/kg, 0-inf) = 11200 ± 2600 ng equiv.h/ml*
^{14}C AUC (iv, 5 mg/kg, 0-inf) = 10700 ± 1900 ng equiv.h/ml*
* data reproduced from reference 1
** determined using individual systemic clearance values following a 5 mg/kg iv dose (see Appendix A)

The sponsor compared the results from the current 60 mg/kg oral dose with previous studies of 5 mg/kg IV and po in the dog. In the sponsor's results below, it may be seen that there is a less than proportional increase in Cmax after the 5 and 60 mg/kg doses and a more than proportional increase in AUC $_{0-\infty}$. The clearance dropped by approximately 50% at the higher dose while the Vd increased. Mean urinary excretion of radioactivity was 32% and mean fecal excretion was 68%. This is the reverse of what has been

Best Possible Copy

reported for most species (predominantly urinary excretion).

Summary of Pharmacokinetic Parameters of Ranolazine in Dog Following Single Intravenous and Single Oral Administration of ^{14}C-Ranolazine

Parameter	5 mg/kg iv* (n=4)	Parameter	5 mg/kg oral* (n=4)	60 mg/kg oral ** (n=3)
C_0 (ng/ml)	4660 ± 983	C_{max} (ng/ml)	265 ± 128	2200 ± 95.4
		t_{max} (h)	0.4 ± 0.2	1.9 ± 1.8
Terminal $t_{1/2}$ (h)	0.56 ± 0.48	Terminal $t_{1/2}$ (h)	1.01 ± 0.55	6.87 ± 3.57
$AUC_{0-infinity}$ (ng.h/ml)	1650 ± 373	$AUC_{0-infinity}$ (ng.h/ml)	471 ± 116#	11800 ± 306
Systemic Clearance (ml/min/kg)	52.6 ± 12.0	Oral Clearance (ml/min/kg)	185 ± 50.0#	84.5 ± 2.25
Volume of Distribution (L/kg)	2.27 ± 1.50	Bioavailability+ (%)	27.6 ± 9.47#	65.3 ± 14.9

* Parameter values calculated from data in reference AT 3407 (SS/038/85). Individual values are given in Appendix A of this report (SS/039/89)
** 60 mg/kg data from this report (SS/039/89)
# n=3
+ Bioavailability values calculated using individual systemic clearance values.

The study reports increased oral bioavailability (from 28% at 5 mg/kg to 65% at 60 mg/kg), decreased clearance, increased volume of distribution and non-linear increases in exposure with increasing oral dose. This is suggestive of saturated clearance, evidenced by a terminal $t_{1/2}$ of 1 hour at 5 mg/kg and 7 hours at 60 mg/kg, or tissue storage. The more rapid loss of parent compound compared to overall drug-derived radioactivity suggests metabolism and, if the pharmacologic effect persists past the disappearance of parent drug, active metabolites.

Other studies:

Summary of the disposition and metabolic effects of RS 43285-193 in animals. AT3840 March 1987. RS 43285 was radiolabeled with ^{14}C on the carbonyl carbon of the acetamide group for these studies.

This is the sponsor's summary of the above metabolism studies. No new information was presented. It was noted that the mean oral AUC was 20% of the IV value. Is this consistent with the levels of radioactivity absorbed in the oral dose studies?

The effect of ranolazine on rat liver cholesterol 7 alpha-hydroxylase activity. AT4961, November 1989

The report notes in the introduction that the study was prompted by the adrenal pathology findings in the 3 month rat study.

Male Sprague-Dawley rats [ ] were euthanized and the livers collected. Microsomes were then prepared and the microsomal protein content determined. Hepatic microsomal 7αhydroxylase activity was measured by isotope incorporation. Concentrations of

Best Possible Copy

ranolazine of 10^{-9}, 10^{-8}, 10^{-7},10^{-6},10^{-5}M were tested for their effect on the enzyme. The effect of metyrapone at 10^{-4}M was used as a positive control.

Results: The positive control caused a 26% decrease in enzymatic activity (0.84±0.06 pmol/min/mg protein for the control vs 0.69±0.14 pmol/min/mg/protein for metyrapone). No consistent effect was discernible for the test compound. One wonders why, for purposes of identification of effect, that the test compound was not tested at the same or a higher concentration than the positive control.

An in vitro investigation of the potential invovlement of cytochrome P450 3A in the hepatic metabolism of ranolazine. CL6906/ SS/035/94 December, 1994

Rat liver microsome from control and dexamethasone-treated male CD/SD rats were prepared previously according to a referenced study. Human liver was obtained frozen from a tissue bank [redacted]. In vitro rate incubations were carried out using ranolazine in water at concentrations from 0.1- 10 µM. Other liver microsome samples were used to try to correlate rate of ranolazine metabolism with CYP3A activity. Testosterone hydroxylase activity was determined by measuring the rates of formation of 2β-, 6β- and 7α-hydroxytestosterone and 4-androsten-3,17-dione. Incubations in the presence of ketaconazole and triacetyloleandomycin (TAO) were performed in a similar manner to the in vitro rate incubations. Ketaconazole and TAO were added to the reaction mixtures at final concentrations of 0.05-100µM and 10-100 µM respectively. It appears from the methods section, that replicates were not uniformly used. An incubation was conducted in a similar manner to the in vitro rate incubations in the presence of TAO and sulphaphenazole at final concentrations of 10 and 50 µM respectively. The reaction proceeded for 30 minutes followed by the addition of ranolazine. The reaction then continued for another 60 minutes. Incubations were also conducted in a similar manner to the in vitro incubations in the presence of untreated and dexamethasone-treated rat liver microsomes at final concentrations of 10µM ranolazine. The reaction was allowed to proceed for 10 minutes. Samples were analyzed by HPLC for ranolazine and metabolite peaks. MS and LC-MS analysis were also employed.

MASS SPECTRAL ANALYSIS OF RANOLAZINE METABOLITES FROM DEXAMETHASONE-TREATED RAT LIVER MICROSOMES AND TAO-INHIBITED HUMAN LIVER MICROSOMES

Ranolazine Metabolite (RS-)	Dexamethasone-Treated Rat Microsomes*	TAO-Inhibited Human Microsomes*	Sulphaphenazole-Inhibited Human Microsomes*
89983	↑	ND	ND
94287	↑↑	↓	↑
88755	↑↑	ND	↑
88681	↑	↓	↑
88640	↑↑	ND	↑
91347	↑	ND	=
88390	↓	=	↑
88772	↑	↓	=
88597	=	=	=
88835	↑	↓	=
89961	↑	↓	=

* Arrows indicate level changes relative to ranolazine level in the sample.
↑ increased level in relation to unchanged ranolazine
↓ decrease level in relation to unchanged ranolazine
= equal level compared to control

ND not detected in human liver microsomes

Results: The presentation of the data is suboptimal. Rate constants were provided for the in vitro rate of metabolism incubations in untreated human liver microsomes, in control and dexamethasone-induced rat liver microsomes, in the presence of ketaconazole and TAO. The values are based on single incubations. A dose-dependent increase in inhibition of ranolazine metabolism was seen with increasing concentrations of ketaconazole. Concentrations of 10 and

100 μM ketaconazole produced ~ 82-83% inhibition of ranolazine metabolism. Concentrations of 10 and 100 μM TAO produced 63% and 82% inhibition of ranolazine metabolism also. Both of these inhibitors were tested without replicates. The mass spectral analysis of ranolazine metabolites is shown here.

It was stated in the text of the report that levels of RS-88390 (O-desmethyl metabolite) and RS-88597 (hydroxylated metabolite) "appeared neither to increase in the CYP3A-induced microsomes nor decrease in the CYP3A-inhibited microsomes." This cannot be evaluated in the data as presented.

Distribution of ranolazine to human blood cells and binding of ranolazine to human plasma, human serum albumin, and human α-1 glycoprotein in vitro by an ultrafiltration method. CVT303.001-N August, 1998.

Human blood was collected from 3 healthy male volunteers. Citrated plasma was collected and used the same day as blood sampling. Purified human serum albumin and α-1acid glycoprotein were purchased from Roche Bioscience. Plasma, human serum albumin or α-1acid glycoprotein was mixed with a fixed amount of [^{14}C]-ranolazine and varying concentrations of unlabelled ranolazine then incubated at 37° for 3 hours. Ultrafiltration was performed on the equilibrated, room temperature incubation systems. Blood (presumably whole) was mixed with [^{14}C] ranolazine and varying concentrations of unlabeled ranolazine and incubated at 37°C for 20 minutes. The blood samples were then centrifuged to separate plasma from rbcs. Samples were analyzed by liquid scintillation counting and/or liquid chromatography.

Results A very small range (0.25 to 10 μg/ml) of ranolazine concentrations was tested. It does not appear that concentrations of protein were varied. It appears from the results that approximately 60-64% of the drug-derived radioactivity is bound in human plasma. An n=1 for α-1 acid glycoprotein binding is presented with insufficient labeling to permit accurate interpretation. The mean percent bound (dpm/ml?) ranges from 47-62% for ranolazine concentrations of 10μg/ml to 0.25μg/ml respectively. The mean percent bound (dpm/ml?) to human serum albumin is reported as approximately 30%. The concentration of ranolazine in blood: concentration in plasma was less than 1 for all 3 volunteers.
Summary: It appears that ranolazine is moderately protein bound and does not sequester in rbcs.

Binding of ranolazine to mouse, rat and dog plasma in vitro by an ultrafiltration method. July 29-Aug.3, 2001; Reported June , 2002. CVT303.019-N
Binding of ranolazine to plasma from mouse, rat and dog was determined in vitro by ultrafiltration methods. [^{14}C]-ranolazine at concentrations of 0.5 -10 μg/ml was incubated with pooled plasma from male CD-1 mice (n=20), male Sprague-Dawley rats (n=6) or male Beagles (n=3) at 37°C for ≥0.5 hours. The animals were drug-free for at least one week prior to blood collection. The unbound fraction in the plasma was separated by centrifugation through a membrane. The radioactivity in plasma and filtrate was determined by liquid scintillation counting.

Results

Mouse: There was no difference in % bound across 0.5 – 10 µg/ml (58.9±0.710 – 57.0±0.445).
Rat: There was no difference in % bound across 0.5 – 30 µg/ml (56.2±0.310 – 53.3±0.971)
Dog: there was no difference in percent bound across 0.5 – 30 µg/ml (47.1±0.580 – 43.7±0.713).
By comparison, binding of ranolazine in human plasma was 60.9-63.9% over the concentration range of 0.25-10 µg/ml.
Ranolzine is moderately plasma protein bound when tested in vitro in the species studied.

PK/TK summary: The studies would be stronger if data was presented to show that there were no endogenous interfering substances in any of the biological matrices.

Ranolazine was rapidly absorbed after oral administration in all non-clinical species. The drug was widely distributed, extensively metabolized and cleared almost equally via urine and feces.

Absorption
Absorption after oral dosing was fairly rapid, with the species studied showing Tmax within 0.5-2 hours.

Distribution
Whole body autoradiography studies following single oral doses, some studies including quantitation by liquid scintillation counting, showed principal tissue levels of radioactivity at 1 hour in the GI tract, liver, adrenals, kidney, thyroid, arterial wall, bone marrow, heart and brain. At 72 hours post-dose, radioactivity was primarily associated with liver, kidney, GI tract and thyroid, although detectable levels were reported for all the original tissues. Single oral doses of ranolazine given to pigmented rats showed that the eyes retained measurable levels of radioactivity. Albino rats showed detectable levels of radioactivity in the eyes (~0.35% x 10^{-3} of the administered dose) that were no longer detectable after 2 days. The elimination half-life for the pigmented eyes was 23 days.

Metabolism

A small percentage of ranolazine appears to be excreted unchanged. The remainder is highly metabolized by CYP450 3A4, CYP450 2D6 and Phase II conjugations. Approximately 40 metabolites were reported in the plasma of rats and more than 80 metabolites found in the urine. Similar findings were reported for dogs. Incomplete resolution of peaks makes it possible that more metabolites exist. There are at least 12 metabolites that regularly appear across species at levels greater than 1% of the AUC for ranolazine. Qualitatively these metabolites are those reported for human plasma samples. Following the metabolites across all studies becomes somewhat challenging as the codes used changed over the course of the studies. The metabolites of interest are shown.

Table 5.5-11 Comparison of Systemic Exposure to Metabolites of Ranolazine in Plasma (AUC) Following a Single Oral Dose of [Carbonyl, Propyl-2 ^{14}C]-Ranolazine in Male CD-1 Mice, Sprague Dawley Rats, Beagle Dogs, and Humans (Cont'd)

	Plasma AUC (µg.h/mL)			
Species	Mouse	Rat	Dog	Human
Reference	CVT303.006-MET	CVT303.003-MET	CVT303.002-MET	CVT303.001-MET
Dose (mg/kg)	50	50	25	500[a]
Ranolazine	8.88	11.2	13.5	7.67
Phase II Metabolites[b,d]				
RS-88390	**3.16**	**3.79**	**2.16**	**4.55**
Ranolazine[d]	**1.32**	**1.08**	**8.13**	**1.46**
RS-88597	**1.98**	**1.37**	**8.70**	**1.02**
RS-89664	0.00	0.00	0.00	0.49
RS-89358	0.36	0.22	**2.81**	0.36
RS-89961	0.18	0.00	0.00	0.35
RS-88835	0.42	0.16	**4.96**	0.27
RS-88640	0.22	0.00	0.46	0.00
RS-88772	0.30	0.00	0.00	0.00
CVT-2534	0.11	0.14	0.00	0.00
Desmethyl RS-88681	**2.54**	0.00	0.00	0.00
RS-89963	**2.06**	0.00	0.15	0.00
RS-88250	0.40	NA[c]	NA	NA
RS-88755	0.28	0.00	0.00	0.00

[a] Humans received a dose of 500 mg ranolazine solution, pH 4.
[b] Bolded values represent those with plasma AUC > 1 µg.h/mL
[c] NA = Not analyzed
[d] All Phase II metabolites were a mixture of glucuronides and sulfates, except for ranolazine, which consisted of only glucuronides.

In one dog study, an oral dose of 25 mg/kg aqueous ranolazine produced signs of lethargy from 4 to 8 hours post-dose. The findings are suggestive of an active metabolite.

In reports CVT303.006.MET (July 2002, mice),CVT303.003.MET amendment dated August 2002 (rats) and CVT303.002.MET (dog) the sponsor cites CVT4786 as a major new metabolite recently identified. In the rat study this accounted for 9.4% of the total radioactivity in urine (parent drug accounted for only 4% of the urinary radioactivity). In the dog study, CVT4786 had an AUC of 14.2% relative to ranolazine.

Excretion

The majority (94-98%) of the radiolabelled material was excreted within the first 48 hours after dosing. The routes of excretion tended to be divided equally between urinary and fecal.

Protein Binding

It appears that 60-64% of drug-derived radioactivity is protein bound in human plasma. Plasma protein binding was slightly lower in the non-clinical species. The average levels in mice, rats and dogs were ~58, 55 and 45% respectively. The data also indicate that the drug does not sequester in rbcs.

PK/TK conclusions: The sponsor detected the drug and derived material, however, the above studies would be stronger for the demonstration of limits of detection and limits of quantitation. The studies would be further strengthened by demonstration that the biological matrices of each species held no endogenous substances that interfered with detection/quantitation. Many of the studies contained small sample sizes and/or incomplete reporting of methodology. Despite these limitations, one comes to the conclusion that the drug was absorbed relatively quickly, with an oral bioavailability that increased from 28% at 5 mg/kg to 65% at 60 mg/kg. The drug is highly metabolized by both Phase I and Phase II routes in all species. CYP3A4 and CYP2D6 have been identified as involved. Major

routes of proposed metabolism include N-dealkylation at both nitrogens of the piperazine, amide hydrolysis and O-demethylation of the methoxy group at the methoxyphenyl ring. O-demethylation followed by glucuronidation and sulfation were reportedly major in humans but minor in dogs. The non-clinical species produce at least a qualitative representation of the major metabolites found in humans. Excretion appears to be essentially complete within approximately 120 hours after ingestion. The majority of drug is excreted within the first 48 hours, divided relatively evenly between urinary and fecal routes.

Other limitations of the characterization of metabolism include lack of plasma level data for the reproductive and developmental toxicology studies. There is also no distribution data for these studies.

IV. GENERAL TOXICOLOGY:

Study title:RS 43285 RBT: Oral EMLD study in rats

Key study findings: A single oral dose of 250 mg/kg produced 40% mortality while a dose of 500 mg/kg produced 60% mortality. Both dose groups showed signs of prostration, dyspnea, convulsions, salivation and ptosis.

Study no: AT3414, SS/051/85
Volume #, and page #: vol 16, p.6
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: April 1985
GLP compliance: no
QA report: yes ( ) no ( )
Drug, lot #, radiolabel, and % purity: lot # 125SS0584,[ ]
Formulation/vehicle:

Methods The purpose of the study was to estimate the mean lethal oral dose. Fasted Sprague-Dawley rats [ ]; 5 males and 5 females per group were given a single oral gavage dose of either 250 or 500 mg/kg of ranolazine. Survivors were euthanized after 14 days with no histopathological assessments.

Results: At the HD, 1/5 m and 2/5f were found dead within 12 minutes of dosing. Clinical signs were seen 2-5 minutes after dosing and included prostration, dyspnea, convulsions, pale extremities (due to vasoconstriction, an observation that was not explained) and salivation.

The animals in both dose groups showed signs within 30 minutes of dosing that included subdued behavior, prostration, convulsions, hyperventilation, piloerection, hunched appearance, ptosis and salivation (HD: 2/5 m, 1/5f; LD: 1/5 m and 3/5 f). No improvement was noted after 2.5 hours, thus the animals were euthanized for humane reasons. It should be noted that in a previous study, animals were dosed at the same levels for a period of three months. The sponsor suggests that fasting the animals in the current study was in part responsible for the poor survival.

Summary: A single oral dose of 250 mg/kg produced 40% mortality while a dose of 500 mg/kg produced 60% mortality. Both dose groups showed signs of prostration, dyspnea, convulsions, salivation and ptosis. The EML oral dose is approximately 250 mg/kg.

Study title:RS43285 VBT: Oral EMLD study in mice

Key study findings: A single oral dose of 250 mg/kg caused severe clinical signs of subdued behavior, hunched stance, piloerection, hyperventilation and prostration in 1/5m and 2/5f. There was no improvement in signs by 2 hours after dosing so the mice were euthanized. A single oral dose of 50 mg/kg produced no clinical signs.

Study no: AT3415, SS/050/85
Volume #, and page #: vol 16, p. 32
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: April 1985
GLP compliance: no
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: lot # 125SS0584, — %
Formulation/vehicle:

Methods: The purpose of the study was to estimate the mean lethal oral dose. Survivors were euthanized after 14 days with no histological assessments made.CD1 mice ([redacted] were assigned to 2 treatment groups with 5 males and 5 females per group. After an overnight fast, the animals received a single oral dose of either 250 or 50 mg/kg.

Results: In the HD group, 1/5 m and 2/5 f showed marked clinical signs beginning approximately 5 minutes after dosing. Signs included "subdued behavior", prostration, hyperventilation, vasoconstriction, hunched appearance and piloerection. There was no improvement in the clinical status two hours after dosing, thus the animals were euthanized. In the surviving animals of this group, the signs remained pronounced until ~ 1 hour after dosing. Marked improvement was seen by 2 hours. No clinical signs were reported for the LD animals.

Summary: A single oral dose of 250 mg/kg caused severe clinical signs in 1/5m and 2/5f. There was no improvement in signs by 2 hours after dosing so the mice were euthanized. A single oral dose of 50 mg/kg produced no clinical signs. The EML oral dose is approximately 250 mg/kg.

Study title: Intravenous EMLD study in mice

Key study findings: A single intravenous dose of 20 mg/kg produced no clinical signs. A single intravenous dose of 30 mg/kg produced clinical signs including hyperventilation, ataxia, piloerection, subdued behavior and prostration. Recovery time was within 1 hour of dosing. The LD50 is >30 mg/kg i.v.

Study no: AT3416, SS/029/85

Volume #, and page #: vol 16, 57
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: April, 1985
GLP compliance: no
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: 125SS0584
Formulation/vehicle: water, sodium hydroxide, dextrose

Methods: Fasted CD1 mice [ ] were assigned to 2 groups of 5m and 5f per group. The mice were given a single intravenous dose of 20 mg/kg or 30 mg/kg.

Results: No signs were reported for the LD group. All mice receiving 30 mg/kg showed signs either immediately or within 10 minutes of dosing. Signs included subdued behavior, ataxia and piloerection. Subdued behavior was the most frequently reported sign. In 3/5m and 1/5f, subdued behavior was accompanied by hyperventilation, prostration and ataxia. Recovery time for all animals was reported to be within 1 hour of dosing.

Summary: A single intravenous dose of 20 mg/kg produced no clinical signs and 100% survival. A single intravenous dose of 30 mg/kg produced clinical signs including hyperventilation, ataxia, piloerection and subdued behavior. Recovery time was within 1 hour of dosing. The LD50 is >30 mg/kg i.v.

Study title: RS-43285: Intravenous EMLD study in rats

Key study findings: No fatalities were reported. However, all animals showed clinical signs that included subdued behavior. Some animals also showed signs of ataxia, prostration, convulsions and hyperventilation. The intravenous LD50 is > 30 mg/kg.

Study no: AT3417, SS/039/85
Volume #, and page #: vol 16, p. 83
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: April 1985
GLP compliance: no
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: 125SS0584, — %
Formulation/vehicle: water, sodium hydroxide, dextrose

Methods : Fasted Sprague-Dawley rats [ ] were assigned to 1 group, with 5 males and 5 females. The rats received a single intravenous dose of 30 mg/kg.

Results: Clinical signs were observed in all animals immediately after dosing. The majority had slightly subdued behavior ± mild ataxia. Marked clinical signs were seen in 2/5m and 2/5f and included subdued behavior, ataxia, prostration, convulsions and hyperventilation. Recovery time was approximately 30 minutes from dosing, however, some animals showed subdued behavior and piloerection for the remainder of the day.

Summary: No fatalities were reported. However, all animals showed clinical signs that included subdued behavior. Some animals also showed signs of ataxia, prostration, convulsions and hyperventilation. The intravenous LD50 is > 30 mg/kg.

RS-43285-193/197/198: Comparative ELD study in rats

Key study findings: All animals showed signs of sedation, prostration, ataxia and dyspnea. The females receiving the racemic mixture had a later onset of signs (1.5 hours vs 12-38 minutes) compared to the animals receiving the enantiomers. The results are inconsistent with other studies that also found salivation, tremors and convulsions as well as earlier onset of signs with the racemic mixture.

Study no: AT6293
Volume #, and page #: Vol 27, p.244
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: August 27, 1992
GLP compliance:
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: racemic mix(lot E6ML001), RS-43285-197 (S-, lot 19) and RS-43285-198 (R+, lot 18)
Formulation/vehicle: distilled water pH adjusted with sodium hydroxide.

Methods: Fifteen male and 15 female .CD(SD)BR rats were allocated to 3 treatment groups of 5 males and 5 females. The animals were given single oral doses of 250 mg/kg of either the racemic mixture or one of the enantiomers. The animals were observed for clinical signs and were weighed on days 1,8 and 15 of the study period. After euthanasia, gross observations were made. No histological examination was made.

Results: One female was euthanized 3 hours after receiving the racemic mixture. The dog showed severe clinical signs of prostration, subdued behavior, ataxia and dyspnea. Signs of subdued behavior and dyspnea began from 12-38 minutes after dosing in most animals except the females receiving the racemic mixture. That group of animals began showing signs approximately 1.5 hours after dosing. Ptosis and subdued behavior were present in most animals to approximately 3 hours. Signs had resolved by Day 2. Body weight data was not presented. Essentially the only data presented was that contained in the text of the report. It is not possible to assess the incidence, duration or severity of the signs from the data presented.

RS-43285 REJ: One month intravenous toxicity study in rats AT3280
Key study findings: Immediate salivation, sedation and convulsions followed iv administration of 25 mg/kg to both sexes of rats. Increased liver weight and decreased uterine weight were seen in drug-treated females. At 25 mg/kg 1/12 males and 1/12 females died. Increased spleen and adrenal weights were seen in the drug-treated males. No NOEL was determined for the organ weight effects in either sex.

Study no: AT3280

Volume #, and page #: volume 15, p.160
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: August, 1984
GLP compliance: statement included (last page of appendices)
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: RS-43285-193, batch number 12, — %
Formulation/vehicle: dextrose adjusted to pH 4. Also used as vehicle control.

Methods: Sprague-Dawley [ ] rats, 12/sex/group were given daily intravenous doses of ranolazine of 0, 1,5 or 25 mg/kg for 28 days. The rats were observed daily for signs, twice a week for body weight and weekly for food consumption. Ophthalmoscopic exams were performed on all rats prior to study commencement and again during the fourth week of the study. Blood samples for hematology and clinical chemistry were collected from six animals per sex from all groupspre-study and from all animals during the 4th week of the study. Urinalysis was performed on samples obtained over a 4 hour period in metabolism cages from 6 animals/sex/group before the study and again during the 4th week of dosing. Full post-mortem exams were conducted on all rats. Certain organs were weighed and a standard list of tissues was collected for histopathological evaluation. Standard sections were stained with hematoxylin and eosin. Frozen sections of formaldehyde fixed liver were stained with Oil Red O for the presence of neutral lipid.

Results: Unscheduled mortality was seen in 1 HD male, one minute after dosing day 4 and 1 HD female immediately after dosing day 26. Signs for the male immediately before death were salivation and convulsions. Signs reported for the female included subdued behavior, salivation and convulsions. Signs were observed regularly for HD males from the first week to the end of the study and from week 2 in HD females to the end of the study. Convulsions, salivation and subdued behavior were seen regularly in a " small number" of animals immediately after dosing with recovery reported to be 5-15 minutes. Records of observation were not provided.

HD males gained on average 6% less than the control group. LD and HD females gained on average 10% and 4% less than the control group, respectively. There was no apparent difference in food consumption between the groups.

RBC count and HCT in MD and HD females were significantly decreased compared to the control and LD groups. This is summarized in the reviewer's table below.

Appears This Way On Original

Reviewer's summary of hematology changes in female rats

dose	RBC x 10^{12}/l	HCT ratio
Pre-dose all	6.72±0.31	0.409±0.020
0	7.24±0.20	0.421±0.017

2	7.22±0.28	0.421±0.016
5	6.85**±0.41	0.403*±0.021
25	6.85**±0.22	0.406*±0.010

There were no toxicologically significant clinical chemistry changes.
Absolute and normalized spleen and adrenal weights were increased in the drug-treated males.
Liver weight was increased and uterine weight decreased in treated females.

IS-43285 REJ : One Month Intravenous Toxicity Study in Rats

Group Mean Organ Weights (g)

Group Number	Dose mg/kg/day		Brain	Heart	Testes	Pituit.	Liver	Prostate	Kidneys	Spleen	Adrenals	Thymus	Thyroids	Bodyweight
MALES														
1	0	Mean	2.02	1.24	4.63	0.013	11.74	0.73	2.69	0.78	0.067	0.62	0.022	333
		SD	0.08	0.13	0.76	0.002	2.11	0.16	0.38	0.11	0.013	0.08	0.003	34
2	1	Mean	2.00	1.25	4.54	0.013	11.46	0.74	2.80	0.81	0.070	0.61	0.023	333
		SD	0.09	0.10	0.64	0.002	1.29	0.26	0.20	0.14	0.015	0.15	0.004	34
3	5	Mean	2.03	1.29	4.71	0.015*	12.64	0.88	2.83	0.88	0.079	0.60	0.024	342
		SD	0.06	0.13	0.43	0.002	1.66	0.26	0.15	0.12	0.014	0.05	0.004	18
4	25	Mean	2.03	1.22	4.60	0.013	11.42	0.79	2.74	0.88	0.075	0.62	0.020	330
		SD	0.09	0.08	0.31	0.002	1.52	0.25	0.29	0.13	0.016	0.09	0.005	25
Group Number	Dose mg/kg/day		Brain	Heart	Ovaries	Pituit.	Liver	Uterus	Kidneys	Spleen	Adrenals	Thymus	Thyroids	Bodyweight
FEMALES														
1	0	Mean	1.85	0.89	0.156	0.015	7.91	0.75	1.83	0.55	0.083	0.49	0.021	217
		SD	0.05	0.04	0.034	0.003	0.92	0.15	0.15	0.07	0.010	0.12	0.004	15
2	1	Mean	1.87	0.86	0.162	0.015	8.18	0.62*	1.88	0.60	0.077	0.52	0.021	215
		SD	0.12	0.08	0.021	0.002	1.06	0.21	0.20	0.08	0.012	0.12	0.003	16
3	5	Mean	1.88	0.87	0.162	0.015	8.77*	0.61*	1.90	0.56	0.075	0.49	0.021	216
		SD	0.07	0.08	0.025	0.002	0.76	0.18	0.17	0.07	0.012	0.08	0.005	16
4	25	Mean	1.84	0.86	0.158	0.014	8.69	0.64	1.88	0.61	0.078	0.52	0.019	217
		SD	0.04	0.08	0.020	0.002	1.14	0.14	0.14	0.11	0.008	0.05	0.005	12

* < 0.05

Best Possible Copy

Appears This Way
On Original

43285 REJ : One Month Intravenous Toxicity Study in Rats

Group Mean Organ Weights as a Percentage of Bodyweight

Group Number	Dose mg/kg/day		Brain	Heart	Testes	Pituitary x1000	Liver	Prostate	Kidneys	Spleen	Adrenals	Thymus	Thyroids x1000	Bodyweight
MALES														
1	0	Mean	0.62	0.37	1.39	3.92	3.51	0.22	0.81	0.23	0.020	0.19	6.70	333
		SD	0.05	0.03	0.19	0.58	0.37	0.04	0.06	0.02	0.004	0.03	0.89	34
2	1	Mean	0.61	0.38	1.37	3.94	3.45	0.22	0.85	0.24	0.021	0.19	6.81	333
		SD	0.06	0.03	0.22	0.96	0.29	0.08	0.06	0.03	0.004	0.04	1.38	34
3	5	Mean	0.60	0.38	1.38	4.38	3.70	0.26	0.83	0.26	0.023	0.17	7.11	342
		SD	0.04	0.04	0.13	0.53	0.51	0.07	0.05	0.04	0.004	0.02	1.03	18
4	25	Mean	0.62	0.37	1.40	3.96	3.46	0.24	0.83	0.27*	0.023	0.19	6.18	330
		SD	0.03	0.02	0.15	0.67	0.36	0.07	0.07	0.04	0.004	0.02	1.73	25

Group Number	Dose mg/kg/day		Brain	Heart	Ovaries	Pituitary x1000	Liver	Uterus	Kidneys	Spleen	Adrenals	Thymus	Thyroids x1000	Bodyweight
FEMALES														
1	0	Mean	0.86	0.41	0.072	5.83	3.67	0.35	0.84	0.26	0.038	0.23	9.54	217
		SD	0.05	0.03	0.015	1.42	0.45	0.08	0.04	0.04	0.005	0.06	1.68	15
2	1	Mean	0.87	0.40	0.076	7.13	3.82	0.29	0.88	0.28	0.036	0.24	8.93	215
		SD	0.05	0.04	0.011	0.90	0.49	0.08	0.07	0.02	0.006	0.04	1.28	16
3	5	Mean	0.87	0.40	0.075	6.98	4.12*	0.28*	0.88	0.26	0.035	0.23	9.78	216
		SD	0.06	0.04	0.008	1.29	0.32	0.08	0.04	0.03	0.004	0.04	2.17	16
4	25	Mean	0.85	0.40	0.073	6.46	4.02*	0.30	0.87	0.28	0.036	0.24	8.73	217
		SD	0.04	0.03	0.008	0.95	0.53	0.07	0.05	0.05	0.003	0.03	2.37	12

Best Possible Copy

The semi-quantitative urinalysis results were presented with acronyms that were undefined. It does not appear however, that there were discernible effects of drug treatment upon the results.

Study title: RS 43285 DCJ: Maximum tolerated intravenous dose study in dogs

Key study findings: Single and repeated doses of 10 and 20 mg/kg/day resulted in the dogs becoming subdued as they received their dose and for approximately 15 minutes thereafter. At 20 mg/kg/day, the sedation was occasionally accompanied by glazed eyes, ataxia and trembling. Vomiting after dosing was recorded on one occasion. The frequency of the clinical signs was reported to diminish over the dosing period, suggesting increased tolerance to the dose, induction of metabolism or increased clearance. A single dose of 40 mg/kg after the 10 and 20 mg/kg doses produced convulsions and collapse immediately post-dosing. The dog was humanely euthanized. Moderate dilation of the right ventricle of the heart was found on gross necropsy. The only data from the ECGs was heart rate. The dose of 20 mg/kg/day was tolerated for 21 days by the 1 female who received it.

Study no: AT3844
Volume #, and page #: vol 17, p. 323

Conducting laboratory and location: Syntex research, Scotland
Date of study initiation: May 31, 1984
GLP compliance: preliminary, non-GLP
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity: RS-43285-193, lot 111SS0284, %
Formulation/vehicle: 2% buffered solution of dextrose, sodium hydroxide and water.

Methods Two pairs of one male and one female Beagles were given once daily intravenous injections on the following schedule:

Pair 1- 7 days at 10 mg/kg/day
7 days at 20 mg/kg/day
Male- 1 day at 40 mg/kg/day
Female- 21 days further at 20 mg/kg/day
Pair 2 – 15 days at 20 mg/kg/day
Body weight was recorded twice weekly, food consumption daily. ECGs were performed at weekly intervals in pair 1 and before dosing and on days 9 and 15 in pair 2. At these times, ECG traces were taken before dosing, 1,6 and 24 hours after dosing. On day 9, the pair 2 traces were taken before dosing and 5 minutes after.

Blood samples were collected pre-study. Pair one was sampled at unspecified intervals while the second pair was sampled day 15 of dosing. Hematology and clinical chemistry parameters were analyzed. All dogs were examined at necropsy. No histopathological analysis was done.
Results: Single and repeated doses of 10 and 20 mg/kg/day resulted in the dogs becoming subdued as they received their dose and for approximately 15 minutes thereafter. At 20 mg/kg/day, the sedation was occasionally accompanied by glazed eyes, ataxia and trembling. Vomiting after dosing was recorded on one occasion. The frequency of the clinical signs was reported to diminish over the dosing period, suggesting increased tolerance to the dose. A single dose of 40 mg/kg after the 10 and 20 mg/kg doses produced convulsions and collapse immediately post-dosing. The dog was humanely euthanized. Moderate dilation of the right ventricle of the heart was found on gross necropsy.
The only data presented from the ECGs was heart rate. Given the very small n of the study, the heart rate, hematology and clinical chemistry data is not particularly helpful.

Study title: RS 43285 DCT: Maximum tolerated oral (intubation) dose study in dogs

Key study findings: Signs reported were sedation, ataxia, muscle tremors, vomiting, salivation and prostration. Convulsions were reported for a male dog who was then euthanized in extremis. Signs lasted for up to 6-7 hours with higher dosages. The dose of 150 mg/kg following the periods of lower dosages was the estimated lethal dose. The 80 mg/kg/day dose is the estimated maximum tolerated dose for an oral dosing study.

Study no: AT 3845
Volume #, and page #: vol 17, p. 359
Conducting laboratory and location: Syntex Research, Scotland

Date of study initiation: May 15, 1984
GLP compliance: preliminary, non-GLP report
QA report: yes ( ) no (x)
Drug, lot #, radiolabel, and % purity:
Formulation/vehicle: sodium hydroxide and distilled water.

Methods Two pairs of Beagles, one male and one female per pair, were intubated orally once each day. Pair one received 50 mg/kg/day for one week followed by 100 mg/kg/day for a week. Both dogs then received an additional single oral dose of 150 mg/kg/day. Pair two was dosed at 100 mg/kg/day for 9 days. This was discontinued due to marked clinical signs. After a seven week recovery period treatment was resumed at 80 mg/kg/day for 2 weeks. Pair 2 was weighed twice weekly during the 2 weeks of 80 mg/kg/day treatment. Food consumption was estimated daily. ECGs were performed before dosing and 1, 6 and 24 hours after dosing in pair 2 on days 1 and 14. Blood samples for hematology and clinical chemistry were collected from the pair 2 dogs pre-test and week 2. After euthanasia, gross necropsy was carried out. No histopathological evaluation was performed.

Results: Single doses of 50 mg/kg and 80 mg/kg caused vomiting within 45 minutes of dosing. A single dose of 100 mg/kg in naïve animals produced no signs. Continued dosing at this level produced combinations of sedation, ataxia, muscle tremors, vomiting, salivation and on one occasion in the female, prostration. On day 9, both dogs were found prostrate and trembling 2 hours after dosing and the male appeared unaware of its surroundings. Partial recovery was seen within 2-4 hours.
A dose of 100 mg/kg following a week at 50 mg/kg caused sedation, muscle tremors, mild ataxia and staining of the mouth in the male. No signs were reported for the female. The male continued to show these signs for the rest of the week at this dose. The female vomited on most occasions within 60 minutes of dosing and showed sedation on several occasions 1-2 hours after dosing. Recovery was within 6-7 hours.
A single oral dose of 150 mg/kg after dosing at 50 and 100 mg/kg caused severe clinical signs in the male within 20 minutes of dosing. Signs included prostration, convulsions, irregular breathing and slightly decreased heart rate. The female showed sedation and muscle tremors with recovery within 4 hours. The male was euthanized in extremis.
Repeated daily doses of 80 and 100 mg/kg/day caused sedation, muscle tremors, mild ataxia, salivation, vomiting and prostration within 1 hour of dosing. Recovery took 3-5 hours.

RS-43285 DEJ: One month intravenous toxicity study in dogs.

Key study findings: This study was suboptimal in reporting and had few apparent findings of significance. The HD (20 mg/kg/day) animals showed signs of sedation, trembling, vomiting and hindlimb ataxia. ECGs were obtained but only raw heart rate data was presented.

Study no: AT3281
Volume #, and page #: Vol 15, p. 279
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: July 1984

GLP compliance: statement included (last page of appendices)
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity: lot number 125ss0584,
Formulation/vehicle: dextrose at pH = 4.

Methods: Three male and three female beagles ( ) per group were given daily intravenous injections of 0, 1,5 or 20 mg/kg/day of ranolazine each day for 28 days. Animals were observed daily for signs, weighed weekly and food consumption estimated daily. All animals had ophthalmic exams pre-study and during week 4 of the study. ECGs were obtained from each animal pre-study, day 1 and during week 4. During the study, ECGs were obtained before dosing, 5 minutes, 1 and 6 hours after dosing. Blood for clinical pathology was collected from each animal pre-study and during the 4th week of the study. Urinalysis was performed at the same time points using metabolism cages. All animals were given a post-mortem examination. Those surviving to scheduled euthanasia were terminated 24 hours after the final dose. As indicated in the histopathology inventory, various organs were weighed and a reasonably standard list of tissues was collected for histopathological examination.

Results: There was no unscheduled mortality. No clinical signs were reported for the control groups. Vomiting before dosing was reported for 1 LD male and 1 LD female day 7. The MD group had reported signs of subdued behavior after dosing on day 1 for 1 male and overnight vomiting day 2 for another male. Signs were reported predominantly for the HD animals. "Subdued behavior" was reported almost daily for all the HD animals. This began immediately after dosing and lasted from 5 minutes to 1 hour. There were also "frequent" reports of vomiting, trembling and hind limb ataxia. One female showed ataxia almost continuously during weeks 3 and 4. Conjunctival congestion was also noted after dosing on day 12 for a HD male and a HD female. No incidence tables were presented for the clinical signs.
Average body weight gain was dose-dependently decreased in the treated males. There was no apparent connection between treatment and body weight for the females. This is summarized in the reviewer's table.

Summary of weight changes

Dose group (mg/kg/day)	Avg male weight gain (kg)	Avg female weight gain (kg)
0	0.43	0.7
1	0.43	-0.1
5	0.17	0.7
20	0	0.4

Food consumption was decreased sporadically in the controls, LD and MD females, significantly so in the last week of the study for the LD and MD females.

ECGs: Only heart rate data was presented. The single animal data was averaged by the reviewer and is presented in the following table.

Reviewer's summary of reported ECG data: Average heart rate (beats per minute) at several times post-dosing

Sex and	Day 1	Week 4

dose (mg/kg)	bd	5 mins	1 hr	6 hr	bd	5 mins	1 hr	6 hrs
Males								
0	112	115	122	124	128	115	128	133
1	131	104	123	121	108	98	110	128
5	146	124	114	153	140	118	119	141
20	133	126	112	140	131	124	111	154
Females								
0	133	129	141	147	126	118	128	140
1	129	111	124	138	116	94	109	130
5	108	123	124	142	129	112	113	143
20	116	149	129	136	113	126	107	133

There do not appear to findings of toxicological significance in the hematology, clinical chemistry or urinalysis data. The urinalysis data was presented with acronyms for which there were no definitions.

Organ weights were presented as single animal data. The reviewer calculated averages for organ weights provided as a percentage of body weight. No significant differences were apparent. However, percentage of body weight is very insensitive for small organs such as the pituitary and adrenal. There were 2 outliers in the control and LD group with regard to uterine weight. Because of this it cannot be determined if there was in fact a dose-related decrease in that organ's weight.

An ophthalmologist's report was not located.
A scant summary of histologic findings showed non-remarkable findings with the exception of meningo-encephalitis in one HD female. The sponsor suggests that this was of viral origin. This raises the question of the standards of care for a viral meningo-encephalitis to have found entry into the colony.

Study title: Oral investigative tolerance study in Beagle dogs with ranolazine administered three times daily.

Key study findings: This study provides limited data. At 60 mg/kg signs after dosing were sedation, salivation, vomiting, ataxia and trembling. Blood vessel dilation (pink ears) was also reported. It is not clear whether hypotension was the sole cause of the subdued behavior, recumbent animals, thrashing and barking.

Study no: AT6436 SS/14/92
Volume #, and page #: vol 28, p. 4
Conducting laboratory and location: Syntex Research, Edinburg, Scotland
Date of study initiation: June 12, 1991
GLP compliance: statement not located
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity: lot 14 E6-ML-001 and E7-ML-001

Formulation/vehicle: possibly given in gelatin capsules

Methods Two male and 2 female dogs were given escalating doses of ranolazine, three times a day on the following schedule:

Days 1-7	25 mg/kg/tid ranolazine
Days 8-14	40 mg/kg/tid ranolazine
Days 15-21	50 mg/kg/tid ranolazine
Days 22-35	60 mg/kg/tid ranolazine

Animals were observed daily for signs, weighed weekly and food consumption was monitored each day. One male and 1 female had ECGs done pre-trial and again on days 1,7,8,14,15,21,22,28,29 and 35 1 and 6 hours after the morning dose.
The same two animals had blood samples collected pre-test, days 7,14, 21,28 and 35. Standard hematology and clinical chemistry were monitored. Animals surviving to the end of the treatment period were euthanized the day after the last dose. No organs were weighed. Histopathological examination was confined to the stomach of one male who was euthanized ahead of schedule. Blood samples for determination of ranolazine in plasma were taken from 1 male and 1 female on days 1,7,14,21 and 28. Day 35 samples were taken from the surviving animals. Timepoints taken were predose, 20 minutes, 40 minutes, 1,2,4,6 and 8 hours after the morning dose. Samples were analyzed by HPLC with fluorometric detection.

Results: One male was euthanized day 29, 24 hours after his last dose due to marked clinical signs of subdued behavior, thrashing legs, trembling and tachypnea lasting ~1.25 hours. The animal remained subdued for another 6 hours. Plasma drug levels were determined but not reported. Clinical signs reported at 25 and 40 mg/kg/tid included green feces and occasional vomiting. At 50 mg/kg/tid, animals occasionally salivated before and/or after dosing. One animal trembled approximately 1 hour after the morning dose. At 60 mg/kg/tid, salivation and peripheral vasodilation (pink ears) became pronounced. Trembling and subdued behavior were also noted. One female was observed on day 35, ~ 1 hour after dosing, lying on its side, legs flaying, barking, trembling and salivating. The sponsor also reported the animal as very subdued. The report is somewhat unclear as it states:

> ...This lasted approximately 4 minutes and the animal appeared to be recovered 3 hours later. A blood sample and ECG were taken for diagnostic purposes only at this time but the results have not been reported here as they did not appear to be drug related.

It is difficult to say if there is a body weight effect as there was no untreated or vehicle group for comparison and a small sample size. There was no apparent change in body weights over the duration of the study.
Only heart rate data was presented from the ECGs. As there was 1 animal per sex for a total of 2 animals, with no untreated control for comparison, no conclusions can be drawn from the data. The hematology and clinical chemistry data were for 1dog/sex. Interpretation is difficult. Given the small sample size, the plasma drug data should not be overinterpreted. It can be said that $AUC_{0\text{-}8}$ (ng.hr/ml) increased with increasing dose.
No gross lesions were reported. The one stomach that was examined histopathologically had multiple small irregular erosions at the gastroesophageal junction that were considered to be unrelated to treatment.

Summary: This underpowered study cannot be given much weight. However, certain observations are interesting: blood vessel dilation (pink ears). It is not clear whether hypotension could be the sole cause of the subdued behavior, recumbent animals, thrashing and barking.

Study title: Four week investigative study in dogs

Key study findings: This single dose study had few findings of toxicological significance.

Study no: AT6543; SS/029/93
Volume #, and page #: vol 28, p.57
Conducting laboratory and location: Syntex Research, Edinburgh, Scotland
Date of study initiation: August 17, 1993
GLP compliance:
QA report: yes () no ()
Drug, lot #, radiolabel, and % purity: batch number E3-NE-002
Formulation/vehicle: tablets

Methods: The study was originally to evaluate local gastrointestinal effects of a sustained release tablet formulation of ranolazine free base. Two male Beagles were assigned to the control group and 4 to the treatment group. The animals were dosed once a day, approximating the target dose of 68.2 mg/kg/day. The sponsor states that this is equivalent to 80 mg/kg/day of the dihydrochloride that was used in a previous 3 month oral study in dogs. Animals were observed for signs, weighed weekly and food consumption measured daily. ECGs were performed pre-trial and on days 3 and 24 of dosing at 3,6 and 24 hours after dosing. Blood samples for plasma ranolazine determination were taken 1,7 and 28 days of dosing at 1,3,5,8,12 and 24 hours after dosing. Samples for clinical pathology were taken before dosing and on day 22 of the study. Animals were euthanized ~3 hours after the last dose, gross observations made, organ weights determined, and samples collected.

Results: There were no apparent differences in weight gain although the treated dogs did eat less than the control animals. Only heart rate data was shown from the ECGs. The rate for the control animals went down in the first 24 hours then increased at two points of determination in week 4. The treated animals showed decreased heart rates day 3. In week 4, 3 continued to show decreased rates while one showed an increase. Organ weight data was provided. However, the small sample size and degree of variability render the data of little value. Plasma level determination of drug exposure showed an increase in AUC $_{0\text{-}24}$ (ng.hr/ml) from day 1 to day 7: 19,500±9450 vs 25,100±13200 respectively. There was a slight decrease evident day 28: 20,500±17300 ng.hr/ml. No gross findings were reported. The only histopathology results provided was the statement "Histopathological examination of the stomach and intestines revealed no pathology."

Summary: It is difficult to say from the data presented whether the decreased heart rates seen in the treated dogs were within the realm of normal variability or treatment-related. This single dose study had few findings of toxicological significance.

Study title: RS-43285 DHT: three month oral toxicity study in dogs

Key study findings: This study was incompletely reported. Although ophthalmic exams were conducted there was no statement from an ophthalmologist. Although ECG tracings were reportedly done, only single animal heart rate data was presented. In both sexes of drug treated animals heart rate was decreased at the 1 hour post-dose observation time in week 1. Clinical signs were noted for doses ≥25 mg/kg, and included salivation, vomiting, ptosis, glazed eyes, conjunctival congestion, sedation, ataxia, trembling and convulsions. "Subdued behavior" was especially apparent in the first month. Inconsistent with the 6 month dog study, the signs reported in this study began from 10-30 minutes after dosing. Significant changes in hematology, clinical chemistry and urinalysis are not apparent. Absolute and normalized testicular and adrenal weight for the treated male dogs was increased over control but not in a dose-dependent fashion. Uterine weight of the drug-treated females was decreased compared to the control group. The presentation of pathology findings was confusing and raised questions as to the consistency of observations made. The manufacturing and stability data in Appendix L noted that placebo analysis indicated an RS-43285-193 content of less than 6.1 x 10^{-5} to less than 1.1 x 10^{-4} % w/w. It is not made clear if this means that ranolazine was not present or was present in levels near the limits of detection/quantitation.

Study no:AT3440, SS/028/85
Volume #, and page #: vol 16, p 109
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: September 11, 1984
GLP compliance: statement included
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity: 125SS0584
Formulation/vehicle: aqueous

Methods: 28 dogs from [redacted] and 2 dogs from [redacted] were assigned to 5 treatment groups of 3 males and 3 females per group. The dogs were dosed once a day for 91-93 days with 0, 5, 25, 60 or 80 mg/kg/day of drug. Animals were monitored daily for signs and food consumption, weekly for body weight, ophthalmoscopy (pre-treatment and week 13), and electrocardiography (pre-treatment, day 1, week 6 and week 13). Tracings were made pre-dose, 1, 6 and 24 hours after dosing. Blood was collected for clinical chemistry and hematology before dosing, and after 4, 8 and 12 weeks of dosing. Urinalysis was performed at the same time points via use of metabolism cages.
Those surviving to the end of the dosing period were euthanized 24 hours after the last dose. Specified organs were collected and weighed.
Results: No unscheduled mortality was reported. Signs were noted for the 3 highest dose groups with the two highest dose groups, 60 and 80 mg/kg, most frequently involved. Subdued behavior was noted frequently, especially in the first month of dosing. Other signs included vomiting, salivation, trembling, conjunctival congestion, ptosis, glazed eyes and ataxia. A combination of these signs was elicited week 13 during the blood sampling, ECG and ophthalmoscopy procedures. Convulsions were limited to the HD group. Other details reported for the HD group included pupils dilated (33%), pupils non-responsive to light (72%) and unusual skin tone of pink or blue (34%). Signs in the 25 mg/kg group were vomiting, ptosis, glazed eyes, ataxia and

subdued behavior. No signs were reported for the 5 mg/kg group. Signs were reported to begin from 10-30 minutes after dosing.

The mean weight change per group was a decrease in body weight. The only exceptions were the 5 mg/kg and 80 mg/kg females who showed slight mean gains.

Reviewer's Summary of Weight Changes (Kg) Calculated from Data Presented

	Dose group (mg/kg/day)				
	0	5	25	60	80
males	-0.44	-0.40	-0.99	-0.89	-0.82
females	-0.73	+0.17	-0.19	-0.05	+0.23

The hematology, clinical chemistry and urinalysis data were presented as single animal data. There were no apparent effects in any of these.

There was no statement from an ophthalmologist as to the ophthalmic findings and no indication that an ophthalmologist had conducted the evaluations.

ECGs- Only single animal HR data was presented. A decrease in heart rate was seen 1 hour after dosing in all drug-treated groups. This effect was apparent at Day 1 in both sexes and week 6 also in females. This is summarized in the reviewer's tables below.

Appears This Way On Original

Reviewer's Summary of Heart Rate Changes for Males

Day 1					
Dose mg/kg	Pre-trial	predose	1hr	6hrs	24hrs
0	118	130	149	115	130
5	106	124	109	104	102
25	95	106	85	108	121
60	116	105	99	101	102
80	157	147	124	122	138

Week 6					
0		130	122	122	131
5		108	96	106	105
25		95	89	102	95
60		108	101	121	110
80		130	126	135	141
Week 13					
0		130	136	124	112
5		100	99	103	109
25		83	81	107	85
60		96	107	94	101
80		117	131	124	118

Reviewer's Summary of Heart Rate Changes for Females

Day 1					
Dose mg/kg	Pre-trial	predose	1hr	6hrs	24hrs
0	127	126	121	138	127
5	125	120	109	106	115
25	133	140	121	136	134
60	125	129	102	129	113
80	130	110	105	106	102
Week 6					
0		105	108	104	107
5		117	97	108	91
25		128	108	144	114
60		136	128	128	149
80		139	111	121	131
Week 13					
0		128	114	124	107
5		92	94	99	90
25		110	120	121	126
60		126	123	114	114
80		111	96	112	125

The histopathology was presented as selected findings for single animals. The protocol states that histopathological evaluation will be performed on all animals of both sexes from all groups (p.211). Clearly this was not done as several of the animals are listed with the designation that no histopathology was done. An amendment to the protocol says that as no compound-related lesions were found in the HD animals, it was decided not to evaluate the other groups. For the control males it was specifically noted that in the testes there was spermatogenesis present and no pathology noted. This designation was not made for every male examined nor are there comments about the testes for every male. The inconsistency is confusing. Does that mean the testes were not examined or were abnormalities not reported? One designation for one HD male notes that there was a localized area of tubular germinal epithelial degeneration which was then immediately qualified as a possible post-mortem artifact.

It was noted that pleural lesions seen at necropsy frequently showed changes consistent with Filaroides hirthi infestation (vol 16, p.128). The presence of this parasite may well confound interpretation of clinical chemistry and hematology results.

Organ weight data was presented for individual animals. In an underpowered study such as this one would not expect to be able to discern differences between groups. The reviewer calculated means for absolute and normalized organ weight from the data presented. This is summarized in the table below. The absolute and normalized weight of testes in the drug-treated groups was more than that of the control group. No pattern was discernible. Absolute and normalized adrenal weight was also increased

Reviewer's summary of absolute and normalized (to body weight) selected organ weights

	Dose mg/kg/day				
	0	5	25	60	80
Absolute (testes)	13.12	14.06	15.2	13.06	15.66
Normalized (testes)	0.95	1.13	1.20	1.02	1.088
Absolute (adrenal)	0.74	0.72	0.835	0.85	0.87
Normalized (adrenal)	0.054	0.058	0.066	0.067	0.060

Uterine weight was also decreased in all drug-treated groups. Adrenal weights in the females were not discernibly affected.

Reviewer's summary of absolute and normalized (to body weight) selected organ weights

	Dose mg/kg/day				
	0	5	25	60	80
Absolute (uterine)	8.05	6.86	3.41	4.78	2.89* 4.03**
Normalized (uterine)	0.75	0.59	0.29	0.40	0.24* 0.34**

* the sponsor had a footnote for one individual measurement "left horn only". ** the asterisked animal was omitted from the calculation

Study title:RS-43285 DJC: Six month oral toxicity study in dogs

Key study findings: Signs were primarily reported for doses ≥25 mg/kg but appeared in all groups with no NOEL identified. Signs included mydriasis with loss of PLR, glazed eyes, ptosis and other signs of sedation. After the first week mydriasis was not seen until 4-5 hours after dosing. After the first month, mydriasis, glazed eyes and ptosis occurred sporadically in the MD and HD groups and was no longer reported for the LD group. Rouleaux was reported for the MD and HD males and 2 HD females. Adrenal weights were slightly increased in the HD males while testicular weight was decreased in the HD group. Pituitary weight was increased in females in a dose-related manner. Although

ECG tracings were obtained only single animal heart rate data was presented with no reporting of ECG intervals.

Study no: AT4050, SS/002/88
Volume #, and page #: vol 19, p 5.
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: October 1986
GLP compliance: statement included in the appendix
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity:
Formulation/vehicle: "various mixtures" containing lactose and magnesium stearate provided in gelatin capsules. Vehicle capsules contained lactose and magnesium stearate

Methods: Sixteen male and 16 female dogs [redacted] from 12-16 months of age, with the exception of male #405 who was 24 months of age, were used. The dogs were assigned to 4 treatment groups with 4 males and 4 females per group. The dogs were dosed orally once a day for 26 weeks. Doses used were 0, 5, 25 and 60 mg/kg/day. ECGs were recorded pretest, day 1, month 3 and month 6. The tracings were taken pre-dose, 1, 6 and 24 hours after dosing. Dogs were sampled for hematology, clinical chemistry and urinalysis pre-dose, 4, 13 and 25 weeks after dosing started. The eyes were examined pre-test and again at 3 and 6 months. Plasma profiles were taken from all dogs to determine evidence of absorption. Samples for this were collected at 0.5, 1,2,4 and 8 hours after dosing. The results were reported separately. At necropsy, various organs were weighed and the standard tissues collected for histopathology.

Results: Signs were mostly at ≥ 25 mg/kg and included mydriasis with decreased or loss of pupillary light reflexes, glazed eyes, ptosis and sedation. After the first week mydriasis was not seen until 4-5 hours after dosing. After the first month, mydriasis, glazed eyes and ptosis occurred sporadically in the MD and HD groups. The mydriasis appeared within several hours of dosing and persisted throughout the working day.
There were no discernible patterns in the body weight changes for either sex.
The only ECG data that was presented was heart rates for individual animals. The reviewer averaged the individual heart rate data provided. The summary is shown below.

Reviewer's Summary of mean heart rate data for the 6 month dog study: males

Dose (mg/kg/ day)	Pre-study	Day 1 (hrs post-dose)				3 months (hrs post dose)				6 months (hrs post-dose)			
[redacted]		BD	1	6	24	BD	1	6	24	BD	1	6	24
0	174	155	137	148	134	125	137	140	132	126	126	137	115
5	154	148	136	137	115	132	112	139	133	143	143	157	151
25	127	123	110	122	121	114	102	117	103	114	103	112	109
60	150	123	107	120	122	103	108	113	110	107	112	114	104

Reviewer's Summary of mean heart rate data for the 6 month dog study: females

Dose (mg/kg/ day)	Pre-study	Day 1 (hrs post-dose)				3 months (hrs post dose)				6 months (hrs post-dose)			
[redacted]		BD	1	6	24	BD	1	6	24	BD	1	6	24
0	133	130	112	125	113	104	112	109	116	117	117	124	110
5	135	128	117	141	138	143	116	128	114	115	124	139	125
25	142	120	113	124	116	114	104	118	117	108	101	125	125
60	149	130	135	132	156	118	122	115	131	105	109	118	112

In the hematology section, rouleaux were noted for 2 MDm dogs, all HD males and 2 MDf. Reticulocytosis appeared sporadically in all groups. One HD female sporadically showed the highest degree of reticulocytosis for the study.

Weight of the adrenal glands and testes were affected in HD males. Pituitary gland was affected in females. Relative organ weights were presented, but without any indication what the comparison was. Organ weight changes are summarized in the reviewer's table below.

Reviewer's summary of organ weight changes

Males				
	Dose group (mg/kg)			
	0	5	25	60
Adrenals absolute weight	0.70±0.07 (left) 0.77±0.09 (rt)	0.70±0.05 (left) 0.80±0.20 (rt)	0.74±0.12(left) 0.76±0.11(rt)	0.81±0.09(left) 0.88±0.12(left)
Relative weight	0.006±0.001(left) 0.007±0.001(rt)	0.006±0.000 0.006±0.001	0.007±0.001 0.007±0.000	0.007±0.001 0.008±0.002
Testes Absolute weight	13.43±.8 (left) 13.52±.52 (rt)	14.31±2.58 (left) 13.18±2.12 (rt)	13.26±1.14 (left) 13.15±1.02 (rt)	10.38±0.68 (left) 10.03±0.76(rt)
Relative weight	0.116±0.007(left) 0.117±0.007(rt)	0.115±0.011 0.106±0.010	0.116±0.011 0.115±0.007	0.090±0.009 0.087±0.009
Females				
Pituitary Absolute weight Relative	0.051±0.015 0.004±0.0001	0.073±0.014 0.0006±0.0000	0.071±0.029 0.0006±0.0003	0.080±0.011 0.0007±0.0002

Summary: Rouleaux formation is a grouping of erythrocytes that resembles a stack of coins. Degree of rouleaux tends to parallel the erythrocyte sedimentation rate, an indicator of inflammation or may be associated with a qualitative change in serum globulins (Duncan and Prasse). Rouleaux may appear to a mild degree in a healthy dog and to a more marked degree in inflammatory or neoplastic processes. The decrease in testicular weight in the HD males is of interest in light of the findings in the fertility study in which male rats showed decreased fertility.

Study title: One year oral toxicity study in dogs

Key study findings: The reporting is suboptimal. The signs in this study, seen primarily at 60 mg/kg/day, were significant in that salivation, sedation, trembling, convulsions, behavioral changes, ataxia and skin conditions were noted. The sponsor ascribes these to hypotension and/or cardiovascular collapse but does not present data to support this. Minimal information was presented concerning a possibly drug-related skin condition in the HD group. Ophthalmic examinations were conducted by a "veterinary consultant" and it is not made clear if this was a veterinary ophthalmologist.

Study no: AT6971/SS/028/94
Volume #, and page #: vol 30, p.3
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: March 23, 1993
GLP compliance:
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity:
Formulation/vehicle: capsules

Methods: Beagles (5/sex/group) were dosed once a day for 1 year with 0,10,25 or 60 mg/kg/day of ranolazine. Dogs were observed each day for signs and food consumption. Body weight was recorded weekly. Ophthalmic exams were performed pre-test and again during months 6 and 12 of the study by an "external veterinary consultant". Electrocardiography was conducted on all animals pre-test, day 1 and during the 5^{th} and 11^{th} month of dosing. ECGs were recorded before dosing and 1,6 and 24 hours after dosing. Blood was collected from all animals for clinical pathology pre-test and again after 1,3,6 and 12 months of dosing. Urinalysis was performed at similar timepoints. Plasma samples were collected after 6 and 12 months of dosing at 0, 0.5, 1,2,5 and 8 hours post dose. At necropsy, gross observations were made, some organ weights taken and tissues collected.

Results: Three out of 5 HD m developed an unspecified skin condition, which after extensive and inconclusive diagnostic work-up was reported to resolve spontaneously. A dermatologist evaluated the cases and came to the conclusion that

Appears This Way
On Original

APPENDIX E
Ranolazine : One Year Oral Toxicity Study in Dogs
Dermalotogists Report

Mr W D Taylor

2.

Routine chemistries, haematology and urinalysis appear unremarkable.

Having ruled out the common causes of a non-pruritic alopecia (i.e. demodicosis, dermatophytosis) diagnosis rests on the histopathological findings. These suggest an inflammatory disease. With the focal epidermal necrosis it would be impossible to rule out the possibility of a drug related toxicity, an immune mediated reaction or external contact or irritant dermatitis. A combination of events or factors may be involved, and to investigate this further a programme of elimination/provocation would be required.

I would be interested to see any further cases of this dermatosis as and when they occur. It is possible that by biopsying an "earlier" lesion we would get a more representative picture of the disease process.

Yours sincerely

Hilary A Farmer BVM&S, CertSAD, MRCVS
Resident in Dermatology

Signs were reported only for the HD group. Salivation was seen most frequently and in the females subdued behavior was also seen frequently (starting 1 hour after dosing and lasting ~ 1 hour). Additional signs were reportedly sporadic in occurrence and included subdued behavior, nervous or wary behavior, ataxia, lying or sitting in the corner of the pen, trembling and watery, glazed or half-closed eyes. Onset was reported as 1-2 hours of dosing with complete or partial recovery 4-6 hours after dosing. Three out of 5 females and 3/5 males in the HD group had convulsions on several occasions, between 21 and 94 minutes after dosing. The convulsions lasted 2-5 minutes during which time the animals were in lateral recumbency, paddling, unaware of the surroundings and panting. One out of 5 LD males convulsed on three occasions, twice before dosing and once 6.5 hours after dosing, in each case in association with some procedure.

There were no differences in body weight gain in the data as reported.

Study Month	Dose mg/kg/day		Na mmol/l	K mmol/l
Pretrial	All Groups	mean	150	4.19
		SD	2	0.24
1 Month	0	mean	145	3.99
		SD	1	0.18
	10	mean	144	3.92
		SD	2	0.17
	25	mean	145	4.13
		SD	1	0.31
	60	mean	145	4.13
		SD	1	0.21
3 Month	0	mean	148	3.92
		SD	1	0.11
	10	mean	147	4.05
		SD	1	0.14
	25	mean	148	3.99
		SD	1	0.14
	60	mean	149	4.09
		SD	1	0.16

Ophthalmic findings were reported as "...three animals, one of which was a control had any findings (corneal opacity, retinal folds and cataract). None of the findings was considered to be related to the treatment..."

Study Month	Dose mg/kg/day		Na mmol/l	K mmol/l
6 Month	0	mean	145	3.72
		SD	1	0.27
	10	mean	144	3.89
		SD	1	0.13
	25	mean	144	3.77
		SD	1	0.20
	60	mean	**148	3.88
		SD	2	0.27
12 Month	0	mean	148	3.7
		SD	0	0.3
	10	mean	147	4.2
		SD	2	0.3
	25	mean	148	4.0
		SD	1	0.2
	60	mean	150	*4.2
		SD	1	0.4

Electrocardiography: only the heart rates were reported. Dose dependent effects were not apparent in the data presented.

Clinical chemistry: there were no changes of biological significance apparent in the hematology data. Serum sodium was slightly increased in the HD males at 3, 6 and 12 months. Serum sodium and potassium were increased in the HD females at 3,6 and 12 months.

Absolute and normalized adrenal weights from all drug-treated groups of males and from the HD females weighed on average more than the control organs. Absolute and normalized kidneys in drug-treated animals of both sexes weighed more than those of the controls. The absolute and normalized weights of the uterus in the MD and HD groups were less than the control weights.

Table 7

Ranolazine: One year Oral Toxicity Study in Dogs

Organ Weights Adjusted for Bodyweight Group Summary - Males

Dose mg/kg/day		Terminal Bodyweight (g)	Adrenal Left	Adrenal Right	Brain	Heart	Kidney Left	Kidney Right	Liver	Pituitary	Prostate	Spleen	Testes Left	Testes Right	Thymus	Thyroid Left	Thyroid Right
0	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	14732	0.696	0.761	81.118	138.348	31.374	31.680	439.222	0.0879	11.691	75.555	13.195	12.831	19.495	0.384	0.381
	se	0.388	0.053	0.061	2.39	12.824	1.85	1.705	21.914	0.0073	2.041	10.203	0.837	0.887	2.166	0.048	0.036
10	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	14450	0.903	0.84	83.939	163.268	37.323	36.098	440.522	0.071	17.388	104.884	11.132	12.183	20.250	0.355	0.345
	se	0.388	0.054	0.062	2.422	12.998	1.874	1.728	22.207	0.0074	2.069	10.339	0.849	0.686	2.195	0.049	0.037
25	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	14858	0.765	0.821	81.016	135.265	32.946	32.875	428.809	0.0594	12.895	102.084	11.854	11.556	19.572	0.374	0.387
	se	0.388	0.053	0.061	2.401	12.884	1.858	1.713	22.015	0.0074	2.051	10.250	0.841	0.690	2.176	0.048	0.036
60	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	14784	0.819	0.84	83.241	138.920	33.783	33.658	466.312	0.0657	12.786	81.119	13.718	13.663	19.540	0.445	0.457
	se	0.388	0.053	0.061	2.393	12.839	1.852	1.707	21.938	0.073	2.044	10.214	0.838	0.688	2.168	0.048	0.036

n = number of animals

LS mean = least square mean

se = standard error of least square mean

Significance level of comparison with control (0 mg/kg/day) using Williams's test * = p<0.05

Table 6
Ranolazine: One year Oral Toxicity Study in Dogs
Organ Weights (g) Group Summary - Females

Dose mg/kg/day		Terminal Bodyweight (g)	Adrenal Left	Adrenal Right	Brain	Heart	Kidney Left	Kidney Right	Liver	Ovary Left	Ovary Right	Pituitary	Spleen	Thymus	Thyroid Left	Thyroid Right	Uterus
0	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	14160	0.71	0.75	74.92	105.31	27.01	26.9	431.15	0.86	1.00	0.088	74.17	16.78	0.34	0.4	13.73
	SD	1253.2	0.08	0.08	6.05	8.64	2.59	3.27	51.77	0.19	0.13	0.015	17.14	5.2	0.08	0.08	6.03
10	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	13314	0.78	0.79	81.66	106.55	28.02	28.25	388.63	1.08	0.9	0.08	87.41	14.51	0.33	0.45	14.34
	SD	1296.7	0.09	0.06	8.4	11	3.74	3.58	48.05	0.59	0.31	0.007	35.5	2.75	0.06	0.08	8.2
25	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	12996	0.66	0.73	82	105.98	27.11	26.96	366.58	0.83	0.77	0.078	67.15				8.39
	SD	2255.2	0.09	0.1	3.98	7.61	3.13	2.79	43.43	0.21	0.16	0.004	24.24				3.99
60	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	14208	0.82	0.91	82.54	124.2	29.26	28.64	484.83	0.8	0.88	0.068	75.43	14.83	0.42	0.44	9.96
	SD	1618.4	0.14	0.14	6.82	14.84	1.57	1.71	81.97	0.25	0.54	0.016	15.46	4.82	0.12	0.07	6.84

Table 6
Ranolazine: One year Oral Toxicity Study in Dogs
Organ Weights (g) Group Summary - Males

Dose mg/kg/day		Terminal Bodyweight (g)	Adrenal Left	Adrenal Right	Brain	Heart	Kidney Left	Kidney Right	Liver	Pituitary	Prostate	Spleen	Testes Left	Testes Right	Thymus	Thyroid Left	Thyroid Right
0	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	14732	0.7	0.76	81.15	138.59	31.41	31.72	440.07	0.068	11.66	75.43	13.17	12.82	19.62	0.36	0.38
	SD	381.2	0.05	0.13	4.86	19.8	3.2	3.88	61.77	0.023	3	27.44	2.69	2.55	6.84	0.1	0.1
10	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	14450	0.89	0.93	83.6	160.86	36.93	35.69	432.17	0.07	17.74	106.08	11.36	12.29	18.98	0.35	0.35
	SD	1066.2	0.17	0.15	4.38	41.36	6.16	4.82	52.78	0.012	6.24	24.70	2.37	1.07	4.53	0.14	0.06
25	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	14858	0.77	0.83	81.22	136.71	33.18	33.12	433.77	0.06	12.69	101.38	11.72	11.49	19.33	0.38	0.38
	SD	533.1	0.11	0.06	6.56	33.3	4.15	4.27	39.33	0.012	4.44	17.13	0.99	0.56	4.92	0.08	0.06
60	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	14784	0.82	0.84	83.34	139.65	33.9	33.78	468.86	0.066	12.68	80.76	13.65	13.63	19.93	0.45	0.45
	SD	201.9	0.15	0.19	5.15	11.71	2.42	2.38	63.68	0.015	4.01	18.98	1.31	1.18	8.43	0.08	0.1

Table 7
Ranolazine: One year Oral Toxicity Study in Dogs
Organ Weights Adjusted for Bodyweight Group Summary - Females

Dose mg/kg/day		Terminal Bodyweight (g)	Adrenal Left	Adrenal Right	Brain	Heart	Kidney Left	Kidney Right	Liver	Ovary Left	Ovary Right	Pituitary	Spleen	Thymus	Thyroid Left	Thyroid Right	Uterus
0	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	14160	0.718	0.755	73.858	103.871	26.499	26.541	425.522	0.658	1	0.088	71.3	15.954	0.336	0.389	14.168
	se	0.742	0.044	0.044	2.591	4.717	1.101	1.259	25.762	0.161	0.155	0.0055	10.574	1.792	0.035	0.026	3.217
10	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	13314	0.759	0.792	*82.555	107.481	28.354	28.485	390.486	1.081	0.899	0.08	89.273	15.049	0.332	0.453	14.058
	se	0.742	0.044	0.043	2.569	4.677	1.092	1.248	25.545	0.16	0.153	0.0055	10.485	1.777	0.035	0.026	3.184
25	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	12906	0.658	0.726	*83.537	108.064	27.843	27.481	374.689	0.84	0.765	0.078	71.299	15.493	0.373	0.356	7.758
	se	0.742	0.045	0.045	2.631	4.791	1.118	1.278	26.165	0.164	0.157	0.0056	10.74	1.82	0.036	0.026	3.261
60	n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	LS mean	14208	0.822	*0.909	*81.378	*122.628	28.708	29.451	478.877	0.792	0.886	0.088	72.289	14.018	0.416	0.424	10.436
	se	0.742	0.044	0.044	2.598	4.73	1.104	1.262	25.835	0.161	0.155	0.0056	10.604	1.797	0.035	0.026	3.22

n = number of animals
LS mean = least square mean
se = standard error of least square mean
Significance level of comparison with control (0 mg/kg/day) using William's test * = p<0.05

Appears This Way On Original

Prostatitis was reported for 0/5 (0), 2/5(LD), 1/5(MD) and 2/5(HD) dogs and aspermatogenesis was reported for 1/5 (LD) dogs and 0 for all other groups. No drug-associated histopath findings were reported for the adrenal glands.

Plasma level drug determination showed that the AUC increased with increasing dose in a greater than linear fashion. Females showed slightly greater exposure than males at the HD.

Flunarizine : One Year Oral Toxicity Study in Dogs
Group Mean Summary of Pharmacokinetic Parameters

Dose (mg/kg/day)	Sex	6 Month Timepoint		12 Month Timepoint	
		C_{max} (ng/ml)	AUC_{0-24h} (ng.h/ml)	C_{max} (ng/ml)	AUC_{0-24h} (ng.h/ml)
10	Male	960 ± 401	2997 ± 472	824 ± 587	3108 ± 533*
	Female	429 ± 412	1687 ± 648	1008 ± 1077	3423 ± 2926**
25	Male	2621 ± 1856	8784 ± 2601	2139 ± 1591	8203 ± 3177*
	Female	2256 ± 824	6864 ± 1990	3505 ± 1762	9073 ± 3924
60	Male	8768 ± 3979	33232 ± 9809	6536 ± 2592	29520 ± 12432
	Female	9716 ± 2811	34796 ± 5016	12816 ± 5688	40409 ± 12199

Values are expressed in terms of flunarizine dihydrochloride.
n=5 except *n=4, **n=2.

Appears This Way On Original

The urinalysis was essentially qualitative. Given this, it does not appear that there were significant findings in the urinalysis data.

The only data provided from the ECGs was heart rate, shown below. It may be seen that average heart rate for the drug-treated males decreased at the 1 hour post-dose observation point. There was no pattern apparent in the data for the females.

Reviewer's summary of mean heart rates (beats/min) males

dose	Pre-trial	Day 1 (hours after dose)				Month 5 (hours after dose)			
		Pre-dose	1	6	24	Pre-dose	1	6	24
0	74	74	78	88	75	73	92	97	85
10	126	114	100	113	104	93	91	121	106
25	122	113	98	125	108	106	97	110	119
60	109	105	104	109	111	106	96	104	104

Summary of heart rates for males continued

dose	Pre-trial	Month 11 (hours after dose)			
		Pre-dose	1	6	24
0	74	98	112	107	104
10	126	111	105	129	126
25	122	113	102	112	119
60	109	111	99	110	129

Reviewer's summary of mean heart rates (beats/min) females

dose	Pre-trial	Day 1 (hours after dose)				Month 5 (hours after dose)			
		Pre-dose	1	6	24	Pre-dose	1	6	24
0	120	104	94	114	93	94	98	123	108
10	109	111	97	112	115	113	103	128	118
25	120	107	89	110	106	104	105	126	120
60	112	108	91	99	100	102	105	119	119

Summary of heart rates for females continued

dose	Pre-trial	Month 11 (hours after dose)			
		Pre-dose	1	6	24
0	120	108	107	123	102
10	109	129	123	143	123
25	120	118	112	133	126
60	112	121	123	134	130

Study title: RS-43285 RHT: Three month oral toxicity study in rats

Key study findings: Unscheduled mortality was seen at 5 (1f), 250 (2m) and 500 (3m, 4f) mg/kg. Clinical signs were seen at doses ≥50 mg/kg. Signs included salivation (≥50 mg/kg), piloerection (≥50 mg/kg), prostration (≥250 mg/kg), hyperpnea (≥250 mg/kg), convulsions(≥250 mg/kg). The HD group also showed ptosis/sedation. The clinical chemistry findings at doses ≥ 250 mg/kg included decreased serum sodium, increased alkaline phosphatase and increased glucose. Absolute and relative liver and adrenal weight was increased at doses ≥50 mg/kg. Relative weight of the heart, pituitary, kidneys and spleen were also increased in both sexes at doses ≥250 mg/kg. Microscopic findings were reported for the HD only and included centrilobular hepatocyte enlargement, pulmonary alveolar foam cell proliferation and adrenal cortical cell vacuolation. Whether the adrenal effect is primary or secondary to effects on electrolytes cannot be determined from the available information. The urinalysis data was not provided nor was the complete pathology record made available as evidenced by textual references to findings in the 250 mg/kg group that were not presented in the pathology data. The manufacturing and stability data (Appendix I), noted that "placebo analysis indicated an RS-43285-193 content of less than 1.15×10^{-5} to less than 7.15×10^{-5} % w/w." It was not made clear if these are the limits of detection and quantitation or if ranolazine was present in the vehicle preparations.

Study no: AT3465, SS/012/85
Volume #, and page #: vol 16, p.224
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: September 1984
GLP compliance: statement included
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity:
Formulation/vehicle: sodium hydroxide and distilled water, pH=4; vehicle was distilled water

Methods: Sprague-Dawley, CD rats [illegible] 2 per sex per group given once daily oral doses of ranolazine at 0, 5, 50, 250 and 500 mg/kg/day for 91 days. Signs were evaluated daily around time of dosing. Body weight and food consumption were recorded weekly. Ophthalmoscopy was performed pre-treatment and during week 12. For hematology and clinical chemistry blood was collected from five animals per sex per group before the dosing phase. Ten rats per sex per group were sampled after 4,8 and 12 weeks of dosing. A four-hour urinalysis was performed for 5 animals per sex per group before dosing started and after 4,8, and 12 weeks of dosing. All animals surviving to scheduled necropsy were euthanized and examined. Specified organs were weighed and collected.

Results:
Unscheduled mortality: 1f @ 5mg/kg; 2m @ 250 mg/kg; 3m and 4f @500 mg/kg. No signs were reported for the lowest dose group. Clinical signs at 50 mg/kg were reported as infrequent and included salivation. Females given 250 mg/kg showed primarily salivation 10-30 minutes after dosing and sometimes prior to dosing. Males in this group were subdued and one also showed salivation and hunched posture. The HD animals showed salivation, subdued behavior, ptosis and mydriasis, prostration, convulsions and hyperventilation. Signs tended to resolve by approximately 6 hours after dosing. Signs were most evident in the first half of the study with females more affected than males, especially in weeks 1 and 2. The mydriasis and ptosis were usually seen in conjunction with convulsions, hyperventilation and in the majority of cases, death. Body condition and appearance was reported to deteriorate in the HD females.

HD males and females gained on average 21% and 8.8% less than their control groups respectively. Food consumption was somewhat decreased in the HD groups.

Ophthalmology: It was reported that there were no treatment-related effects. There was no evidence that a qualified ophthalmologist had made that decision or had evaluated the animals.

Hematology: There were some slight changes in hematology parameters that were marked as statistically significant. The biological significance, if any, is doubtful.

Clinical Chemistry: In both sexes, serum sodium was significantly decreased at 8 and 12 weeks in the two highest dose groups. Glucose was increased in the 2 highest dose groups of females as shown here. There were scattered changes in other parameters, but for the most part lacking in dose dependence or identifiable patterns.

Males Females

Time Point	Group Number	Dose mg/kg/day		Na mEq/l	K mEq/l	Urea mg/dl	Glucose mg/dl
Pre-dose	All		Mean	139	5.3	11.0	85
			SD	1	0.5	2.4	10
4 Weeks	1	0	Mean	152	5.1	15.6	123
			SD	1	0.3	2.0	19
	2	5	Mean	151	5.0	16.1	124
			SD	1	0.2	2.2	12
	3	50	Mean	151	4.9	15.2	135
			SD	1	0.3	2.4	25
	4	250	Mean	149**	4.8	18.4	137
			SD	2	0.3	5.7	37
	5	500	Mean	148**	5.1	16.5	125
			SD	2	0.4	2.7	15
8 Weeks	1	0	Mean	147	5.5	16.7	161
			SD	1	0.8	2.9	28
	2	5	Mean	146	5.3	19.0	206
			SD	1	0.6	1.7	41
	3	50	Mean	146	5.0	18.4	164
			SD	1	0.5	1.5	52
	4	250	Mean	145**	5.1	19.3	176
			SD	2	0.4	6.7	35
	5	500	Mean	144**	5.4	18.5	167
			SD	1	0.9	3.1	42
12 Weeks	1	0	Mean	145	4.8	13.3	191
			SD	1	0.4	0.9	48
	2	5	Mean	147	4.9	13.5	168
			SD	2	0.4	1.9	51
	3	50	Mean	146	5.2	14.9	174
			SD	1	0.6	1.7	40
	4	250	Mean	146	5.5	16.0**	165
			SD	1	0.4	1.7	32
	5	500	Mean	145	4.7	16.0**	157
			SD	1	0.5	2.9	28

* p < 0.05
* p < 0.01

Time Point	Group Number	Dose mg/kg/day		Na mEq/l	K mEq/l	Urea mg/dl	Glucose mg/dl
Pre-dose	All		Mean	139	4.9	15.5	101
			SD	1	0.4	1.8	13
4 Weeks	1	0	Mean	148	5.4	22.1	120
			SD	1	0.9	2.3	16
	2	5	Mean	149	4.9	20.4	153
			SD	2	0.7	2.8	37
	3	50	Mean	148	5.0	20.7	123
			SD	2	0.8	3.0	8
	4	250	Mean	147	4.8	22.7	149**
			SD	1	0.6	3.9	24
	5	500	Mean	147	4.3**	18.8*	207**
			SD	1	0.4	2.9	17
8 Weeks	1	0	Mean	149	5.2	21.1	161
			SD	1	0.6	3.1	28
	2	5	Mean	150	4.6	20.5	207
			SD	1	0.5	2.6	30
	3	50	Mean	149	4.8	22.1	161
			SD	0	0.7	3.8	19
	4	250	Mean	148*	5.3	17.9*	209**
			SD	1	0.4	2.0	33
	5	500	Mean	148*	4.6**	17.5*	210**
			SD	1	0.4	0.4	29
12 Weeks	1	0	Mean	145	5.3	19.8	137
			SD	1	0.6	2.8	38
	2	5	Mean	145	4.5	20.3	157
			SD	1	0.7	2.6	28
	3	50	Mean	144**	4.8	20.4	123
			SD	1	0.7	1.7	13
	4	250	Mean	143**	4.9	19.3	165*
			SD	1	0.8	2.6	28
	5	500	Mean	142**	4.7	18.7	168*
			SD	2	0.5	2.4	29

* p < 0.05
** p < 0.01

Appears This Way
On Original

Alkaline phosphatase was increased in both sexes at the two highest doses.

Reviewer's summary of Alkaline Phosphatase Values.

timepoint	Dose mg/kg/day	Alkaline phosphatase (IU/l)	
		females	males
predose	all	128±26	214±31
4 weeks	0	65±17	95±18
	5	61±13	99±20
	50	65±15	102±20
	250	85*±19	109±31
	500	129**±58	150**±33
8 weeks	0	50±15	74±14
	5	50±15	73±16
	50	57±19	75±19
	250	65±24	85±17
	500	84**±25	111*±43
12 weeks	0	35±15	56±11
	5	29±9	55±12
	50	43±14	57±18
	250	55*±19	68±17
	500	63**±24	91**±35

Organ weights: absolute liver and adrenal weight was increased in both sexes at doses ≥ 50 mg/kg. Absolute spleen weight was increased in the females while kidney weight was increased in the males. Relative weight of the heart, pituitary, liver, kidneys, spleen and adrenals were increased in both sexes.

RS-61285 SAT : Three Month Oral Toxicity Study in Rats

Organ Weights as a Percentage of Bodyweight Group Summary

Group Number	Dose mg/kg/day		Brain	Heart	Testes	Pituitary x1000	Liver	Prostate	Kidneys	Spleen	Adrenals	Thymus	Thyroids x1000	Body Weight (g)
MALES														
1	0	Mean	0.45	0.32	1.23	3.46	3.01	0.21	0.70	0.18	0.014	0.08	6.07	491
		SD	0.05	0.02	0.12	0.49	0.21	0.06	0.08	0.03	0.002	0.01	1.32	42
2	5	Mean	0.46	0.32	1.14	3.28	2.98	0.22	0.69	0.19	0.017	0.09	6.65	490
		SD	0.09	0.02	0.22	0.44	0.25	0.07	0.04	0.02	0.003	0.02	0.82	35
3	50	Mean	0.47	0.32	1.25	3.21	3.11	0.22	0.72	0.19	0.018*	0.09	5.91	482
		SD	0.03	0.02	0.12	0.30	0.33	0.04	0.07	0.02	0.002	0.01	1.67	33
4	250	Mean	0.44	0.33	1.21	2.93**	3.52***	0.24	0.77**	0.28	0.024***	0.11***	6.34	502
		SD	0.05	0.02	0.14	0.47	0.32	0.05	0.07	0.02	0.005	0.02	1.14	47
5	500	Mean	0.49	0.39*	1.38*	3.66	3.99***	0.19	0.89***	0.23***	0.031***	0.09	7.66**	433
		SD	0.03	0.05	0.07	0.46	0.42	0.04	0.07	0.04	0.006	0.02	0.93	25

Group Number	Dose mg/kg/day		Brain	Heart	Ovaries	Pituitary x1000	Liver	Uterus	Kidneys	Spleen	Adrenals	Thymus	Thyroids x1000	Body Weight (g)
FEMALES														
1	0	Mean	0.71	0.38	0.057	6.42	3.33	0.36	0.80	0.21	0.030	0.12	9.16	277
		SD	0.06	0.03	0.010	1.09	0.54	0.07	0.09	0.03	0.006	0.03	1.56	23
2	5	Mean	0.72	0.40	0.062	6.96	3.17	0.31	0.80	0.21	0.030	0.13	8.34	280
		SD	0.07	0.03	0.010	1.15	0.31	0.06	0.06	0.03	0.006	0.02	1.61	22
3	50	Mean	0.75	0.41	0.067	7.83**	3.61	0.35	0.85	0.24	0.035	0.13	10.26	273
		SD	0.06	0.05	0.016	1.50	0.38	0.10	0.10	0.04	0.006	0.02	1.63	25
4	250	Mean	0.73	0.43*	0.072*	6.94	4.15***	0.31	0.86	0.26**	0.048***	0.15**	9.95	272
		SD	0.05	0.03	0.019	1.37	0.50	0.07	0.06	0.05	0.006	0.02	1.58	11
5	500	Mean	0.77**	0.44**	0.059	7.77*	4.54***	0.39*	0.91**	0.29***	0.087***	0.15*	10.01	248
		SD	0.04	0.03	0.009	0.85	0.52	0.10	0.10	0.05	0.010	0.03	2.05	17

* p 0.05
** p 0.01
*** p 0.001

Best Possible Copy

TABLE 5

RS-01285 NHP : Three Month Oral Toxicity Study in Rats

Organ Weights Group Summary (g)

Group Number	Dose mg/kg/day		Brain	Heart	Testes	Pituitary	Liver	Prostate	Kidneys	Spleen	Adrenals	Thymus	Thyroids	Body-Weight (g)
MALES														
1	0	Mean	2.19	1.58	5.97	0.017	14.75	1.03	3.39	0.91	0.071	0.49	0.030	491
		SD	0.11	0.09	0.43	0.002	1.37	0.27	0.27	0.14	0.010	0.07	0.007	42
2	5	Mean	2.32	1.55	5.61	0.016	14.65	1.09	3.38	0.91	0.081*	0.46	0.029	490
		SD	0.32	0.10	1.12	0.002	1.76	0.31	0.22	0.11	0.014	0.10	0.005	35
3	50	Mean	2.25	1.55	6.01	0.015	14.56	1.08	3.46	0.89	0.085**	0.44	0.028	482
		SD	0.10	0.11	0.50	0.002	1.57	0.21	0.32	0.08	0.011	0.08	0.008	33
4	250	Mean	2.18	1.64	6.01	0.015**	17.65***	1.20	3.86***	1.02	0.122***	0.55***	0.032	502
		SD	0.08	0.12	0.39	0.002	2.03	0.29	0.26	0.18	0.025	0.10	0.006	47
5	500	Mean	2.12	1.70*	5.98	0.016	17.37***	0.85	3.85***	1.01	0.136***	0.41	0.033	435**
		SD	0.07	0.25	2.39	0.002	2.13	0.19	0.27	0.22	0.030	0.12	0.004	35

Group Number	Dose mg/kg/day		Brain	Heart	Ovaries	Pituitary	Liver	Uterus	Kidneys	Spleen	Adrenals	Thymus	Thyroids	Body-Weight (g)
FEMALES														
1	0	Mean	1.95	1.06	0.157	0.018	9.18	0.82	2.21	0.57	0.083	0.33	0.025	277
		SD	0.13	0.07	0.029	0.003	1.41	0.21	0.30	0.08	0.014	0.09	0.004	23
2	5	Mean	2.04	1.11	0.177	0.020	8.99	0.86	2.27	0.60	0.086	0.37	0.023	283
		SD	0.18	0.08	0.043	0.003	1.25	0.13	0.18	0.08	0.020	0.04	0.004	22
3	50	Mean	2.03	1.11	0.182	0.021**	9.82	0.96	2.31	0.64	0.096	0.34	0.026	273
		SD	0.10	0.13	0.039	0.004	1.17	0.22	0.20	0.10	0.013	0.07	0.005	25
4	250	Mean	1.99	1.16*	0.197*	0.019	11.30***	0.84	2.33	0.70**	0.130***	0.41*	0.027	272
		SD	0.14	0.09	0.057	0.003	1.43	0.18	0.18	0.12	0.017	0.05	0.004	11
5	500	Mean	1.91	1.08	0.146	0.019	11.30**	0.96	2.25	0.72**	0.166***	0.36	0.025	248***
		SD	0.10	0.11	0.022	0.002	1.64	0.24	0.25	0.12	0.028	0.09	0.005	17

* p < 0.05
** p < 0.01
*** p < 0.001

Appears This Way On Original

Necropsy/microscopic findings were reported only for the highest dose group of each sex. These are summarized below in the reviewer's table.

Reviewer's summary of microscopic findings

finding	Males	Females
Centrilobular hepatocyte enlargement	0/12(c), 1/12 hd	0/12(c), 7/12 hd
Pulmonary alveolar foam cell proliferation	1/12(c), 6/12 hd	0/12(c), 2/12 hd
Adrenal cortical vacuolation	0/12(c), ¾ (250 mg/kg), 12/12 hd	0/12(c), 7/12 hd
Adrenal cortical necrosis	0/0 (c), 0/0 (c)	0/0 (c), 1/12 hd

The sponsor states in the text (p.251) that adrenal cortical vacuolation occurred in 3/3 males in the 250 mg/kg group. In the tables of microscopic findings presented on pages 264-266, findings are presented for control and 500 mg/kg groups only. Individual animal pathology data was reported pages 312 to 339 but for fewer animals than were in each group. The data was not complete pathology records but selected findings. Was this the sum total of what was generated? Why was this not summarized in the incidence tables also?

Summary: The urinalysis data was not provided and it was not clear if the complete pathology data was provided. Unscheduled mortality was seen at 5, 250 and 500 mg/kg. Clinical signs were seen at doses ≥50 mg/kg. Signs included salivation (≥50 mg/kg), piloerection (≥50 mg/kg), prostration (≥250 mg/kg), hyperpnea (≥250 mg/kg), convulsions (≥250 mg/kg). The clinical chemistry (decreased serum sodium and increased alkaline phosphatase) and microscopic findings (adrenal vacuolation) are supportive of adrenal involvement. Whether the adrenal effect is primary or secondary to effects on electrolytes cannot be determined from the available information. Hepatic enlargement was noted both in the organ weight data and in the microscopic findings. Another finding of note was an increased incidence of pulmonary alveolar foam cell proliferation. A complete assessment of the drug-associated effects cannot be made from the report.

Study title: RS43285 RJT: Six month oral toxicity study in rats with a one month recovery period

Key study findings:
Clinical signs were reported for doses of ≥50 mg/kg/day and included the salivation, sedation, prostration, tachypnea or shallow breathing, hunched appearance, ataxia, piloerection and convulsions noted in other studies. Signs were usually present within 1-2 hours after dosing and showed partial to complete resolution by 4-6 hours post-dose. Target cells, reported in the hematology results, may be associated with altered cholesterol and phospholipid content of RBC membranes, usually due to changes in liver function. Serum sodium, potassium, glucose and cholesterol were affected in both sexes. In the HD groups of both sexes, LDH and HDBH (hydroxybutyrate dehydrogenase) were increased. Results for pathology, ophthalmoscopy and urinalysis were poorly presented and not susceptible to easy interpretation. However, adrenal organ weights and histopathology support the adrenal gland as a target organ as well as alterations in liver function and possibly cardiac muscle damage.

Study no: AT3935, SS//047/87
Volume #, and page #: vol 18., p.3
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: August 1986
GLP compliance: statement included
QA report: yes () no (x)
Drug, lot #, radiolabel, and % purity: lot number 15 aka 153SS0785
Formulation/vehicle: control was dH_2O

Methods: Sprague-Dawley CD rats [redacted] were assigned to groups with 30 males and 30 females in the control and HD groups and 20 males and females in each of the LD and MD groups. All rats were dosed for at least 182 days by oral gavage. At the end of the dosing period, all LD and MD animals and the first 20 rats in the control and HD groups were

euthanized. The remaining 10 rats in the control and HD groups were given a 30 day drug-free recovery period. Doses used were 0,5,50 and 200 mg/kg.

Blood for determination of plasma level drug values was taken at one, three and six months from animals not sampled for clinical pathology (approximately 10/sex/group). Clinical signs were monitored each day. Bodyweight and food consumption were recorded weekly. Ophthalmoscopy was performed before dosing started and again in weeks 12 and 25. Ten animals per sex per group were sampled for clinical pathology before dosing, weeks 4, 13 and 25 of dosing. Urinalysis was performed on samples obtained over a 4 hour period in metabolism cages (8 animals per sex per group) before dosing started and again at 4,13 and 25 weeks of dosing.

All animals that died on study were examined as were the animals surviving to scheduled termination. The latter group of animals were euthanized 24 hours after the last dose. Organ weights and microscopic evaluation were made for a specified list of tissues. Adrenals from all recovery group animals were examined. The sponsor stated on p. 22 that " As treatment-related changes at the terminal kill were confined to the adrenals and liver Oil-Red-O, histopathological examination of altered tissue in the recovery groups was considered unnecessary."

Liver samples were taken at euthanasia from 8 animals/sex for the control and MD groups. Hepatic levels of CYP450 and microsomal protein were reported separately. Adrenals from all recovery group animals were examined. The sponsor states

Recovery Groups
Adrenals from all recovery group animals in groups 1 and 4 were examined. As treatment-related changes at the terminal kill were confined to the adrenals and liver Oil-Red-O, histopathological examination of altered tissue in the recovery groups was considered unnecessary.

Results: One LDf and 3HDm died or were euthanized in extremis. Clinical signs included salivation, seen within 10 minutes of dosing in the MD and HD groups. Signs in the HD group included subdued behavior, hunched appearance, ataxia, prostration, tachypnea, piloerection, ptyalism and convulsions. Signs usually occurred within 1-2 hours of dosing with partial to complete resolution of signs 4-6 hours post-dose.
Bodyweights: baseline values were not provided. It does appear that in the first month the treated rats gained less than the control group. The sponsor's table is shown here.

RS 43285 RJT : Six Month Oral Toxicity Study in Rats

Group Mean Bodyweights (g) - Day 1 and 24 Weeks - Groups 1 and 4

Group No./Sex	Dose mg/kg/day	Sub-Group	Day 1	24 Weeks
MALES				
1	0	Non-recovery	257	590
		Recovery	252	602
4	200	Non-recovery	256	605
		Recovery	251	533
FEMALES				
1	0	Non-recovery	186	326
		Recovery	188	319
4	200	Non-recovery	185	318
		Recovery	175	309

Best Possible Copy

Food consumption appeared to be comparable between the groups.
Hematology: Target cells were reported for the HD animals of both sexes (7/10 males, 4/10 females) at 25 weeks. The sponsor suggests this as a cause for the slight increase in MCV. Neutrophil counts were consistently higher in drug-treated males compared to the control group. The same trend was not apparent in the females.

Reviewer's summary of neutrophil counts x 10^9/l in male rats

Dose group Mg/kg	Time point				
	pre	4weeks	13weeks	25 weeks	Recovery
0	1.2±0.5	1.9±0.6	2.7±0.9	2.4±1.0	2.3±0.6
5		2.5±1.1	2.6±1.8	4.1±4.6	
50		2.8±1.3	2.3±1.3	3.1±1.2	
200		3.2*±1.0	3.4±3.1	4.4±2.6	2.8±0.9

Clinical chemistry: in both sexes, Na, K, glucose and cholesterol were affected. Of concern also is that at the HD in both sexes, LDH and HBDH were also elevated. The elevation of CK, LDH and HBDH together is suggestive of cardiac muscle damage. While CK was not measured, the consistent elevation of LDH and HBDH in the HD, both sexes, is noteworthy.

RS 41285 BJT : Six Month Oral Toxicity Study in Rats

Clinical Chemistry Group Summary - Males

Week	Group Number	Dose mg/kg/day		Na mEq/l	K mEq/l	Urea mg/dl	Glucose mg/dl	ALAT IU/l	ASPAT IU/l	LDH IU/l	HBDH IU/l	Alk.P IU/l	Cholest. mg/dl	Trig. mg/dl	NEFA mEq/l
25	1	0	Mean	144	4.9	32.4	155	23	41	125	36	96	91	115	0.8
			SD	1	0.4	4.6	29	5	5	38	10	18	11	46	0.3
	2	5	Mean	144	5.1	30.6	155	24	38	122	36	96	101	112	0.9
			SD	1	0.5	2.6	39	6	5	23	8	26	19	47	0.3
	3	50	Mean	146***	5.0	31.6	129	24	39	137	38	109	107	108	1.0
			SD	1	0.4	2.9	35	5	8	24	6	20	27	42	0.3
	4	200	Mean	144	5.3	31.0	136	23	38	191**	51**	106	123**	108	0.8
			SD	1	0.3	4.6	32	6	5	50	13	34	24	32	0.2
4 Recovery	1	0	Mean	146	5.2	29.8	124	27	31	81	26	93	103	171	1.2
			SD	1	0.4	4.6	15	7	4	32	9	36	17	69	0.3
	4	200	Mean	145	5.2	34.0	159	24	32	87	28	112	94	172	1.0
			SD	1	0.4	4.0	42	5	3	47	13	29	27	107	0.4

** p less than 0.01
*** p less than 0.001

Clinical Chemistry Group Summary - Males

Week	Group Number	Dose mg/kg/day		Na mEq/l	K mEq/l	Urea mg/dl	Glucose mg/dl	ALAT IU/l	ASPAT IU/l	LDH IU/l	HBDH IU/l	Alk.P IU/l	Cholest. mg/dl	Trig. mg/dl	NEFA mEq/l
Pre-dose	All	-	Mean	144	5.2	25.9	97	21	44	103	31	367	118	108	1.2
			SD	1	0.5	5.2	18	4	4	33	9	73	21	36	0.2
4	1	0	Mean	152	5.0	35.0	146	21	38	113	32	215	92	101	1.3
			SD	1	0.4	3.8	37	3	3	53	14	30	13	27	0.2
	2	5	Mean	152	5.0	33.4	123	23	40	110	31	202	100	97	1.5
			SD	1	0.4	3.1	16	3	4	25	7	45	13	41	0.2
	3	50	Mean	152	4.9	34.9	115*	23	38	129	35	217	99	81	1.5
			SD	1	0.3	4.0	18	4	2	62	15	44	17	18	0.2
	4	200	Mean	151**	5.1	33.3	125*	25*	40	140	37	206	123**	93	1.5
			SD	1	0.4	3.1	17	5	2	22	6	47	22	33	0.3
13	1	0	Mean	147	5.0	37.0	124	22	43	111	33	124	92	97	0.9
			SD	2	0.3	7.0	22	4	11	33	10	18	11	41	0.2
	2	5	Mean	146	5.2	34.2	131	21	38	122	34	109	95	91	0.9
			SD	1	0.4	2.4	22	3	3	39	11	26	16	51	0.2
	3	50	Mean	149*	4.9	35.7	119	20	36	120	32	121	98	79	1.0
			SD	1	0.2	4.1	26	3	3	23	6	19	23	23	0.2
	4	200	Mean	148*	5.1	32.8	119	21	38	137	37	123	121**	94	1.1
			SD	2	0.3	3.1	18	5	2	49	12	33	21	33	0.2

* p less than 0.05
** p less than 0.01

RS 43285 RJT : Six Month Oral Toxicity Study in Rats

Clinical Chemistry Group Summary - Females

Week	Group Number	Dose mg/kg/day		Na mEq/l	K mEq/l	Urea mg/dl	Glucose mg/dl	ALAT IU/l	ASPAT IU/l	LDH IU/l	HBDH IU/l	Alk.P IU/l	Cholest. mg/dl	Trig. mg/dl	NEFA mEq/l
Pre-dose	All	-	Mean	141	5.1	35.5	93	16	42	173	48	246	99	57	0.8
			SD	1	0.5	5.3	14	2	4	58	15	44	20	15	0.2
4	1	0	Mean	146	5.3	37.3	107	20	40	128	35	112	90	56	1.4
			SD	2	0.5	3.7	12	2	4	41	14	14	11	20	0.4
	2	5	Mean	147	4.8*	36.5	107	24	43	124	34	116	103	58	1.5
			SD	1	0.5	4.5	12	8	9	51	14	21	11	20	0.2
	3	50	Mean	147	4.9	40.1	105	28	42	106	30	121	118**	62	1.2
			SD	2	0.5	5.2	13	21	13	30	9	23	22	15	0.4
	4	200	Mean	145	4.6**	43.0**	130	29**	41	190*	51*	125	157**	59	1.1
			SD	1	0.5	4.6	44	6	5	92	23	23	27	17	0.3
13	1	0	Mean	148	4.8	37.7	113	22	42	113	34	58	99	72	1.2
			SD	1	0.5	5.5	17	9	13	30	8	14	16	17	0.2
	2	5	Mean	147	5.0	39.0	103	28	41	109	32	61	112	73	1.2
			SD	1	0.4	9.5	8	15	9	35	9	10	11	18	0.2
	3	50	Mean	148	4.8	35.3	116	18	35	102	30	55	125**	76	1.2
			SD	1	0.3	3.9	23	5	3	20	5	10	22	18	0.2
	4	200	Mean	148	5.1	36.7	147*	22	33*	148	42	63	164**	79	1.2
			SD	1	0.6	5.5	38	5	4	79	20	14	23	26	0.5

* p less than 0.05
** p less than 0.01

TABLE 4

RS 43285 RJT : Six Month Oral Toxicity Study in Rats

Clinical Chemistry Group Summary - Females

Week	Group Number	Dose mg/kg/day		Na mEq/l	K mEq/l	Urea mg/dl	Glucose mg/dl	ALAT IU/l	ASPAT IU/l	LDH IU/l	HBDH IU/l	Alk.P IU/l	Cholest. mg/dl	Trig. mg/dl	NEFA mEq/l
25	1	0	Mean	140	5.1	34.9	122	42	59	124	35	39	122	95	n/a
			SD	1	0.5	4.3	28	25	35	12	8	8	17	33	
	2	5	Mean	140	4.6*	36.8	125	73	84	126	35	51*	138	104	1.1
			SD	1	0.5	7.3	23	50	49	34	9	13	34	31	0.5
	3	50	Mean	140	4.3*	33.3	133	44	50	113	32	48*	146*	110	1.0
			SD	1	0.5	4.0	30	37	23	26	7	10	24	22	0.3
	4	200	Mean	138**	4.7*	36.1	134	42	42	153	37	47*	198**	129	1.1
			SD	1	0.7	1.8	22	31	18	97	23	9	29	65	0.5
4 Recovery	1	0	Mean	143	5.1	36.7	169	50	52	126	38	59	150	168	1.4
			SD	1	0.5	4.4	28	20	20	64	17	23	27	41	0.4
	4	200	Mean	143	5.1	33.6	131	33	38	121	36	46	137	204	1.4
			SD	1	0.5	4.4	29	18	11	54	14	19	20	117	0.4

n/a - Mean and SD not applicable. Sample size equal to 3

* p less than 0.05
** p less than 0.01

Ophthalmoscopy and plasma drug levels were not presented. The report does not specify who actually performed the ophthalmic exam, that is, was it a staff veterinarian or a veterinary ophthalmologist?

Urinalysis data was presented for single animals in a form that did not lend itself to easy interpretation. Single letter abbreviations were used to indicate the parameter being measured with no key. Despite this, there appeared to be a slight increase in ketones, bile pigments, blood pigments and urobilinogen in the HD males at 13 and 25 weeks. There was also an increase in the incidence and degree of changes in the "A" column. The HD females showed a slight increase in urinary bile pigments.

The sponsor reports gross adrenal enlargement in 1/20 MDf, 1/20 HDm, 9/20HDf.
Organ weights: liver, adrenal, heart and kidney weights were increased in both sexes, with the effects more pronounced in the females. This is summarized in the reviewer's table below.

Reviewer's summary of organ weight data

Males : dose mg/kg/day				
	0	5	50	200
adrenal	0.063±0.01	0.065±0.01	0.065±0.011	0.089**±0.016
heart	1.65±0.18	1.75±0.18	1.75±0.28	1.78±0.25
Kidney	3.73±0.40	3.50±0.53	3.75±0.40	4.03±0.52
Liver	16.23±2.96	15.42±2.16	16.08±2.11	17.66±2.71
Females: dose mg/kg/day				
adrenal	0.074±0.011	0.071±0.012	0.083±0.019	0.108**±0.019
heart	1.13±0.11	1.04*±0.11	1.11±0.09	1.22**±0.13
Kidney	2.35±0.30	2.22±0.22	2.33±0.24	2.58*±0.33
Liver	9.48±1.09	9.08±0.95	9.99±1.22	11.04**±1.51
spleen	0.54±0.06	0.54±0.08	0.59*±0.08	0.65**±0.07
pituitary	0.017±0.004	0.017±0.003	0.020*±0.004	0.021**±0.004

*p<0.05, **p<0.01

There were no apparent organ weight effects in the recovery males. Liver and adrenal weight was still increased in the females at the end of the recovery period.

Reviewer's summary of end of recovery weights for females

	Control group at recovery end	HD at recovery end
Adrenal gland	0.067±0.017	0.082*±0.011
liver	8.92±0.63	9.64±1.23

*p<0.05

The only microscopic findings of apparent significance concerned the adrenal gland.
Vacuolation was reported only for males. Cytoplasmic foaminess was reported only for females.
This is summarized in the reviewer's table below.

Reviewer's summary of adrenal gland findings

	Dose group (mg/kg/day)			
	0	5	50	200
Diffuse vacuolation of the zona fasiculata (males only)	0/20	6/20	9/20	18/18
Diffuse cytoplasmic foaminess of the zona fasiculata (females only)	0/20	0/20	0/20	17/20

Of the 4 unscheduled deaths (p.32), 3 showed moderate/marked diffuse cytoplasmic vacuolation of the fasciculata cells. These 3 animals included a recovery male. The female was the1/4 with no histologic evidence of change. The sponsor also notes that these animals were not tabulated. In the recovery males, vacuolation of the zona fasciulata was still present in all HD animals but was "reduced." In one animal the findings were "marked."

Summary: Target cells may be associated with altered cholesterol and phospholipid content of RBC membranes, usually due to changes in liver function. Serum sodium, potassium, glucose and cholesterol were affected in both sexes. In the HD groups of both sexes, LDH and HDBH (hydroxybutyrate dehydrogenase) were increased. Results for pathology, ophthalmoscopy and urinalysis were poorly presented and not susceptible to easy interpretation. However, adrenal organ weights and histopathology support the adrenal gland as a target organ as well as alterations in liver function and possibly cardiac muscle damage. In the Discussion section (p.36), the sponsor states that:

> The normal foamy appearance of cells of the zona fasciculata is due to the presence of lipid which may serve as a precursor of steroid biosynthesis. The vacuolation and increased foamy appearance of these cells after administration of RS 43285 is the probable result of accumulation of cytoplasmic lipid. Similar changes have been observed with a number of compounds which inhibit steroidogenesis[9,10]. However, in contrast to such compounds as aminoglutethimide, amphenone and other corticosteroid inhibitors administration of RS 43285 for six months does not appear to induce cortical cell degeneration, atrophy or necrosis[10]. Following the one month recovery period the effect was diminished in top dose males and was no longer evident in top dose females.

> Although the changes in these rat adrenals may be representative of a functional disturbance in the cells of the zona fasciculata it should be emphasised that no firm evidence of adrenocortical insufficiency was observed even at the highest dose level in this six month study with RS 43285.
>
> It may be concluded that oral administration of RS 43285 for six months produced no adverse effects in female rats at 5 mg/kg/day. Evidence of altered activity in adrenal cells was observed in male rats at 5 mg/kg/day. However the changes are reversible and may be attributable to the pharmacological activity of the compound.

The PK data for this study was not located in the submission. A study conducted in 1994 used doses of 2,5, 50 and 150 mg/kg daily for 6 months (AT6811). Doses of ≥50 mg/kg/day showed a tendency towards increasing plasma exposure over time.

Summary of comparable pk

	Females 150 mg/kg/day			Males 150 mg/kg/day		
	Rat AUC_{0-24} ng.hr/ml	Human AUC_{0-24}	Rel exposure	Rat AUC_{0-24} ng.hr/ml	Human AUC_{0-24}	Rel exposure

Day 1	99800	Tid dosing	1.48X	59500	33700	0.89
Day 92	126000	33700	1.87	63600		0.95
Day 183	167000		2.48	127000		1.89

While the sponsor identifies a NOEL for females at 5 mg/kg and for males at 2mg/kg, this does not equal the therapeutic exposure that a human could expect to achieve.

Study title: One year oral toxicity study in rats

Key study findings: Signs seen ≥50 mg/kg included salivation and/or heavy staining of the muzzle, subdued behavior, ataxia, gasping or irregular breathing and half closed eyes. Onset was generally within 1-2 hours of dosing with complete or partial recovery 4-6 hours after dosing. Convulsions were reported for a 20 mg/kg female and a 200 mg/kg male.

The HD males had gained on average 15% less body weight than the control group ($p<0.05$ by Student's t test). The females in the 20, 50 and 200 mg/kg groups gained on average 12-8% more than the control group (NS). The weekly food consumption data does not indicate significant differences between the treatment groups.
Both sexes at the HD showed slight decreases in Hb and MCHC and slight increases in reticulocyte count. Both sexes at the HD also showed slight increases in platelet count. The adrenal gland was again a target organ in the females. A drug effect was seen in increased incidences of pneumonia (described by the sponsor as inhalational) in both sexes of drug-treated animals. The sponsor hypothesized the possibility of a long-term effect on pharyngeal/esophageal muscle tone.

Study no: AT6544/ SS/003/93
Volume #, and page #: vol 28, 113
Conducting laboratory and location: Syntex Research, Edinburgh, Scotland
Date of study initiation: November 5, 1991
GLP compliance:
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity: E6-ML-001
Formulation/vehicle: aqueous buffered solution. Water was used as the vehicle control

Methods Male and female rats CD(SD)BR), 20/sex/group were orally gavaged once a day for 1 year with 0,20,50 or 200 mg/kg/day ranolazine. Rats were observed daily for signs. Body weights were recorded weekly and food consumption was estimated weekly. Blood was collected for clinical pathology pre-test and after 4, 8 and 12 months of dosing. Blood for plasma level determination of drug was collected after 6 and 12 months of dosing. Samples were collected from 10 rats/sex/group. Those not sampled for clinical pathology were sampled for plasma level drug determination at 30 mins., 1.5,3,6 and 24 hours after dosing. Urinalysis was performed at similar timepoints. At time of necropsy, tissues were collected from all animals but examined only from the HD, control groups and premature decedents. Lungs and adrenals were examined from all animals.

Results: Four animals were euthanized (1FC, 2 M 2 mg/kg, 1F 50 mg/kg) were euthanized due to the size of SQ masses. 12 animals were found dead with little to no reported signs: 2m/2f (controls); 1m (20 mg/kg);1m/1f (50 mg/kg); 4f/1m (200 mg/kg). 10 more animals were euthanized due to marked clinical signs including hunched appearance, weight loss, labored or irregular breathing, convulsion, piloerection and subdued behavior. The total number of unscheduled mortalities was summarized by the sponsor as shown below:

Summary of deaths

Dose of ranolazine (mg/kg/day)	Number of animals M/F	Deaths M/F
0	20/20	4/3
2	20/20	2/0
20	20/20	1/0
50	20/20	1/3
200	20/20	5/7

Signs seen at doses ≥50 mg/kg included salivation and/or heavy staining of the muzzle, subdued behavior, ataxia, gasping or irregular breathing and half closed eyes. Onset was generally within 1-2 hours of dosing with complete or partial recovery 4-6 hours after dosing. Convulsions were reported for a 20 mg/kg female and a 200 mg/kg male.

By week 52, the HD males had gained on average 15% less body weight than the control group ($p<0.05$ by Student's t test). The females in the 20, 50 and 200 mg/kg groups gained on average 12-8% more than the control group (NS). The weekly food consumption data does not indicate significant differences between the treatment groups.

Hematology: Both sexes at the HD showed slight decreases in Hb and MCHC and slight increases in reticulocyte count. Both sexes at the HD also showed slight increases in platelet count. From the text of the report it was noted that an eosinophilia was apparent in 5 HD animals and 1 MD male at week 17. It was also noted that increased numbers of eosinophils were more apparent in the males at MD and HD. At week 33, the sponsor states that "...although only one male receiving 50 mg/kg/day and one male receiving 200 mg/kg/day recorded an eosinophilia, increased numbers of eosinophils continued to be seen. No obvious increase in granulocytes was apparent throughout the study."

Appears This Way
On Original

Table 3
Ranolazine One Year Oral Toxicity Study in Rats
Haematology Group Summary - Females

Study Week	Group No	Dose mg/kg/day		Hb g/dl	RBC 10^{12}/l	HCT ratio	RBC Indices MCH pg	MCHC g/dl	MCV fl	Retics %	Plats 10^9/l	PT sec	PTT sec
0	All	0	Mean	14.4	7.07	0.458	20.3	31.4	64.8	8.6	970	-	-
			SD	0.9	0.49	0.027	0.8	0.4	1.8	1.2	119	-	-
17	1	0	Mean	15.5	8.24	0.471	18.7	32.8	57.2	1.8	800	19.0	20.0
			SD	0.2	0.32	0.010	0.8	0.4	2.2	0.4	117	1.5	2.0
	2	2	Mean	15.6	8.29	0.480	18.8	32.5	57.9	1.3	839	19.7	20.5
			SD	0.5	0.27	0.016	0.4	0.5	1.4	0.5	118	2.8	3.3
	3	20	Mean	15.2	8.03	0.474	18.9	*32.0	*58.1	1.5	753	19.1	21.1
			SD	0.6	0.26	0.017	0.6	0.6	1.8	0.5	86	2.3	4.0
	4	50	Mean	15.2	8.04	0.473	18.9	*32.2	*58.8	1.5	904	21.2	22.7
			SD	0.6	0.40	0.020	0.4	0.4	1.3	0.4	112	3.1	3.6
	5	200	Mean	14.9	**7.79	0.468	19.1	*32.0	**59.8	1.8	**1016	19.7	*24.3
			SD	0.7	0.30	0.020	0.4	0.6	1.4	0.4	79	1.6	4.0
33	1	0	Mean	15.1	8.32	0.479	18.2	31.5	57.6	1.2	781	23.7	24.8
			SD	0.5	0.30	0.017	0.6	0.4	2.1	0.3	79	1.9	1.9
	2	2	Mean	15.6	8.54	0.494	18.3	31.7	57.8	1.1	777	21.0	25.9
			SD	0.5	0.43	0.017	0.5	0.3	1.4	0.2	109	0.0	5.1
	3	20	Mean	15.3	8.35	0.490	18.3	31.2	58.7	1.2	742	23.6	27.4
			SD	0.9	0.50	0.028	0.6	0.3	1.9	0.2	124	3.4	3.9
	4	50	Mean	15.4	8.42	0.491	18.4	31.5	58.4	1.4	777	27.9	28.2
			SD	0.9	0.50	0.028	0.3	0.5	1.6	0.3	203	7.6	3.1
	5	200	Mean	14.8	8.03	0.481	18.5	**30.9	**58.9	**1.7	*915	25.3	**34.6
			SD	0.8	0.39	0.024	0.3	0.5	1.3	0.2	120	3.6	4.1
49	1	0	Mean	14.9	8.16	0.482	18.3	31.0	58.9	1.5	726	20.2	26.8
			SD	0.6	0.33	0.021	0.6	0.4	1.9	0.5	98	4.2	4.1
	2	2	Mean	15.5	8.49	0.499	18.3	31.1	58.8	1.0	718	18.1	19.3
			SD	0.6	0.39	0.019	0.4	0.6	1.6	0.4	94	1.6	2.5
	3	20	Mean	15.3	8.33	0.501	18.4	30.6	60.2	1.5	672	19.2	20.0
			SD	0.7	0.42	0.021	0.6	0.5	1.8	0.8	111	1.4	1.7
	4	50	Mean	15.1	8.17	0.487	18.5	30.9	59.8	1.8	728	18.5	20.5
			SD	1.1	0.67	0.033	0.4	0.4	1.8	0.8	135	0.8	2.4
	5	200	Mean	14.4	7.76	0.468	18.6	30.8	60.3	1.7	793	**17.3	19.2
			SD	1.0	0.60	0.032	0.3	0.4	1.5	0.5	134	1.8	2.3

Significance level of comparison with 0 mg/kg/day using William's t-test * = p<0.05, ** = p<0.01

Best Possible Copy

Table 3
Ranolazine One Year Oral Toxicity Study in Rats
Haematology Group Summary - Males

Study Week	Group No	Dose mg/kg/day		Hb g/dl	RBC 10^{12}/l	HCT ratio	RBC Indices MCH pg	MCHC g/dl	MCV fl	Retics %	Plats 10^9/l	PT sec	PTT sec
0	All	0	Mean	13.2	8.43	0.432	20.7	30.6	67.8	10.6	760	-	-
			SD	0.6	0.33	0.018	0.7	0.4	2.0	1.8	104	-	-
17	1	0	Mean	14.8	8.31	0.460	17.8	32.1	55.4	2.7	876	20.8	20.9
			SD	0.7	0.47	0.020	0.7	0.2	2.1	0.8	199	1.8	2.6
	2	2	Mean	15.0	8.59	0.467	17.5	32.2	54.3	1.9	890	21.4	20.8
			SD	0.6	0.33	0.014	0.3	0.4	0.9	0.7	121	1.1	1.4
	3	20	Mean	15.0	8.27	0.465	18.1	32.2	56.2	1.7	846	21.4	20.2
			SD	0.5	0.34	0.011	0.6	0.5	1.9	0.8	189	1.7	1.9
	4	50	Mean	14.8	8.53	0.484	17.4	32.0	54.5	2.0	900	21.7	21.4
			SD	0.5	0.37	0.018	0.6	0.4	1.9	0.8	168	2.9	2.5
	5	200	Mean	14.2	8.24	0.451	17.3	**31.6	54.9	2.9	**1081	21.0	22.6
			SD	0.9	0.65	0.026	0.7	0.6	2.4	1.6	192	3.4	6.4
33	1	0	Mean	15.0	8.84	0.487	17.0	30.8	55.1	1.1	839	26.5	23.1
			SD	0.4	0.38	0.016	0.7	0.8	2.3	0.2	124	5.5	4.3
	2	2	Mean	15.0	8.86	0.485	17.0	30.9	54.8	1.2	883	29.8	29.6
			SD	0.6	0.29	0.013	0.3	0.5	0.9	0.3	152	8.2	9.9
	3	20	Mean	14.9	8.51	0.482	17.6	31.0	56.6	1.2	946	30.9	29.1
			SD	0.5	0.33	0.013	0.5	0.4	1.6	0.4	129	7.3	7.5
	4	50	Mean	14.9	8.73	0.478	17.0	31.1	54.8	1.4	945	36.4	32.9
			SD	0.4	0.31	0.014	0.6	0.5	2.1	0.3	96	4.0	2.9
	5	200	Mean	14.4	8.46	*0.468	17.1	30.9	55.4	**1.7	**1143	30.5	*26.8
			SD	1.0	0.83	0.031	1.0	0.5	2.8	0.8	227	6.4	3.9
49	1	0	Mean	15.5	9.23	0.512	16.8	30.2	55.5	2.1	836	20.3	17.8
			SD	0.6	0.35	0.019	0.7	0.5	2.2	0.5	249	2.1	2.5
	2	2	Mean	15.1	9.13	0.503	16.6	30.1	55.0	1.9	941	19.4	17.4
			SD	0.5	0.33	0.014	0.9	0.5	1.0	0.5	159	1.1	1.3
	3	20	Mean	15.0	8.57	0.496	17.3	30.2	57.2	1.8	843	20.7	18.2
			SD	0.6	0.36	0.014	0.6	0.5	1.8	0.5	150	1.4	2.1
	4	50	Mean	15.3	9.11	0.505	16.8	30.3	55.5	2.1	983	19.7	18.2
			SD	0.4	0.35	0.013	0.7	0.4	2.4	0.4	155	1.3	1.9
	5	200	Mean	**14.7	8.65	0.493	16.6	29.8	55.6	2.5	**1087	22.0	19.4
			SD	1.2	0.70	0.035	0.8	0.8	2.1	1.0	178	3.6	4.4

Significance level of comparison with 0 mg/kg/day using William's t-test * = p<0.05, ** = p<0.01

Table 4
Ranolazine : One Year Oral Toxicity Study in Rats
Clinical Chemistry Group Summary - Males

Study Week	Group No	Dose mg/kg/day		Na mmol/l	K mmol/l	Urea mmol/l	Gluc mmol/l	Chol mmol/l	Trig mmol/l	Creat µmol/l	ALT IU/l	AST IU/l	LDH IU/l	HBDH IU/l	Prot g/l
0	All	0	Mean	140	5.78	8.1	9.9	2.6	1.5	43	66	55	109	33	57
			SD	1	0.38	0.7	1.1	0.4	0.6	9	9	6	43	11	2
17	1	0	Mean	143	4.42	5.6	10.9	2.8	1.3	57	42	48	98	25	69
			SD	1	0.40	0.6	2.4	0.6	0.3	4	12	15	36	9	4
	2	2	Mean	144	4.36	5.9	10.7	2.7	1.3	+50	38	44	90	24	70
			SD	1	0.46	0.9	1.6	0.6	0.5	3	8	10	25	6	4
	3	20	Mean	143	4.22	6.2	12.4	2.6	1.4	58	39	46	101	27	70
			SD	1	0.47	0.6	0.9	0.4	0.5	3	12	13	32	7	3
	4	50	Mean	**144	4.11	6.2	13.0	2.4	1.4	+61	32	38	89	24	71
			SD	1	0.35	0.7	1.0	0.6	0.6	3	4	6	28	7	3
	5	200	Mean	**144	4.34	7.1	12.1	+3.5	**2.0	60	34	42	106	29	**73
			SD	1	0.40	2.5	1.6	1.1	0.7	5	17	19	47	12	3
33	1	0	Mean	146	4.53	5.2	10.6	2.8	1.6	51	52	54	99	28	71
			SD	1	0.52	0.7	2.1	0.9	0.8	3	30	27	22	5	3
	2	2	Mean	145	4.79	5.2	+9.4	2.9	1.8	54	55	60	138	+38	71
			SD	3	0.57	0.8	0.8	0.8	0.8	2	28	23	45	11	3
	3	20	Mean	145	4.48	5.4	10.5	2.8	1.4	53	46	55	129	35	71
			SD	1	0.47	0.5	1.7	0.6	0.5	3	14	17	37	8	2
	4	50	Mean	145	4.84	5.4	+9.0	2.7	2.0	53	39	44	152	30	72
			SD	2	0.37	0.5	1.3	0.9	1.0	3	9	11	131	10	2
	5	200	Mean	146	4.77	6.8	10.6	+3.8	*2.4	+55	@@0	39	108	31	73
			SD	1	0.37	3.2	1.8	1.7	0.9	4	4	10	49	12	3
49	1	0	Mean	146	4.50	5.0	9.6	3.3	1.6	53	55	60	83	28	71
			SD	1	0.50	0.7	2.1	1.0	0.5	3	29	28	30	10	4
	2	2	Mean	146	4.67	5.1	9.3	3.2	1.7	+57	36	47	+167	+48	71
			SD	2	0.51	0.5	1.8	1.0	0.5	2	6	9	130	35	4
	3	20	Mean	**144	4.33	5.5	9.9	3.3	1.6	55	48	55	129	46	71
			SD	0	0.49	0.6	1.7	0.8	0.5	2	15	14	35	9	2
	4	50	Mean	**145	4.77	6.4	8.8	3.4	2.4	55	36	46	134	40	72
			SD	1	0.44	0.8	1.8	1.4	1.1	3	12	14	55	16	2
	5	200	Mean	**144	4.36	**8.1	10.2	3.8	1.8	++57	@@27	39	134	40	73
			SD	1	0.60	0.7	1.9	1.0	0.5	4	6	10	62	16	3

Significance level of comparison with 0 mg/kg/day using William's t-test * = p<0.05, ** = p<0.01
Student's t-test + = p<0.05, ++ = p<0.01 Shirley's test @ = p<0.05, @@ = p<0.01

Slight changes in the differential were marked as statistically significant but are of questionable biological significance.

Clinical chemistry showed slight decreases in serum sodium (m+f), increased cholesterol (m+f) and triglycerides for the HD males and increased creatinine concentrations for the drug-treated males. Simultaneous increases in LDH and HDBH were noted in the drug-treated males also.

Table 4
Ranolazine : One Year Oral Toxicity Study in Rats
Clinical Chemistry Group Summary - Females

Study Week	Group No	Dose mg/kg/day		Na mmol/l	K mmol/l	Urea mmol/l	Gluc mmol/l	Chol mmol/l	Trig mmol/l	Creat µmol/l	ALT IU/l	AST IU/l	LDH IU/l	HBDH IU/l	Prot g/l
0	All	0	Mean	142	4.95	6.9	9.3	2.5	1.4	45	43	43	103	30	63
			SD	1	0.40	1.0	1.0	0.4	0.4	9	7	4	30	8	3
17	1	0	Mean	143	4.57	5.7	9.0	2.2	1.5	64	39	51	113	31	71
			SD	1	0.38	0.7	1.6	0.6	0.6	5	12	24	43	10	3
	2	2	Mean	143	4.37	5.9	10.1	2.5	1.4	63	54	55	115	33	70
			SD	1	0.32	0.6	3.1	0.7	0.7	4	21	13	33	8	3
	3	20	Mean	143	4.26	*6.4	10.4	2.6	2.0	62	39	48	117	33	70
			SD	1	0.54	0.7	1.8	0.4	0.7	5	7	13	44	11	0
	4	50	Mean	143	4.47	*6.4	8.9	2.5	1.5	63	37	42	118	33	71
			SD	1	0.41	0.8	1.4	0.3	0.8	2	4	5	45	10	2
	5	200	Mean	144	4.27	**6.8	10.1	**3.8	1.2	64	34	38	123	34	71
			SD	1	0.37	0.8	2.0	0.7	0.5	5	8	11	36	9	4
33	1	0	Mean	149	4.97	6.3	8.6	2.8	2.0	62	49	61	145	40	75
			SD	2	0.54	0.9	1.8	0.5	0.7	3	18	21	43	10	4
	2	2	Mean	151	4.75	6.1	8.9	2.9	1.8	63	66	92	190	60	75
			SD	3	0.60	0.8	2.3	1.2	1.1	4	23	31	89	14	5
	3	20	Mean	149	+4.20	6.6	10.6	3.0	2.8	63	45	60	150	42	75
			SD	3	0.23	0.7	1.5	0.3	1.8	3	16	29	97	24	7
	4	50	Mean	150	4.70	6.6	8.9	3.0	1.9	64	48	59	167	45	77
			SD	1	0.43	1.2	1.1	0.6	0.8	4	13	24	63	16	4
	5	200	Mean	*147	4.70	6.4	9.5	**4.5	1.8	61	35	45	177	47	75
			SD	2	0.69	0.9	0.8	1.4	1.0	3	18	26	73	18	4
49	1	0	Mean	148	4.13	6.5	10.0	3.2	2.5	57	54	71	114	35	79
			SD	2	0.33	0.9	1.9	0.5	1.3	3	18	29	48	12	7
	2	2	Mean	145	4.45	6.0	9.8	3.3	2.5	58	58	85	148	46	78
			SD	1	0.86	1.1	2.4	1.7	1.4	3	29	41	90	23	5
	3	20	Mean	+145	4.11	7.2	10.0	3.1	2.2	60	45	59	115	34	79
			SD	1	0.43	1.1	1.5	0.3	0.9	5	18	25	54	17	4
	4	50	Mean	++144	4.22	6.3	9.7	3.1	2.3	59	40	56	107	34	82
			SD	1	0.29	0.8	1.6	0.7	1.2	1	16	27	40	11	5
	5	200	Mean	146	4.32	6.1	9.0	**5.8	2.0	+54	*37	*41	68	22	78
			SD	2	0.50	0.5	1.8	2.1	0.7	4	13	18	26	7	5

Significance level of comparison with 0 mg/kg/day using William's t-test * = p<0.05, ** = p<0.01
Student's t-test + = p<0.05, ++ = p<0.01

A brief statement indicates that there were ophthalmic findings of corneal pitting, cataracts and corneal opacities. An ophthalmologist's report was not located. The sponsor felt the findings were unrelated to drug.

Best Possible Copy

The absolute and normalized adrenal weights were increased in males and females at >50 mg/kg. Absolute and normalized liver and kidney weights were increased in both sexes at the HD.

Group Mean Organ weights adjusted for terminal body weights: males

Dose (mg/kg/day)		Terminal Bodyweight	Adrenal#	Brain	Heart	Kidney	Liver
0	n	16	15	15	16	16	16
	mean	779.5	0.0664	2.3184	2.1891	4.5993	25.8529
	se	25.8	0.0030	0.0269	0.0516	0.1064	0.7506
2	n	18	18	18	18	18	18
	mean	804.1	0.0745	2.3378	2.2180	4.7580	27.1544
	se	24.3	0.0031	0.0250	0.0493	0.1016	0.7173
20	n	19	19	19	19	19	19
	mean	749.1	0.0698	2.3159	2.2902	4.8594	27.1437
	se	23.7	0.0029	0.0239	0.0474	0.0977	0.6894
50	n	19	19	19	19	19	19
	mean	783.3	0.0695	2.3003	2.1562	4.7422	26.4786
	se	23.7	0.0028	0.0240	0.0475	0.0978	0.6899
200	n	15	15	15	15	15	15
	mean	700.5	0.1216**	2.3156	2.2243	5.3114**	29.7724**
	se	26.6	0.0056	0.0278	0.0551	0.1135	0.8010

Key: Significance level of comparison with control using William's test: * = p<0.05, ** = p<0.01
Significance level of comparison with control using Student's t-test: + = p<0.05, ++ = p<0.01
= Back transformed means and standard errors; data were logarithmically transformed prior to analysis.

Best Possible Copy

Group mean organ weights adjusted for terminal body weight: females

Dose (mg/kg/day)		Terminal Bodyweight	Adrenal#	Brain	Heart	Kidney	Liver
0	n	17	17	17	17	17	17
	mean	448.7	0.0995	2.1270	1.5993	3.2263	16.5225
	se	15.8	0.0049	0.0216	0.0353	0.1151	0.5511
2	n	20	20	20	20	20	20
	mean	442.6	0.0885	2.1344	1.5406	3.0773	16.3145
	se	14.6	0.0040	0.0201	0.0328	0.1059	0.5212
20	n	20	20	20	20	20	20
	mean	479.4	0.0955	2.1418	1.5300	3.1151	16.6844
	se	14.6	0.0045	0.0199	0.0326	0.1062	0.5178
50	n	17	17	17	17	17	17
	mean	479.0	0.1128	2.1215	1.5532	3.2257	16.8120
	se	15.8	0.0055	0.0216	0.0353	0.1150	0.5609
200	n	13	13	13	13	13	13
	mean	474.2	0.1501**	2.1230	1.5857	3.8291**	19.5521**
	se	18.1	0.0084	0.0248	0.0402	0.1311	0.6392

Key: Significance level of comparison with control using William's test: * = p<0.05, ** = p<0.01
Significance level of comparison with control using Student's test: + = p<0.05, ++ = p<0.01
= Back transformed means and standard errors, data were logarithmically transformed prior

Gross and histopathologic findings included adrenal, pituitary and pulmonary changes. The sponsor does not separate the results into those who died ahead of scheduled euthanasia and those surviving to scheduled termination. Lungs and adrenals were analyzed from the LD and MD groups also.

Reviewer's summary of gross and histopathological changes

	Dose of ranolazine mg/kg/day				
(n=20 per group/sex)	0	2	20	50	200
Enlarged adrenal gland- f	0	0	1	2	7
Enlarged pituitary gland- f	2	2	4	5	10
Adrenal cortical vacuolation-f	4	2	2	3	19
m	13	15	6	19	20
Inhalation pneumonia f	0	0	1	3	8
M	2	0	0	5	15
Alveolar foam cell proliferation	2	5	2	2	7

Pk data showed that plasma levels increased with increasing dose. The increase in AUC values was greater than proportional from 2 to 20 mg/kg and from 20 to 50 mg/kg.

At the lowest dose, the AUC increased from 6 to 12 months, suggesting accumulation. There was slight increase in AUC from 6 to 12 months at 50 and 200 mg/kg, suggesting either accumulation or saturation of clearance.

Dose	C_{max} (ng/ml)		$AUC_{0\text{-}24\ h}$ (ng.h/ml)	
2 mg/kg/day	Male	Female	Male	Female
6 months	118 ± 32.4	334 ± 117	369	809
12 months	124 ± 31.8	500 ± 196	841	780

Dose	C_{max} (ng/ml)		$AUC_{0\text{-}24\ h}$ (ng.h/ml)	
20 mg/kg/day	Male	Female	Male	Female
6 months	3330*	5740 ± 700	7690	17600
12 months	2990*	6950 ± 410	7630	26800

Dose	C_{max} (ng/ml)		$AUC_{0\text{-}24\ h}$ (ng.h/ml)	
50 mg/kg/day	Male	Female	Male	Female
6 months	8900 ± 3260	8940 ± 191	28200	64500
12 months	5520 ± 3370	9020 ± 672	30100	71300

Dose	C_{max} (ng/ml)		$AUC_{0\text{-}24\ h}$ (ng.h/ml)	
200 mg/kg/day	Male	Female	Male	Female
6 months	17500 ± 11800	26800 ± 1840	132000	232000
12 months	27500*	29800*	202000	-

Values expressed in terms of ranolazine dihydrochloride.

C_{max} values were determined from the mean plasma level data and AUC values were calculated from these mean profiles.

C_{max} values are quoted as Mean ± SD for n=2 rats except for * where n=1.

The urinalysis data was presented in a format that was difficult to interpret. The mostly qualitative data was presented as single animal data. In the drug-treated males, starting with the first determination at 16 weeks, there appear to be increased incidence and severity of something found in the microscopy. This appears to be sperm, crystals and combinations of the two as well as some unspecified material in the "abno" column. There were findings of crystals for some of the HD males at 32 weeks. At the final determination, 48 weeks, there were microscopic findings of apparently dose-related frequency and severity for each of the groups of males.

Study title: 28-Day Repeated dose toxicity study of ranolazine free base containing RS94287 in Sprague-Dawley rats

Key study findings: RS94287, also known as [ ] is an impurity listed in the specifications for ranolazine, occurring at not more than [ ] w/w (Item 4, vol 1, p. 56). The study was inadequate in design. This is essentially an interaction study between the impurity and the drug. The dose of drug used was a fraction of the human exposure and clinically irrelevant. There was no untreated group for comparison. Despite this, the data presented here showed a slight decrease in HCT in both sexes, accompanied in males by an increase in spleen weight and accompanied in females by an increase in MCV, suggestive of hemolysis and a regenerative response respectively. Organ weight data also showed a decreased weight of testes with increased concentration of impurity. In females, the MD and HD showed statistically significant increases in serum sodium and decreases in serum potassium. From the data available it cannot be concluded that this material is without biological effects.

Study no: 124-007
Volume #, and page #: vol 11, p.4
Conducting laboratory and location: [ ]
Date of study initiation: November 12, 1999
GLP compliance: statement included
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity: RS-94287 (also known as [ ] lot # 4007, ranolazine free base, lot E4-NE-002
Formulation/vehicle: control material was distilled water.

Methods: [ ] is an impurity listed in the specifications for ranolazine, occurring at not more than [ ] w/w (Item 4, vol 1, p. 56). Ranolazine free base containing — was given by oral gavage each day for 28 days to Sprague-Dawley rats . CD® (SD)BR VAF, [ ] The study design is summarized in the table below.

Group designation *(percentage based on dosage level	Dosage level(mg/kg)	# of animals	
		Males	females

	Control (Ranolazine)	20	5	5
	Ranolazine + 1% —	20	5	5
	Ranolazine + 2.5% —	20	5	5
	Ranolazine + 5.0% —	20	5	5

It is not expressly stated what the dose of ranolazine is, but it is a reasonable assumption that 20 mg/kg is the dosage used. The sponsor notes in their table that the percentage of — was based on the dosage level. Since the dosage of ranolazine was the same for all groups, this is not quite clear. It is also not clear to what the percentage of the impurity refers.

Animals were observed twice a day for morbidity and mortality. Body weights were recorded pre-test, approximately weekly, the day before necropsy and the day of necropsy. Ophthalmic exams were performed pre-test and prior to necropsy by a staff veterinarian. Blood samples were obtained prior to necropsy and analyzed for hematology and clinical chemistry parameters. Semi-quantitative urinalysis was performed on all animals at some time prior to necropsy. Study day 29, animals were euthanized and gross observations made. Organ weights were recorded for brain, liver, kidneys, testes, ovaries, adrenal glands, spleen and thymus. Histopathology was conducted for the control and 5% — group on the following tissues: adrenals, aorta, bone marrow, brain, cervix/vagina, epididymides, esophagus, eyes with optic nerve, femur, gross lesions, heart, cecum, colon, rectum, duodenum, ileum, jejunum, kidneys, liver, lungs, mesenteric and mandibular lymph nodes, mammary gland, ovaries, oviducts, pancreas, pituitary gland, prostate gland, mandibular salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, tongue, trachea, uterus and urinary bladder.

Results: There were no treatment related signs. The male rats treated with — showed less weight gain than the rats treated with ranolazine alone. This is summarized in the reviewer's table. There was no apparent effect on female weights.

Summary of weight changes in male rats.

	Ranolazine alone	Ranolazine + 1% —	Ranolazine + 2.5% —	Ranolazine + 5% —
Absolute gain from baseline (g) Days 1-29	152 ± 31	138± 31	124± 28	134± 8
% of baseline	70%	65%	58%	63%

There was a slight decrease in HCT in both sexes. Mean cellular volume decreased slightly in males and increased significantly in females:

MCV(% cu microns pico grams) in female rats

ranolazine	56.8±1.51
Ranolazine + 1% —	58.5±1.44
Ranolazine + 2.5% —	61.1**±2.36 ($p<0.01$)
Ranolazine + 5% —	60.1*±1.60 ($p<0.05$)

There were no significant effects in the clinical chemistry results for the males. There were however, significant effects on Na and K in the female results, as shown in the reviewer's table below.

Significant Clinical Chemistry Results for Female Rats

group	Na mmol/l	K mmol/l
Ranolazine	144±0.4	7.2±1.07
Ranolazine + 1%	145±1.5	6.9±1.33
Ranolazine + 2.5%	148**±1.3	5.4*±0.63
Ranolazine + 5 %	147**±0.8	6.0±0.89

There were several organ weight effects in the male rats. This is summarized in the reviewer's table below.

Summary of organ weight effects in male rats

	ranolazine	Ranolazine 1%	Ranolazine 2.5%	Ranolazine 5%
Spleen as % body weight	0.188±0.0179	0.213±0.0162	0.229**±0.013	0.206±0.0206
Testes as % of body weight	0.902±0.0873	0.861±0.0379	0.865±0.0927	0.753±0.304
Liver as % of brain weight	626.7±82	661±132	590±72	581±41
Spleen as % of brain weight	34±3.6	39±1.4	40*±3.0	36±4.1
Testes as % brain weight	163±22	156±12	151±16	131±53

*significant at $p<0.05$, **$p<0.01$

The only histopathological finding possibly related to the organ weights was 1/5 HD males showed epididymal atrophy. In an appendix, it was noted that this was secondary to testicular degeneration.

There was no apparently drug-related effect in the urinalysis data.

Summary: The study was inadequately performed. This is essentially an interaction study between the impurity and the drug. However, the dose of drug used was a fraction of the human exposure and clinically irrelevant. Despite this, the data presented here showed a slight decrease in HCT in both sexes, accompanied in males by an increase in spleen weight and accompanied in females by an increase in MCV, suggestive of hemolysis and a regenerative response respectively. Organ weight data also showed a decreased weight of testes with increased concentration of impurity. In females, the MD and HD showed statistically significant increases in serum sodium and decreases in serum potassium. From the data available, it cannot be concluded that this material is without biological effects.

Appears This Way On Original

Study title: 28-Day repeated dose toxicity study of ranolazine free base containing RS88778 in Sprague-Dawley rats.

Key study findings: RS88778, also known as [redacted], is an impurity occurring at NMT [redacted] w/w (Item 4, vol 1, p.56). The hematology data was presented twice: once under the heading of hematology, once under the "Clinical Chemistry" section. The clinical chemistry data was found under the "Organ Weight" heading. The organ weight summary was not found although individual organ weights were found in Appendix 9(p.158). The urinalysis summary was found as an addendum on page 195. The sloppiness of the report makes one somewhat uncomfortable. The dose of ranolazine is clinically irrelevant. There was no untreated control group for comparison. The MD and HD males showed 11% and 16% less weight gain than the ranolazine controls. The study is poorly designed and inadequate; however, the difference in weight gain in the males makes it impossible to say that the compound is without biological effect.

Study no: 124-005
Volume #, and page #: vol 10, p.4
Conducting laboratory and location: [redacted]
Date of study initiation: October 7, 1999
GLP compliance: statement was included
QA report: yes (x) no ()
Drug, lot #, radiolabel, and % purity: RS88778 [redacted] lot # EE-315-64, ranolazine free base lot # E4-NE-002
Formulation/vehicle: deionized water

Methods: Male and female ~ CD® (SD) BR VAF /Plus out-bred albino rats [redacted] were given daily oral doses of ranolazine or ranolazine + [redacted] at 1%, 2.5% or 5% (percent of what we are not sure). The study design is shown in the table below.

Summary of study design

Group	Dose of ranolazine (mg/kg/)	# of animals	
		males	females
Ranolazine (control)	20	5	5
Ranolazine + 1% [redacted]	20	5	5
Ranolazine +2.5% [redacted]	20	5	5
Ranolazine +5 % [redacted]	20	5	5

Observations for mortality and moribundity were made twice daily. Body weights were recorded pretest, approximately weekly, on the day prior to necropsy and day of necropsy

An ophthalmological exam was conducted pre-test and prior to necropsy by a staff veterinarian. Blood samples were collected prior to necropsy and analyzed for standard hematological and clinical chemistry parameters. Semi-quantitative urinalysis was performed at some point prior to necropsy. Day 29, All animals were euthanized and gross observations made. Organ weights were recorded for brain, liver, kidneys, testes, ovaries, adrenal glands, spleen and thymus. Samples for histopathology were collected from adrenal glands, aorta, bone marrow, brain, cervix/vagina, epididymides, esophagus, eyes with optic nerve, femur, gross lesions, heart, cecum, colon, rectum,duodenum, ileum, jejunum, kidneys, liver, lungs, mandibular and mesenteric lymph nodes, mammary gland, ovaries, pancreas, pituitary gland, prostate gland, mandibular salivary gland,

sciatic nerve, seminal vesicles, skeletal muscles, skin,spinal cord, spleen, stomach, testes, thymus, thyroid, tongue, trachea, uterus and urinary bladder.

Results: There were no clinical signs referable to drug treatment. There was no apparent affect upon female weight but male weight was decreased with increasing dose of the impurity as summarized in the reviewer's table.

Summary of weight changes

	ranolazine	Ranolazine + 1% [redacted]	Ranolazine + 2.5%[redacted] p	Ranolazine+ 5% [redacted]
Absolute change from baseline (g) Days 1-29	119.2± 44.7	128.6±29.9	95.8±43.6	90.4±21.0
% difference from baseline	48	53	37	32

There were no significant effects apparent in the hematology data for either sex. The clinical chemistry data was presented under the organ weight section, apparently in error as the hematology data was shown twice, once under the clinical chemistry heading and individual animal hematology data was presented in the organ weight section. The organ weight summary was not found. Individual animal data was presented in Appendix 9. Urinalysis data was found as an addendum to the report. There were no findings of significance in the data as presented.

TOXICOLOGY SUMMARY:

Ranolazine is the racemic mixture of the (+)R enantiomer (RS43285-198) and the (-)S enantiomer (RS43285-197). There was no presentation of a systematic, organized characterization and comparison of the relative toxicities of the racemate versus the enantiomers. It has been proposed to market the drug as a racemic mixture based upon the assumption that the enantiomers possess equal pharmacological and toxicological characteristics. The data to support that assumption was not found in the data presented. The drug is also highly metabolized. The pharmacology/toxicology characterization of the metabolites is incomplete.

Reports were presented for:

Acute oral and intravenous dosing studies in mice, rats and dogs
One month oral and iv dosing studies in rats and dogs
3 month oral study in mice
3, 6 and 12 month oral dosing studies in rats
3, 6 and 12 month oral dosing studies in dogs

Special toxicology studies included:

Acute adrenal function in rats
A subsequent modified acute adrenal function study
One month adrenal function in rats
In vitro studies of adrenal steroid release ± ACTH stimulation and ± steroidal precursors

Studies in mice

Oral EMLD study in mice: A single oral dose of 250 mg/kg caused severe clinical signs of subdued behavior, hunched stance, piloerection, hyperventilation and prostration in 1/5m and 2/5f. There was no improvement in signs by 2 hours after dosing. A single oral dose of 50 mg/kg produced no signs.

Intravenous EMLD in mice: A single intravenous dose of 20 mg/kg produced no signs. A single intravenous dose of 30 mg/kg caused signs including hyperventilation, ataxia, piloerection, subdued behavior and prostration. Recovery was reported to occur within 1 hour of dosing.

Three-month oral dose ranging study in mice: Animals were dosed with 0, 5, 50, 100 or 200 mg/kg/day of ranolazine. The study was terminated at 8 days due to the deaths of animals at 50(3/10m), 100(1/10m, 3/10f) and 200(2/10m, 4/10 f) mg/kg. Clinical signs reported in this study-included sedation, hunched stance and prostration. The 100 and 200 mg/kg males gained on average 45% and 61% less than the control males.

Three-month oral dose ranging study in mice: mice received oral doses of 0, 5, 15, 25 or 35 mg/kg/day ranolazine. Final body weight was inconsistently affected and the results cannot be given any toxicological significance. There was a very slight decrease in hemoglobin, RBC and hematocrit in the 15, 25 and 35 mg/kg-treated animals. The hematology results are remarked upon only because they are consistent with similar minimal changes noted in other studies in other species. There were few findings of toxicological significance in this study.

Studies in rats

Oral EMLD study in rats: Five male and five female rats per group received single oral doses of either 250 or 500 mg/kg. The lower dose produced 40% mortality while the 500-mg/kg dose caused 60% mortality. Both dose groups showed signs of prostration, dyspnea, convulsions, salivation and ptosis. Survivors were euthanized after 14 days with no gross or histopathological observations made.

Intravenous EMLD study in rats: The rats received a single intravenous dose of 30 mg/kg. No fatalities were reported. However, all animals showed clinical signs that included subdued behavior. Some animals also showed signs of ataxia, prostration, convulsions and hyperventilation. The intravenous LD50 is greater than 30 mg/kg.

Comparative EMLD study in rats: Five male and five female rats per group were given single oral doses of 250 mg/kg or either the racemic mixture or one of the enantiomers. All animals showed signs of sedation, prostration, ataxia and dyspnea. The females receiving the racemic mixture had a later onset of signs (1.5 hours vs 12-38 minutes) compared to the animals receiving the enantiomers. The results were inconsistent with other studies that also found salivation, tremors and convulsions as well as earlier onset of signs with the racemic mixture.

One month intravenous toxicity study in rats: Twelve rats per sex per group were given daily intravenous doses of ranolazine of 0, 1, 5 or 25 mg/kg for 28 days. Immediate salivation,

sedation and convulsions followed iv administration of 25 mg/kg to both sexes of rats. At 25 mg/kg, 1/12 males and 1/12 females died. Increased liver weight (3%, 11% and 10% of control from LD to HD) and decreased uterine weight (-17%, -19% and –12% of control, from LD to HD respectively) were seen in drug-treated females. The uterine weight change was significant in the LD and MD groups at $p<0.05$. Increased spleen (4%, 13% and 13% of control from LD to HD respectively) and adrenal weights (4%, 18% and 13% from LD to HD) were reported for the drug-treated males. No NOEL was determined for the organ weight effects in either sex.

Three month oral toxicity data in rats: Twelve rats per sex per group were given once daily oral doses of ranolazine at 0, 5, 50, 250 and 500 mg/kg/day for 91 days. Unscheduled mortality was seen at 5 (1f), 250 (2m) and 500 (3m, 4f) mg/kg. Clinical signs were seen at doses ≥50 mg/kg. Signs included salivation (≥50 mg/kg), piloerection (≥50 mg/kg), prostration (≥250 mg/kg), hyperpnea (≥250 mg/kg) and convulsions (≥250 mg/kg). The HD group also showed ptosis/sedation. Clinical chemistry findings in both sexes included increased serum sodium at the two highest doses at 8 and 12 weeks and increased alkaline phosphatase. Glucose was increased in the two highest dose groups of females. There were histopathological findings of adrenal vacuolation, centrilobular hepatocyte enlargement and pulmonary alveolar foam cell proliferation. The incidences are summarized in the table below.

Reviewer's summary of reported microscopic findings

finding	Males	Females
Centrilobular hepatocyte enlargement	0/12 ©, 1/12 HD	0/12 ©, 7/12 (HD)
Pulmonary alveolar foam cell proliferation	1/12©, 6/12 HD	0/12©, 2/12 (HD)
Adrenal cortical cell vacuolation	0/12 ©, 12/12 HD	0/12©, 7/12(HD)

*The sponsor stated in the text that vacuolated adrenal cortical cells were found in "a number of" 250 mg/kg (MD) males.

A complete assessment of the toxicological effects and independent interpretation of the study cannot be made from the material presented.

Six month oral toxicity study in rats with a one-month recovery period: Sprague-Dawley rats were assigned to groups with 30 males and 30 females in the control and HD groups. There were 20 males and 20 females in each of the LD and MD groups. At the end of the dosing period, all LD and MD and the first 20 rats in the control and HD groups were euthanized. The remaining 10 rats in the control and HD groups were given a 30 day drug-free recovery period. Doses used were 0, 5, 50 and 200 mg/kg.

Clinical signs were reported for doses ≥50 mg/kg/day and included the salivation, sedation, prostration, tachypnea or shallow breathing, hunched appearance, ataxia, piloerection and convulsions noted in other studies.Signs were usually observed within 1-2 hours after dosing and showed partial to complete resolution by 4-6 hours post-dose. Target cells reported in the hematology results may be associated with altered cholesterol and phospholipid content of RBC membranes, usually due to changes in liver function. Serum sodium, potassium, glucose and cholesterol were affected in both sexes as has been seen before in this species. In the HD groups of both sexes, LDH and HDBH (hydroxybutyrate dehydrogenase) were increased. Results for pathology, ophthalmoscopy and urinalysis were not susceptible to easy interpretation. The

adrenal and liver weights were increased in both sexes as summarized in the table below. It may be seen that liver and adrenal weight increases persisted into the recovery phase for the females. There were no apparent organ weight effects in the recovery males.

Reviewer's summary of organ weight data

Males: dose mg/kg/day				
	0	5	50	200
Adrenal	0.063±0.01	0.065±0.01	0.065±0.011	0.089**±0.016
Liver	16.23±2.96	15.42±2.16	16.08±2.11	17.66±2.71
Females				
Adrenal	0.074±0.011	0.071±0.012	0.083±0.019	0.108**±0.019
liver	9.48±1.09	9.08±0.95	9.99±1.22	11.04**±1.51
Female weights at end of recovery				
adrenal	0.067±0.017			0.082*±0.011
liver	8.92±0.63			9.64±1.23

*p<0.05, **p<0.01

The histopathology results were not entirely consistent with the previous study in that adrenal vacuolation was reported only for the males. The adrenal effects are summarized in the table below.

Reviewer's summary of adrenal gland findings

	Dose group (mg/kg/day)			
	0	5	50	200
Diffuse vacuolation of the zona fasiculata (males only)	0/20	6/20	9/20	18/18
Diffuse cytoplasmic foaminess of the zona fasiculata (females only)	0/20	0/20	0/20	17/20
Gross adrenal enlargement	1/20 MD f, 9/20 HD f 1/20 HD m			

One year oral toxicity study in rats : Male and female rats, 20/sex/group were orally gavaged once a day for 1 year with 0, 20, 50 or 200 mg/kg/day ranolazine. Signs were reported for doses ≥50 mg/kg included salivation and/or heavy staining of the muzzle, subdued behavior, ataxia, gasping or irregular breathing and half-closed eyes. Onset was generally within 1-2 hours of dosing with complete or partial recovery 4-6 hours after dosing. Convulsions were reported for a 20 mg/kg female and a 200 mg/kg male. The HD males gained on average 15% less body weight than the control group ($p<0.05$ by Student's test). The females in the 20, 50 and 200 mg/kg groups gained on average 12-8% more than the control group (NS). The weekly food consumption data does not indicate significant differences between the treatment groups. Both sexes at the HD showed slight decreases in HB and MCHC and slight increases in reticulocyte count. Both sexes at the HD also showed slight increases in platelet count. Adrenal weight was increased in HD males and females at 50 and 200 mg/kg. Adrenal cortical vacuolation was increased in the HD females. Liver weight was increased in the HD groups of both sexes. Findings are summarized in the tables below.

Reviewer's summary of organ effects

	Dose group (mg/kg)				
N=20 per group per sex	0	2	20	50	200
Enlarged adrenal gland- females	0	0	1	2	7
Enlarged pituitary gland- females	2	2	4	5	10
Adrenal cortical vacuolation- females	4	2	2	3	19
Males	13	15	6	19	20
Inhalation pneumonia- females	0	0	1	3	8
males	2	0	0	5	15
Alveolar foam cell proliferation	2	5	2	2	7

Reviewer's summary of organ weight changes

Dose group	Adrenal weight		Liver weight	
Mg/kg/day	males	females	males	females
0	0.0066±0.003	0.095±0.005	25.85±0.75	16.52±0.56
2	0.0745±0.003	0.089±0.004	27.15±0.72	16.31±0.52
20	0.0698±0.003	0.099±0.005	27.14±0.69	16.38±0.52
50	0.0695±0.003	0.1128±0.006	26.48±0.69	16.51±0.66
200	0.1216**±0.006	0.1501**±0.008	29.77**± 0.80	19.55**± 0.70

The multiples of human therapeutic doses were calculated from 5 days of orally dosed 1000 mg of ranolazine, t.i.d. The human AUC at this dose was 33700 ng.hr/ml.

Summary of exposure relative to humans

Rat dose	AUC_{0-24} ng.hr/ml At 12 months		Multiple of human maximum dose	
	Males	Females	males	Females
2	841	780	0.013x	0.01x
20	7630	26800	0.11x	0.40x
50	30100	71300	0.45x	1.07x
200	202000	Not given		

The urinalysis data indicated a dose-related change in the samples from the males. Incidence and severity of sperm and crystals present in the urine was noted. From the data presented it is not known if the sperm were normal or abnormal in morphology.

Studies in dogs

Maximum tolerated intravenous dose study in dogs: Two pairs of 1 male and 1 female were given once daily intravenous injections on the following schedule:
Pair 1: 7 days at 10 mg/kg/day
7 days at 20 mg/kg/day

Male: 1 day at 40 mg/kg/day
Female: 21 days further at 20 mg/kg/day

Pair 2: 15 days at 20 mg/kg/day

Single and repeated doses of 10 and 20 mg/kg/day resulted in the dogs becoming subdued as they received the dose and for approximately 15 minutes thereafter. At 20 mg/kg, the sedation was occasionally accompanied by glazed eyes, ataxia and trembling. Vomiting after dosing was recorded on one occasion. The frequency of the clinical signs was reported to diminish over the dosing period, suggesting either increased tolerance to the dose or induction of metabolism. A single dose of 40 mg/kg after the 10 and 20 mg/kg doses produced convulsions and collapse immediately post-dosing. The dog was humanely euthanized. Moderate dilation of the right ventricle was found on gross necropsy. While ECG tracings were generated, only heart rate data was provided. The dose of 20 mg/kg/day was tolerated for 21 days by the 1 female who received it.

Maximum tolerated oral (intubation) dose study in dogs: Two pairs of Beagles, one male and one female per pair were orally intubated once each day. Pair one received 50 mg/kg/day for one week followed by 100 mg/kg/day for a week. Both dogs then received an additional single oral dose of 150 mg/kg/day. Pair two was dosed at 100 mg/kg for 9 days. This was discontinued due to marked clinical signs which included combinations of sedation, ataxia, muscle tremors, vomiting, salivation and on one occasion in the female, prostration. On day 9, both dogs were found prostrate and trembling 2 hours after dosing. The male appeared unaware of his surroundings. Partial recovery was seen within 2-4 hours. After a 7 week recovery period, dosing was resumed at 80 mg/kg/day for 2 weeks.

Single doses of 50 and 80 mg/kg caused vomiting within 45 minutes of dosing. Repeated doses of 80 mg/kg caused sedation, muscle tremors, mild ataxia, salivation, vomiting and prostration within 1 hour of dosing. Recovery took 3-5 hours.

A single dose of 100 mg/kg in naïve animals produced no signs. Continued dosing at this level produced the signs already noted above. When the 100 mg/kg dose followed a week of dosing at 50 mg/kg, the signs included sedation, muscle tremors, mild ataxia and staining of the mouth (salivation?) in the male and no reported signs in the female. The female vomited on most occasions within 60 minutes of dosing and showed sedation on several occasions 1-2 hours after dosing. Recovery was within 6-7 hours.

One month intravenous toxicity study in dogs: Three male and three female beagles per group were given daily intravenous injection of 0, 1,5 or 20 mg/kg/day of ranolazine.

There was no unscheduled mortality. Signs were reported predominantly for the HD group and included "subdued behavior" almost daily for all HD animals. This began immediately after dosing and lasted from 5 minutes to 1 hour. There were also reports of vomiting, trembling and hind limb ataxia. One female showed ataxia almost continuously during weeks 3 and 4. Conjunctival congestion was reported for a HD male and a HD female. No incidence tables were given. Average body weight was dose-dependently decreased in the males but apparently unaffected in females despite sporadically decreased food consumption and significantly decreased food consumption in the last week in LD and MD females.

Although ECG tracings were reportedly generated, only single animal heart rate data was presented. The study is underpowered to find patterns, although there is a suggestion of a decrease in heart rate in treated animals at 5 minutes and 1 hour after dosing.

A brief pathological report showed only a mention of meningo-encephalitis in one HD female. The sponsor suggests that this was of viral origin. This raises concerns as to the standards of care used in the animal facility if viral meningo-encephalitis (canine distemper against which dogs are routinely vaccinated) found entry.

Oral investigative tolerance study in Beagle dogs with ranolazine administered three times daily: Two male and 2 female dogs were given escalating doses of ranolazine three times a day on the following schedule:

Days 1-7: 25 mg/kg tid ranolazine
Days 8-14 40 mg/kg tid
Days 15-21 50 mg/kg tid
Days 22-35 60 mg/kg tid

One male was euthanized day 29 some 24 hours after his last dose due to marked clinical signs of subdued behavior, thrashing legs, trembling and tachypnea. Plasma drug levels were determined but not reported. Signs reported at 25 and 40 mg/kg tid included green feces and occasional vomiting. Salivation was mild at 50 mg/kg/tid and more pronounced at 60 mg/kg. At 60 mg/kg, peripheral vasodilation as evidenced by the ears was also pronounced as were trembling and subdued behavior. Day 35, one female was observed in lateral recumbency, legs flaying, barking, trembling and salivating. This lasted some 4 minutes with recovery ~3 hours later.

Of the ECG data, only heart rates were provided. The hematology and clinical chemistry data were for 1 dog/sex. This underpowered study provides little information. The sponsor proposes hypotension as a cause of the clinical signs but presents no data to support this. It is not clear that hypotension alone would cause a recumbent dog to thrash and bark.

Four week investigative study in dogs:
This study was originally to evaluate local gastrointestinal effects of a sustained release tablet. Two male Beagles were assigned to the control group and 4 to the treatment group. The animals were dosed once a day, approximating the target dose of 68.2 mg/kg/day. The sponsor states that this is equivalent to the 80 mg/kg/day of the dihydrochloride which was used in a 3 month oral study in dogs.

This single dose study with suboptimal reporting had few findings of toxicological significance.

Three month oral toxicity study in dogs: Clinical signs were noted for doses ≥25 mg/kg, and included salivation, vomiting, ptosis, glazed eyes, conjunctival congestion, sedation, ataxia, trembling and convulsions. "Subdued behavior" was especially apparent in the first month. Significant changes in hematology, clinical chemistry and urinalysis are not apparent. Absolute and normalized testicular and adrenal weight for the treated male dogs was increased over

control but not in a dose-dependent fashion. Uterine weight of the drug-treated females was decreased compared to the control group. The presentation of pathology findings was confusing and raised questions as to the consistency of observations made. Although ophthalmic exams were conducted there was no statement from an ophthalmologist. Although ECG tracings were obtained, only single animal heart rate data was presented. In both sexes of drug treated animals heart rate were decreased at the 1 hour post-dose observation time in week 1.

Organ weight data was presented for individual animals. In an underpowered study such as this one would not expect to be able to discern differences between groups. The reviewer calculated means for absolute and normalized organ weight from the data presented. This is summarized in the table below. The absolute and normalized weight of testes in the drug-treated groups was more than that of the control group but no pattern was discernible. Absolute and normalized adrenal weight was also increased

Reviewer's summary of absolute and normalized (to body weight) selected organ weights

	Dose mg/kg/day				
	0	5	25	60	80
Absolute (testes)	13.12	14.06	15.2	13.06	15.66
Normalized (testes)	0.95	1.13	1.20	1.02	1.088
Absolute (adrenal)	0.74	0.72	0.835	0.85	0.87
Normalized (adrenal)	0.054	0.058	0.066	0.067	0.060

Uterine weight was also decreased in all drug-treated groups. Adrenal weights in the females were not discernibly affected.

Reviewer's summary of absolute and normalized (to body weight) selected organ weights

	Dose mg/kg/day				
	0	5	25	60	80
Absolute (uterine)	8.05	6.86	3.41	4.78	2.89* 4.03**
Normalized (uterine)	0.75	0.59	0.29	0.40	0.24* 0.34**

* the sponsor had a footnote for one individual measurement "left horn only". ** the asterisked animal was omitted from the calculation

Decreased uterine weight was also seen in the one month intravenous dose study in rats.

Six month oral toxicity study in dogs: Dogs were assigned to 4 treatment groups with 4 males and 4 females per group. The dogs were dosed once a day for 26 weeks with 0, 5, 25 or 60 mg/kg/day. Signs were primarily reported for doses ≥ 25 mg/kg and included mydriasis with loss of pupillary light reflexes, glazed eyes, ptosis and other signs of sedation. After the first week mydriasis was not seen until 4-5 hours after dosing. After the first month, mydriasis, glazed eyes and ptosis occurred sporadically in the MD and HD groups. Rouleaux were reported for the MD and HD males and 2 HD females.

Adrenal weights were slightly increased in the HD males while testicular weight was decreased in the HD group. Pituitary weight was increased in females in a dose-related manner.

Reviewer's summary of organ weight changes: Percent change from the control group

Males				
	Dose group (mg/kg)			
	0	5	25	60
Adrenals	-	0	+6	+16
Testes	-	0	0	+23
Females				
Pituitary	-	+43	+39	+57

One year oral toxicity study in dogs: Five Beagles per sex per group were dosed once a day with 0, 10, 25 or 60 mg/kg/day. The signs in this study were significant in that salivation, sedation, trembling, convulsions, personality changes, ataxia and skin conditions (¾ HD males) were noted. The sponsor ascribes these to hypotension and/or cardiovascular collapse but presents no data to support this. The sponsor also stated that the skin condition was unrelated to drug treatment. The dermatologist's report stated that a drug-related effect could not be ruled out based on information available. The minimal information regarding the skin condition indicates an immune-mediated condition. A tissue distribution study in albino and pigmented rats showed preferential distribution to the retina and the skin of pigmented rats. Reports exploring this have not been found in this file. Does the apparent predilection for melanin binding have any bearing upon the skin pathology?

ECG tracings were reportedly obtained but only heart rates were presented.

Ophthalmic examinations were conducted by a "veterinary consultant". It was not made clear if the consultant was an ophthalmologist.

Urinalysis data was essentially qualitative. Acronyms were used without definition, making interpretation difficult.

Absolute and normalized uterine weight of the MD and HD females was decreased compared to the controls. Prostatitis was reported for 0/5(control), 2/5 (LD), 1/5(MD and 2/5(HD) males. Organ results are summarized below.

Reviewer's summary of organ weight changes: percent difference from control

Dose of ranolazine (mg/kg/day)	Adrenal		prostate	testes	uterus
	male	female			
10	+25	0	+52	-9	+4
25	+10	0	+9	-11	-39
60	+14	+21	+9	+5	-27

There was no difference in $AUC_{0\text{-}24}$ between 6 months and 12 months. Females showed greater plasma levels than males at the HD. In both sexes the increase in plasma level with increasing dose was greater than linear. The human equivalent doses are summarized in the table below.

Summary of multiples of human therapeutic exposure

Dog dose	$AUC_{0\text{-}24}$ ng.hr/ml	Multiple of human dose

(mg/kg)	male	female	male	female
10	3108±567	3423±2926	0.09x	0.10x
25	8203±3177	8873±3924	0.25x	0.27x
60	29520±12432	40409±12199	0.89x	1.21x

Special Toxicology Studies

The sponsor states that the "...in vitro and in vivo studies conducted to delineate the observation of adrenalcortical hypertrophy in rats showed ranolazine has no effects of potential toxicological relevance (vol 2, p.40)." The reviewer feels that the studies provide potentially useful pharmacological and toxicological information. From the Special Toxicology Studies (see below in section VIII), the individual study results may be summarized as follows:

RS 43285 RQT(3): Acute adrenal function study in rats

After two oral doses of 300 mg/kg ranolazine, plasma ACTH and corticosterone were decreased. Tissue levels of pregnenolone, progesterone, corticosterone and aldosterone, all expressed as ng/gland, were decreased compared to the control by 31%, 60%, 80% and 63% respectively. The acute increase in tissue steroids with decreased plasma ACTH and corticosterone is not consistent with stress but is suggestive of an acute drug effect.

RS 43285 RQT(2): Acute adrenal function study in rats

From the data as presented, it appears that prior to the addition of a stressful event, the mean basal ACTH, plasma corticosterone and adrenal corticosterone were lower in the drug-treated animals compared to the controls. Plasma ACTH, corticosterone and adrenal corticosterone increased as did plasma cholesterol levels. Therefore, the drug-treated animals did in fact mount an appropriate response to the stressor. We do not have plasma drug levels provided, hematology (was there an appropriate stress-induced neutrophilia), clinical chemistry or histopathology (effects upon lymphoid organs) to assess the relative contribution of stress. From the data presented it can be concluded that there is some form of drug effect upon the adrenal gland after acute administration of ranolazine.

RS 43285 RQT: One month adrenal function study in rats

There was little difference between the treatment groups in terms of basal plasma ACTH and corticosterone. Following a defined stress, plasma and adrenal corticosterone increased to a greater extent in all drug-treated animals. Only the HD group showed slight increases both in serum cholesterol and triglycerides and in adrenal weight.

RS-43285: Investigative study in rat adrenal cells in-vitro

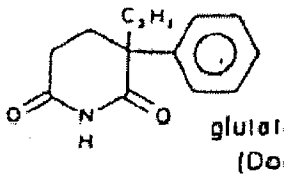
Under the conditions of the assay, ranolazine added to rat adrenal cells treated with ACTH produced a decrease in detected corticosterone. The study would be stronger for the inclusion of comparator compounds. However, the results as presented suggest either a physical or pharmacological interaction with ACTH or some non-competitive effect on the adrenal cells.

CL5566 In-vitro investigation of effects on adrenal tissue in rat, dog and man
Ranolazine inhibited both basal and ACTH-stimulated steroid (cortisol, coricosterone and aldosterone) secretion by rat, dog and human adrenocortical cells at concentrations $\geq 10^{-5}$ mol/l. Some effects were also seen at concentrations of 5×10^{-6}, 10^{-6} and 10^{-7} mol/l. Ranolazine also inhibited basal steroid release and release in the presence of precursors at essetially the same concentrations. It is not clear if the effect is from non-specific enzymatic interaction, cytotoxicity, or involvement in a step prior to production of 22-hydroxycholesterol such as delivery of cholesterol to CYP450 or the ACTH segment of the pathway.

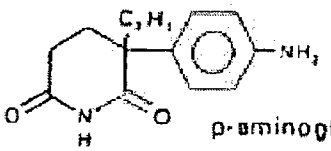
Summary of Special Toxicology Studies

The studies indicate both acute and chronic effects of ranolazine on the adrenal gland. The basal circulating corticosterone levels were generally suppressed while tissue (adrenal) levels were increased. Although this effect is not consistent with a general stress response, hematology, clinical chemistry as well as histopathology of the lymphoid system are needed to support fully that conclusion. When a known, defined stress, usually physical restraint, was applied to the animals, an appropriate response of increased plasma corticosterone was seen in the drug-treated rats. The increase was however, less than the control group response in one study and greater than the control group in a second study. The studies in CL5566 showing that ranolazine caused a decrease in release of adrenal steroids in the basal state, after ACTH stimulation and in the presence of precursors were consistent with the other in vitro work. The laboratory that conducted that work also compared the ranolazine structure to that of known adrenocortical inhibitors such as glutethimide, p-aminoglutethimide, amphenone B, o,p-DDD(mitotane) and metyrapone. The sponsor's diagram is shown below.

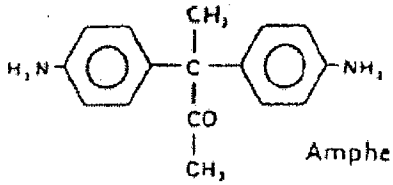
Appears This Way
On Original



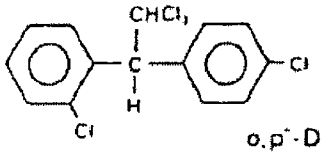
glutethimide
(Doriden)



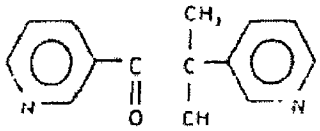
p-aminoglutethimide
(Elipten)



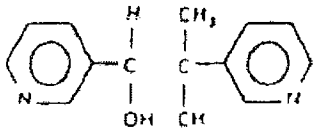
Amphenone B



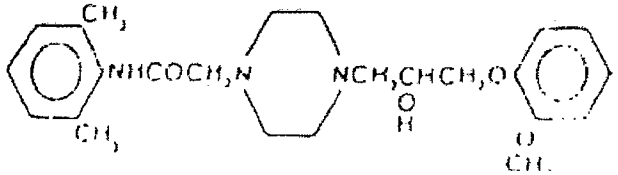
o,p'-DDD
(Mitotane)



Metyrapone
(SU4885)



Metyrapol
(reduced Metyrapone)



IRS 13285

Initiated by the findings of adrenal lesions in two species in the general toxicology studies, the special studies indicate ranolazine's ability to influence adrenal gland function in the preclinical species. It is not clear whether this effect is direct or indirect. One possible indirect mechanism is opioid receptor binding. According to Goodman and Gilman (9th edition), opioid receptor binding can cause a decrease in ACTH secretion. Drolet et al., (Prog Neuro-Psychopharmacol &Biol Psychiat 2001, vol 25, pp729-741) noted that opioids can diminish stress-induced neuroendocrine and autonomic responses and may stimulate these effector systems in the non-stressed state. When the adrenal effects are taken into consideration with receptor binding studies, the neurological signs shown by the animals and the safety pharmacology studies, the combined data suggests a possibility that either the parent drug or a metabolite may be contributing opioid receptor binding to the pre-clinical picture. It is also possible that the adrenal effects are indirect support for the sponsor's proposed mechanism of pharmacologic action.That is, rate limiting components of adrenal steroid biosynthesis are mobilization and delivery of cholesterol to the inner mitochondrial matrix. Somehow ACTH stimulates this translocation by methods that are incompletely described. The rate limiting conversion in steroidal synthesis is the conversion of cholesterol to pregnenolone, another mitochondrial event (Goodman and Gilman 9th ed). If ranolazine does in fact alter the mitochondrial fatty acid oxidation process, are these events somehow secondarily or indirectly changed?

Overall Summary

There are several points of concern common to all the toxicology studies.

1. There is no margin of safety between the plasma levels causing adverse effects in the non-clinical species and the therapeutic plasma levels in humans. Wherever possible, the reviewer calculated exposure relative to humans using the ratio of AUC. The human AUC value should be multiplied by 2 to reflect the exposure that occurs with the proposed twice a day dosing.
2. There is evidence from the distribution studies conducted in pigmented rats that indicates melanin binding in the retinal pigmented epithelium and pigmented skin.
   Skin problems were reported in one of the dog studies. The dermatologist's report stated that a drug effect could not be ruled out.
   No ophthalmologist's report was located in any of the toxicology reports. There is no evidence that a qualified veterinary ophthalmologist has evaluated the eyes of the animals used in the toxicology studies. Of particular interest are the dogs, the only pigmented animals used.
3. Although it was stated in a number of studies that ECG tracings were obtained, the only data presented was single animal heart rate data.

4. The reproductive and developmental toxicology studies indicated effects on fertility. This was supported in several studies: a dose-related increase in pituitary size in female rats (1 year oral study) and a decrease in uterine weight was noted in a 1 month iv dosing rat study; a dose-related decrease in the absolute and normalized uterine weight in female dogs (3 month study). A dose-related decrease in absolute and normalized uterine weight was also seen in the 1 year oral toxicity study in dogs. In males, an increase in absolute and normalized testicular weight was reported in the 3 month dog study and the 6 month dog study. In the 1 year study, the weight of

the prostate was increased at all doses while testicular weight was decreased at LD and MD and increased at HD. The urinalysis data in the one year rat study indicated a dose-related change in the presence of sperm and crystals. There is insufficient information to evaluate the relevance of this finding to the question of fertility.

5. In both rats and dogs, adrenal weight was increased. Where pathology was reported, histopathological findings included diffuse vacuolation (males only), diffuse cytoplasmic foaminess of the zona fasiculata (females only). The mechanism of the adrenal changes is not clear and the relevance to humans is unknown at this time.
6. Neurologic signs were reported for essentially every study. Signs included sedation, ataxia, ptosis, salivation, dyspnea, tachypnea, piloerection, hunched appearance and convulsions. Loss of pupillary light reflex was noted in 2 dogs studies. The sponsor attributes these signs to hypotension but presents no data to support this. The sponsor does not use the word "sedation" to describe the animals' "subdued behavior and ptosis." The neurological evaluation of the safety pharmacology clearly indicated CNS effects of ranolazine, including sedation. Why is the word not used in the toxicology studies?

Toxicology conclusions: Preclinically, ranolazine has neurologic, cardiovascular and reproductive toxicities. The contribution of the metabolites to these adverse effects is unknown. The apparent melanin binding of drug and long elimination half-life raises a question as to the long-term effect upon the pigmented structures of the eye and pigmented skin. If the mechanism of action of ranolazine is a primary down-regulation of mitochondrial fatty acid oxidation, what effect will accumulation of drug have upon the mitochondria-rich retinal pigment epithelium? The study reports presented are suboptimal and leave questions unanswered as to the preclinical characterization of ranolazine.

Appears This Way
On Original

Histopathology Inventory for NDA

Study	SS/028/85	SS/012/85	SS/047/87	AT6971	AT3280	AT3281	AT4050	AT6544	AT6543
Species	dog	Rat	rat	Dog	Rat		Dog	Rat	dog
Adrenals	X*	X*	X*	X*	X*	X*	X*	X*	X*
Aorta	x	X	X	X	x	x	x	X	X
Bone Marrow smear	x		X		x	x	x	X	X
Bone (femur)		X	X		x				
Brain	X*	X*	X	X*	X*	X*	X*	X*	X*
Cecum									X
Cervix	x	X	X	x	x	x	x	X	
Colon	X	X	X	x	x	x	x	X	X
Duodenum	X	X	X	x	x	x	x	X	X
Epididymis	X	X	X	x	x	x	x	X	
Esophagus	X	X	X	x	x	x	x	X	X
Eye	X	X	X	x	x	x	x	X	X
Fallopian tube									
Gall bladder	X			x		x	x		X
Gross lesions	X	X	X	x	x	x	x	X	X
Harderian gland			X					X	
Heart	X*	X*	X*	X*	x	X*	X*	X*	X*
Ileum	X	X	X	x	x	x	x	X	X
Injection site					x	x			
Jejunum	X	X	X		x	x	x	X	X
Kidneys	X*	X*	X*	X*	X*	X*	X*	X*	X*
Lachrymal gland				x			x		X
Larynx									
Liver	X*	X*	X*	X*	X*	X*	X*	X*	X*
Lungs	X	X	X	x	x	x	x	X	X
Lymph nodes, cervical									
Lymph nodes mandibular			X		x			X	
Lymph nodes, mesenteric	X	X	X	x	x	x	x	X	X
Mammary Gland	X	X	x	x	x	x	x	X	X
Nasal cavity									
Optic nerves	X			x	x	x	x		
Ovaries	X*	X*	X	x	x	X*	X*	X*	
Pancreas	X	X	X	x	x	x	x	X	X
Parathyroid			X	x				X	x
Peripheral nerve									
Pharynx									
Pituitary	X*	X*	X*	X*	X*	X*	X*	X*	X*
Prostate	X*	X*	X*	X*	X*	X*	X*	X*	X*
Rectum									X
Salivary gland	X	X	X	x	x	x	x		X
Sciatic nerve	X	X	X	x	x	x	x	X	X
Seminal vesicles									
Skeletal muscle	X	X	X	x	x	x	x	X	X
Skin	X	X	X	x	x	x	x	X	X
Spinal cord	X		X	x	x	x	x	X	X
Spleen	X*	X*	X*	X*	x	x	X*	X*	X*

Sternum	X				x	x			
Stomach	X	X	X	x	x	x	x		X
Testes	X*	X*	X*	X*	X*	X*	X*	X*	X*
Thymus	X*	X	X	X*	X*	X*	X*	X*	X*
Thyroid	X*	X	X*	X*	X*	X*	X*	X*	X*
Tongue			X				x	X	
Trachea	X	X	X	x	x	x	x	X	x
Urinary bladder	X	X	X	x	x	x	x		X
Uterus	X*	X*	X*	X*	X*	X*	X*	X*	
Vagina	X	X	x	x			x		
Zymbal gland									
Standard List									

X, histopathology performed
*, organ weight obtained

V. GENETIC TOXICOLOGY:

Study title: Bacterial reverse mutation assay for [ ]

Key findings: [ ] is an impurity present in the drug product at NMT [ ], w/w. This study is inconclusive. There was a disagreement in the interpretation of results between a technician and the study director that the study director chose to resolve by "reviewing selected plates that could be recovered following disposal of the plates " and doing replicate plating. It would be preferable to repeat the study de novo.

Study no: AA60LA.503.BTL
Volume #, and page #: Vol 14, p. 207
Conducting laboratory and location: [ ]
Date of study initiation: June 18, 2002
GLP compliance: statement included
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: SAR-85-118, >[ ]

Methods: [ ] was tested in the bacterial reverse mutation assay using Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 and E. coli WP2*uvr*A ±S9 activation. In the initial assay, the maximum concentration tested was 5000 µg per plate. Concentrations from 0.05 to 1.5 mg/ml were soluble and clear. Concentrations from 4-100 mg/ml were cloudy. Undissolved particles were present from 1.5 – 100 mg/ml. Concentrations tested were 2.5, 7.5,25, 75, 200, 600, 1800 and 5000 µg per plate. Precipitate was observed at concentrations ≥600 µg per plate. The concentrations tested in the confirmatory assay were 2.5, 7.5, 25, 75, 200, 600, 1800 and 5000 µg per plate. Precipitate was again observed at concentrations ≥ 600 µg per plate. The vehicle used was DMSO. Appropriate positive controls were used. Criteria for a positive response was set to include a "...dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3.0-times the mean

vehicle control value. Datasets for the remaining tester strains were required to have a 2-fold increase in revertants.

Results: The study director apparently did not agree with the results generated by the technician conducting the study and so retrieved some plates from the biohazard waste and re-read them. To confirm the point, replicate plates were made of the plates retrieved from the garbage. The details are provided in the sponsor's paragraph below.

In Experiment B2 (Confirmatory Mutagenicity Assay), increases in the apparent revertant counts, consistent with the criteria for positive responses, were observed with tester strains TA100 and TA1537 in the absence of S9 activation and with TA100 and TA1535 in the presence of S9 activation. Since these responses were drastically different than those observed in Experiment B1, the Study Director reviewed selected plates from Experiments B1 and B2 that could be recovered following disposal of the plates. His initial assessment based on observation of the recovered plates was that intermediate colonies were present in the plates from Experiment B2, especially at the upper dose levels. No intermediate colonies were observed in the plates from Experiment B1. Intermediate colonies are colonies that are smaller than normal revertants but larger than background lawn colonies. This is a phenomenon that is seen occasionally and quite often intermediate colonies are not true revertants. To confirm this hypothesis, selected, recovered plates from Experiment B2 were replicate-plated onto histidine-free medium. The results of these replicate-platings are presented in parentheses next to the corresponding original plate counts in Tables 13 to 16. The results show that the apparent increases in revertant counts observed with TA100 are not true revertants; this doesn't appear to

Bacterial Mutation Assay
Summary of Results

Table 32

Test Article Id : [ ]
Study Number : AA60LA.503.BTL Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	11 ± 1	162 ± 5	32 ± 1	8 ± 1	11 ± 1
2.5	12 ± 2	172 ± 20	22 ± 4	7 ± 1	9 ± 2
7.5	9 ± 0	199 ± 2	26 ± 1	7 ± 2	8 ± 2
25	14 ± 1	194 ± 44	23 ± 3	9 ± 4	10 ± 1
75	10 ± 1	249 ± 68	22 ± 2	14 ± 5	11 ± 3
200	12 ± 2	370 ± 37	48 ± 19	16 ± 4	10 ± 2
600	9 ± 1	356 ± 98	71 ± 19	21 ± 10	9 ± 1
1800	10 ± 3	486 ± 66	74 ± 7	27 ± 3	10 ± 2
5000	13 ± 1	764 ± 135	82 ± 8	27 ± 4	6 ± 3
Positive	104 ± 20	629 ± 85	591 ± 81	936 ± 170	115 ± 16

Liver Microsomes: Rat liver S9

Dose (ug/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	16 ± 1	163 ± 10	14 ± 2	6 ± 2	11 ± 3
2.5	23 ± 4	169 ± 22	15 ± 1	8 ± 5	15 ± 5
7.5	14 ± 1	150 ± 12	10 ± 1	8 ± 2	9 ± 2
25	20 ± 3	183 ± 9	15 ± 5	6 ± 2	10 ± 3
75	17 ± 2	187 ± 36	9 ± 3	5 ± 1	11 ± 2
200	25 ± 3	192 ± 48	18 ± 3	7 ± 2	9 ± 1
600	20 ± 1	235 ± 39	29 ± 16	6 ± 1	10 ± 2
1800	16 ± 2	250 ± 15	29 ± 1	6 ± 2	7 ± 1
5000	21 ± 2	440 ± 58	182 ± 10	12 ± 3	7 ± 2
Positive	294 ± 24	585 ± 53	151 ± 61	56 ± 7	1015 ± 140

Vehicle = Vehicle Control
Positive = Positive Control
Plating aliquot: 50 µL

Bacterial Mutation Assay
Summary of Results

Table 33

Test Article Id : L J
Study Number : AA60LA.503.BTL Experiment No : B3

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	13 ± 2	137 ± 9	14 ± 3	6 ± 2	12 ± 1
2.5	11 ± 1	163 ± 12	17 ± 4	6 ± 3	18 ± 3
7.5	11 ± 1	148 ± 10	18 ± 4	6 ± 3	12 ± 1
25	11 ± 2	166 ± 13	15 ± 1	8 ± 1	16 ± 3
75	15 ± 4	199 ± 34	12 ± 4	6 ± 3	11 ± 1
200	14 ± 3	250 ± 57	31 ± 7	10 ± 3	12 ± 3
600	11 ± 3	157 ± 19	32 ± 9	12 ± 3	14 ± 1
1800	13 ± 3	200 ± 33	29 ± 5	15 ± 3	12 ± 4
5000	13 ± 4	139 ± 28	22 ± 7	11 ± 3	10 ± 1
Positive	100 ± 4	631 ± 16	202 ± 8	555 ± 148	84 ± 6

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	21 ± 3	132 ± 5	14 ± 3	6 ± 3	12 ± 2
2.5	23 ± 3	152 ± 23	17 ± 7	9 ± 2	14 ± 2
7.5	24 ± 2	113 ± 11	17 ± 3	5 ± 2	11 ± 1
25	18 ± 2	157 ± 25	17 ± 3	4 ± 2	14 ± 1
75	13 ± 5	157 ± 20	12 ± 2	6 ± 2	14 ± 3
200	14 ± 4	147 ± 26	15 ± 5	7 ± 3	14 ± 3
600	20 ± 3	184 ± 35	17 ± 6	5 ± 2	11 ± 1
1800	15 ± 5	173 ± 48	23 ± 2	6 ± 1	16 ± 2
5000	19 ± 2	201 ± 11	18 ± 2	5 ± 1	12 ± 2
Positive	187 ± 47	667 ± 27	80 ± 13	80 ± 7	251 ± 64

Vehicle = Vehicle Control
Positive = Positive Control
Plating aliquot: 50 µL

One may ask several questions. Apparently, the technician who originally generated the results either did not feel that intermediate colonies were present or was unaware of the phenomenon. Was an inadequately trained technician conducting the study and if so how much reliance can be placed on the work? Or, was this a disagreement of interpretation? In either case, the study should have been repeated de novo rather retrieving discarded plates.
The study is inconclusive.

Study title: Mutagenicity evaluation of RS43285 batch #11 in the Ames Salmonella/Microsome plate test

Key findings: Under the conditions of the assay RS43285 did not cause an increase in revertants.

Study no: AM0203, [illegible] Number 20988
Volume #, and page #: Vol 14, p. 275
Conducting laboratory and location: [illegible]
Date of study initiation: April 10, 1984
GLP compliance: statement included
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: RS43285, batch 11, purity [illegible] %

Methods: Strains used were TA-1535, TA-1537, TA-1538, TA98 and TA-100. Doses used were determined from a preliminary assay, the results of which were not shown. Doses of RS43285 dissolved in DMSO were 1,10,100, 500, 1000, 2500, 5000 and 10000 (not dissolved but solid) µg per plate. The highest dose was reported to cause 100% toxicity. The compound was tested ±S9 activation. The drug solvent was used as a negative control. Positive controls for -S9 studies were reported as sodium azide, 2-Nitrofluorene and 9-aminoacridine. The positive control for +S9 was 2-amino-anthracene. Criteria for a positive result was observation of a dose-response over three test concentrations and an increase in revertants equal to or greater than three times the solvent control value at the peak of the dose response. It was also required that strains derived from the same parental strain both show similar responses. The sponsor also stated that positive results not confirmed by repetition were not considered positive.

Results: Cytotoxicity was not shown. A summary of concentrations tested, appearance of background lawn and # of colonies per plate is presumably from the dose-ranging study The positive control did not work for TA-100 (-S9) and produced an insipid result with activation. These results were found again in a repeat study. In a third study using only TA98 and TA100, the positive control finally produced an acceptable response. The assay with the two strains was repeated and the results confirmed.

Conclusion: To be completely in accordance with contemporary standards either S. typhimurium strain TA102 or E. coli wp2*uvr*A ±pKM101 should have been included in the tester strains used. However, the study is reasonable. Under the conditions of the assay, RS43285 did not cause an increase in revertants.

Study title: Mutagenicity evaluation of RS-43285-193 in the Ames Salmonella/microsome and mitotic gene conversion assay with yeast strain D4 plate test

Key findings: Cytotoxicity results were not apparent. Under the conditions of the assay, no positive findings were apparent.

Study no: AM0219 Syntex #917-Y-84-43285-193-VO/MU/AM
Volume #, and page #:vol 14, p.298

Conducting laboratory and location: [illegible] J
Date of study initiation: October 26,1984
GLP compliance: a statement was included
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity:RS-43285-193,[illegible] J%

Methods:
Salmonella strains used were TA-1535, TA-1537, TA-1538, TA-98 and TA-100.
Doses used were determined from a preliminary assay, the results of which were not shown. Doses of RS43285 dissolved in DMSO were 1,10,100, 500, 1000, 2500, 5000 and 10000 (not dissolved but solid) µg per plate. The highest dose was reported to cause 100% toxicity. The compound was tested ±S9 activation. The drug solvent was used as a negative control. Positive controls for -S9 studies were reported as sodium azide, 2-Nitrofluorene and 9-aminoacridine. The positive control for +S9 was 2-amino-anthracene. Criteria for a positive result was observation of a dose-response over three test concentrations and an increase in revertants equal to or greater than three times the solvent control value at the peak of the dose response. It was also required that strains derived from the same parental strain both show similar responses. The sponsor also stated that positive results not confirmed by repetition were not considered positive.

Doses used were selected from a preliminary toxicity test performed on the D4 strain. The doses chosen were 1,10,100, 500, 1000, 2500, 5000 and 10000 (not dissolved but solid) µg per plate. Samples were processed ±S9 activation. The positive control for –S9 was N-methyl-N-nitro,N-nitrosoguanidine and for +S9 was sterigmatocystin. The negative control was the vehicle of DMSO. Criteria for a positive result was observation of a positive dose response over three concentrations with the highest increase equal to twice the solvent control value. A positive result that was not confirmed by repetition was not considered significant.

Results and Summary: Cytotoxicity results were not apparent. Under the conditions of the assay, no positive findings were apparent.

Study title: Mutagenicity Evaluation of RS-43285-193 in the in vivo mouse micronucleus assay

Key findings: The presentation of the study does not conform to contemporary standards. Two different methods of euthanasia were used and the report does not specify which animals were euthanized by which method. Control values were presented only for the 24 hour samples. Plasma values or some other indicator of systemic exposure were neither provided nor was a reference given. While the reviewer is inclined to think that the test article did not cause an increase in the number of micronuclei under the assay conditions, the study is equivocal.

Study no:AM0225 918-Y-84-43285-193-MU-MN
Volume #, and page #: vol 14, p. 326
Conducting laboratory and location: [illegible] J
Date of study initiation: January 21, 1985
GLP compliance: statement was included
QA reports: yes (x) no ()

Drug, lot #, radiolabel, and % purity: lot #12, purity —%

Formulation/vehicle: water

Methods: Male and female ICR mice [ ] 5/sex/group were used. Single oral doses of 0 (vehicle control of deionized water), 30, 100 and 300 mg/kg and a positive control of triethylmelamine were used. Animals were euthanized by either CO_2 or cervical dislocation at 24, 48 and 72 hours after dosing. Criteria for a positive response included statistically significant dose-related increase in micronucleated PCEs or the detection of a reproducible and statistically significant dose-response for at least one dose-level. The sponsor states that the final decision was based on "scientific judgement".

Results: Control data was shown only for the 24 hour time point. As presented, the test article did not cause an in crease in micronuclei.

Summary: The presentation of the study does not conform to contemporary standards. Two different methods of euthanasia were used and the report does not specify which animals were euthanized by which method. Control values were presented only for the 24 hour samples. Plasma values or some other indicator of systemic exposure were neither provided nor was a reference given. While the reviewer is inclined to think that the test article did not cause an increase in the number of micronuclei under the assay conditions, the study is equivocal.

Study title: AM0304: Mutagenicity test on RS-43285-193 in an in vitro cytogenetic assay measuring chromosomal aberration frequencies in Chinese hamster ovary (CHO) cells. Amended final report.

Key findings: The study is inadequate in design and yet positive results reproduced with metabolic activation.

Study no: [ ] # 9737-0-437, Syntex protocol 939-Y-86-43285-193-MU-CHO
Volume #, and page #: vol 15, page 1
Conducting laboratory and location: [ ]
Date of study initiation: February 10, 1987
GLP compliance: statement included
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: RS-43285-193, —.

Formulation/vehicle: Test article was dissolved in McCoys 5a culture medium.

Methods: Duplicate cultures were used at each dose level for the test article. Single cultures were used for the negative control, solvent control and at each of two doses of the positive control. Chromosomal aberrations were analyzed from the four highest doses from which results could be obtained and from only one of the positive control doses. Positive controls were mitomycin c (MMC) for –S9 and cyclophosphamide (CP) for +S9.

For –S9, cells were exposed to the test article until 2.5 hours prior to harvest. The cells were treated with colcemid for that 2.5 hour period. +S9, there was a 2 hour incubation with drug followed by washing and reincubation with culture medium for the appropriate interval of time. Colcemid was added 2.5 hours before the termination of the cultures.

Concentrations used in the range-finding study ran from 161 ng/ml to 4.84 mg/ml. Without activation, 161 µg/ml and 484 µg/ml caused 10% and 40% decreases in monolayer confluence and dose-related decreases in visible mitotic cells. The range of 161 mg/ml to 4.84 mg/ml caused "complete toxicity". A concentration range of 45µg/ml through 600 µg/ml was tested in a 20 hour assay. Concentrations reported were 150, 300, 450 and 600 µg/ml. With metabolic activation, the concentrations of 1.61 mg/ml and 4.84 mg/ml caused toxicity with no monolayer remaining. At 484 µg/ml, no toxicity was reported. Ten (100 µg/ml – 600 µg/ml) and 20 (400 µg/ml – 1.6 mg/ml) hour harvests were used for the aberration assay. Concentrations reported were 192, 288, 384 and 576 µg/ml for the 10 hour assay and 400 and 800 µg/ml for the 20 hour assay. One hundred metaphases were analyzed.

One hundred cells from each duplicate culture at four dose levels of drug and from each of the negative and solvent control cultures were analyzed for aberrations. From one positive control culture 25 cells were scored for aberrations. Gaps were not recorded.

Criteria for a positive control included consideration of overall aberration frequency, percentages of cells with any or more than aberrations, positive dose response, and ultimately, scientific judgement.

Results: Cytotoxicity was not shown in the same table with the aberration data. An increase in # of aberrations per cell and % cells with aberrations was reported for 576µg/ml +S9, 10 hour incubation. This was not seen in the 400 or 800 µg/ml concentrations incubated for 20 hours.

Appears This Way
On Original

CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS
(Results from Individual Cultures)

Assay No.: 3737 Trial No.: I Lab Code Cy: 2177 Activation: With [X] Without [ ]

Compound: RS-43285-193

TREATMENT	CELLS SCORED	NUMBER AND TYPE OF ABERRATION: NOT COMPUTED TG	NOT COMPUTED SG	SIMPLE TB	SIMPLE SB	SIMPLE DM	COMPLEX ID	COMPLEX TR	COMPLEX QR	COMPLEX CR	COMPLEX D	COMPLEX R	COMPLEX CI	OTHER PU	OTHER GT	NO. OF ABERRATIONS PER CELL	% CELLS WITH ABERRATIONS	% CELLS WITH >1 ABERRATIONS
CONTROLS NEGATIVE: McCoy's 5a	100	8		1	2											0.03	3.0	0.0
SOLVENT: McCoy's 5a 10 µl/ml	100	7	2													0.00	0.0	0.0
POSITIVE: Cyclophosphamide 50 µg/ml	25	1	2		6	4		4		3			1			0.68	44.0	16.0
TEST COMPOUND: RS-43285-193																		
192 µg/ml A	100	5			1											0.01	1.0	0.0
192 µg/ml B	100	6			1				1			1				0.03	3.0	0.0
288 µg/ml A	100	13			2				1			1				0.04	4.0	0.0
288 µg/ml B	100	12	3		1	1											1.0	1.0
384 µg/ml A	100	5			1							1					1.0	1.0
384 µg/ml B	100	8															0.0	0.0
576 µg/ml A	100	3	6		6	10										0.16	15.0	
576 µg/ml B	100	2	6		4	4										0.08	7.0	1.0

A dose response was seen in a repeat +S9 study. This result was repeated.

TABLE 5A CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS
Cells Fixed 10.0 Hours After Treatment

Assay No.: 3737 Trial No.: III Lab Code Cy: 3113 Activation: With [X] Without [ ]

Compound: RS-43285-193

TREATMENT	CELLS SCORED	NUMBER AND TYPE OF ABERRATION: NOT COMPUTED TG	NOT COMPUTED SG	SIMPLE TB	SIMPLE SB	SIMPLE DM	COMPLEX ID	COMPLEX TR	COMPLEX QR	COMPLEX CR	COMPLEX D	COMPLEX R	COMPLEX CI	OTHER PU	OTHER GT	NO. OF ABERRATIONS PER CELL	% CELLS WITH ABERRATIONS	% CELLS WITH >1 ABERRATIONS
CONTROLS NEGATIVE AND SOLVENT:	200	13	1								3					0.02	1.5	0.0
POSITIVE: Cyclophosphamide 50 µg/ml	25	2	1		2			2	1	5						0.32	24.0*	8.0*
TEST COMPOUND: RS-43285-193																		
598 µg/ml	200	4			1	1					1					0.03	1.5	0.0
898 µg/ml	200	11			2	4			1		1					0.04	2.5	1.0
798 µg/ml	200	24	1		6	14			1	2						0.13	11.0*	1.0

*Significantly greater than the pooled negative and solvent controls, p<0.01

Note: TB=chromatid break
SB= chromosome break

Best Possible Copy

TABLE 5B CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS
(Results from Individual Cultures)

Assay No.: 9373 Trial No.: III Lab Code Cy: 3117 Activation: With [X] Without [ ]

Compound: RS-43285-193

TREATMENT	CELLS SCORED	NUMBER AND TYPE OF ABERRATION																NO. OF ABERRATIONS PER CELL	% CELLS WITH ABERRATIONS	% CELLS WITH >1 ABERRATIONS
		NOT COMPUTED			SIMPLE			COMPLEX							OTHER					
		TG	SG		TB	SB	DM	ID	TR	QH	CR	D	R	CI		PU	GT			
CONTROLS NEGATIVE: McCoy's 5a	100	5	1									2						0.02	2.0	0.0
SOLVENT: McCoy's 5a 10 µl/ml	100	5										1						0.01	1.0	0.0
POSITIVE: Cyclophosphamide 50 µg/ml	25	2	1		2			2	1	3								0.32	24.0	8.0
TEST COMPOUND: RS-43285-193																				
598 µg/ml A	100	2			1	1						1						0.03	3.0	0.0
598 µg/ml B	100	2																0.00	0.0	0.0
698 µg/ml A	100	5				1						1						0.02	2.0	0.0
698 µg/ml B	100	6			2	3			1									0.06	3.0	2.0
798 µg/ml A	100	14	1		4	6			1	2								0.13	11.0	1.0
798 µg/ml B	100	10			4	8												0.12	11.0	1.0

9737-0-437

Best Possible Copy

Summary: For an acceptable protocol, at time of harvest, the highest concentration should produce a significant decrease in degree of cell confluency, mitotic index or cell count (all greater than 50%). The results reported here cannot be attributed to excessive cytotoxicity as cell culture confluence was reported as 100% of the control for the tested range of concentrations with activation and 63% at the highest concentration tested without activation. With and without activation, cells should be exposed to drug for 3-6 hours and sampled at a time equivalent to approximately 1.5 normal cell cycle lengths (~24 hours for CHO cells) after the beginning of treatment. In this study, cells were exposed for ~17 hours to drug (-S9) or for 2hours (+S9). Harvesting for the –S9 cells was ~2.5 hours later (total 20 hours, slightly less than 1 cell cycle). Harvesting for the +S9 cells was ~8 hours later (total of 10 hours) or 18 hours later (total of 20 hours). The study is inadequate in design and yet positive results reproduced with metabolic activation. The results should be considered positive.

Study title:AM0393 Escherichia coli WP2 *uvr*A reverse mutation preincubation assay

Key findings: Under the conditions of the assay, none of the tested concentrations produced an increase in revertants, either with or without metabolic activation.

Study no: 934-Y-91-43285-193-MU-EC, lab study number TA217.502041
Volume #, and page #: Volume 15, page 41
Conducting laboratory and location: [redacted]
Date of study initiation: 11/26/91

GLP compliance: statement was included
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: RS-43285-193, lot#E9-ML-002/01, —%

Formulation/vehicle: DMSO

Methods: RS-43285-193 was tested using the E. coli tester strain WP2*uvr*A ±S9 metabolic activation. A dose range-finding study examined concentrations of 0.3, 1.0, 3.3, 10, 33, 100, 333, 1000, 3333 and 5000 µg per plate. Reduction in colonies >50% was reported for 3333 and 5000 µg/plate +S9. No toxicity was reported for any concentration –S9. The same concentrations were used in the definitive assay. The positive control with metabolic activation was 2-aminoanthracene and the control without activation was methylmethanesulfonate.

Criteria for a positive response included a dose-response effect and at least a 2-fold increase in the number of revertants per plate over the mean number of revertants per plate of the appropriate vehicle control.

Results: Under the conditions of the assay, none of the tested concentrations produced an increase in revertants, either with or without metabolic activation.

Study title:AM0394 CHO/HGPRT Mutation Assay

Key findings: Under the conditions of the assay, the test article did not cause an increase in mutations, either with or without S9 metabolic activation.

Study no: lab # TA217.332016, sponsor # 935-Y-91-43285-193-MU-HGPRT
Volume #, and page #: vol 15, p. 76
Conducting laboratory and location: [ ]
Date of study initiation: Nov. 22, 1991
GLP compliance: statement included
QA reports: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: RS-43285-193, lot #E9-ML-002/01, — %

Formulation/vehicle: DMSO

Methods:
In the preliminary assay, cells were exposed to concentrations of 0.1, 0.3, 1,3,10, 30, 100, 300 and 1000 µg/ml for 5 hours. Cloning efficiency was ≥that of the vehicle control. In the definitive assay, the concentrations used were 200, 400, 600, 800 and 1000µg/ml±S9. Relative cloning efficiency was decreased only in the positive controls.
Positive controls were ethyl methanesulfonate (EMS) and benzo (a) pyrene (B (a) P).
Criteria for a positive result included a dose-dependent increase in mutant frequencies with at least two consecutive doses showing mutant frequencies which are elevated above 40 mutants per 10^6 clonable cells.

Results: Under the conditions of the assay, the test article did not cause an increase in mutations, either with or without S9 metabolic activation.

Study title 0434 Salmonella/mammalian-microsome preincubation mutagenicity assay (Ames test) and Escherichia coli WP2*uvr*A reverse mutation assay

Key findings: Cytotoxicity was reported for 3500 µg and 5000 µg per plate without S9 activation and at 5000µg per plate with S9 activation. The toxicity reported for the highest concentration was moderate to complete. None of the tester strains showed an increase in revertants following exposure to the test article.

Study no: sponsor's # 900-Y-94-43285-003-MU-AMEC
Volume #, and page #: vol 15, p. 108
Conducting laboratory and location: C J
Date of study initiation: 9/20/93
GLP compliance: statement included
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: RS-43285-003, lot 30933-P-100,

Formulation/vehicle: DMSO

Methods: RS-43285-003 was tested in the *Salmonella* and *E. coli* mutagenicity assay using tester strains TA98, TA100, TA1535, TA1537, TA1538 and WP2*uvr*A ±S9 activation. The positive control for the plates –S9 were: 2-nitrofluorene, sodium azide,9-aminoacridine and methyl methanesulfonate. Positive controls for the plates +S9 was 2-aminoanthracene. Concentrations tested were 250, 500, 1000, 2000, 3500 and 5000 µg per plate.

Criteria for a positive assay were listed as 1) a minimum of three non-toxic dose levels required to evaluate the assay data (toxicity criteria also described) 2) at least a two-three - fold increase depending upon the tester strain in the number of revertants per plate over the mean revertants per plate of the appropriate control 3)accompanied by a dose-response.

Results: Cytotoxicity was reported for 3500 µg and 5000 µg per plate both ± S9 activation. The toxicity reported for the highest concentration was moderate to complete. None of the tester strains showed an increase in revertants following exposure to the test article.

Genetic toxicology summary: RS-43285 has been tested in several versions of the Ames assay including Salmonella strains, yeast D4 strains and E. coli WP2*uvr*A. There are several early versions of the Ames assay that were done according to GLP protocols but are not acceptable by contemporary standards due to insufficient number of tester strains used. The most recent *Salmonella sp.* and *E. coli* WP2*uvr*A Ames assays were acceptable by current standards and show no positive responses in the test system. The drug has also been tested in an *in vivo* mouse micronucleus assay, chromosomal aberration assay using CHO cells and a CHO/HGPRT mutation assay.

Under the conditions of the CHO/HGPRT mutation assay, the test article did not cause an increase in mutations, either with or without S9 metabolic activation.

The presentation of the mouse in vitro micronucleus study does not conform to contemporary standards. Two different methods of euthanasia were used and the report does not specify which animals were euthanized by which method. Control values were presented only for the 24 hour samples. Plasma values or some other indicator of systemic exposure were neither provided nor was a reference given. While the reviewer is inclined to think that the test article did not cause an increase in the number of micronuclei under the assay conditions, the study is equivocal.

The CHO chromosomal aberration assay was not done according to contemporary standards yet positive results were obtained. For an acceptable protocol, at time of harvest, the highest concentration should produce a significant decrease in degree of cell confluency, mitotic index or cell count (all by greater than 50%). The results reported here cannot be attributed to excessive cytotoxicity as cell culture confluence was reported as 100% of the control for the tested range of concentrations. With and without activation, cells should be exposed to drug for 3-6 hours and sampled at a time equivalent to approximately 1.5 normal cell cycle lengths (~24 hours for CHO cells) after the beginning of treatment. In this study, cells were exposed for ~17 hours to drug (-S9) or for 2 hours (+S9). Harvesting for the –S9 cells was ~2.5 hours later (total 20 hours, slightly less than 1 cell cycle). Harvesting for the +S9 cells was ~8 hours later(total of 10 hours) or 18 hours later (total of 20 hours). Positive results reproduced with metabolic activation.

Genetic toxicology conclusions: There was no evidence of genotoxicity either in the Salmonella/E.coli WP2uvrA reverse mutation assay either with or without S9 activation or in the CHO/HGPRT assay. However, the in vitro mouse micronucleus assay was inadequate and equivocal. Positive results were found in the CHO chromosomal aberration assay. The genotoxicity of this drug is incompletely characterized.

Labeling recommendations: C
J

VI. CARCINOGENICITY:

Study Title: Ranolazine: Three month oral dose ranging study in mice.

Key study findings: The study was terminated after 8 days due to the deaths of animals at 50, 100 and 200 mg/kg. Clinical signs reported in this study included sedation, hunching and prostration.

Study number: AT6424
Volume #, and page #: vol 27, p. 280
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: February 17, 1987
GLP compliance: statement included
QA report: yes () no (x)
Drug, lot #, and % purity:

Methods: CD-1(ICR)BR mice, 10/sex/group, were given daily oral doses of ranolazine at 0,5,50, 100 and 200 mg/kg/day for 8 days. The original intention was to dose for 3 months. Body weights were recorded weekly and food consumption was estimated weekly. Post-mortem examinations were conducted on those who died. Surviving animals were euthanized and discarded without further examination at the termination of the study.

Results: Fourteen animals died in the first week of dosing. Eleven of these deaths were considered to be treatment related. The deaths are summarized in the reviewer's table below.

Reviewer's summary of unscheduled mortality

Dose of ranolazine (mg/kg/day	# of animals M/F	Deaths M/F	comments
0	10/10	1/0	Malintubation
5	10/10	0/0	
50	10/10	3/0	1 malintubation
100	10/10	1/3	1 male was malintubated
200	10/10	2/4	

Signs were reported for all dose groups.
At 5 mg/kg: 1 m subdued and hunched before dosing and for 2.5 hours after days 5-7
50 mg/kg: 1 m subdued with staining on head
100 mg/kg: subdued behavior
200 mg/kg: 4m/3f subdued day 1, 1m/2f ptosis, 2 more females had a rough coat. There were other instances of subdued behavior and prostration.

In the eight days of the study, the 100 and 200 mg/kg/day males gained on average 45% and 61% less than the control males. Body weights in the females showed no recognizable pattern. Food intake amongst all groups was apparently unaffected. Due to the high number of treatment related deaths it was decided to terminate the study prematurely.

Study title: Three month oral dose ranging study in mice

Key study findings: There is a very slight decrease in hemoglobin, RBC and hematocrit in the 15, 25 and 35 mg/kg-treated males. The hematology results are remarked upon only because they are consistent with similar minimal changes noted in other studies in other species. There are few findings of toxicological significance in this study.

Study number: AT5989 SS/030/90
Volume #, and page #: vol 23, p 4
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: May 1987
GLP compliance:
QA report: yes () no ()
Drug, lot #, and % purity:

CAC concurrence:

Study Type (2 yr bioassay, alternative model etc.): dose ranging

Formulation/vehicle: water
Drug stability/homogeneity:
Methods: Five treatment groups of 10 mice/sex/group were established with :CD-1 (ICR)BR animals [ ] Mice received oral doses of 0, 5,15,25 or 35 mg/kg/day each day for 91-92 days. Body weight and food consumption were recorded weekly. There was daily observation for clinical signs. Blood samples were collected from all surviving mice from all groups under anesthesia after 12 weeks of dosing. Samples from the first 5 animals per group were used for clinical chemistry and samples from the last five per group were used for hematology. Mice were euthanized 24 hours after the last dose. Gross observations were recorded. Tissues weighed were: adrenals, heart, kidneys and liver. Tissues collected for histopath were adrenals, heart, kidneys, liver, lung, altered tissue.

Results:
The information regarding clinical signs and mortality is somewhat unclear. The most frequently observed sign was subdued behavior. The sponsor states that "In those animals that died and were found to have been **misdosed** most were subdued and later cold to the touch for up to three days before death." There was no further elaboration on the term "misdosed." Eight animals were reported to die from malintubation. Seven animals died either during or within a day of the terminal bleed (6 controls, 1 @5 mg/kg, 2 @15 mg/kg, 3 @ 25 mg/kg and 2 @ 35 mg/kg).

Weight was inconsistently affected in all treatment groups. There were no apparent differences in food consumption.

Percent difference in final weight from the untreated control group

	Dose mg/kg/day			
	5	15	25	35
Males	-10	-22	+10	+10
females	+7	-7	-12	-11

There is a very slight decrease in hemoglobin, RBC and hematocrit in the 15, 25 and 35 mg/kg-treated males.
There were no significant differences or trends in the organ weight data.

Summary: There are few findings of toxicological significance in this study. The hematology results are remarked upon only because they are consistent with similar minimal changes noted in other studies in other species.

The summary of the Carcinogenicity studies that was presented to the Exec CAC is attached as Appendix I.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Study title: Oral Fertility and Reproduction Study in Rats with RS-43285-193

Key study findings: This study was marked by suboptimal design, methodology and reporting. However, fertility was decreased in both sexes. The decreased fertility in males was repeated twice. Decreased implantation index was noted in females. Developmental delays were seen in the offspring in all drug-treated groups. These delays included eye opening, negative geotaxis, vaginal opening and survival. Therefore, even without maternal toxicity, there were detrimental effects upon the offspring.

Study no.: AT4136 116-R-86-43285-PO-RMF
Volume #, and page #: Volume 19, page 175
Conducting laboratory and location: Syntex Research, Palo Alto, CA
Date of study initiation: September 19, 1986
GLP compliance: no statement was located in the study, however, pages are signed and there is a QA statement.
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: — %
Formulation/vehicle: sterile water with sodium hydroxide and pH adjusted to pH=4

Methods:

Four groups of 20 male and 40 female rats (CD® (SD)BR:VAF PLUS, [illegible] were given once daily oral doses of 5, 40 or 300 mg ranolazine/kg. Doses were based on the 3 and 6 month rat toxicology studies. After males had been dosed for 80 days and females for 14 days, the sexes were cohabited. Dosing of both sexes continued during the cohabitation period and until weaning of the F1 pups. After weaning the F1 pups, half the control P1 males and all LD and MD P1 males were euthanized and necropsied. Because of an apparent reduction in fertility the remaining control males and the surviving HD males were allowed to survive and were given two additional mating trials with nondosed females. The two additional mating trials followed dosing for 156 days and again after a 32 day recovery period. In the main mating study, animals were cohabited in the ratio of 2 females to 1 male. Due to the deaths of a number of the HD animals, 6 mating groups were housed in a ratio of 1 male and three females. Cohabitation was continued until at least 30 P1 females in each group showed evidence of mating.

The first 12-15 P1 females in each group with evidence of mating were euthanized on GD13, 14 or 15 for midgestation evaluations. The remaining P1 females, with or without evidence of mating, were allowed to litter.

The second generation phase of the study was conducted without further drug treatment.

Randomly selected male and female F1 pups within each dose group were mated at time of sexual maturity. They were then designated P2. Fifteen mating units per treatment group consisted of 2 females and 1 male per unit. Observations were made for the pups of P2 animals (F2) until weaning at 21 days post-partum. The cohabitation period was listed as " continued until at least 18 P2 females in each group showed evidence of mating."

F1 pups were examined for post natal developmental landmarks on specific days. However, "If all pups in a litter did not show a positive response on the first day of observation, the pups were again observed for these characteristics at the next scheduled day of observation for general clinical condition. ... Postweaning developmental tests were conducted only on F1 pups selected for the P2 generation. Each weanling was tested individually on the day indicated (±2 days). If the weanling did not show a positive response at the age indicated, the test was repeated approximately 7 days later or until all animal in the group showed maturation characteristics."

Results:

Seven out of 20 HD males died, from 0.5 – 3 hours after dosing, usually following signs of collapse, convulsions, inactivity and with pulmonary lesions suggestive of gavage errors (p.190). There were ""infrequent recordings" of decreased respiration and pallor. HD females showed signs of inactivity, salivation and collapse. In both sexes, the signs lasted for up to 1 hour. From week 5 to the end of the study, most HD males were reported to show consistent salivation. Day 3 of the study, a number of animals (males) in all groups began to show signs consistent with sialodacryoadenitis. This was confirmed via the sentinel animals. The sponsor reports that approximately 40% manifested this sign by the end of week 1 and approximately 90% by the end of week 6. Residual clinical signs were reported to be gone by week 17. The sponsor states that 4/20 HD females were reported to have died also, presumably of other causes (gavage errors).

In the first 5 weeks of the study the HD males gained on average 24% less than the control group. By week 32, the end of the study, HD males had overall gained 5% less weight than the vehicle controls. The sponsor reported that there were no significant effects on body weight of either sex (p.180). The LD group showed inconsistent patterns of weight gain, possibly due to the swelling associated with the sialodacryoadenitis.

The body weight history for the females is somewhat confusing. The table titled " Summary of P1 pregnant female body weight " starts at week 13 pregnant female weights and goes to week 19. Since the gestation period for the rat is considerably less than 6 weeks, the summary is not clear. The next table is titled "Summary of P1pregnant midgestation sacrifice female body weights." The post-mating day 1 numbers do not correspond to any of the weights in the "summary of pregnant weights" table. Were the numbers for the interim sacrifice animals kept separate from the other body weights? A footnote says that the midgestation sacrificed females were not included in the computations after study week 14.

Sponsor's summary of reproduction status (reviewer's percentages in parentheses)

	group

Dose received (mg/kg/day)	0	5	40	300
P1 males				
Number cohabited	20	20	20	15*
# with evidence of mating (%)	18(90)	19(95)	19(95)	15 (100)
# impregnating at least 1 female* **	**19(95)**	**20**	**20**	**12 (80)**
P1 females				
Number cohabited	40	40	40	36**
# with evidence of mating (%)	**30(75)**	**30(75)**	**31(77)**	**25(69)**
Dams sacrificed at midgestation				
# pregnant	**14(100)**	**14(100)**	**14(93)**	**8(67)**
# not pregnant (% of total)	**0 (0)**	**0 (0)**	**1(7)**	**4 (33%)**
Dams allowed to litter				
# littered	14	16	14	8
# not littered and not pregnant	**2(12.5)**	**0 (0)**	**2(12.5)**	**5 (38)**
Number without evidence of mating	10/40(25)	10/40(25)	9/40(23)	11/36(31)
Number littered	2	3	6	
# not littered and not pregnant	8/40(20)	7/40	3/40	8/36(22)

*five males died prior to initial breeding
**four females died prior to breeding
***includes females with and without evidence of breeding

The two additional studies done due to the initial indications of decreased male fertility are summarized below:
Study 176-R-87 Male rats were given RS-43285 orally at 300 mg/kg/day for at least 133 days before mating to untreated females. There was a reduction in fertility: 100 % of control males impregnated females compared to 69% of HD males.

Study 195-R-87 Male rats were given RS-43285 orally at 300 mg/kg/day for at least 156 days, followed by a 32 day recovery period before mating to untreated females. There was a decrease in male fertility: 100% of control males impregnated females compared with only 69% of HD males.

The sponsor goes on to state that there were several specific males who contribute to these findings:
Study 116-R-86 3 males, #413, #415 and #419
Study 176-R-87 3 males, #415, #419 and #435
Study 195-R-87 4 males #407, #415, #419 and #435

#413 died prior to subsequent matings and no histopathology was provided. The other rats showed histologic evidence of atrophic changes of the testes and/or epididymides. The sponsor felt that these results were incidental. For rats # 407, 415, 419 and 435 to be the sole cause of the 31% decrease in fertility in the last two studies, there could have been no more than 13 animals in the HD group. Given that there were only 15 animals surviving the initial period of dosing, this is entirely possible. However, an effect found in 31% of the animals cannot be dismissed out of hand as unrelated to drug treatment. It must also be emphasized that even with a month of drug-free recovery and no significant weight differences, the decreased male fertility, seen three times in this series of studies did not disappear, reverse or mitigate. The sponsor states that in the 3-

month rat oral tox study there were no changes in the male sex organs after dosing at 500 mg/kg/day. The histopath for the 3 month study was incomplete with no incidence or summary tables. Histopathology results for the 6 month rat study were not susceptible to easy interpretation.

The summary of P1 gestation indices for the midgestation sacrifice group showed a dose-related decrease in percent pregnant and a 25% increase in % pregnant with resorptions. There was a non-significant decrease in litter size and implantations with drug-treatment.

TABLE 4 (1 OF 2)
SUMMARY OF P1 GESTATION INDICES FOR MIDGESTATION SACRIFICE GROUP

GROUP	NO. DAMS	NO. PREG	% PREG	# WITH RESORP.	% PREG WITH RESORP.	# W/ALL FETUSES RESORB.
VEHICLE CONTROL	14	14	100.0	7	50.0	0
5 MG/KG/DAY RS43285-193	14	14	100.0	11	78.6	0
40 MG/KG/DAY RS43285-193	15	14	93.3	11	78.6	0
300 MG/KG/DAY RS43285-193	12	8	66.7	6	75.0	0

VARIABLE	GROUP	N	MEAN	STD DEV	P-LEVEL*
LITTER SIZE	VEHICLE CONTROL	14	14.2	2.29	0.567
	5 MG/KG/DAY RS43285-193	14	13.4	2.28	
	40 MG/KG/DAY RS43285-193	14	13.6	3.34	
	300 MG/KG/DAY RS43285-193	8	13.8	2.92	
TOTAL RESORPTIONS	VEHICLE CONTROL	14	1.0	1.11	0.295
	5 MG/KG/DAY RS43285-193	14	1.1	1.00	
	40 MG/KG/DAY RS43285-193	14	1.1	0.92	
	300 MG/KG/DAY RS43285-193	8	1.1	0.83	
IMPLANTATIONS	VEHICLE CONTROL	14	15.2	1.69	0.735
	5 MG/KG/DAY RS43285-193	14	14.5	2.31	
	40 MG/KG/DAY RS43285-193	14	14.6	3.27	
	300 MG/KG/DAY RS43285-193	8	14.9	3.27	

* - JONCKHEERE DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
N = NUMBER OF PREGNANT FEMALES
NOTE: P1 SIRES WERE DOSED 82 DAYS BEFORE COHABITATION THROUGH WEEK 23
P1 DAMS WERE DOSED 14 DAYS BEFORE COHABITATION UNTIL SACRIFICE

SOURCE: IRDM J08$0473 (11/05/87) J08$0469 (11/05/87) SYW (11/18/87)

Appears This Way
On Original

Summary of P1 Gestation Indices for Midgestation Sacrifice Group

VARIABLE	GROUP	N	MEAN	STD DEV	P-LEVEL*
CORPORA LUTEA	VEHICLE CONTROL	14	13.8	2.33	0.692
	5 MG/KG/DAY RS43285-193	14	13.7	2.16	
	40 MG/KG/DAY RS43285-193	14	13.1	2.16	
	300 MG/KG/DAY RS43285-193	8	14.8	1.28	
RESORPTION INDEX	VEHICLE CONTROL	14	6.9	7.88	>.389
	5 MG/KG/DAY RS43285-193	14	7.3	6.62	
	40 MG/KG/DAY RS43285-193	14	7.9	6.55	
	300 MG/KG/DAY RS43285-193	8	7.0	5.25	
IMPLANTATION INDEX	VEHICLE CONTROL	14	113.1	21.18	>.180
	5 MG/KG/DAY RS43285-193	14	107.3	18.79	
	40 MG/KG/DAY RS43285-193	14	112.9	25.51	
	300 MG/KG/DAY RS43285-193	8	100.3	19.75	

* - JONCKHEERE DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
N = NUMBER OF PREGNANT FEMALES
NOTE: P1 SIRES WERE DOSED 82 DAYS BEFORE COHABITATION THROUGH WEEK 23
P1 DAMS WERE DOSED 14 DAYS BEFORE COHABITATION UNTIL SACRIFICE
RESORPTION INDEX = (TOTAL RESORPTIONS/IMPLANTATIONS) X 100
IMPLANTATION INDEX = (IMPLANTATIONS/CORPORA LUTEA) X 100

SOURCE: IRDM JOB$0473 (11/05/87) JOB$0469 (11/06/87) SYW (11/18/87)

The summary indices for midgestational sacrifice group (dosed males mated with untreated females) shows decreased percentage pregnancies and fewer implantations.

TABLE 4A (2 OF 2)
SUMMARY OF GESTATIONAL INDICES FOR MIDGESTATION SACRIFICE GROUP
FOR 176-R-87-43285-193-P0-MR

VARIABLE	GROUP	N	MEAN	STD DEV	P-LEVEL*
RESORPTION INDEX	VEHICLE CONTROL	10	8.6	8.51	
	300 MG/KG/DAY RS43285-193	8	3.9	4.80	0.913
IMPLANTATION INDEX	VEHICLE CONTROL	10	117.5	17.57	
	300 MG/KG/DAY RS43285-193	8	90.6*	27.37	0.015

RESORPTION INDEX = (TOTAL RESORPTIONS/IMPLANTATIONS) X 100
IMPLANTATION INDEX = (IMPLANTATIONS/CORPORA LUTEA) X 100

* - MANN-WHITNEY TEST ONE SIDED P-VALUE ON ALL GROUPS

* - INDICATES SIGNIFICANTLY LOWER THAN VEHICLE CONTROLS VIA THE MANN-WHITNEY TEST AT P = 0.05

NOTE: UNDOSED FEMALES MATED WITH DOSED MALES. N = NUMBER OF MALES WITH AT LEAST 1 FEMALE PREGNANT.

SOURCE: IRDM JOB$0255 (09/11/87) SYW$2599 (09/08/87)

When treated males were mated to treated females, the HD group had a pregnancy rate of 53% compared to 75% for the control group.

HD male rats treated for 133 days before mating with untreated females: 69% of the HD males impregnated females compared to 100% for untreated control males. Male rats treated for 156 days followed by a 32-day drug-free recovery period were mated to untreated females. 69% of the HD males impregnated females compared to 100% of the untreated control males.

The testicular and epididymal pathology results show that in the HD males who survived to necropsy, 2 out of 13 showed epididymal atrophy with 0-few spermatozoa present. One additional animal showed a decreased amount of spermatozoa. Incidence of these findings in all other groups was 0. Four/13 HD rats showed atrophy of the seminiferous tubules compared to 2/20 control rats. There was no quantitative examination of sperm numbers, motility or morphology apparent in the report. There was no mention of prostate gland or the seminal vesicles. No organ weights were listed.

The 4-day survival index was significantly decreased in the HD group and non-significantly decreased in the MD group: Control and LD groups: 100% survival, MD: 99% survival and HD: 95.59% survival ($p<0.05$ by Mann-Whitney). Survival index was also decreased day 7 (MD and HD), in all drug-treated groups days 14 and 21.

TABLE 5 (2 OF 2)
SUMMARY OF P1 GESTATION & F1 NEONATAL INDICES**

VARIABLE	GROUP	N	MEAN	STD DEV	P-LEVEL*
SURVIVAL INDEX DAY 7	VEHICLE CONTROL	16	100.00	0.000	0.016
	5 MG/KG/DAY RS43285-193	19	100.00#	0.000	
	40 MG/KG/DAY RS43285-193	20	99.38	2.795	
	300 MG/KG/DAY RS43285-193	11	96.59+	8.083	
SURVIVAL INDEX DAY 14	VEHICLE CONTROL	16	100.00	0.000	0.052
	5 MG/KG/DAY RS43285-193	19	94.74	22.942	
	40 MG/KG/DAY RS43285-193	20	99.38	2.795	
	300 MG/KG/DAY RS43285-193	11	96.59	8.083	
SURVIVAL INDEX DAY 21	VEHICLE CONTROL	16	100.00	0.000	0.030
	5 MG/KG/DAY RS43285-193	19	94.74	22.942	
	40 MG/KG/DAY RS43285-193	20	97.50	6.538	
	300 MG/KG/DAY RS43285-193	11	96.59+	8.083	
LACTATION INDEX	VEHICLE CONTROL	16	100.00	0.000	0.310
	5 MG/KG/DAY RS43285-193	19	94.74	22.942	
	40 MG/KG/DAY RS43285-193	20	98.13	6.117	
	300 MG/KG/DAY RS43285-193	11	100.00	0.000	

* - JONCKHEERE DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
+ - INDICATES SIGNIFICANTLY DIFFERENT FROM VEHICLE CONTROLS VIA THE MANN-WHITNEY TEST AT P=0.05
# - OBSERVATIONS FOR THIS GROUP EQUAL TO CONTROLS, NO COMPARISONS WERE MADE
** - FOR LITTERED FEMALES ONLY
DAY(I) SURVIVAL INDEX=(PUPS ALIVE DAY(I)/PUPS ALIVE DAY 1 AFTER CULLING) X 100
LACTATION INDEX=(PUPS ALIVE DAY 21/PUPS ALIVE DAY 4) X 100
NOTE: P1 SIRES WERE DOSED 82 DAYS BEFORE COHABITATION THROUGH WEEK 23
P1 DAMS WERE DOSED 14 DAYS BEFORE COHABITATION UNTIL SACRIFICE

SOURCE: IRDM JQB$0403 (10/27/87) JQB$0456 (11/04/87)

Mean pup weights in the HD group were decreased compared to control at all points of determination and reporting.

TABLE 6 (1 OF 2)
SUMMARY OF F1 MEAN PUP WEIGHTS (GRAMS) PER LITTER

MALES

AGE	GROUP	N	MEAN	STD DEV	P-LEVEL*
DAY 4	VEHICLE CONTROL	15	11.17	1.087	0.266
	5 MG/KG/DAY RS43285-193	18	11.23	0.930	
	40 MG/KG/DAY RS43285-193	20	11.20	1.066	
	300 MG/KG/DAY RS43285-193	11	10.57	0.937	
DAY 7	VEHICLE CONTROL	15	16.73	1.517	0.658
	5 MG/KG/DAY RS43285-193	19	16.75	1.943	
	40 MG/KG/DAY RS43285-193	20	16.98	1.542	
	300 MG/KG/DAY RS43285-193	11	16.08	1.826	
DAY 14	VEHICLE CONTROL	15	33.61	2.621	0.323
	5 MG/KG/DAY RS43285-193	18	33.93	1.852	
	40 MG/KG/DAY RS43285-193	20	33.56	2.490	
	300 MG/KG/DAY RS43285-193	11	32.39	2.946	
DAY 21	VEHICLE CONTROL	15	53.01	4.810	0.571
	5 MG/KG/DAY RS43285-193	18	55.10	3.870	
	40 MG/KG/DAY RS43285-193	20	55.23	5.401	
	300 MG/KG/DAY RS43285-193	11	51.21	4.288	

* - JONCKHEERE DOSE RESPONSE TWO SIDED P-VALUE ON ALL GROUPS
N = NUMBER OF LITTERS WITH SURVIVING MALE PUPS
NOTE: P1 SIRES WERE DOSED 82 DAYS BEFORE COHABITATION THROUGH WEEK 23
P1 DAMS WERE DOSED 14 DAYS BEFORE COHABITATION UNTIL SACRIFICE

SOURCE: FROM JOB$0404 (10/27/87)

Appears This Way
On Original

Developmental parameters show that eye opening was delayed in all drug-treated litters compared to the control. Incisor eruption was delayed in the LD and HD groups. Negative geotaxis was also delayed in a dose-related manner.

TABLE 7 (1 OF 4)

SUMMARY OF F1 PHYSICAL AND BEHAVIORAL CHARACTERISTICS

CHARACTERISTIC (TEST NO: AGE)	GROUP	N	PROP$ OF LITTERS	P-* LEVEL	PROP# PER LITTER MEAN	PROP# PER LITTER STD DEV	P-** LEVEL
PINNA DETACHMENT (TEST 1:DAY 4)	VEHICLE CONTROL	16	1.00		1.00	0.000	
	5 MG/KG/DAY RS43285-193	18	1.00		1.00	0.000	
	40 MG/KG/DAY RS43285-193	20	1.00		1.00	0.000	
	300 MG/KG/DAY RS43285-193	11	1.00		1.00	0.000	
PINNA DETACHMENT (TEST 2:DAY 7)	VEHICLE CONTROL	16	1.00		1.00	0.000	
	5 MG/KG/DAY RS43285-193	19	1.00		1.00	0.000	
	40 MG/KG/DAY RS43285-193	20	1.00		1.00	0.000	
	300 MG/KG/DAY RS43285-193	11	1.00		1.00	0.000	
INCISOR ERUPTION (TEST 1:DAY 10)	VEHICLE CONTROL	16	0.13		0.46	0.356	
	5 MG/KG/DAY RS43285-193	18	0.06		0.33	0.357	
	40 MG/KG/DAY RS43285-193	20	0.10		0.43	0.343	
	300 MG/KG/DAY RS43285-193	11	0.09	0.434	0.19	0.351	0.077
INCISOR ERUPTION (TEST 2:DAY 14)	VEHICLE CONTROL	16	1.00		1.00	0.000	
	5 MG/KG/DAY RS43285-193	18	1.00		1.00	0.000	
	40 MG/KG/DAY RS43285-193	20	1.00		1.00	0.000	
	300 MG/KG/DAY RS43285-193	11	1.00		1.00	0.000	
FUR DEVELOPMENT (TEST 1:DAY 7)	VEHICLE CONTROL	16	1.00		1.00	0.000	
	5 MG/KG/DAY RS43285-193	19	1.00		1.00	0.000	
	40 MG/KG/DAY RS43285-193	20	1.00		1.00	0.000	
	300 MG/KG/DAY RS43285-193	11	1.00		1.00	0.000	
EYE OPENING (TEST 1:DAY 14)	VEHICLE CONTROL	16	0.25		0.57	0.345	
	5 MG/KG/DAY RS43285-193	18	0.06		0.38	0.322	
	40 MG/KG/DAY RS43285-193	20	0.10		0.46	0.340	
	300 MG/KG/DAY RS43285-193	11	0.09	0.122	0.24#	0.360	0.021

N = NUMBER OF LITTERS TESTED ON DESIGNATED DAY, - = NOT APPLICABLE
$ = PROPORTION OF LITTERS WITH ALL PUPS HAVING THE CHARACTERISTIC ON OR BEFORE THE DESIGNATED TEST DAY
# = PROPORTION OF PUPS IN A LITTER WITH THE CHARACTERISTIC ON OR BEFORE THE DESIGNATED TEST DAY
* - ARMITAGE DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
**- JONCKHEERE DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
- INDICATES SIGNIFICANTLY DIFFERENT FROM VEHICLE CONTROLS VIA THE ONE-SIDED MANN-WHITNEY TEST AT P=0.01

NOTE: P1 SIRES WERE DOSED 82 DAYS BEFORE COHABITATION THROUGH WEEK 23
P1 DAMS WERE DOSED 14 DAYS BEOFRE COHABITATION UNTIL SACRIFICE
SOURCE: FROM SYW$3173 (11/16/87), SYW$3133 (11/13/87), SYW$3162 (11/16/87), SYW$3187 (11/17/87), SYW$3183 (11/17/87), SYW$3206 (11/17/87)

TABLE 7 (2 OF 4)

SUMMARY OF F1 PHYSICAL AND BEHAVIORAL CHARACTERISTICS

CHARACTERISTIC (TEST NO: AGE)	GROUP	N	PROP$ OF LITTERS	P-* LEVEL	PROP# PER LITTER MEAN	PROP# PER LITTER STD DEV	P-** LEVEL
EYE OPENING (TEST 2:DAY 21)	VEHICLE CONTROL	16	1.00		1.00	0.000	
	5 MG/KG/DAY RS43285-193	18	1.00		1.00	0.000	
	40 MG/KG/DAY RS43285-193	20	1.00		1.00	0.000	
	300 MG/KG/DAY RS43285-193	11	1.00	-	1.00	0.000	
SURFACE RIGHTING REFLEX (TEST 1:DAY 7)	VEHICLE CONTROL	16	0.38		0.85	0.144	
	5 MG/KG/DAY RS43285-193	19	0.47		0.88	0.159	
	40 MG/KG/DAY RS43285-193	20	0.50		0.80	0.228	
	300 MG/KG/DAY RS43285-193	11	0.36	0.555	0.84	0.167	0.393
SURFACE RIGHTING REFLEX (TEST 2:DAY 10)	VEHICLE CONTROL	16	0.88		0.97	0.100	
	5 MG/KG/DAY RS43285-193	19	0.89		0.98	0.063	
	40 MG/KG/DAY RS43285-193	20	0.95		0.99	0.028	
	300 MG/KG/DAY RS43285-193	11	1.00	0.942#	1.00	0.000	0.918
SURFACE RIGHTING REFLEX (TEST 3:DAY 14)	VEHICLE CONTROL	16	1.00		1.00	0.000	
	5 MG/KG/DAY RS43285-193	19	0.95		0.99	0.029	
	40 MG/KG/DAY RS43285-193	20	1.00		1.00	0.000	
	300 MG/KG/DAY RS43285-193	11	1.00	0.758#	1.00	0.000	0.658
NEGATIVE GEOTAXIS (TEST 1:DAY 7)	VEHICLE CONTROL	16	0.25		0.62	0.375	
	5 MG/KG/DAY RS43285-193	19	0.21		0.58	0.347	
	40 MG/KG/DAY RS43285-193	20	0.10		0.54	0.297	
	300 MG/KG/DAY RS43285-193	11	0.00	0.024	0.41*	0.281	0.034
NEGATIVE GEOTAXIS (TEST 2:DAY 10)	VEHICLE CONTROL	16	0.75		0.97	0.061	
	5 MG/KG/DAY RS43285-193	18	0.83		0.95	0.130	
	40 MG/KG/DAY RS43285-193	20	0.75		0.98	0.085	
	300 MG/KG/DAY RS43285-193	11	0.73	0.388	0.96	0.066	0.384

N = NUMBER OF LITTERS TESTED ON DESIGNATED DAY, - = NOT APPLICABLE
$ = PROPORTION OF LITTERS WITH ALL PUPS HAVING THE CHARACTERISTIC ON OR BEFORE THE DESIGNATED TEST DAY
# = PROPORTION OF PUPS IN A LITTER WITH THE CHARACTERISTIC ON OR BEFORE THE DESIGNATED TEST DAY
* - ARMITAGE DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
**- JONCKHEERE DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
- EXACT DOSE RESPONSE ONE SIDED P-VALUE ON ALL GROUPS
* - INDICATES SIGNIFICANTLY DIFFERENT FROM VEHICLE CONTROLS VIA THE ONE-SIDED MANN-WHITNEY TEST AT P=0.05

NOTE: P1 SIRES WERE DOSED 82 DAYS BEFORE COHABITATION THROUGH WEEK 23
P1 DAMS WERE DOSED 14 DAYS BEOFRE COHABITATION UNTIL SACRIFICE
SOURCE: FROM SYW$3173 (11/16/87), SYW$3133 (11/13/87), SYW$3162 (11/16/87), SYW$3187 (11/17/87), SYW$3183 (11/17/87), SYW$3206 (11/17/87)

Vaginal opening was delayed in the MD and HD F1 females. While 100% of the control and LD offspring were reported to have opened between days 33-36, only 98% and 86% of the MD and HD rats respectively opened during that time. With a range of three days for the observation, some sensitivity is lost.

Fertility as evidenced by mating shows only 75% of the P1 untreated control females pregnant (30/40) and 53% of the HD females (19/36). In the P2 generation, only 67% of the untreated control P2 females (10/15) were reported pregnant. Of the drug-treated P2 offspring, 100% were reported pregnant. The untreated controls in each case show substantially lower mating and fertility values than the drug-treated animals of both generations.

Sponsor's Summary of P2 Reproductive Status (Reviewer's Percentages)

	Group			
Parental Dose mg/kg/day	0	5	40	300
P2 males				
Number cohabited	15	14*	15	15
Number with evidence of mating	14	13	15	14
Number impregnating at least 1 female	10	14	15	15
P2 Females				
Number cohabited	29*	30	30	30
# with evidence of mating	22(76)	24(80)	27(90)	18 (60)
# littered	15(52)	21(70)	25(50)	17(57)
# not littered and not pregnant	7	3	2	1
# without evidence of mating	7(24)	6	3	12(40)
#littered	1	4	0	2
# not littered	6(21)	2	3	10 (30)
#pregnant	0	0	0	1
#not pregnant	6	2	3	9

*one male and 1 female died prior to breeding.

Summary:
The study has several problems:

1. No organ weight data was provided.
2. No information was provided for the seminal vesicles or prostate gland
3. The cohabitation period (found in an Appendix) was listed as 5 days to 3 weeks if necessary. No detail was given in the report as to the time of cohabitation actually used for the different treatment groups or how many matings were necessary on average for the different groups.
4. The protocol did not specify and the report did not provide any information as to the cyclicity of the females.
5. The report did not specify methods of fixation or sectioning for what little pathology was done.
6. Sperm assessment including numbers, motility and morphology was not specified in the protocol nor mentioned in the report.
7. Any female that did not litter was euthanized and examined by necropsy approximately 1 week after the expected time of parturition for determination

of pregnancy status- not the most sensitive time for determination of fertility problems.

8. No table of data was found for those P1 females euthanized at the end of gestation rather than at midgestation.
9. Day 3 of the study, a number of males in all groups developed swelling in the ventrum of the neck. Approximately 40% of the males manifested this sign by the end of the first week and approximately 90% by the end of week 6. Sialodacryoadenitis (SDA) was diagnosed. Residual clinical signs were reported as absent by week 17 of the study. Why was the study continued at all given the incidence and duration of the disease outbreak? When an outbreak of SDA occurs, it typically goes through the entire colony with a single animal having signs for 7-10 days. There are two publications that report the virus causes irregular reproductive cycles in females (Macy et al. 1996. " Reproductive abnormalities associated with a coronavirus." Lab Anim Sci, vol 46, p 129-132; Utsumi 1991" Reproductive disorders in female rats infected with SDA". Exp Animal, vol 40, p 361-365.) as well as other reproductive and developmental problems.

Despite the suboptimal methodology, that there was a decrease in male fertility determined on the basis of dosing and breeding, a very unusual circumstance. This decrease was seen not once, but three times: in the initial P1 cohabitation/breeding(HD males impregnating females was 80% compared to the control group, 95%) the first extended dosing period of 133 days(HD males impregnated 69% of untreated females compared to the control males impregnating 100% of females) and in the group given the 32-day drug-free recovery period (69% of HD males impregnated untreated females compared to untreated males who impregnated 100% of untreated females). Because the rat has a large functional reserve, to see a decrease in fertility in such an insensitive parameter as breeding is a cause for concern. In the dose gap between 40 mg/kg and 300 mg/kg may well be a dose-response curve without parental clinical signs. The sponsor states that the decreases in male fertility in the HD group were due solely to 4 animals. Since the total group size for the surviving HD males was n=14, the 4 males in question represented 31% of the group. Although 2/20 control males were found to have atrophy of the seminiferous tubules, that group of males impregnated 100% of the females with whom they were cohabited.

It should also be noted that even without the data from the P1 end-of-gestation data, there was an apparent decrease in female fertility. The sponsor states in the summary that 53% of the HD P1 females were pregnant compared to the untreated controls where 75% were pregnant. The control fertility is low, but the HD females show a decrease that appears to go beyond the controls. At the midgestational euthanasia, there was 100% pregnancy in the control and LD groups, 93% pregnancy in the MD (with no maternal toxicity) and 67% pregnancy in the HD group. There was also an increase in percent pregnant with resorptions in all the drug-treated groups: 25-28% greater than the control group. Mean litter size was decreased in the drug-treated groups. Mean implantations were decreased in the midgestational data for the drug-treated groups, at the term euthanasia and in the extended study (176-R-87-43285-193-PO-MR, 133 days of dosing, a 27% decrease in implantation index compared to controls) in which untreated females were mated with HD males. There was no difference in implantation index when males were given a 32-day drug-free recovery period before mating. This is suggestive of a detrimental effect upon the quality of DNA in the sperm.

Developmental effects were also apparent. Pre-weaning mortality was significantly different from the control group in the HD pups on post-natal days 1,4,7 and 21. Eye opening and negative geotaxis were delayed in all drug-treated groups compared to the control pups. Vaginal opening was also delayed in MD and HD female pups. Given the insensitive nature of the method used to examine the developmental endpoints, the detection of any differences is impressive.

Study title: Oral Teratology Study in Rats with RS-43285-193

Key study findings: The study is suboptimal in design by contemporary standards. The HD produced such severe toxicity that there was an inadequate number of litters for evaluation if one uses the current guidelines. The sponsor argues that under the 1966 guidelines in place at the time of conduct, the study was acceptable. There was an increase in the incidence of reduced fetal ossification of the pelvic bones: 24% of the control litters vs 53% of the HD litters were affected. Misshapen sternebrae were also increased: control 52% of litters, LD 73% of litters, MD 69% of litters and HD 80% of litters. The sponsor appears to have analyzed the fetuses as the unit of comparison instead of the litter. If one looks at the above two parameters by the sponsors methods, the pelvic bone effect was control: 4%, LD 3%, MD 3%, HD 16%. Sternebrae: control 10%, LD 11%, MD 12% and HD 21%. Dysmorphic cranial ossification was increased in the LD and MD groups: control 52% of litters, LD 62%, MD 69% and high dose 33%. A HD that had not killed so many of the dams and had left more litters for evaluation may have shown completion of the dose response. No blood levels of drug are provided or referenced. From other studies (see the Carcinogenicity report) it is known that the LD and MD used produced fractional human equivalent doses. Based on a comparison of $AUC_{0\text{-}8}$, a 150 mg/kg dose in rats gave 0.7x the exposure of a human taking 30 mg/kg/day. Assuming a 70 kg person, this is approximately 2000 mg total dose or 1000 mg given bid. Given the inability to achieve a substantial multiple of human exposure in the animals, the conservative interpretation of this study would seem the most prudent. That is, that the excessive mortality at the HD prevented the clear delineation of the dose response curve and it should be interpreted that the drug causes teratogenic effects at low multiples of human exposure.

Study no.:AT3758 51-R-86-43285-193-PO-TT
Volume #, and page #: vol 17, p. 5
Conducting laboratory and location: Syntex Research, Palo Alto, CA
Date of study initiation: May 27, 1986
GLP compliance: no
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: lots AT258SA2420, AT258SA320A, AT258/259SA224C aka AT258/259SA321A; [illegible] %
Formulation/vehicle: sterile water with sodium hydroxide and pH adjusted to pH=4

Methods: Sprague-Dawley rats [illegible] CD(SD)BR:VAF/Plus, from) with evidence of mating were randomly assigned to 4 groups of 27 females each. Rats were dosed with 0 (vehicle control), 5, 40 or 400 mg/kg/day RS-43285 by oral gavage from GD7 through GD16. After

3 days of once daily dosing the regimen was changed to twice daily dosing (half the dose given at each dosing) for all animals due to high mortality in the HD group. The rats were euthanized GD21. Females were observed daily with observations recorded on GD1, 7,14,16, 21, and days when a significant change was noted. Food intake was measured and recorded days 1,7,14 and 21. After euthanasia, the uterus was weighed and corpora lutea counted, live fetuses, early and late resorptions recorded. One-third of live fetuses were euthanized and processed for visceral examination. The remaining fetuses were processed for skeletal examination.

Results: 10 of 27 HD females died between study days 8-17 (4 deaths occurring after the switch to twice daily dosing). Other signs reported for this group included significantly lower food intake, listless and significantly lower gestational weight (28% lower than the control group), inactivity, labored respiration, slight to moderate ptosis, lack of mobility, unthrifty, cold, cyanotic and vocalizing post-dosing. Eight of 27 HD rats collapsed and had clonic convulsions after dosing (p.14). Four other HD rats collapsed post-dosing also. Mydriasis was also seen post-dosing as well as gasping, salivation and ocular and nasal discharge. There were no other reported signs of significance for the other drug-treated groups. Weight gain was comparable for the control, LD and MD groups.

There were no pregnant dams without fetuses in the control, LD or MD groups. There were however 9 pregnant dams without live fetuses in the HD group.

In the Summary of Gestational Indices (p.128), the "live litter size" is reported as:

group	Minimum live litter size	Maximum live litter size
Control	5.0	18.0
LD	6.0	19.0
MD	1.0	19.0
HD	1.0	19.0

Fetal weight was significantly ($p<0.005$) decreased in the HD group: 2.8 g vs 3.6 g for the control pups.

Number of corpora lutea showed a dose-related pattern even though dosing did not start until after the establishment of pregnancy:

Summary of corpora lutea(p.129)

group	Minimum corpora lutea	Maximum corpora lutea
Control	13.0	21.0
LD	13.0	20.0
MD	7.0	24.0
HD	4.0	21.0

Gestation survival index for all groups was shown as 100%. This is confusing in light of the 9 HD litters listed as "without live fetuses." The resorption index (total resorptions/implantations) was 11.3 for the HD group compared to 6.1 for the controls.

GROSS GROUP FETAL INCIDENCE TABLE FOR RATS GIVEN RS 43285-193

EXPERIMENT REGIMEN
(2 DAILY ORAL DOSE(S) DURING DAY 7 THRU DAY 16 OF GESTATION)
GROUP 0100 - VEHICLE CONTROL
GROUP 0200 - 5.000 MG/KG/DAY RS 43285-193
GROUP 0300 - 40.000 MG/KG/DAY RS 43285-193
GROUP 0400 - 400.000 MG/KG/DAY RS 43285-193
(FEMALES WERE NECROPSIED ON POST-MATING DAY 21)

GROUP	0100		0200		0300		0400	
PREGNANT DAMS IN GROUP	25		26		26		24	
PREGNANT DAMS WITHOUT LIVE FETUSES							9	
LIVE FETUSES IN GROUP	353		381		380		207	
EXTERNAL EXAMS IN GROUP	353		381		380		207	
SKELETAL EXAMS IN GROUP	231		250		253		134	
VISCERAL EXAMS IN GROUP	122		131		127		73	

	LITTER #	LITTER %	FETUS* #	FETUS* %	LITTER #	LITTER %	FETUS* #	FETUS* %	LITTER #	LITTER %	FETUS* #	FETUS* %	LITTER #	LITTER %	FETUS* #	FETUS* %
NO. WITH ONE OR MORE CHANGES	25	100.0	181	51.3	26	100.0	187	49.1	26	100.0	183	48.2	15	100.0	127	61.4
EXTERNAL EXAMINATION																
NO ANOMALIES OBSERVED																
NO. OF FETUSES	25	100.0	351	99.4	26	100.0	379	99.5	25	96.2	378	99.7	15	100.0	307	100.0
AT 4Y	1	4.0	1	.3												
DECREASED SIZE	1	4.0	1	.3	1	3.8	1	.3								
EDEMA									1	3.8	1	.3				
EYE/S																
ANOPHTHALMIA					1	3.8	1	.3								
PALLOR									1	3.8	1	.3				
RACHISCHISIS																
W/ EXENCEPHALY					1	3.8	1	.3								
SKELETAL EXAMINATION																
NO ANOMALIES OBSERVED																
NO. OF FETUSES	18	76.0	53	22.8	19	73.1	68	27.2	18	69.2	73	28.8	4	26.7	9	6.7
CRANIAL																
OSSIFICATION																
DYSMORPHIC	13	52.0	40	17.3	16	61.6	44	17.6	18	69.2	41	16.2	6	33.3	8	5.7
REDUCED	8	32.0	13	5.6	8	30.8	11	4.4	6	23.1	11	4.3	6	40.0	11	8.2
HYOID																
OSSIFICATION																
NON OSSIFIED	9	36.0	19	8.2	13	50.0	23	9.2	13	50.0	28	11.1	9	60.0	13	9.7
REDUCED	8	32.0	29	12.6	9	34.6	24	9.6	15	57.7	44	17.4	8	53.3	9	6.7

08/05/86 KEY: *-IN CASE OF LITTERING, THESE FIGURES INCLUDE PUPS

Significant effects on fetal weight were seen at the HD with median fetal weight 78% that of the control value. The incidence of reduced ossification of the pelvic bones was 16% in the HD group vs 4% in the controls using the sponsor's incorrect method of comparing fetuses rather than comparing litters. Misshapen sternebrae were increased across the drug-treated groups.

Summary

The study is inadequate. The HD produced such severe toxicity that there was an inadequate number of litters for evaluation. There is in the data a suggestion of several dose-related effects upon the developing fetus. A HD that produced only mild maternal toxicity might have helped to elucidate any fetal effects. The mortality that occurred has potentially obscured any teratological liability. No blood levels of drug are provided or referenced. From other studies (see the Carcinogenicity report) it is known that the LD and MD used produced fractional human equivalent doses.

Study title: Oral Teratology Study in Rabbits with RS-43285-193

Key study findings: Excessive maternal toxicity produced an inadequate number of litters for evaluation by contemporary standards. It may be seen in the existing data that in the absence of maternal toxicity, there was no NOEL for the observation of decreased implantation index.

Summary of implantation indices

Implantation index	87.7 (c)	73.8(LD)	64.3*(MD)	58.4**(HD)

(implantations/corpora lutea)				

significantly different from the controls by the Mann-Whitney test at *p=0.05 and **p=0.01

Reduced ossification of sternebrae was present in 54% of control litters and 77% of HD litters. There was a similar finding in the rat study. In this case, the effect is probably due to maternal toxicity. The LD and MD groups consumed the same amount of food as the control group but gained on average 38 and 66% more body weight than did the control animals.

Study no.: AT3802 92-B-86-43285-193-PO-TT
Volume #, and page #: Vol. 17, p. 158
Conducting laboratory and location: Syntex Research, Palo Alto, CA
Date of study initiation: September 29, 1986
GLP compliance: no
QA reports: yes () no ()
Drug, lot #, radiolabel, and % purity: lot numbers AT278SA331B,AT278SA248E, AT278SA244I, [illegible]%
Formulation/vehicle: sterile water with sodium hydroxide and pH adjusted to pH=4

Methods: 80 New Zealand White (NZW) rabbits [illegible] were randomly assigned to 4 groups of 20 females each and were then artificially inseminated (GD1). The groups were orally gavaged once daily with doses of 0 (vehicle control), 6, 45 or 150 mg/kg/day from GD7 through GD19. Dams were euthanized GD29. Body weights were recorded on gestation days 1, 8, 15, 22 and 29. Measurement of food intake began on the day following insemination and was recorded daily through GD29.

Results: Clinical signs were reported primarily for the HD animals and included increased respiration (10/20) and inactivity in 6/20, dyspnea, ataxia and collapse. Signs were reported to begin within 5-10 minutes of dosing and last for 30-90 minutes. In the first 3 weeks of the study, the HD group consumed approximately half of the amount eaten by the other groups. The records of food consumption did not show differences between the control, LD and MD groups.

Weight: LD and MD rabbits gained more weight than the control animals. HD dams gained less.

Reviewer's Summary of body weight changes (p.279)

Dose group	Δ from GD1 to GD29 in grams	% difference from control
0	274	
6 mg/kg	378	38
45 mg/kg	456	66
150 mg/kg	185	-32

Mortality: One MD and 3 HD females died within 2 minutes of dosing, possibly from incorrect intubation. Two HD animals died for whom pregnancy status was unknown. The data from these two animals was excluded from all summaries.

Abortions: 1 control, 1 LD, 3 HD females

Summary of maternal indices showing effects

	Dose (mg/kg/day of RS-43285)			
	0	6	45	150
No. females inseminated	20	20	20	20
No. females died prior to scheduled sacrifice	1	1	2	6
No. pregnant	0	0	1	1
No. not pregnant	0	0	1	0
No. pregnancies not determined	0	0	0	2
No. of females aborted	1	1	0	3
No. pregnant at term	13	14	14	13
Implantation index (implantation's/corpora lutea)	87.7	73.8	64.3*	58.4**

significantly different from the controls by the Mann-Whitney test at *p=0.05 and **p=0.01

Summary: Excessive maternal toxicity produced an inadequate number of litters for evaluation by contemporary standards. It may be seen in the existing data that in the absence of maternal toxicity, there was no NOEL for the observation of decreased implantations.

Study title: Oral perinatal and postnatal reproduction study in rats with RS-43285-193

Key study findings: The methods state that only healthy animals were used in the study and then asserts that 1 LD dam was euthanized for health problems apparent before the start of dosing. There was no apparent maternal toxicity. General condition for pups on PN day 1 was recorded in error for approximately 40% of the litters. No data was presented for developmental landmarks. The study is inadequate.

Study no.: 10-R-88-43285-193-PO-PP/AT4822
Volume #, and page #: vol 22, p.54
Conducting laboratory and location: Syntex Research, Palo Alto, CA
Date of study initiation: February 11, 1988
GLP compliance: hard to tell. There was a QA statement but no GLP statement
QA reports: yes (x) no ()
Drug, lot #, radiolabel, and % purity: LD: ATSA239F; MD: AT342 SA320D; HD: AT342 SA223J
Formulation/vehicle: water, adjusted to pH=4

Methods: Female CD rats CD®BR:VAF/Plus) with evidence of mating were randomly assigned to 4 groups of 28 females each. The rats were given 0 (water control), 10, 40 or 200 mg/kg/day by oral gavage from GD15 to weaning at PN21. Litters were culled on PN 1 to 8 pups of 4 of each sex where possible. PN days 4,7,14 and 21, the litters were observed for weight number live and sex.

Results: No compound related signs were reported for any animals. One LD dam was euthanized due to poor health before dosing. One control and 1 HD dam were euthanized due to loss of all pups in their litters. There was no significant difference in average weight gain from post-mating day 1 to post-mating day 29 between the treatment groups.

General condition of pups on PN 1 was recorded in error for approximately 40% of the litters. The sponsor stated that this did not impact the "study conduct or interpretations; the data are not presented in this report."
There were no differences in the data as reported for the gestation and neonatal indices. There were no significant differences in pup weight or survival for either sex. No data was presented for developmental indices. The pups that died in the first 4 days were examined for anomalies. The data was not presented in such a way as to be able to determine the litter incidence.

Summary: The methods state that only healthy animals were used in the study and then asserts that 1 LD dam was euthanized for health problems apparent before the start of dosing. There was no apparent maternal toxicity. Observations on general condition for PN 1 for approximately 40% of the litters were recorded in error. No data was presented for developmental landmarks. The study provides little information as to the post-natal effects of the drug.

Reproductive and developmental toxicology summary:
It was communicated to the sponsor after the filing meeting that the reproductive toxicity studies were inadequate due to the disease outbreak in the fertility study and in other studies due to excessive mortality resulting in insufficient numbers for comparison. The sponsor replied in writing that they disagreed with this interpretation and in their opinion, the studies were adequate and interpretable. This reply is part of the record for this NDA. Based upon the evidence available in the studies the following interpretations were reached.

Fertility: For reasons detailed in the review it must be concluded that drug-treatment caused a profound decrease in male rat fertility that was not alleviated with a drug-free recovery period. Decreased implantation (NS) was noted in female rats and a statistically significant dose-related decrease in implantation index was also reported in the rabbit Seg II study. No data was provided on female cyclicity.

Teratogenicity: The rat study was inadequate by contemporary standards but the sponsor argues that the study is acceptable by the 1966 standards in place at the time of conduct. Dose-related skeletal malformations were reported at all doses, even without maternal toxicity in the rats: LD 5 mg/kg(0.066x human exposure), MD 40 mg/kg (1.14 x human exposure) and HD 400 mg/kg (no data available for estimation). A skeletal effect was apparent in the surviving HD litters of rabbits. However, in this study, the effect may well have been due to maternal toxicity.
Exposure relative to humans can only be estimated by surface area comparisons: Rabbit HD of 400 mg/kg ÷3= 133 x37=4933 mg/m^{2}HED. The MRHD in terms of surface area is 1200 mg/m^2. Therefore, 4933 ÷1200 = 4.1X MRHD on the basis of surface area.

Better selection of doses might have better demonstrated a dose-response effect. As plasma levels were not provided, it is difficult to estimate the relative human exposure. What is troubling to the reviewer is how in the EMLD toxicology studies, a single oral dose of 250 mg/kg caused 40% mortality and a single oral dose of 500 mg/kg caused 60% mortality. The three-month rat study showed 30% mortality in females dosed with 500 mg/kg/day. The rat teratology study also showed approximately 30% maternal mortality at the HD of 400 mg/kg as did the rabbit study (1/20 control, 1/20 LD, 2/20 MD and 6/20HD). Why were the studies started with doses known to produce such severe effects?

Development: Two studies in rats examined post-natal development. The combination fertility-post-natal development study used an insensitive method of assessing developmental landmarks. Despite this, a dose-related delay in negative geotaxis was reported. Delays were also noted in eye opening, incisor eruption and vaginal opening although these effects did not show a dose-response relationship. The second development study had several problems: General condition of the pups on PN1 was reportedly recorded in error for 40% of the litters. The methods state that only healthy animals were used, but 1 dam assigned to the LD group was euthanized for health problems that had been apparent prior to the start of dosing. The reliability of the report is a moot point as no data whatsoever was reported for developmental landmarks, simply a statement that there were no effects. Results of the first study must then predominate and it must be concluded that the drug caused developmental delays at doses with no apparent maternal toxicity.

Overall summary: The sponsor feels that the studies were done to meet the standards existing at the time of conduct and are therefore adequate. The reviewer has stated points of concern in detail in the various reviews. As requested by the sponsor, these studies have been interpreted based upon the data presented.

Reproductive and developmental toxicology conclusions: The drug causes a decrease in male fertility, is embryotoxic, caused an increase in spontaneous bone defects (sternum) and causes developmental delays in the absence of maternal toxicity.

Labeling recommendations: "Carcinogenesis, Mutagenesis, Impairment of Fertility" the sponsor's statement should be changed to read that ranolazine [redacted]

[redacted]

"Pregnancy—Category C" The sponsor's first two sentences in this section should be removed and replaced with a statement [redacted]

[redacted]

VIII. SPECIAL TOXICOLOGY STUDIES:

Study title: RS 43285 RQT(3): Acute adrenal function study in rats

Key study findings: After two oral doses of 300 mg/kg ranolazine, plasma ACTH and corticosterone were decreased. Tissue levels of pregnenolone, progesterone, corticosterone and aldosterone, all expressed as ng/gland, were decreased compared to the control by 31%, 60%, 80% and 63% respectively.

Study no: AT4437, SS/072/88
Volume #, and page #: vol 22, p 5.

Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: December, 1987
GLP compliance:
QA reports: yes () no ():
Drug, lot #, radiolabel, and % purity: E6-ML-001
Formulation/vehicle:

Methods: Male Sprague-Dawley rats (CD(SD)BR) were assigned to 2 groups of 10 rats each. One group received the vehicle of water, the other group received 300 mg/kg ranolazine. The rats were given 2 doses of ranolazine within a 24 hour period. The rats received the second dose approximately 2 hours prior to blood sampling. Stress tests and euthanasia were conducted between 10.00 and 12.00 hours when then sponsor states that ACTH and corticosterone levels are considered to show least variation due to diurnal rhythm. Each rat was stressed by placing it for 2 minutes in a hollow perspex tube on 2 occasions 20 minutes apart. The animals were then euthanized by decapitation 20 minutes after being placed in the perspex tube for the second time. The blood samples were then analyzed for ACTH and corticosterone. The adrenal glands were weighed. The left adrenal was then assayed for pregnenolone, progesterone, corticosterone and aldosterone. The right adrenal was collected for histopathology.

RS 43285 ROT(3) : Adrenal Function Study (Acute) in Rat

Plasma Results

Dose mg/kg/day		ACTH pg/ml	Corticosterone ng/ml
0	Mean	54.2	255.8
	SD	25.8	95.1
	Mean	48.5	173.3*
	SD	7.9	31.5
		8.54	31.77

SED - Standard error of the difference between means

Statistical significance of differences from the control based on 2-sided t-tests.

* p less than 0.05

Results: Plasma ACTH and corticosterone levels were decreased in the drug-treated animals. Tissue levels of pregnenolone, progesterone, corticosterone and aldosterone were decreased by statistically significant amounts.

Appears This Way On Original

Sponsor's summary of adrenal tissue results

Dose Mg/kg	Weight (mg)	Pregnenolone Ng/gland	Progesterone Ng/gland	Corticosterone Ng/gland	Aldosterone Ng/gland
0	18.7±3.6	80.7±23.8	171.0±70.9	742.6±415.4	6.36±2.19

300	19.6±2.5	56.0*±15.5 (31%)	69.0*±40.1 (60%)	152.0**±114.3 (80%)	2.38**±0.68 (63%)
Sed		9.12	26.07	136.4	0.73

Sed= standard error of the difference between means
Statistical significance of differences from the control based on 2-sided t-tests: *p<0.05, **p<0.01, ***p<0.001
Numbers in parentheses are reviewer's calculation of percent difference from the control value.
After two oral doses of 300 mg/kg ranolazine, plasma ACTH and corticosterone were decreased. Tissue levels of pregnenolone, progesterone, corticosterone and aldosterone, all expressed as ng/gland, were decreased compared to the control by 31%, 60%, 80% and 63% respectively.

Study title: RS 43285 RQT(2): Acute adrenal function study in rats

Key study findings: From the data as presented, it appears that prior to the addition of a stressful event, the mean basal ACTH, plasma corticosterone and adrenal corticosterone were lower in the drug-treated animals compared to the controls. Plasma ACTH, corticosterone and adrenal corticosterone increased as did plasma cholesterol levels. Therefore, the drug-treated animals did in fact mount an appropriate response to the stressor. We do not have plasma drug levels provided, hematology (was there an appropriate stress-induced neutrophilia), clinical chemistry or histopathology of the lymphoid organs. From the data presented it can be concluded that there is some form of drug effect upon the adrenal gland after acute administration of ranolazine.

Study no: AT4436 SS/071/88
Volume #, and page #: volume 21, p 266
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: November 1987
GLP compliance: statement included
QA reports: yes (x) no ():
Drug, lot #, radiolabel, and % purity: E6-ML-001
Formulation/vehicle:

Methods: Following sham dosing for 16-18 days, Ranolazine was given twice within 24 hours at doses of 0 and 300 mg/kg to groups of 24 male Sprague-Dawley (:CD(SD)BR,] rats. Each group was divided into 3 subgroups. The measurements made for each sub-group are shown in the sponsor's summary below.

	Sub-groups		
	1(pre-stress)	2(2 min post-stress)	3 (20 min post-stress)
Plasma ACTH	+	+	-
Plasma corticosterone	+	-	+
Adrenal corticosterone	+	-	+
Plasma lipids/glucose	+	+	+

Total and free cholesterol, triglycerides, non-esterified fatty acids and glucose were assayed in all rats. Stress tests and euthanasia were performed between 10.00 and 11.20 hours to minimize diurnal variation in ACTH and corticosterone. Each rat was restrained for one minute in a

perspex tube to induce stress. It was killed 2 minutes after first being put in the tube and blood for plasma ACTH determination was collected. Adrenal glands were collected and weighed. The left adrenal gland from subgroups 1 and 3 were individually homogenized and lipid extracts assayed for corticosterone content. The adrenals from subgroup 2 were frozen.

Results: The sponsor's summary tables are shown below.

	Dose mg/kg/day	Plasma ACTH pg/ml	Corticosterone Plasma ng/ml	Corticosterone Adrenal ng/gland	Corticosterone Adrenal ng/mg
Basal	0	24.2	4.8	76.3	4.90
	300	21.8	3.0*	34.8*	2.35*
After Stress	0	95.7	235.7	1090.9	73.16
	300	71.6	177.4	444.5**	26.13*
Selr		0.17	0.20	0.27	0.27
Selr (AS)			0.21	0.28	0.28

Selr - Standard error of ln (ratio) between basal groups.

Selr (AS) - Standard error of ln (ratio) between stressed groups.

Statistical significance of differences from the control based on 2-sided t-tests.

* p less than 0.05
** p less than 0.01

Appears This Way
On Original

Group Mean Plasma Lipids and Glucose Results

	Dose mg/kg/day	Cholesterol Total mg/dl	Cholesterol Free mg/dl	Cholesterol Ester mg/dl	Triglyceride mg/dl	NEFA mEq/L	Glucose mg/dl
Basal	0	82.6	19.4	63.3	74.3	0.241	163.7
	300	87.9	21.0	66.9	91.6*	0.265	164.6
2 min After Stress	0	78.6	19.8	58.9	120.0	0.329	158.7
	300	100.5**	24.3	76.3**	82.3***	0.195	173.4
20 min After Stress	0	88.7	22.7	66.0	109.3	0.397	164.1
	300	101.0	25.5	75.5	81.8*	0.349	174.1
S.E.D.			2.94				8.0
S.E.D (20)			3.05				8.3
Selr		0.079		0.075	0.104	0.249	
Selr (20)		0.082		0.078	0.107	0.258	

S.E.D. - Standard error of the difference between means
S.E.D.(20) - Standard error of the difference between groups 20 minutes after stress.

Selr - Standard error of ln (ratio) between any two groups.
Selr (20) - Standard error of ln (ratio) between groups 20 minutes after stress.

Statistical significance of differences from the control based on 2-sided t-tests.

* p less than 0.05
** p less than 0.01
*** p less than 0.001

	Dose mg/kg/day	Plasma ACTH pg/ml	Corticosterone Plasma ng/ml	Corticosterone Adrenal ng/gland	Corticosterone Adrenal ng/mg
Basal	0	24.2	4.8	76.3	4.90
	300	21.8	3.0*	34.8*	2.35*
After Stress	0	95.7	235.7	1090.9	73.16
	300	71.6	177.4	444.5**	26.13*
Selr		0.17	0.20	0.27	0.27
Selr (AS)			0.21	0.28	0.28

Selr - Standard error of ln (ratio) between basal groups.

Selr (AS) - Standard error of ln (ratio) between stressed groups.

Statistical significance of differences from the control based on 2-sided t-tests.

* p less than 0.05
** p less than 0.01

It may be seen that after dosing with drug, but before being stressed, the ranolazine animals had slightly lower "basal" ACTH and lower plasma and tissue corticosterone levels. After the stressor, plasma and tissue levels of all the above-mentioned parameters increased. However, the drug-treated animals did not show the same mean magnitude of increase as did the control group. Unfortunately, the results shown did not include the standard deviations of the means. We do not know what variability was inherent in the assay or in the animals and thus do not know if the values shown are real or fall within the variability of the assay and lab. Looking at the plasma lipid values, it may be seen that the basal levels of the two groups are essentially the same with the exception of slightly higher triglyceride levels in the drug-treated group. Was 2 minutes post-stress sufficiently long enough to see the full magnitude of response? At both time points after the stressor, the cholesterol levels were higher in the treated vs control groups. Triglyceride levels were somewhat lower. Again, standard deviations were not shown.

Summary
From the data as presented, it appears that prior to the addition of a stressful event, the mean basal ACTH, plasma corticosterone and adrenal corticosterone were lower in the drug-treated animals compared to the controls. Plasma ACTH, corticosterone and adrenal corticosterone increased as did plasma cholesterol levels. Therefore, the drug-treated animals did in fact mount an appropriate response to the stressor. We do not have plasma drug levels provided, hematology (was there an appropriate stress-induced neutrophilia) or histopathology. It can be concluded that there is some form of drug effect upon the adrenal gland after acute administration of ranolazine. According to Goodman and Gilman (9th edition), opioid receptor binding can cause a decrease in ACTH secretion. Drolet et. al. also noted (Prog Neuro-Psychopharmacol &Biol Psychiat 2001, vol 25, pp729-741) that opioids can diminish stress-induced neuroendocrine and autonomic responses and may stimulate these effector systems in the non-stressed state.

Study title: RS 43285 RQT: One month adrenal function study in rats

Key study findings: There was little difference between the treatment groups in terms of basal plasma ACTH and corticosterone. Following a defined stress, plasma and adrenal corticosterone increased to a greater extent in all drug-treated animals. Only the HD group showed slight increases both in serum cholesterol and triglycerides and in adrenal weight.

Study no: AT4372 SS/061/88
Volume #, and page #: vol 21, p. 164
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: September 8, 1987
GLP compliance:
QA reports: yes () no ():
Drug, lot #, radiolabel, and % purity: E6-ML-001
Formulation/vehicle: water

Methods: Ranolazine was given orally to groups of n=20 male Sprague-Dawley CD(SD)BR)rats at doses of 0, 5, 50 and 300 mg/kg/day for 29 days. At the end of this time,

plasma and tissue (adrenal) assays for corticosterone and lipids were performed as well as plasma ACTH. EM and light microscopy examination of adrenal tissue was also carried out. All rats were euthanized 2 hours after dosing. The first 10 rats per group were used to evaluate basal parameters. The last 10 rats at each dose were subjected to stress stimuli (restraint in a perspex tube) and euthanized within 20 minutes of being placed in the tube to evaluate physiologic markers of stress.

Results: Basal ACTH and corticosterone did not differ significantly between groups and given the apparent variability in the measurements, did not differ at all.

Summary of basal plasma ACTH and Corticosterone

Dose mg/kg/day	ACTH pg/ml	Corticosterone ng/ml
0	28±10	21±29
5	39±32	5±9
50	38±17	15±24
300	34±8	10±11

N=10 per group. Numbers are mean ±SD

Following the stressor, plasma corticosterone was increased in both control and treated animals. The increase in ranolazine-treated rats was slightly greater than that seen in control rats.

Summary of adrenal function tests: stress induced corticosterone

Dose	Plasma	Adrenal		
mg/kg/day	Ng/ml	Weight mg	Ng/gland	Ng/mg
0	92±32	24±2	844±853	36±36
5	128±59	25±4	1181±648	46*±23
50	163±130	25±4	1395±900	55*±32
300	191±96	35±4	1869±960	53*±26

*$p<0.05$

The sponsor's data on plasma lipids indicates little difference if any between the stressed an unstressed states when one compares within a given treatment group. The drug-treated animals had slightly higher cholesterol levels than the controls. Given the normal range of cholesterol levels that may be found in rats, the results may also fall within the range of normal variability.

RS 43285 RQT : One Month Adrenal Function Study In Rats

Group Summary - Plasma Lipids

Unstressed

	Dose mg/kg/day	Cholesterol Total mg/dl	Cholesterol Free mg/dl	Cholesterol Ester mg/dl	Triglyceride mg/dl	NEFA mEq/L
Mean	0	83	18	65	120	0.18
SD		16	5	12	27	0.06
Mean	5	90	20	70	133	0.20
SD		9	3	7	28	0.08
Mean	50	86	18	69	111	0.36**
SD		13	3	11	30	0.20
Mean	300	111***	26***	85***	93**	0.22
SD		17	5	14	22	0.11

Stressed

	Dose mg/kg/day	Cholesterol Total mg/dl	Cholesterol Free mg/dl	Cholesterol Ester mg/dl	Triglyceride mg/dl	NEFA mEq/L
Mean	0	88	24	64	114	0.19
SD		15	3	15	24	0.10
Mean	5	90	26	64	119	0.31*
SD		16	4	14	19	0.13
Mean	50	100	28*	72	119	0.47***
SD		16	5	12	19	0.21
Mean	300	117***	35***	82***	103	0.63***
SD		15	4	12	21	0.11

* p less than 0.05
** p less than 0.01
*** p less than 0.001
n = 10

The lipids within the adrenal gland show more marked changes at the HD for total, free and esterified cholesterol as well as triglycerides and NEFA.

RS 43285 RQF : One Month Adrenal Function Study in Rats

Group Summary - Adrenal Lipids

	Dose	Cholesterol						Triglyceride		NEFA	
		Total		Free		Ester					
	mg/kg/day	mg/gland	%	mg/gland	%	mg/gland	%	mg/gland	%	mg/gland	%
Mean	0	0.265	1.13	0.064	0.27	0.202	0.86	0.226	0.94	0.020	0.08
SD		0.052	0.22	0.013	0.06	0.047	0.19	0.106	0.36	0.010	0.03
Mean	5	0.284	1.13	0.078	0.30	0.206	0.82	0.260	1.02	0.021	0.08
SD		0.057	0.23	0.018	0.05	0.041	0.19	0.103	0.38	0.010	0.04
Mean	50	0.298	1.21	0.082*	0.33*	0.216	0.88	0.264	1.07	0.020	0.08
SD		0.044	0.16	0.013	0.05	0.035	0.13	0.112	0.47	0.007	0.02
Mean	300	0.581***	1.65***	0.128***0.37**		0.453***1.29***		0.380***	1.09	0.043***	0.12**
SD		0.161	0.36	0.054	0.14	0.144	0.33	0.165	0.44	0.017	0.04

* p less than 0.05
** p less than 0.01
*** p less than 0.001
n = 10

[redacted]

The HD group also showed a greater weight of adrenal gland compared to the other treatment groups.

Summary of adrenal organ weights (n=20)

Dose mg/kg/day	Body weight	Absolute adrenal weight	Adrenal weight normalized to body weight [x]
0	359±30	50±7	0.139
5	369±33	52±7	0.141
50	356±42	50±7	0.140
300	371±28	68**±9	0.183

[x]reviewer's calculation from available data

Gross and microscopic observations are summarized below. Changes in the zona fasciculata were most pronounced in the HD group.

Summary of gross and microscopic adrenal changes

	Dose group (mg/kg/day)			
	0	5	50	300
Adrenal enlargement	1/20	1/20	2/20	3/20
Adrenal pallor	1/20	1/20	3/20	14/20
Adrenal discoloration	0/20	0/20	0/20	1/20
Zona fasciculata cytoplasmic vacuolation	6/20	6/20	7/20	16/19
Zona fasciculata cytoplasmic foaminess				
Minimal	7	6	7	3
Slight	11	11	10	10
Moderate	2	3	3	6
marked	0	0	0	19

Electron microscopy changes were reported to include an increase in the number and size of intracytoplasmic membrane-bound vesicles, some so large that the nucleus was compressed. Fusion between vesicles was reported to happen commonly. Some contained a homogeneous, lipid-like substance but more were reported to have a fine, floccular nature. Mitochondria were reported to be enlarged, some cavitated and some containing a flocculent substance. Fusion with the vesicles was also noted. Increased numbers of lysosomes were also reported in the drug-treated animals. The sponsor interpreted the results as evidence of enhanced cellular activity, possibly a normal secretory process.

Study title:RS-43285: Investigative study in rat adrenal cells in-vitro

Key study findings: Under the conditions of the assay, ranolazine added to rat adrenal cells treated with ACTH produced a decrease in detected corticosterone.

Study no: AT6435
Volume #, and page #: vol 27, p.318
Conducting laboratory and location: Syntex Research, Scotland
Date of study initiation: February 2, 1993
GLP compliance: statement included
QA reports: yes (x) no ():
Drug, lot #, radiolabel, and % purity: RS-43285 lot E9-ML-001
Formulation/vehicle:

Methods: Adrenal glands were harvested from 2 male Sprague-Dawley CD(SD)BR) rats and monolayer cultures prepared. After 2 days of incubation at 37°C, the culture medium was removed and replicates of 4 wells were incubated with the following:

- Control medium
- 10^{-9}M ACTH ± 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}M RS-43285
- 2.5x10^{-6}M pregnenolone± 10^{-4} RS-43285
- 2.5x10^{-6}M progesterone ± 10^{-4}M RS-43285
- 2.5x10^{-6}M deoxycorticosterone (DOC) ± 10^{-4} M RS-43285

All incubations were for 2 hours except for DOC, which was 20 minutes.The report stated that the incubation times had been previously shown to allow linear kinetics to ensure that enyzme inhibition would be fully expressed. Corticosterone and cAMP were measured with radio-immunoassays.

Results: RS-43285 at concentrations of 10^{-7}M and 10^{-6}M had no effect on corticosterone secretion from ACTH-treated cells. At 10^{-5}M and 10^{-4}M ranolazine decreased corticosterone secretion from ACTH-treated cells by 15% and 87% respectively. Cyclic AMP values were shown only for the ACTH-treated cells and 1 concentration of ranolazine. The sponsor's data is shown below.

Mean Corticosterone and Cyclic AMP results (n=4)

TABLE 1

Treatment group	Medium 199 with	Corticosterone secreted (pmol/well±SEM)	Cyclic AMP secreted (fmol/well±SEM)
A	No additions	0.8 ± 0.27	5.5 ± 0.6
B	10^{-9}M ACTH	11.8 ± 0.65	263 ± 26
C	10^{-9}M ACTH, 10^{-7}M RS-43285	11.6 ± 0.97	-
D	10^{-9}M ACTH, 10^{-6}M RS-43285	14.1 ± 1.55	-
E	10^{-9}M ACTH, 10^{-5}M RS-43285	10.0 ± 0.77	-
F	10^{-9}M ACTH, 10^{-4}M RS-43285	1.5 ± 0.22	426 ± 39
G	2.5x10^{-6}M Pregnenolone	25.5 ± 1.90	-
H	2.5x10^{-6}M Pregnenolone, 10^{-4}M RS-43285	24.8 ± 1.30	-
I	2.5x10^{-6}M Progesterone	25.5 ± 2.20	-
J	2.5x10^{-6}M Progesterone, 10^{-4}M RS-43285	22.1 ± 1.41	-
K	2.5x10^{-6}M Deoxycorticosterone	34.0 ± 1.56	-
L	2.5x10^{-6}M Deoxycorticosterone,10^{-4}M RS-43285	29.8 ± 3.25	-

Under the conditions of the assay, ranolazine added to cells treated with ACTH produced a decrease in detected corticosterone.

Study title:RS-43285: Investigative study in rat adrenal cells in-vitro

Key study findings: Under the conditions of the assay, ranolazine added to rat adrenal cells treated with ACTH produced a decrease in detected corticosterone.

Study no: CL5566, SS/141/90
Volume #, and page #: vol 35, p. 333
Conducting laboratory and location: [redacted]
Syntex Research
Date of study initiation: february 1988 to March 1989
GLP compliance: no
QA reports: yes () no (x):
Drug, lot #, radiolabel, and % purity: E6 M1001
Formulation/vehicle: Krebs-bicarbonate Ringer solution

Methods: A series of in vitro studies were commissioned from [redacted] and carried out as pilot investigations. The primary objectives of the studies were:

a) to assess whether ranolazine directly inhibited coricosteroid synthesis in rat adrenal tissue
b) to assess whether similar effects occurred in dog and human adrenal tissue
c) to investigate sites of corticosteroid synthesis inhibition in rat adrenal tissue.

The results of the effects on rat, dog and human adrenal tissue and the site of inhibition studies were presented. Since the time of conduct of those studies, Syntex developed in house expertise and developed an alternative interpretation of the studies. That interpretation did not concur with [redacted]'s interpretation. Therefore, that alternative interpretation was also presented in the report. It was noted that additional experiments had been commissioned, were ongoing [redacted] and would be reported elsewhere.

Rat adreanl tissues were obtained from female Wistar rats (~2 months old). Suspensions of adrenal zona glomerulosa and fasciculata/reticularis cells were prepared by established methods.

Human adrenal tissue was prepared from renal transplant donors.

Dog adrenal tissue was obtianed from 2 male Beagles (~8 and 9 months old).

Four series of experiments were performed:

a) Effect of Rs-43285 on steroid secretion by rat adrenocortical cells: RS-43285 was added to incubations of rat adrenal zona glomerulosa and inner zone cells in the presence or absence of a maximally stimulating dose of ACTH(10^{-9}mol/l). Doses of RS-43285 used were 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/l. Control incubations of rat adrenal zona glomerulosa and inner zone cells containing either no additives or ACTH alone were also included.
b) Effect of RS-43285 on steroid secretion by dog adrenocortical cells. RS-43285 was added to incubations of dog adrenal cells in the presence and absence of ACTH 10^{-9}mol/l. Doses of RS-43285 used were 10^{-9}, 10^{-8}, 10^{-7}, 5 x10^{-7}, 10^{-6}, 5x10^{-6}, 10^{-5}, 5x10^{-5}, and 10^{-4} mol/l. Control preparations with no ACTH or additives were also prepared.

c) Effect of RS-43285 on steroid secretion by human adrenocortical cells. RS-43285 was added to incubations of human adrenal cells ±ACTH (10^{-9}mol/l). Doses of RS-43285 were 10-8, 10-7, 10-6, 10-5 and 10-4 mol/l. Control incubations of human cells with no ACTH or other additives were also included.
d) Effect of RS88597 on steroid secretion by rat adrenal cells. RS-88597 was added to incubations of rat adrenal zona glomerulosa and inner zone cells ± ACTH(10^{-9}mol/l). Doses of RS-43285 used were 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/l. Control incubations of rat adrenal zona glomerulosa and inner zone cells containing either no additives or ACTH alone were also included.

Steroid analysis: The sponsor assayed the major corticosteroid in the chosen species. Corticosterone, aldosterone (all experiments) and cortisol (dog and human only) were measured by radioimmunoassay. 18-hydroxycorticosterone and 18-hydroxycorticosterone were measured by gas liquid chromatography after derivitization.

Results

Rat adrenal zona glomerulosa cells: aldosterone secretion was concentration-dependently decreased from ACTH-stimulated cells with little effect at concentrations $<10^{-7}$. Basal aldosterone secretion was minimally affected at the highest concentration although the graphical presentation is difficult to assess quantitatively.
Significant decreases were seen at 10^{-5}($p<0.01$) and 10^{-4} mol/l ($p<0.001$). ACTH-stimulated corticosterone-release was also decreased at concentrations of $>10^{-6}$ mol/l. There was a spurious decrease at 10^{-8}mol/l. Effects were significant at 10^{-5} ($p<0.01$) and 10^{-4}($p<0.001$) mol/l.
Basal corticosterone was significantly ($p<0.05$) decreased at the highest concentration and appears to show a moderate decrease at the next lower concentration.

Rat zona fasciculata cells: ACTH-stimulated corticosterone was variabily decreased at all concentrations $\geq 10^{-8}$ mol/l with no solid dose-response apparent. The highest concentration of ranolazine produced a slight but significant ($p<0.05$) decrease in ACTH-stimulated 18-OH-DOC secretion. Basal 18-OH-DOC secretion was decreased at concentrations of 10^{-7}, 10^{-5} and 10^{-4} mol/l ($p<0.01$ for each) but with no apparent dose-response. There was a decrease in basal corticosterone secretion at concentrations $\geq 10^{-8}$ mol/l with no concentration response.

Dog adrenal cells: RS-43285 showed a concentration-dependent decrease in ACTH-stimulated release of both aldosterone and cortisol. The effective concentrations were $\geq 5 \times 10^{-7}$mol/l with a flat line at the 3 concentrations $\geq 10^{-5}$mol/l. ACTH-stimulated corticosterone release was also concentration-dependently decreased at concentrations $\geq 10^{-6}$ mol/l. Basal aldosterone and cortisol secretion were also decreased but with little difference in effect between the concentrations from 10^{-6} and 5×10^{-5} mol/l. Basal corticosterone was decreased at concentrations $\geq 10^{-6}$ mol/l with significant decreases $\geq 5 \times 10^{-6}$ mol/l.

Human adrenal cells: ACTH-stimulated aldosterone and cortisol was concentration-dependently decreased at $\geq 10^{-6}$ mol/l. The effect was significant at 10^{-5} and 10^{-4} mol/l with $p<0.001$ for both concentrations. Corticosterone was decreased at concentrations of 10^{-5} (NS) and 10^{-4} ($p<0.001$).

Basal aldosterone and corticosterone release were concentration-dependently decreased at $\geq 10^{-6}$ mol/l. Basal cortisol secretion showed a lesser decrease at the same concentrations.

The effects on 18-)H-β showed a great deal of variability. There were significant ($p<0.01$) decreases in ACTH-stimulated levels at 10^{-5} and 10^{-4} mol/l but no clear concentration-response was evident. Secretion of 18-OH-DOC was significantly ($p<0.001$) decreased at the 2 highest concentrations with essentially no visible effect at the lower concentrations.

As the sponsor states regarding RS88597: The compound decreased ACTH stimulated aldosterone and 18-OH-β secretion by rat adrenal zona glomerulosa (AZG) cells at concentrations of 10^{-5} and 10^{-4} mol/l. Corticosterone and 18-OH-DOC secretion by rat AZG cells was decreased only by the highest concentration of the drug. Basal aldosterone and 18-OH-DOC secretion by AZG cells was decreased by the highest concentration of compound only while corticosterone secretion was decreased by RS-88597 at concentrations of 10-5 and 10-4 mol/l. Rs-88597 also decreased ACTH-stimulated corticosterone secretion by rat adrenal inner zone cells at a concentration of 10^{-4} mol/l. ACTh-stimulated 18-OH-DOC secretion was decreased at 10^{-4} and 10^{-5} mol/l. There was no effect on basal corticosterone or 18-OH-Doc secretion by rat adrenal inner zone cells.

This reviewer concurs with the interpretation of the person who conducted the study and found that:

> Taken together these results show that RS-43285 inhibits both basal and ACTH-stimulated steroid secretion by rat, dog and human adrenocortical cells at concentrations ≥10-5 mol/l. Some effects are also seen at the lower concentrations...It is unlikely that the apparent inhibition at the lower concentration, which occurred when no effect was observed at 10-6 mol/l in the same experiment is attributable to the action of the compound. The metabolite of RS-43285, RS-88597 may be slightly less potent than RS-43285 itself. The maximum concentration of this drug to affect steroid secretion was 10-5 mol/l and in some experiments no effect was seen.

The sponsor of the studies disagreed with this interpretation for the stated reasons that (quoted directly from p. 337, vol 35)

1. The experimental systems used suffer by comparison with those of other laboratories.
2. The sponsor considered the response to ACTH poor and the baseline corticosteroid high which "Consequently, the latitude for convincingly demonstrating inhibitory effects is more limited in [redacted]'s system than in others."
3. The sponsor felt that the rat zona fasciculata/reticularis cells did not produce a credible dose response.
4. The sponsor felt that the dog adrenal cells showed an IC50 for inhibition of corticosterone synthesis between 10^{-6} and $5x10^{-6}$M and that the difference in the extent of inhibtion between cortisol synthesis (~35%) and corticosterone synthesis (~90%) was difficult to explain.

Overall the sponsor agrees that the results indicate inhibition of corticosterone/cortisol synthesis in vitro. They question whether this was a specific enzymatic effect or whether it was due to cytotoxicity.

Second Set of Studies Within This Report

Adrenal tissue was obtained from female Wistar rats. RS-43285 at concentrations of 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/l was added to incubations of rat adrenocortical cells in the presence and absence of added

- 22-hydroxycholesterol (cytochrome P-450 side chain cleavage precursor)
- pregnenolone (3-hydroxysteroid dehydrogenase Δ4-5 isomerase precursor)
- progesterone (21-hydroxylase precursor)
- deoxycorticosterone (11β-hydroxylase precursor)

10 nmol of each precursor was added. Control incubations containing no RS43285 were also included for each added precursor. It was anticipated that significant amounts of deoxycorticosterone would be formed so the analysis for this compound was included. Corticosterone and aldosterone were measured by radioimmunoassay. Deoxycorticosterone and 18-hydroxydeoxy-corticosterone (18-OH-DOC) were measured by gas-liquid chromatography.

Results:
RS-43285 caused a dose-dependent decrease in the secretion of corticosterone and aldosterone by a mixed population of rat adrenocortical cells. The decrease was significant at concentrations of 10^{-5} and 10^{-4} mol/l. A small non-significant decrease in 18-OH-DOC secretion was seen at these concentrations also. DOC secretion was increased by addition of ranolazine at all concentrations. The addition of precursors increased the secretion of steroids.

Added 22-hydroxycholesterol: ranolazine significantly inhibited the secretion of deoxycorticosterone and aldosterone at concentrations of 10^{-5} and 10^{-4} mol/l while corticosterone was decreased by RS-43285 at 10^{-4}mol/l.

Added pregnenolone: aldosterone and corticosterone secretion was decreased at ranolazine concentrations of 10^{-4}mol/l while DOC secretion was decreased by 10^{-5} mol/l but not at 10^{-4} mol/l. There was a non-significant decrease in 18-OH-DOC secretion at 10^{-4} mol/l.

Added progesterone: ranolazine decreased aldosterone secretion at a concentration of 10^{-4} mol/l and coricosterone at 10^{-7}, 10^{-5} and 10^{-4} mol/l but not at 10^{-6} mol/l. A concentration response was not seen for corticosterone. Neither 18-OH-DOC nor DOC secretion were affected.

Added DOC: Only 18-OH-DOC secretion was decreased at the highest concentration of 10^{-4} mol/l.

Basal secretion of corticosterone, aldosterone and to some extent 18-OH-DOC were decreased by added ranolazine. Steroid secretion in the presence of added precursors was also decreased by the addition of ranolazine. The sponsor felt that the results of this study "...should be discounted as suitable substrate concentrations and incubation times were not determined for

each precursor(p. 338, vol 35)". The conducting laboratory addressed this in their conclusion saying that arbitrary amounts of precursors were added and that too great an amount may have overcome the inhibitory effects of ranolazine. Further experiments using a range of concentrations would be necessary to investigate this further. Suboptimal concentrations is one possible explanation for the variability of results and the differences in effect of RS-43285 on steroid secretion. The conducting laboratory suggested other possibilities also. For example:

It may be that the drug is acting non-specifically in vitro with the enzymes to produce the observed effects but the major site of action is at a step in the pathway prior to the conversion of 22-hydroxycholesterol to pregnenolone (such as the hydroxylation of cholesterol, catalyzed by CYP450). Steroid biosynthesis is also controlled by the delivery of cholesterol to CYP450 and that this delivery of cholesterol is increased by ACTH. Ranolazine may interact with this multi-step system.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: There are areas where the pre-clinical database could be strengthened.

General Toxicology Issues: What is the contribution, if any, of the major human metabolites to the adverse effects seen? Will reproductive toxicology studies that meet current standards confirm and even extend the observed reproductive and developmental liability? What is the long term effect, if any, of ranolazine on the pigmented structures of the eye where it and/or a metabolite persist(s) with a half-life of approximately 8 days?

Recommendations:There are several pre-clinical areas that would benefit from further elucidation.

1) The capacity of ranolazine and several of the major human metabolites to bind to cardiac ion channels requires investigation as to potential for causing repolarization abnormalities. The effects upon cardiac contractility and total peripheral resistance seen in the cardiovascular safety study also require further investigation.

2) Clarification of how the sponsor eliminated the various possible mechanisms of action to come to the conclusion that partial inhibition of fatty acid oxidation is the primary mechanism.

3)There are several metabolites that are found in humans at concentrations ≥1%. Systematic characterization of the pharmacology/toxicology of these metabolites would improve the characterization of ranolazine. At a minimum, the metabolites of interest could be characterized in a standard receptor binding screen. Safety pharmacology studies would also be a beginning in the determination of which metabolites are pharmacologically or toxicologically active.

4)The questions regarding the potential reproductive toxicology could be addressed with a combined fertility-development study (such as was submitted), but this time using a HD that does not cause the severe toxicity seen in the existing studies and conforming to the current guidances. A thorough investigation of the potential fertility effects would include but not be limited to: adequate histopathology of both males and females (following the current guidelines from the Society for Toxicologic Pathology), assessment of sperm motility, morphology and number, data regarding the cyclicity of

females and adequate description of developmental parameters using a sensitive detection method.
5)Plasma levels and distribution of drug in the pregnant female and the fetus as well as partitioning into the fetus are a vital part of the pre-clinical characterization.
6)The melanin binding properties of ranolazine and potential effects upon the pigmented structures of the eye require elucidation.

Labeling with basis for findings: see above

X. APPENDIX/ATTACHMENTS:

Addendum to review:

Other relevant materials (Studies not reviewed, appended consults, etc.):

- Studies Not Reviewed:
- The identification of the metabolites of ranolazine in human plasma CL6943
- In vitro metabolism of ranolazine by human liver microsomes and identification of major human cytochrome P450 isozymes involved in the hepatic metabolism of ranolazine CVT303.009-N
- The P170-glycoprotein transporter as a potential site of the ranolazine/digoxin drug interaction. CVT303.010-N
- Determination of the potential inhibitory effects of commonly prescribed drugs on the metabolism of ranolazine by human liver microsomes in vitro. CVT303.011-N
- AT3237:The effects of RS-43285 on the Electrocardiographic Changes Produced by Transient Myocardial Ischaemia in the Dog
- AT 3238: The Effects of RS- 43285 on Regional Myocardial Blood Flow Changes Produced by Transient Myocardial Ischaemia in the Dog
- AT 3239: The Effect of RS- 43285 on the Response of the Isolated Guinea- Pig Atria to Isoprenaline
- AT 3240: The Effect of RS- 43285 on the Response of the Isolated Guinea- Pig Ileum to Acetylcholine and Histamine
- AT 3241: Affinity of RS- 43285 for M1 and M2 Muscarinic Subtypes
- AT 3242: In Vitro Affinity of RS- 43285 for Alpha- Adrenoceptors
- AT 3243: In Vitro Affinity of RS- 43285 for Beta- Adrenoceptors
- AT 3244: In Vitro Affinity of RS- 43285 for 5HT -Receptors
- AT 3245: The Effect of RS- 43285 on the Cardiovascular Haemodynamics of the Chloralose Anaesthetised Cat
- AT 3246: The Effect of RS- 43285 on the Response of the Isolated Guinea- Pig Tracheal Strip to Isoprenaline
- AT 3247: The Effect of RS- 43285 on High Affinity Uptake of Noradrenaline and 5-Hydroxytryptamine into Brain Synaptosomes
- AT 3249: Affinity of RS- 43285 for D1 and D2 Dopamine Receptors of Rat Striatal Membranes

- AT 3250: The Effect of RS- 43285 on Nicotinic Responses to Acetylcholine in the Frog Rectus Abdominis Muscle
- AT 3251: The Effect of RS- 43285 on the Response of the Isolated Guinea- Pig Atria to Histamine
- AT 3252: Effects of RS- 43285 on Sodium Currents in the Guinea- Pig Myocardium AT 3253: The Effect of RS- 43285 on Adenosine Binding to Rat Frontal Cortex
- AT 3254: The Effects of RS- 43285 on Intra- Atrial, Atrio- Ventricular and Intra- Ventricular Conduction in the Isolated Langendorff Perfused Rabbit Heart
- AT 3258: Possible Involvement of Myocardial Adenylate Cyclase in the Action of RS- 43285
- AT 3261: The Effects of RS- 43285 on the General Haemodynamic Changes Produced by Transient Myocardial Ischaemia in the Dog

- AT 3267: Calcium Entry Blocking Effects of RS- 43285 in the Guinea- Pig Myocardium

- AT 3284: Effects of RS- 43285 on Potassium- Induced Contractures of Guinea- Pig Mesenteric Artery, Portal Vein and Porcine Coronary Artery

- AT 3285: Effects of RS- 43285 on Calcium- Dependent Slow Action Potentials Recorded from the Guinea- Pig Myocardium
- AT 3286: Effects of RS- 43285 on Active Potassium Currents and Membrane Permeability of the Guinea- Pig Myocardium
- AT 4708: The Effects of Ranolazine on the Ischaemia- Induced Increase in Alpha1- Adrenoceptor Density in the Rat Left Ventricle
- AT 4765: Effect of Ranolazine on Reperfusion- induced Cardiac Fibrillation and Survival After Various Periods of Ventricular Ischemia in Working Rat Hearts in Vitro
- AT 4766: Effect of Oral and Intravenous Ranolazine on Coronary Arterial Ligation and Reperfusion- induced Cardiovascular Morbidity and Mortality in Anaesthetised Rats
- AT 4828: The Effect of RS- 43285- 193 and Its Two Enantiomers (RS- 43285- 197 and RS- 43285- 198) on Rat Cardiac Cyclic AMP Phosphodiesterase
- AT 4991: The Effects of Ranolazine on the Blood Pressure Response to Haemorrhagic Hypovolaemia in the Anaesthetised Rat
- AT 5032: Effects of Ranolazine (RS- 43285) on the Energy Metabolism of the Rat Isolated Heart Subjected to a Period of Temporary Ischemia: Study Using Phosphorus- 31 NMR Spectroscopy
- AT 5298: Effect of Propranolol or Ranolazine Infused Alone or Together as a Mixture on the Performance of Rat Working Hearts Subjected to Left Ventricular Ischaemia and Reperfusion
- AT 5299: On the Role of Oxygen- Derived Free Radicals in the Production of Ischaemia and Reperfusion Injury and Ranolazine's Protective Effect in Working Rat Hearts In Vitro
- AT 5304: Effects of Ranolazine on Ouabain- Induced Toxicity in the Guinea- Pig Papillary Muscle Preparation
- AT 5307: The Action of Ranolazine at Thromboxane (TP) Receptors in Isolated Guinea Pig Aorta
- AT 5425: Effects of RS- 43285- 193 on Guinea- Pig Cardiac Ventricular Action Potentials "In Vitro" under Normal and Ischaemic Conditions

- AT 5450: The Binding of [3H]- RS- 43285- 193 to Rat Cardiac Mitochondria
- AT 5458: Comparison of Vasodilatation in Rat Aorta Evoked by Sodium Nitroprusside and RS- 43285- 193
- AT 5712: Reduction of Myocardial Enzyme Release by RS- 43285 (Ranolazine) in a Subhuman Primate Model of Ischaemia with Reperfusion: Post- Reperfusion Treatment with RS- 43285- 193 (racemate) and its Enantiomers RS- 43285- 197 (S- isomer) and RS- 43285- 198 (R- isomer)
- AT 5713: Limitation of Myocardial Enzyme Release by Ranolazine (RS- 43285- 193) in a Subhuman Primate Model of Ischaemia with Reperfusion: Pre- Ischaemia Treatment with RS- 43285- 193
- AT 5714: The Effect of RS- 43285- 193 on Adenosine Uptake Sites in Guinea- Pig Brain
- AT 5787: Effects of Ranolazine (RS- 43285- 193) and Nitrendipine in a Rat Model of Calcinosis
- AT 5800: The Effects of Ranolazine on Organ Preservation in Porcine Renal Autotransplantation

- AT 5993: Effects of Ranolazine on Inotropic Responses in the Guinea- Pig Papillary Muscle Preparation
- AT 6130: The Effect of Ranolazine on Myocardial Infarct Size in a Canine Model of Regional Myocardial Ischaemia and Reperfusion. Estimation of the Degree of Infarct Size Reduction.
- AT 6488: Test of RS- 43285- 193 (Ranolazine) for Inhibition of Lipid Peroxidation in Primary Rat Blood Monocytes (RBM) Using the Fluorescent Polyunsaturated Fatty Acid, Cis- Parinaric Acid
- AT 6632: Effects of Ranolazine and its R- and S- isomers on the Consequences of Coronary Artery Ligation of Rat Hearts In Vitro and In Vivo
- AT 6733: Measurement of Plasma Ranolazine and Myocardial Enzyme Markers in Samples from Lucchesi Dog Infarct Study
- AT 7001: Effects of Ranolazine on Positive Inotropic Responses to Forskolin and High Stimulation Intensity in the Electrically- Paced Guinea- Pig Papillary Muscle Preparation
- AT 7006: Effects on Ranolazine on L- Type Calcium Channel Currents in Single Guinea- Pig Ventricular Myocytes
- AT 7007: Further Studies on the Effects of Ranolazine on Metabolic Substrate Utilisation in the Isolated Rat Heart
- AT 7011: Effects of Ranolazine in In Vitro Neurotoxicity Models
- AT 7012: Pilot Studies on the Effects of Ranolazine on High- Energy Phosphate Content and Intracellular pH in Ischaemic Langendorff- Perfused Rat Hearts as Assessed Using 31 P- NMR
- AT 7023: Beneficial Effects of Ranolazine on Porcine Renal Preservation
- AT 7025: Effects of Chronic Ranolazine Treatment on Exercise Performance and Other Parameters in Rats With Myocardial Infarction and Chronic Heart Failure
- AT 7026: Preliminary Studies on the Effects of Ranolazine in a Rat Model of Myocardial Infarcted Congestive Heart Failure
- AT 7039: Protective Effects of Ranolazine on Ventricular Fibrillation Induced by KATP Activation in Isolated Rabbit Hearts During Hypoxia and Re- Oxygenation
- AT 7080: Effects of Ranolazine on Myocardial Metabolism in Anesthetized Swine

- CL 5832: In Vitro Effects of Ranolazine (RS- 43285- 193) on the Uptake and Catabolism of Adenosine in Human Red Blood Cells
- CL 5973: The Lack of Effect of RS- 43285- 193 on the Oxidative Burst of Human Neutrophils
- CL 5985: Ranolazine (RS- 43285), a Putative Anti- ischaemic Agent, Inhibits Complex I of the Mitochondrial Respiratory Chain
- CL 6073: Analysis of Actions of Ranolazine on Hormone Regulated Adenylyl Cyclases CL 6208: Studies on the Effect of Ranolazine on the Cyclic AMP Signal Induction Pathway
- CL 6482: Tests of RS- 43285- 193 (Ranolazine) for Inhibition of Lipid Peroxidation in Human Low Density Lipoproteins Using the Fluorescent Polyunsaturated Fatty Acid, Cis-Parinaric Acid
- CL 7067: Effects of Ranolazine on Membrane Lipid Peroxidation

- CL 7068: Ranolazine Inhibition of Respiratory Complex I: Evidence for Greater Potency in Broken or Uncoupled than in Coupled Mitochondria
- CVT303.023- N: The Effects of Ranolazine on Palmitate and Butyrate Oxidation in the Rat Heart
- CVT303.024- N: A Study of the Effects of Ranolazine on AV Nodal Conduction Time in Guinea Pig, Rat and Mice Hearts Using His- Bundle Electrograms
- CVT303.025- N: Effect of Selected Fatty Acid Oxidation Inhibitors on Palmitate and Glucose Oxidation in Rat Isolated Hearts
- CVT303.023- P: Effects of Ranolazine on Infarct Size Following Regional Ischemia and Reperfusion of the Rat Heart
- CVT303.039- P: Effects of Ranolazine on Action Potentials from Human Ventricular Myocytes
- CVT303.044- P: Effects of Acute Intravenous Ranolazine on Cardiac Function in Dogs With Advanced Heart Failure: A Dose Escalation Study
- CVT303.048- P: Electrophysiologic Effects of Ranolazine in the Guinea- Pig Heart In Vivo
- CVT303.004- R: Studies on the Effect of R, Sand RS Ranolazine on Crotonase Activity

The following studies are for impurities that are either not longer present in the drug product or are present in levels low enough that qualification is not needed.

- 124- 006: 28- Day Repeated Dose Toxicity Study of Ranolazine Free Base Containing RS-88056 in Sprague- Dawley Rats
- 124-013: 28- Day Repeated Dose Toxicity Study of Ranolazine Free Base Containing CVT - 2458 [ ] in Sprague- Dawley Rats
- 124-014: 28- Day Repeated Dose Toxicity Study of Ranolazine Free Base Containing CVT - 245.9 [ ] in Sprague- Dawley Rats
- 124- 015: 28- Day Repeated Dose Toxicity Study of Ranolazine Free Base Containing CVT - 2511 [ ] in Sprague- Dawley Rats
- 124- 016: 28- Day Repeated Dose Toxicity Study of Ranolazine Free Base Containing CVT - 3379 [ ] in Sprague- Dawley Rats
- 124- 018: 28- Day Repeated Dose Toxicity Study of Ranolazine Free Base Containing CVT - 4795 [ ] in Sprague- Dawley Rats

- 124- 019: 28- Day Repeated Dose Toxicity Study of Ranolazine Free -Base Containing CVT -2728 [redacted] in Sprague- Dawley Rats

Any compliance issues:

Appears This Way
On Original

APPENDIX II Carcinogenicity Summary

[redacted]

[redacted]

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Elizabeth Hausner
9/2/03 03:58:44 PM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
9/3/03 12:35:08 PM
PHARMACOLOGIST

Statistical Review and Evaluation

Review of Carcinogenicity Data

IND Number:	43,735
Applicant:	CV Therapeutics
Drug Name:	Ranolazine®(RS-43285-003) Sustained Release
Indication:	Alzheimer's disease
Document reviewed:	Carcinogenicity Study Reports and Data Sets
Date of submission:	Data submitted on 11/6/2001
Pharmacology Reviewer:	Elizabeth Hausner, Ph. D. (HFD-110)
Statistical Reviewer:	John Lawrence, Ph. D. (HFD-710)

1. Introduction

A carcinogenicity study was conducted in mice to assess the carcinogenic potential of Ranolazine®. The study was designed as a 2-year study. Mice were randomly divided into five groups stratifying by gender (50 males and 50 females per group) – two controls and three separate dosage level groups (5, 15, and 50 mg/kg/day administered in the diet). Since the two control groups are theoretically identical, the results are reported from the analyses with the control groups combined. The data that was provided did not distinguish the two control groups and therefore, the analysis could not be performed with each control group separately.

A second 90-week study was done in rats. Rats were randomly divided into five groups stratifying by gender (60 males and 60 females per group) – two control and three separate dosage level groups (5, 50, 150 mg/kg/day administered in the diet). The sponsor's statistical report did not indicate the reason why the study was terminated after 90 weeks.

In both studies, all analyses were performed separately by gender. After the treatment period, all surviving animals were sacrificed and various hematological and pathological examinations were performed.

2. Summary of Sponsor's Analysis

In the mouse study, no significant differences were found in the survival times between any dose in males or females compared to the controls. With regards to body weight, a number of significant differences were observed during the earlier stages of the

study (Weeks 0 to 26), but no consistent pattern emerged to suggest evidence of a drug effect in either males or females. There was no evidence of tumorigenicity for the compound administered to mice at dose levels up to 50 mg/kg/day for at least 104 weeks.

In the rat study, survival was shorter in the highest dose group than in the control groups in males but there was no apparent dose-related decrease in survival in females. There was evidence of an increasing trend in tumor incidence with dose for benign follicular adenoma of the thyroid gland, interstitial cell tumors of the testes, and combined incidence of follicular adenoma and hyperplasia of the thyroid for males. The FDA review did not include an analysis of combined incidence of follicular adenoma and hyperplasia of the thyroid, but the sponsor's results appear to be reasonable. For females, there were significant increasing trends for benign phaeochromocytoma and cortical adenoma of the adrenal gland and malignant sarcoma of the subcutaneous tissue.

3. Reviewer's Analysis of Mouse Study

The number of mice in each group who died in different time intervals appears in Table 3.1. The Kaplan-Meier estimates of the survival curve appear in Figures 3.1a and 3.1b. Both Table 3.1 and Figure 3.1 show a suggested trend in males but no trend in females.

The p-values from the dose-mortality trend tests appear in Table 3.2. The results of these tests confirm what is visually apparent from the Kaplan-Meier curves and the number of deaths per time interval. There is a suggestion of a trend for decreased survival in male mice from the Kaplan-Meier plots and the p-values for both of the dose-mortality trend tests are near 0.05. None of the p-values for female mice are significant.

The entire table of comparisons of organ specific tumors appears in the appendix. In females and in males, there are no sites with a significant trend.

Appears This Way
On Original

Table 3.1 Number of deaths per treatment group in different time intervals.

Week	Group				
	Control	5 mg	15 mg	50 mg	Total
0-52	9	4	2	5	20
53-78	20	8	14	13	55
79-91	4	7	5	7	23
92-104	17	7	10	8	42
105-106	50	24	19	17	110
Total	100	50	50	50	250
0-52	8	7	8	2	25
53-78	27	4	13	13	57
79-91	15	10	7	5	37
92-104	18	14	6	9	47
105-106	32	15	16	21	84
Total	100	50	50	50	250

Table 3.2 Dose-Mortality Trend Tests. This test is run using Trend and Homogeneity Analyses of Proportions and Life Table Data Version 2.1, by Donald G. Thomas, National Cancer Institute.

Method	Time-Adjusted Trend Test	Statistic	P-value
Cox	Dose Mortality Trend	3.84	0.0502
	Depart from Trend	0.60	0.7392
	Homogeneity	4.44	0.2177
Kruskal-Wallis	Dose Mortality Trend	3.23	0.0722
	Depart from Trend	0.39	0.8227
	Homogeneity	3.62	0.3051
Cox	Dose Mortality Trend	1.78	0.1817
	Depart from Trend	0.67	0.7165
	Homogeneity	2.45	0.4843
Kruskal-Wallis	Dose Mortality Trend	1.74	0.1872
	Depart from Trend	1.76	0.4150
	Homogeneity	3.50	0.3210

Figure 3.1a Kaplan-Meier estimates of survival curves for male mice by treatment group.

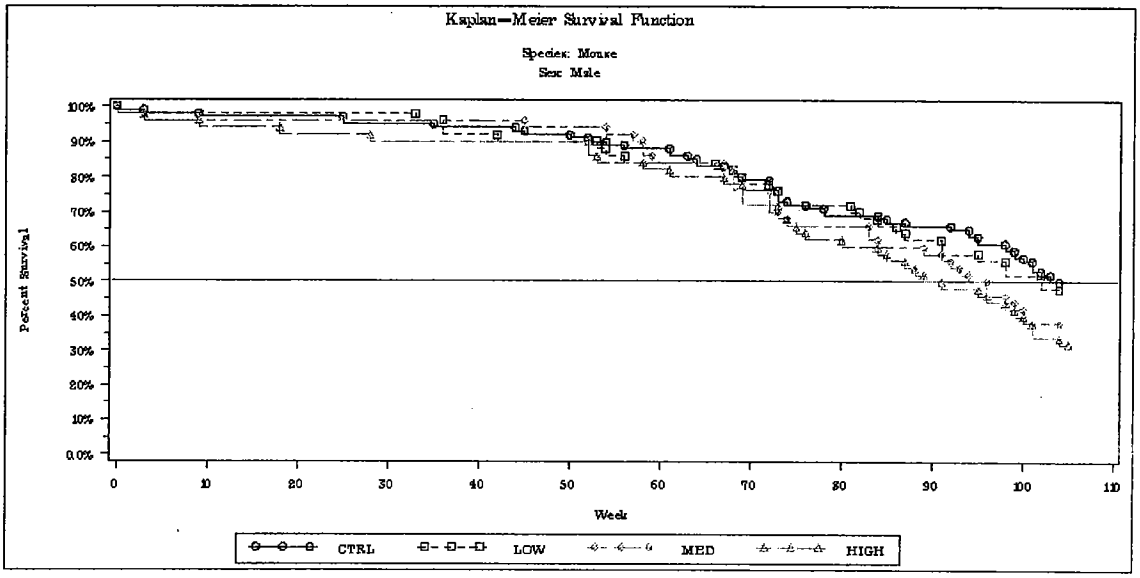
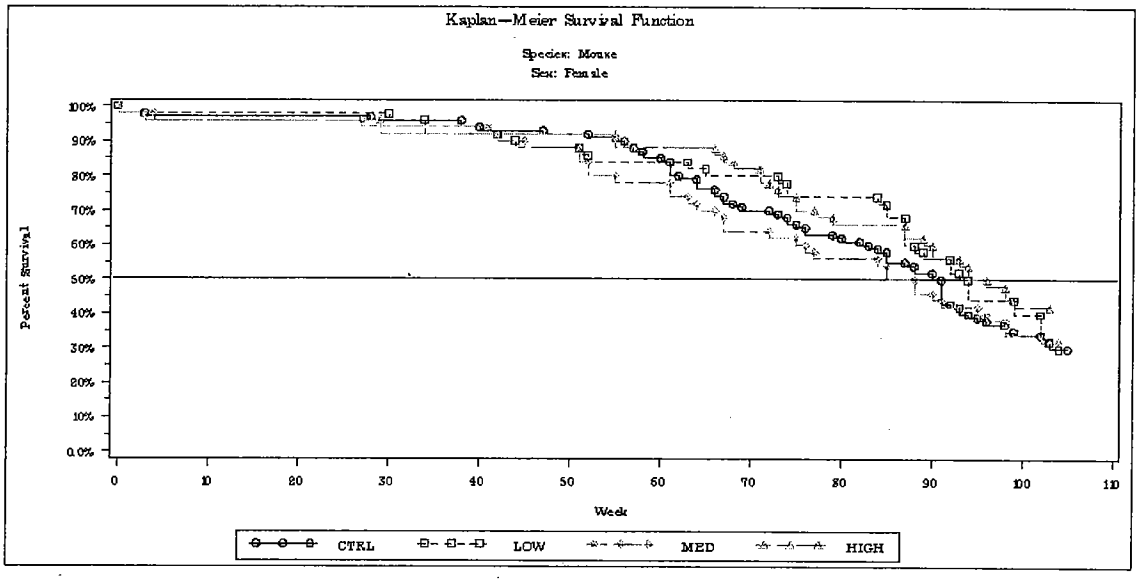


Figure 3.1b Kaplan-Meier estimates of survival curves for female mice by treatment group.



4. Validity of Mouse Studies

Among male mice, there were a sufficient number of mice alive in the high dose at the end of the study to present tumors. The 15 mg and 50 mg groups had significantly greater mean body weight gains relative to the control group through Week 8. During Weeks 8 to 13, the body weight gain in all three active treatment groups was significantly less than the control. The average body weights in all treatment groups remained roughly the same as the control groups throughout the remainder of the study. However, the numerically reduced survival, which is close to statistical significance, would indicate that the high dose was close to the maximum tolerated dose for male mice.

Among female mice, there were a sufficient number of mice alive in the high dose at the end of the study to present tumors. The 15 mg and 50 mg groups had significantly greater mean body weight gains relative to the control group during Weeks 0-4 and also during Weeks 8-13. During Weeks 4-8, the body weight gain in all three active treatment groups was significantly less than the control. The average body weights in all treatment groups remained roughly the same as the control groups throughout the remainder of the study. These findings on weight together with no apparent dose mortality trend indicate that the high dose may not have reached the maximum tolerated dose for female mice.

5. Reviewer's Analysis of Rat Study

The number of rats in each group who died in different time intervals appears in Table 5.1. Since the length of the study was 90 weeks, the FDA partition of the time intervals for a 2-year study was used by the sponsor with the last time interval truncated at 90 weeks. This partition was also used in this review. The Kaplan-Meier estimates of the survival curve appear in Figures 5.1a and 5.1b. Both Table 5.1 and Figure 5.1 show a trend toward shorter survival among the dose groups in males, but no trend in females.

The p-values from the dose-mortality trend tests appear in Table 5.2. The results of these tests confirm what is visually apparent from the Kaplan-Meier curves and the number of deaths per time interval. In male rats, there is decreased survival with increasing dose and the p-values from both tests for dose-mortality trend are statistically significant, but no other p-values for male or female rats are significant.

The entire table of comparisons of organ specific tumors appears in the appendix. Exact p-values are used unless mixed tumor types are found. P-values are compared to 0.025 or 0.005 for rare and common tumors respectively. In males, there are significant findings for benign follicular adenoma of the thyroid gland (p-value = 0.0027) and interstitial cell tumors of the testes (p-value = 0.0013). For females, there was a significant finding for cortical adenoma of the adrenal gland (p-value = 0.0225). The sponsor's report indicates significant findings for malignant sarcoma of the subcutaneous tissue and benign phaeochromocytoma of the adrenal gland in female rats. However, the p-values (0.038 and 0.0549 respectively) do not reach significance per the FDA standard.

Table 5.1 Number of deaths per treatment group in different time intervals.

Week	Group				
	Control	5 mg	50 mg	150 mg	Total
0-50	8	2	4	9	23
51-80	26	17	19	19	81
81-89	25	4	8	8	45
90-90	61	37	29	24	151
Total	120	60	60	60	300
0-50	5	3	3	5	16
51-80	51	20	21	25	117
81-89	15	8	19	10	52
90-90	49	29	17	20	115
Total	120	60	60	60	300

Table 5.2 Dose-Mortality Trend Tests. This test is run using Trend and Homogeneity Analyses of Proportions and Life Table Data Version 2.1, by Donald G. Thomas, National Cancer Institute.

Method	Time-Adjusted Trend Test	Statistic	P-value
	Male		
Cox	Dose Mortality Trend	5.52	0.0188
	Depart from Trend	1.28	0.5265
	Homogeneity	6.81	0.0783
Kruskal-Wallis	Dose Mortality Trend	6.72	0.0096
	Depart from Trend	0.81	0.6681
	Homogeneity	7.52	0.0570
	Female		
Cox	Dose Mortality Trend	1.39	0.2391
	Depart from Trend	2.47	0.2909
	Homogeneity	3.86	0.2775
Kruskal-Wallis	Dose Mortality Trend	0.65	0.4187
	Depart from Trend	2.35	0.3092
	Homogeneity	3.00	0.3914

Figure 5.1a Kaplan-Meier estimates of survival curves for male rats by treatment group.

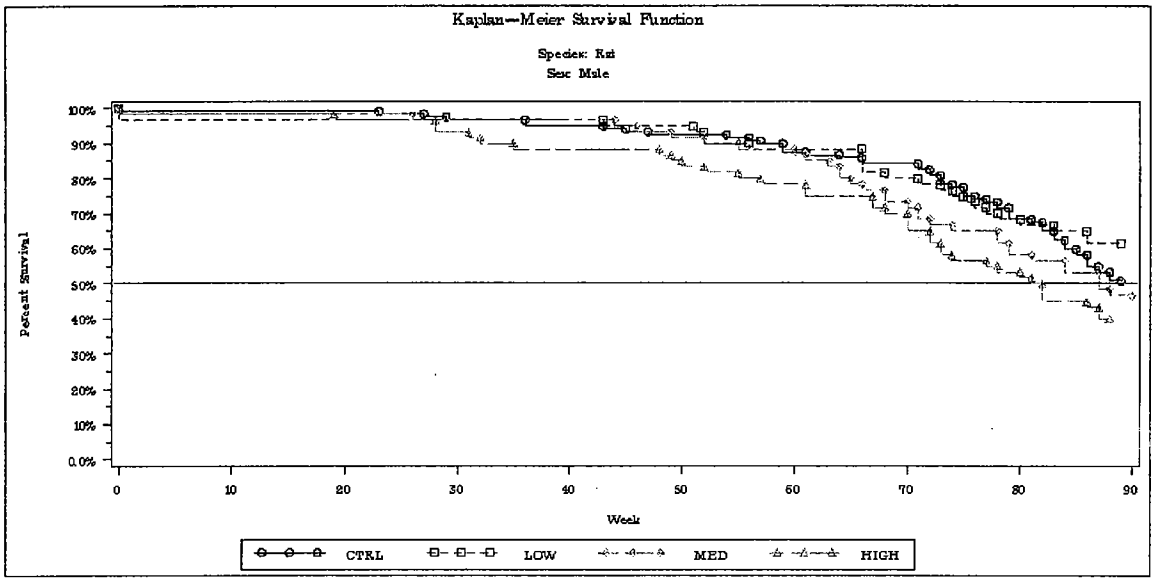
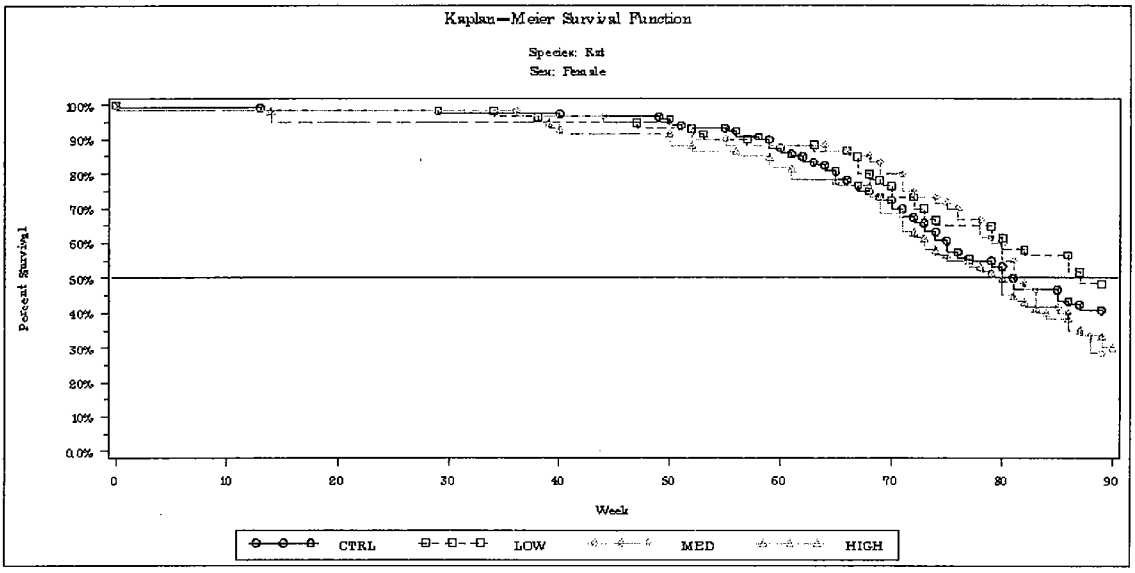


Figure 5.1b Kaplan-Meier estimates of survival curves for female rats by treatment group.



6. Conclusions

In male mice, there was a marginally statistically significant relationship between dose and decreased length of survival. However, there did not appear to be a dose-related effect on mortality in female mice. There were no significant tumor findings in either male or female mice. For male mice, the high dose appears to be close to the maximum tolerated dose because of the apparent reduced survival. The validity of the female mouse study is questionable because of the lack of significant tumor findings, the mortality and body weight data taken in totality.

In male rats, a dose-related trend toward decreased survival was suggested, but no dose-related diffence in survival was found in females. In males, there is a significant dose-tumor positive linear trend for benign follicular adenoma of the thyroid gland (p-value = 0.0027) and interstitial cell tumors of the testes (p-value = 0.0013). For females, there was a significant increasing trend for cortical adenoma of the adrenal gland (p-value = 0.0225).

The findings in this review differ slightly from the sponsor's report. The sponsor found no evidence of a dose-related survival trend in male mice. The sponsor did not discuss the issue of the validity of the mouse study. Finally, the sponsor's report indicates significant findings for malignant sarcoma of the subcutaneous tissue and benign phaeochromocytoma of the adrenal gland in female rats, but the FDA analysis determined that the observed p-values (0.038 and 0.0549 respectively) for these tumors were not statistically significant.

Appears This Way
On Original

Appendix

Test for Dose-Tumor Positive Linear Trend

Source: Female Mouse Data

Organ Name	Tumor Name	Nat. Rate	0	5	15	50	Tumor type	pValue (Exact)	pValue (Asymp)
HAEMOPOIETIC	HISTIOCYTIC	8%	8	6	2	4	MX	0.7026	0.7058
HAEMOPOIETIC	LYMPHOMA	13%	13	5	7	7	MX	0.4924	0.4980
HARDERIANGL	ADENOMA	8%	8	3	3	4	MX	0.4554	0.4616
ADRENALGLAN	PHEOCHROMOCY	.0%	0	1	2	1	IN	0.2580	0.2810
ADRENALGLAN	CORTICALADE	.0%	0	0	0	1	IN	0.2500	0.0499
LIVER	HEPATOCELLUL	1%	1	0	0	0	IN	1.0000	0.7655
LUNG	ALVEOLAR/BRO	10%	10	4	5	3	IN	0.8349	0.8314
LUNG	ALVEOLAR/BRO	8%	8	2	1	5	MX	0.3375	0.3397
MAMMARYGLA	ADENOCARCINO	2%	2	5	3	0	FA	0.9617	0.9457
MAMMARYGLA	ADENOMA	1%	1	1	1	0	FA	0.7322	0.7901
MAMMARYGLA	FIBROADENOMA	.0%	0	0	0	1	FA	0.2083	0.0322
OVARY	LUTEOMA	.0%	0	1	0	0	IN	0.6190	0.7196
OVARY	TUBULARADEN	1%	1	0	0	2	IN	0.1528	0.0711
OVARY	CYSTADENOMA	.0%	0	0	0	1	IN	0.2500	0.0499
PANCREAS	SARCOMA(UNKN	.0%	0	1	0	0	IN	0.6170	0.6756
PARATHYROID	ADENOMA	.0%	0	1	0	0	IN	0.6190	0.7196
PITUITARYGL	ADENOMA	3%	3	3	2	2	MX	0.5625	0.5796
SKIN	KERATOACANTH	1%	1	0	0	0	FA	1.0000	0.7802
SKIN	PAPILLOMA	.0%	0	1	0	0	FA	0.6190	0.7196
SKIN	SARCOMA(UNKN	.0%	0	0	1	0	FA	0.4184	0.5155
SKIN	BASALCELLT	.0%	0	1	0	0	FA	0.6108	0.7040
STOMACH	ADENOMA	1%	1	0	0	0	IN	1.0000	0.7655
SUBCUTTISSU	FIBROSARCOMA	1%	1	0	0	0	FA	1.0000	0.7912
BRAIN	MENINGIOMA	1%	1	0	0	0	FA	1.0000	0.7787
THYROIDGLAN	FOLLICULARA	1%	1	0	0	0	IN	1.0000	0.7964
UTERUS	FIBROMA	.0%	0	1	0	0	IN	0.6170	0.6756
UTERUS	LEIOMYOMA	5%	5	0	0	2	MX	0.5308	0.5428
UTERUS	LEIOMYOSARCO	.0%	0	0	0	1	FA	0.2234	0.0384
UTERUS	POLYP	6%	6	0	3	2	MX	0.5664	0.5808
CERVIX	LEIOMYOFIBRO	.0%	0	1	0	0	FA	0.6154	0.7037
CERVIX	LEIOMYOMA	1%	1	1	0	0	IN	0.8456	0.8087
CERVIX	MUCO-EPIDERM	1%	1	0	0	0	IN	1.0000	0.7964
CERVIX	POLYP	.0%	0	0	1	0	IN	0.3243	0.4140
CERVIX	STROMALSARC	1%	1	0	1	1	MX	0.3070	0.2915
COLON	ADENOMA	.0%	0	0	1	0	IN	0.4405	0.5345
CRANIUM	SARCOMA(UNKN	1%	1	0	0	0	FA	1.0000	0.7807

Test for Dose-Tumor Positive Linear Trend

Source: Male Mouse Data

Organ Name	Tumor Name	Nat. Rate	0	5	15	50	Tumor type	pValue (Exact)	pValue (Asymp)
ABDOMINALCA	SARCOMA(UNKN	.0%	0	1	0	0	FA	0.6008	0.6909
HAEMOPOIETIC	HAEMANGIOMA	3%	3	0	1	1	MX	0.4651	0.4577
HAEMOPOIETIC	HAEMANGIOSAR	.0%	0	1	0	0	FA	0.5920	0.6793
HAEMOPOIETIC	HISTIOCYTIC	1%	1	1	1	0	MX	0.7443	0.7875
HAEMOPOIETIC	LYMPHOMA	5%	5	3	4	1	FA	0.7613	0.7639
HARDERIANGL	ADENOCARCINO	.0%	0	0	0	1	FA	0.1801	0.0223
HARDERIANGL	ADENOMA	10%	10	4	8	3	MX	0.6723	0.6764
KIDNEY	TUBULARADEN	.0%	0	1	0	0	IN	0.5455	0.6549
KIDNEY	CORTICALCAR	2%	2	0	0	1	MX	0.4101	0.3200
KIDNEY	CYSTADENOMA	1%	1	0	0	0	IN	1.0000	0.7540
ADRENALGLAN	PHEOCHROMOCY	.0%	0	0	1	1	IN	0.0991	0.0609
ADRENALGLAN	CORTICALADE	6%	6	6	1	2	IN	0.8062	0.8026
LIVER	HEPATOCELLUL	12%	12	4	5	4	IN	0.5811	0.5859
LIVER	HEPATOCELLUL	10%	10	3	3	6	MX	0.2809	0.2804
LUNG	ALVEOLAR/BRO	12%	12	8	6	8	IN	0.1628	0.1568
LUNG	ALVEOLAR/BRO	21%	21	6	6	7	MX	0.7009	0.7031
PENIS	PAPILLOMA	1%	1	0	0	0	IN	1.0000	0.7540
PITUITARYGL	ADENOMA	1%	1	0	1	0	IN	0.6286	0.6988
SKIN	SARCOMA(UNK	.0%	0	0	0	1	FA	0.1545	0.0140
SKIN	SARCOMA(UNKN	.0%	0	0	0	1	IN	0.1545	0.0140
STOMACH	ADENOMA	1%	1	0	0	0	IN	1.0000	0.7540
SUBCUTTISSU	HAEMANGIOMA	1%	1	0	0	0	IN	1.0000	0.7540
SUBCUTTISSU	SARCOMA(UNKN	1%	1	0	0	0	FA	1.0000	0.7781
TAIL	FIBROMA	.0%	0	0	0	1	FA	0.1545	0.0140
TESTES	INTERSTITIAL	5%	5	1	2	5	IN	0.0276	0.0175
TESTES	TUBULARADEN	.0%	0	0	0	1	IN	0.2364	0.0454
BRAIN	MENINGIOMA	.0%	0	1	0	0	FA	0.5578	0.6665
THYROIDGLAN	FOLLICULARA	1%	1	1	0	0	IN	0.9296	0.8576

Appears This Way
On Original

Test for Dose-Tumor Positive Linear Trend

Source: Female Rat Data

Organ name	Tumor name	0	5	50	150	Tumor type	pValue (Exact)	pValue (Asymp)
ABDOMINALCA	LIPOMA(TA)	0	0	0	1	IN	0.1739	0.0194
COLON	LEIOMYOMA	0	0	1	0	IN	0.3246	0.3973
KIDNEY	LIPOMA	0	1	0	0	IN	0.5739	0.7070
ADRENALGLD.	CORTICALADE	0	0	1	2	IN	0.0225	0.0076
ADRENALGLD.	CORTICALCAR	1	0	0	1	MX	0.3189	0.1889
ADRENALGLD.	PHAEOCHROMOC	2	1	1	3	IN	0.0549	0.0338
LIVER	HEPATOCELLUL	3	2	0	1	IN	0.6680	0.6790
LYMPH/HAEMOP	HISTIOCYTIC	3	0	1	0	FA	0.8311	0.8094
LYMPH/HAEMOP	LYMPHOMA	1	1	0	0	MX	0.8296	0.8133
MAMMARYGLD.	FIBROADENOMA	83	36	33	34	MX	0.5076	0.5116
MAMMARYGLD.	FIBROMA(TA)	4	1	1	1	MX	0.6494	0.6395
MAMMARYGLD.	ADENOCARCINO	12	8	8	2	MX	0.9108	0.9051
MAMMARYGLD.	LIPOADENOMA	0	0	1	0	FA	0.3333	0.3961
MAMMARYGLD.	LIPOMA	0	0	1	0	FA	0.4023	0.4355
MAMMARYGLD.	ADENOMA(TA)	13	7	8	4	MX	0.6616	0.6671
NASALCAVITY	SQUAMOUSCEL	0	0	1	0	IN	0.3217	0.3952
OVARY	GRANULOSATH	1	0	0	1	MX	0.3549	0.1983
OVARY	SERTOLICELL	0	1	0	0	IN	0.5739	0.7070
PANCREAS	ISLETADENOM	1	0	2	0	IN	0.5185	0.5495
PARATHYROID	ADENOMA	1	1	0	0	IN	0.8410	0.8192
PITUITARYGL	ADENOCARCINO	4	3	0	2	MX	0.5325	0.5662
PITUITARYGL	ADENOMA(TA)	86	40	47	48	MX	0.0658	0.0638
SKIN	FIBROMA(TA)	1	0	0	0	FA	1.0000	0.7649
SKIN	FIBROSARCOMA	2	0	0	0	FA	1.0000	0.8279
SKIN	PAPILLOMA	1	0	1	0	FA	0.5617	0.6041
SUBCUT.TISS	FIBROMA(TA)	2	1	0	0	FA	0.9293	0.8614
SUBCUT.TISS	FIBROSARCOMA	1	1	0	0	FA	0.8418	0.8121
SUBCUT.TISS	LIPOMA	0	0	1	1	FA	0.0809	0.0481
SUBCUT.TISS	SARCOMA	0	0	0	2	FA	0.0380	0.0035
THYMUSGLD.	THYMOMA	0	0	0	1	IN	0.1714	0.0184
THYROIDGLD.	CCELLADENO	3	3	4	3	IN	0.2083	0.2084
THYROIDGLD.	FOLLICULARA	1	0	1	1	IN	0.3509	0.2672
UTERUS	POLYP	3	1	5	0	IN	0.7925	0.7760
UTERUS	STROMALSARC	0	1	0	2	FA	0.0511	0.0283
BRAIN	GRANULARCEL	1	0	0	0	IN	1.0000	0.8134
BRAIN	ASTROCYTOMA	0	0	1	0	FA	0.4121	0.4308
CERVIX	FIBROMA	0	0	0	1	IN	0.1739	0.0194
CERVIX	POLYP	0	1	1	0	IN	0.4461	0.6065
CLITORALGLD	ADENOMA	0	0	0	1	IN	0.1739	0.0194

Test for Dose-Tumor Positive Linear Trend

Source: Male Rat Data

Organ name	Tumor name	0	5	50	150	Tumor type	pValue (Exact)	pValue (Asymp)
ABDOMINALCA	CARCINOMAOF	1	0	0	0	FA	1.0000	0.7571
ABDOMINALCA	FIBROMA	1	0	0	0	FA	1.0000	0.7486
ABDOMINALCA	SARCOMAOFU	0	1	0	0	FA	0.5897	0.7089
DUODENUM	ADENOCARCINO	0	0	0	1	IN	0.1589	0.0159
KIDNEY	TUBULARADEN	1	0	0	0	IN	1.0000	0.7446
L.N.,MESENT	HAEMANGIOMA	3	5	0	0	IN	0.9821	0.9647
ADRENALGLD.	CORTICALADE	1	2	1	1	IN	0.3153	0.3909
ADRENALGLD.	CORTICALCAR	0	1	0	0	IN	0.5960	0.7136
ADRENALGLD.	PHAEOCHROMOC	17	5	10	12	IN	0.0183	0.0144
LIVER	HEPATOCELLUL	4	2	0	1	IN	0.7190	0.7262
LIVER	HEPATOCELLUL	3	0	3	1	MX	0.4248	0.4006
LUNG	ALVEOLAR/BRO	0	1	0	0	IN	0.5960	0.7136
LUNG	ALVEOLAR/BRO	1	0	0	0	IN	1.0000	0.7446
LYMPH/HAEMOP	HISTIOCYTIC	2	1	1	3	FA	0.0596	0.0376
LYMPH/HAEMOP	LYMPHOMA	8	2	5	1	MX	0.7925	0.7848
LYMPH/HAEMOP	MYELOIDLEUK	1	0	1	1	MX	0.2392	0.1900
MAMMARYGLD.	FIBROADENOMA	2	0	1	0	FA	0.7352	0.7275
MAMMARYGLD.	FIBROMA(TA)	0	1	0	0	FA	0.5847	0.7237
MAMMARYGLD.	ADENOCARCINO	1	0	0	0	FA	1.0000	0.7446
ORALCAVITY	PAPILLOMA	1	0	0	0	IN	1.0000	0.7422
ORALCAVITY	AMELOBLASTOM	0	0	1	0	FA	0.3929	0.4306
PANCREAS	EXOCRINEADE	2	0	1	2	IN	0.1114	0.0677
PANCREAS	EXOCRINECAR	0	0	0	1	IN	0.1589	0.0159
PANCREAS	ISLETADENOM	6	5	4	5	IN	0.1424	0.1426
PARATHYROID	ADENOMA	1	3	0	0	IN	0.8524	0.8795
PITUITARYGL	ADENOCARCINO	0	1	0	0	FA	0.5631	0.7139
PITUITARYGL	ADENOMA(TA)	55	28	34	22	MX	0.5385	0.5408
PROSTATEGLD	ADENOCARCINO	1	1	0	0	FA	0.8232	0.8139
PROSTATEGLD	ADENOMA	0	0	0	1	IN	0.1589	0.0159
SALIVARYGLD	ADENOMA	0	0	1	0	IN	0.3510	0.3908
SKIN	FIBROMA(TA)	20	10	1	6	MX	0.8327	0.8331
SKIN	FIBROSARCOMA	1	0	0	1	FA	0.3222	0.1703
SKIN	PAPILLOMA	3	2	0	0	FA	0.9572	0.9155
SKIN	BASALCELLT	4	2	0	0	MX	0.9729	0.9353
SPLEEN	HAEMANGIOSAR	0	0	0	1	IN	0.1589	0.0159
BONE	OSTEOSARCOMA	0	0	2	0	FA	0.3594	0.3752
SUBCUT.TISS	FIBROMA(TA)	4	4	3	4	FA	0.1155	0.1123
SUBCUT.TISS	FIBROSARCOMA	0	1	0	1	FA	0.1729	0.1459
SUBCUT.TISS	LIPOMA	9	5	4	0	MX	0.9824	0.9700
SUBCUT.TISS	SARCOMA	0	0	1	0	FA	0.3993	0.4396
TAIL	FIBROMA	0	0	1	0	FA	0.3510	0.3908
TESTES	INTERSTITIAL	5	3	4	8	MX	0.0030	0.0013
TESTES	MESOTHELIOMA	2	0	1	0	IN	0.7295	0.7238
THYMUSGLD.	THYMOMA	0	1	0	0	IN	0.5960	0.7136
THYROIDGLD.	CCELLADENO	6	4	2	2	IN	0.7533	0.7616
THYROIDGLD.	CCELLCARCI	2	1	0	0	MX	0.9304	0.8602

THYROIDGLD.	FOLLICULARA	3	1	2	6	IN	0.0027	0.0008
URINARYBLAD	LEIOMYOSARCO	0	0	0	1	IN	0.1589	0.0159
URINARYBLAD	LIPOMA	0	1	0	0	IN	0.5960	0.7136
URINARYBLAD	PAPILLOMA	0	0	1	0	IN	0.4691	0.4897
ZYMBAL'SGLA	SEBACEOUSSQ	0	0	0	1	FA	0.1595	0.0162
ZYMBAL'SGLA	SQUAMOUSCEL	0	1	1	0	MX	0.4993	0.6438
BRAIN	GRANULARCEL	3	0	0	1	MX	0.5350	0.4740
BRAIN	RETICULOSIS	0	0	0	1	FA	0.1675	0.0185
BRAIN	ASTROCYTOMA	1	1	0	1	MX	0.3399	0.3095

cc: IND #43,735
HFD-110/Mr. Fromme
HFD-110/Dr. Gordon
HFD-110/Dr. Hausner
HFD-710/Dr. Chi
HFD-710/Ms. Kelly
HFD-110/Dr. Hung
HFD-110/Dr. Lawrence
HFD-110/chron

LAWRENCEJ/594-5375/report.doc/12/3/01

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

John Lawrence
12/3/01 03:38:11 PM
BIOMETRICS

Roswitha Kelly
12/4/01 08:24:55 AM
BIOMETRICS

George Chi
12/5/01 01:22:33 PM
BIOMETRICS

Executive CAC
Date of Meeting: January 15, 2002
Mouse/Rat Carcinogenicity Study

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
Robert Osterberg, Ph.D.,HFD-520 Alternate member
John Leighton,Ph.D., HFD-150 Alternate Member
Al DeFelice, Ph.D., HFD-110Team Leader (not present)
Elizabeth Hausner, D.V.M., HFD-110 Presenting Reviewer

Author of Draft: Elizabeth Hausner

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

IND 43,735
Drug Name: Ranolazine
Sponsor: CV Therapeutics
Note: All p values provided are those from the CDER statistical reviewer.

Background: Ranolazine is a fatty acid oxidation inhibitor for the indication of angina.

Mouse Carcinogenicity Study: A trend to decreased survival in males (p=0.05) was seen. Testicular interstitial cell tumors were found at incidences of 5/100(Combined controls), 1/50(LD), 2/50(MD) and 5/49(HD), p= 0.0276 by CDER analysis.

Rat Carcinogenicity Study: A trend to decreased survival in males was seen (p=0.02). Testicular cell tumors were also seen at incidences of 5/120(combined controls), 3/60(LD), 4/60(MD) and 8/60(HD), p=0.003 by CDER analysis. Other tumors seen in male rats were thyroid follicular cell adenoma (p=0.0027) and malignant histiocytic sarcoma (p=0.059). When the control groups are separated, the incidences of thyroid tumors in the male rats are 3/60(C1), 0/60(C2), 1/60(LD), 2/58(MD) and 6/60(HD). Tumors reported for female rats were adrenal cortical cell adenomas(p=0.0225), adrenal pheochromocytoma (p=0.0549) and malignant subcutaneous sarcoma (p=0.059).

Executive CAC Recommendations and Conclusions: It was concluded that there were no noteworthy findings.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, HFD-110
/ADeFelice, HFD-110
/EHausner, HFD-110
/EFromm, HFD-110
/ASeifried, HFD-024

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

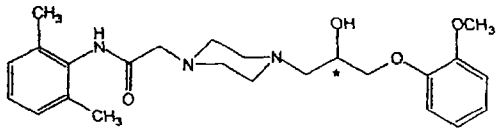
/s/

Joe Contrera
1/29/02 10:16:01 AM

CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT AND FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET
Review of Carcinogenicity Study Results

P/T Reviewer(s): Belair, Patel, Barry, Hausner
Date: January 4, 2002
IND/NDA: IND43,735, related IND 30,205
Drug Code #RS-43285-003
CAS#: 95635-55-5
Division: Cardio-Renal Drug Products, HFD-110
Drug Name: ranolazine
Chemical Structure:

1-Piperazineacetamide, N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy) propyl]-, (±)-



* chiral center

Molecular weight: 427.54
Molecular formula: $C_{24}H_{33}N_3O_4$
Sponsor: CV Therapeutics
Carcinogenicity Report Date: Rat Study received 10/31/91, final report12/28/95,
Electronic dataset July 26, 2001
Mouse Study dated 2/26/93, electronic dataset 7/26/2001
Therapeutic category: anti-anginal
Pharmacologic classification: fatty acid oxidation inhibitor

Mutagenic/Genotoxic: Negative in Ames, CHO/HGPRT assay, the Saccharomyces assay for mitotic gene conversion, mouse micronucleus assay and possibly dominant lethality as part of the male fertility study (records and reviews for the dominant lethality have not yet been located).

Comment: CDER statistical analysis indicated a trend toward decreased survival in both male mice and rats. Significant neoplastic findings are summarized in the table below.

Summary of tumor incidences

species	sex	Tumor type	Incidence per dose group				P value	
			C	LD	MD	HD	sponsor	FDA*
Rat	m	Thyroid, follicular adenoma	3/120	1/60	2/58	6/60	<0.001**	0.0027
rat	f	Adrenal, cortical adenoma	0/120	0/60	1/60	2/60	0.032[4]	0.0225
Rat	m	Testes, interstitial (b)	5/120	3/60	4/60	8/60	0.001[3]	0.0030
mouse	m	Testes, interstitial	5/100	1/50	2/50	5/49	0.019**	0.0276

*p value from Exact test, **one-tailed prevalence trend-test
[3] one-tailed combined trend p-value, [4] one-tailed exact trend test

The sponsor reported significant findings of malignant sarcoma of subcutaneous tissue and benign pheochromocytoma of the adrenal gland, both tumor types in female rats. The p values do not reach significance per the FDA standard.

Summary of tumor incidences

species	sex	Tumor type	Incidence per dose group				P value	
			C	LD	MD	HD	sponsor	FDA*
Rat	F	Adrenal pheochromocytoma	1/120	1/60	1/60	3/60	0.013**	0.0549
Rat	f	Malignant sarcoma,SC	0/120	0/60	0/60	2/60	0.040[4]	0.0380
rat	m	Malignant histiocytic sarcoma	2/120	1/60	1/60	3/60	0.037[5]	0.059

*p-value from exact test, [4] one-tailed exact trend test,[5] one-tailed time to tumor trend p-value

Relative human exposure: Based on AUC comparison, total exposure at the highest dose tested in rats was 0.7-4X the human plasma exposure. The highest mouse dose tested produced ≤ 0.25X the human therapeutic exposure based on AUC.

Adequacy of studies: Both studies were adequate. In males, a true MTD appears to have been used based upon body weight changes and decreased survival.. In females, there were no effects upon body weight and no significant toxicological findings. However, available records indicate that there was a very small margin between the MTD in the rodents and doses that produced CNS signs and death. The sensitivity of the rodent species to the CNS effects of the drug precluded higher doses.

MOUSE CARCINOGENICITY STUDY

Mouse study duration: 104 weeks
Study Starting Date: January 1989
Study Ending Date: February 1991
Mouse Strain: CD-1(ICR)BR
Route: oral gavage
Number of Mice:
- Control-1 (C1): 50m, 50f
- Control-2(C2): 50m, 50f
- Low dose (LD): 50m, 50f
- Middle Dose(MD): 50m, 50f
- High Dose-1 (HD1): 50m, 50 f
- High Dose-2 (HD2): na

Mouse Dose Levels (mg/kg/day):
- Low Dose: 5
- Middle Dose: 15
- High Dose: 50

Basis for Doses selected: 50 mg/kg/day was MTD based upon a 3 month dose-ranging study that lasted 1 week at 200 mg/kg and 3 months at 35 mg/kg according to the

sponsor. The review found discusses the 1-week period. The high dose appears to be close to the MTD for male mice on the basis of reduced survival and weight gain. There were no apparent findings to support this as an MTD for the female mice.

Prior FDA concurrence: 11/18/88, record of Dr. E.J. Belair agreeing to doses.
Mouse Carcinogenicity: By sponsor's analysis, testicular interstitial cell tumors in male mice were significant at $p \leq 0.05$. Distribution was 2/50 (C1), 3/50(C2), 1/50 (LD), 2/50(MD), 6/50(HD)

Mouse Study Comments: Dr D.G. Patel who reviewed the mouse study commented on the HD: Issue of HD resolved on basis of a dose-ranging study, which showed very little margin of safety between the MTD in mice and lethality.

CDER statistical analysis indicates: Marginally significant relationship between dose and decreased length of survival for male mice. No significant tumor findings in either sex of mice. Our statistician questioned the adequacy of the female study due to the lack of effects on body weight, significant toxicologic findings or mortality.

RAT CARCINOGENICITY STUDY

Rat Study Duration: 90 weeks
Study Starting Date: January 1992
Study Ending Date: October 1993
Rat Strain: Sprague Dawley CD(SD)BR]

Route: oral gavage
NUMBER OF RATS:

- Control 1(C1): 60 m, 60 f (distilled water)
- Control 2(C2): 60 m, 60 f (distilled water)
- Low Dose (LD): 60m, 60 f
- Middle Dose (MD): 60 m, 60 f
- High Dose (HD): 60 m, 60 f

RAT DOSE LEVELS (mg/kg/day):

- Low dose: 5
- Middle Dose: 50
- High Dose: 150

Basis for selected dose: MTD
Prior FDA Dose Concurrence: yes, from Division, Dec 18, 1991. Telephone conversation record (12/3/91) and memos to the file from E.J. Belair dated 12/4/91 and 12/6/91.

Rat Carcinogenicity: Sponsor shows statistically significant trends in males for thyroid follicular adenomas ($p \leq 0.001$), testicular interstitial cell tumors ($p \leq 0.001$) and in females for pheochromocytomas ($p=0.0549$) and adrenal cortical adenomas ($p=0.032$). Malignant subcutaneous fibrosarcoma was reported at an incidence in females of 0/120(C),

LD(0/60), MD (0/60) and HD (2/60), p=0.038. Histiocytic sarcoma in males was reported at an incidence of 2/120(C), 1/60(LD), 1/60(MD) and 3/60 (HD), p=0.037.

CDER statistical analysis indicates: Dose-related trend towards decreased survival in males suggested. Males: benign follicular adenoma of the thyroid (p=0.0027), interstitial cell tumor of the testes (p=0.0013). Females: cortical adenoma of the adrenal gland (p=0.0225). No other statistically significant findings.

Comments: the sponsor states increased incidence of thyroid follicular adenoma or hyperplasia was also found. In previous toxicology studies the adrenal gland was shown to be the target organ for toxicity. Dose-response for adrenal pathology was shown with regard to increased size, weight and vacuolation.

CARCINOGENICITY SUMMARY FOR RANOLAZINE
TABLE OF CONTENTS

I. BACKGROUND

Ranolazine has been under development since ~1987. IND 30,205 was presented for an intravenous formulation for the indication of angina. The current IND, 43,735, is a sustained release oral tablet for treatment of angina. The drug is a racemic mixture of two enantiomers, (+)R and (-)S-ranolazine, which have similar pk in humans. The mechanism of action is unclear but may involve inhibition of β-oxidation of fatty acids with stimulation of glucose oxidation. There is evidence that ranolazine has effects upon the Ca2+, Na+ and K+ channels in cardiac muscle, giving it the properties of Class I and Class III antiarrhythmic drugs.

There are records of telephone discussions between the sponsor and the agency regarding the protocols for the carcinogenicity studies. Concurrence with the doses chosen was reached. When CV Therapeutics, the current sponsor, assumed control of development, they requested that the CAC address the early termination of the rat study conducted by the previous sponsor. This was discussed in the minutes of the December 8, 1998 Executive CAC meeting. The original sponsor felt that survival was poor enough to warrant termination at 21 months instead of 24 months. Based upon the mortality data presented, the CAC did not concur with the action of early termination. The table presented below is taken from the minutes of the December 8, 1998 CAC meeting, prepared by Dr C. Resnick.

NUMBER OF ANIMALS SURVIVING TO TERMINAL SACRIFICE

Group No.	mg/kg/day	No.Surviving Males		No.Surviving Females	
1	000	34/60	(81 wks)[3]	27/60	(78 wks)[3]
2	000	27/60	(81 wks)[3]	22/60	(78 wks)[3]
3	005	37/60	(82 wks)[3]	29/60	(80 wks)[3]
4	050	[1]28/60	(79 wks)[3]	17/60	(79 wks)[3]
5	150	24/60	(75 wks)[3]	[2]18/60	(76 wks)[3]

[1]EXCLUDES ANIMAL FOUND DEAD DURING WEEK 90.
[2]EXCLUDES 2 ANIMALS WHICH DIED UNDER ANESTHESIA DURING TERMINAL BLEED.
[3]PARENTHESES ENCLOSE MEAN DURATION OF TREATMENT. FOR GROUPS 1 & 2, VALUES GIVEN ARE THE AVERAGE OF BOTH GROUPS.

In 4Q of 2001, the sponsor submitted an electronic dataset to allow CDER statisticians to evaluate the data from the mouse and rat carcinogenicity studies.

Comparison of Metabolism: Humans, Rats, Mice

The most recent data provided by the sponsor shows approximately the same degree of protein binding (57-64%) in rats, mice and humans. Metabolism is more extensively characterized in rats than mice. Both plasma and urine have been studied in rats while only urine has been studied in mice. Oral bioavailability is~71% in male rats and ~63% in female rats. Clearance is ~twice as great in male rats as in female rats: 54.8ml/min/kg vs 21.8 ml/min/kg respectively.

Qualitatively, the metabolites present in human plasma and urine are represented in rat plasma and urine also. Of the 22 metabolites listed for human plasma and urine, 5 are listed as present in mouse urine. That number includes parent drug and 2 of the 4 main metabolites. The main route of excretion is via the urine.

A ^{14}C radiolabel study was done in albino SD(CD) rats and pigmented Long-Evans rats. The areas of greatest concentration of radiolabel included the adrenal glands, kidney, liver and pituitary gland. The lowest levels of radioactivity were reported to occur in the lungs, muscle (including myocardium), bone marrow and testes.

were given one week of ranolazine 400 mg tid, atenolol 100 mg qd and placebo tid in random order. At the end of each week, a study day was scheduled (each study day 7-10 days apart) with exercise testing done 1 hour after drug administration at the same time of day at each clinic visit. Exercise testing was to be performed on a bicycle (2 sites) or a treadmill (the other study sites). No interim washout period was planned between treatment periods.

Table 1. RAN 080: Inclusion/exclusion criteria

Inclusion criteria	Exclusion criteria
1. Informed consent; 2. Males or females, 18-75 years old; 3. Chronic stable angina responding to medical therapy (beta-blockers, calcium channel-blockers or long-acting nitrates)[13] 4. Sinus rhythm with ECG signs of ischemia ($\geq$ 0.1 mV ST depression in one lead during prestudy stress test) within 3-9 minutes after start of exercise; 5. Coronary artery disease confirmed by angiography or proven MI.	1. Termination of prestudy exercise test for reasons other than angina; 2. Clinically significant arrhythmias, or CHF; 3. Unstable angina or MI less than 1 month before placebo run-in; 4. Pregnant/breastfeeding/females of childbearing potential unless sterilized or taking adequate contraception; 5. Investigational drug in previous 28 days; taking part in another clinical study; previously entering this study; 6. Use of anticonvulsants/enzyme-inducing medication; 7. Alcohol/narcotic abuse; Hepatic/renal dysfunction; Unable to stop beta-blocker therapy; Sensitivity/allergy to beta-blocker; History of cerebral hemorrhage, thrombosis or aneurysm; Pulmonary hypertension; Surgically curable hypertension or malignant hypertension; COPD; Pacemaker.

Concomitant medication:
Allowed medications included long and short-acting nitrates and calcium antagonists except verapamil (and other cardiac depressant calcium antagonists). Prohibited medications were to be discontinued 24 hours prior to beginning placebo washout. Short-acting nitrates were not to be taken within 6 hours of the exercise test.

Exercise testing:
All exercise testing was planned 1 hour post-dose.

Bicycle:
Bicycle testing was performed with a starting load of 20 watts, increasing by 20 watts every minute until typical ST depression and angina occur.

Treadmill:
Treadmill testing was planned using a Bruce protocol[14], beginning at 1.7 mph/0% grade, increasing to 1.7 mph/5% grade and 1.7 mph/10% grade at 3 minute stages.

Efficacy evaluations (during exercise testing):
1. Heart rate;
2. Blood pressure;
3. Rate-pressure product
4. Time to angina;

[13] The protocol (p.3) specifically defined improvement with medical therapy as: 1. patients whose medical treatment was optimized using available exercise testing; 2. newly diagnosed patients with at least 30 sec improvement in time to angina on repeat exercise testing after a standard dose of beta-blocker or calcium antagonist; 3. secondary referral patients with at least 30 sec decrease in time to angina after withdrawal of one anti-anginal medication.
[14] Reviewer: this is actually a modified Bruce protocol. A standard Bruce protocol would have involved a 10% grade (not 0%) at Stage 1.

5. Time to 0.1 mV ST depression;
6. Maximal ST depression (mV);
7. Exercise duration;
8. ST depression after 1 and 5 minutes of recovery;

Additional efficacy evaluation included nitroglycerin consumption and the number of anginal attacks (via diary).

A full ECG analysis of the whole stress test was done as a quality control.

Statistics:
The primary efficacy variable was time to angina (other variables: exercise duration, time to 1 mm ST depression, maximum ST depression, integrated ST depression). With a 90% power and significance level of 5%, 82 patients were felt needed to detect a 0.5 minute difference between any pair of treatments in the time to angina; this calculation was based on an estimated within patient SD of 0.983 (from study RAN 072).
Patients who fail to reach angina on all 3 study days will be excluded, while those who fail to reach angina on one or two study days may be substituted for time to angina, depending on how many patients this involves. A secondary analysis will be performed which substitutes exercise duration for all patients who fail to reach angina on any study day. If a large number of patients are protocol violators or noncompliant, this secondary analysis will be repeated, excluding those patients.
The number of angina attacks and nitroglycerin use will be listed and summarized but not formally analyzed.
The exercise protocols were designed to ensure that angina will occur at approximately the same time for a patient, regardless of the method of testing. Since some sites will use bicycle testing, center will be included in the analysis.
Data from those patients who fail to complete one or more phases of the study will still be included in the analysis.
The integrated ST depression will be calculated as the area under the ST depression/time curve. Rate pressure product will be calculated as heart rate x SBP.
Efficacy variables will be analyzed using ANOVA model, including treatment, period, center, subject within center and the treatment by center interaction. If the number of subjects within centers is small, the centers may be pooled.
All statistical tests were two-tailed, with a 5% level of significance. No adjustments for multiple comparisons were made.

Interim assessment: An interim assessment of safety was initially planned when data were available on approximately half of the total patients recruited. If assessment indicated unsatisfactory tolerability, then the study could be terminated or the ranolazine dose reduced to 320 mg tid for the remaining patients (see Protocol Amendment).

Protocol Amendments:
1. (March 26, 1992): Added double-blind placebo blister packs to that drugs, for patients who withdraw prior to active treatment phase, can be reused. Changed inclusion criterion for angiographic confirmation of CAD to include angiography prior to 12 months, or history of proven MI.
2. (May 14, 1992): Added bilirubin to testing. Interim safety assessment plan changed to occur instead when an excess of adverse events was noted on a blinded safety review. No statistical testing was planned during this interim assessment. If tolerability to ranolazine was unsatisfactory, then the study would be terminated or the dose reduced to 320 mg tid for the remaining patients.
3. (December 8, 1992): Allowed the use of long-acting nitrates.

Results:

Patient Disposition:
A total of 163 patients were enrolled, and 158 patients were randomized; 155 received ranolazine, 154 received atenolol and 154 received placebo. Of the 158 patients, 117 were considered to be evaluable for

the primary efficacy variable. Most common reasons for non-evaluability were: missing time to angina on all 3 active treatment studies, and failing to complete all 3 active treatment phases. A total of 152 patients completed all three study days. Four patients (2 on ranolazine, 2 on placebo) were terminated due to AE[15]; one patient (atenolol) was lost to follow-up, and one patient (ranolazine) was inappropriately enrolled. Of those randomized, 135 patients entered RAN081 follow-up study.

Baseline characteristics:
The study population was 89% male and 99% Caucasian, with mean age 59 yrs, weight 79 kg, BP 139/83 mm Hg, pulse 71 bpm. Ninety nine percent were on concomitant medications at any visit. Of 158 patients, 74% percent were taking aspirin; about 31% took concomitant nitroglycerin, 24% were on isosorbide dinitrate, 54% were on calcium channel blockers (27% were on diltiazem).
Over 85% of patients experienced 4-10 day intervals between study visits. No imbalances between treatment groups was seen.

Efficacy:
Of note, 43 patients were tested with the bicycle method (3 sites); 74 patients were tested with the treadmill method.

Primary Efficacy Variable: Time to onset of angina is shown below. The mean difference between ranolazine and placebo was 51 sec with a 95% CI (34.2, 67.8) over zero and a p-value of < 0.001. A significant improvement in time to angina was also seen with atenolol. Similar results were seen with an analysis of evaluable patients (in the evaluable population, treatment by method interaction = NS and treatment by investigator interaction p=0.08).

Table 2. RAN 080: Time to Onset of Angina (all patients)

	Baseline (N=158)	Ranolazine (N=153)	Atenolol (N=153)	Placebo (N=152)
Mean time to onset angina (sec)* (SE)	342	409 (6)	398 (6)	358 (4)
Range (sec)	91-720	120-900	138-780	60-780

Source: Table 10. *Mean was adjusted for imbalance in number of patients receiving each treatment in each center. Treatment by investigator interaction $p < 0.001$; treatment by method interaction p=0.01

A **first period analysis** of the time to onset of angina also showed a significant improvement with ranolazine (mean difference 39 sec, 95% CI (7, 72), p=0.02) vs. placebo. A significant improvement vs. placebo was also seen with atenolol.

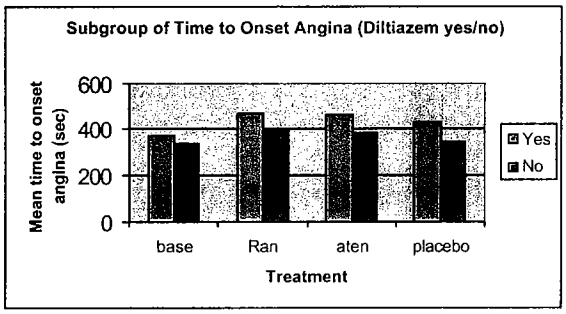


Figure 1. Time to onset angina by Diltiazem use

Please note that the number of patients taking diltiazem is 35-40 per treatment group, and the number not on diltiazem is 116-118 per treatment group. The means are not adjusted. There was no stratification for diltiazem use and no prespecified subgroup analysis.

[15] The ranolazine AE were leukemia (patient #225) and chest pain (#105). The two placebo AE were chest pain (#130) and angina (#227).

Other variables:

Exercise duration:

The mean difference in exercise duration for ranolazine-placebo was 37.1 sec (95% CI 22.2, 52; p<0.001). A significant improvement in exercise duration was also seen with atenolol. There were also significant treatment by investigator ($p < 0.001$) and treatment by method interactions (p=0.02). Three sites (56 patients) used bicycle exercise tests; the other six sites (97 patients) used the treadmill. While the two groups are numerically unequal, it appears that treatment effects are not as obvious with bicycle testing. Even on placebo, duration of exercise is shorter.

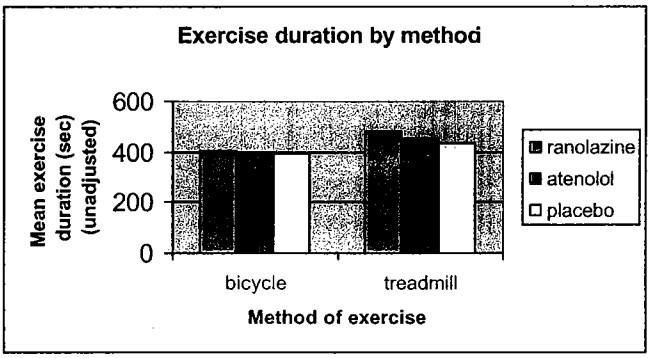


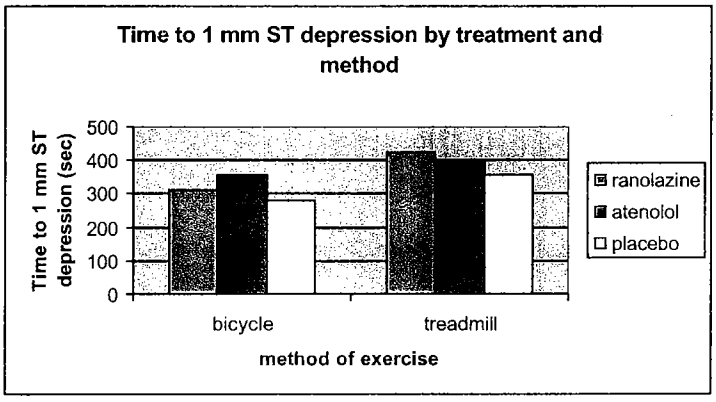
Figure 2. Exercise duration by method of testing.

Time to 1 mm ST depression

The mean difference in time to 1 mm ST depression for ranolazine vs. placebo was 52.6 sec (95% CI 34.8, 70.5; $p < 0.001$). Results for atenolol were favorable and statistically significant as well. There were significant treatment by investigator ($p < 0.001$) and treatment by method (p=0.002) interactions. Results for the evaluable population were similar.

Figure 3. Time to 1 mm ST depression by method

Means are unadjusted. Time to ST depression is longer with the treadmill method for all groups, including placebo.



Ranolazine vs. atenolol:

For the primary endpoint, time to 1 mm ST depression, and exercise duration (evaluable patients) there were no significant differences between ranolazine and atenolol. For the parameter exercise duration (all

patients), there was a significant[16] improvement with ranolazine vs. atenolol (mean difference = 21.1 sec, 95% CI = 6.2, 36.0, p-value =0.006). However, results were not consistent across centers (p <0.001). In one site (Dr. Cocco's center[17]), the difference between ranolazine IR and atenolol was 107 seconds in ranolazine's favor.[18]
There were also significant differences in heart rate (greater mean decrease in heart rate in the atenolol group) both at rest, onset of angina, and end-exercise. Significant differences were seen vs. atenolol with respect to ST segment value at rest and maximum ST depression (greater depressions with atenolol).

Reviewer: For the primary endpoint, no superiority over atenolol was demonstrated. In addition, for ST segment value at rest, as well as maximum ST depression, there were significant treatment by investigator interactions of these measurements (p=0.001) indicating heterogeneity of results.

Heart rate: A significant decrease in heart rate was seen with respect to atenolol-treated patients compared to those on placebo. The mean difference for ranolazine-placebo was 1.5 bpm (p=NS). A significant (p< 0.001) treatment by investigator interaction was noted. Similar results were obtained with respect to heart rate at the end of exercise.

Blood Pressure (BP):
The mean ranolazine-placebo difference for resting systolic BP was –0.5 mm Hg (p=NS) and for diastolic BP 0.2 mm Hg (p=NS) ; there was a statistically significant reduction, vs. placebo, in resting SBP and DBP for atenolol-treated patients. At end of exercise, mean SBP was higher with ranolazine compared to placebo. The mean R-P difference was 4.7 mm Hg (95% CI 0.8, 8.7; p=0.02). The treatment by investigator interaction was significant at p < 0.001. For DBP the mean R-P difference was 0.6 mm Hg (p=NS).

Rate Pressure Product (RPP): Compared to placebo, the mean RPP was significantly reduced with atenolol and mildly (not significantly) increased with ranolazine. At end of exercise, the RPP was significantly increased in the ranolazine group (mean difference vs. placebo was 1261 bpm*mmHg, p=0.005) and significantly decreased in the atenolol group (mean difference vs. placebo was –6797 bpm*mm Hg, p<0.001). The treatment by investigator interaction was significant (both at rest and end of exercise) at p < 0.001).

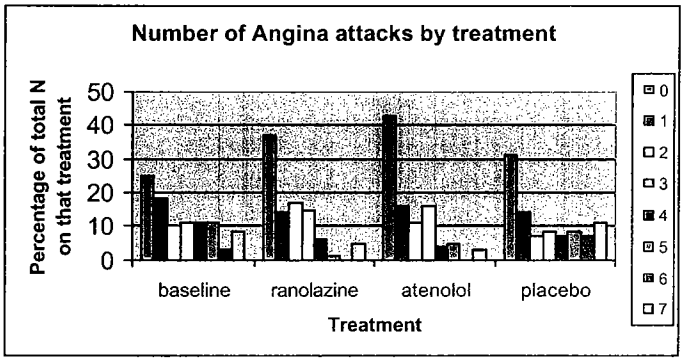


Figure 4. Angina attacks by treatment.

The legend refers to number of anginal attacks. No adjustments were made to allow for different lengths of time on treatments.

[16] The p-values were calculated from pairwise comparisons from ANOVA models appropriate to 3 period crossover design; no adjustments were made for multiple comparisons.

[17] Dr. Cocco's study site was also noted in Study RAN 081.
[18] In fact, if one looks at the data from Dr. Cocco's site, the mean baseline exercise duration is 389 (149.3) seconds. The group on atenolol experienced a mean exercise duration of 389.1 (139.6) seconds.

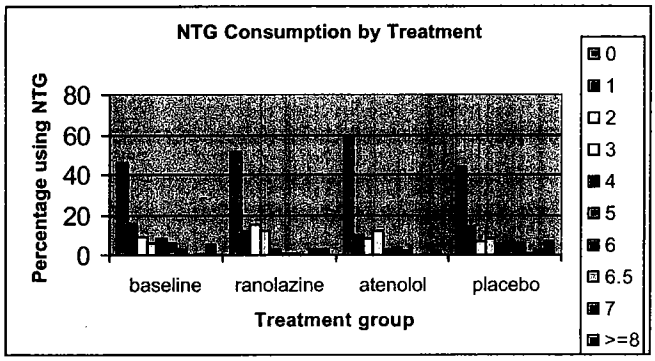


Figure 5. Nitroglycerin consumption by treatment

The legend refers to the number of NTG used. No adjustments have been made to allow for differing lengths of time on treatment.

Plasma Levels:
There were a total of 143 plasma concentrations from the 155 patients on ranolazine IR. The mean plasma concentration was 2039 ng/ml (range < 5 to 5750 ng/ml). One patient (number 199) had a concentration below detection. For 9 patients, no plasma concentrations were available because of interfering peaks in the samples. Three patients did not provide plasma samples.
Forty patients (28%) were on concomitant diltiazem; the overall mean plasma concentration for this group was 2529 (range 47-5750) ng/ml. One hundred three (72%) were not taking concomitant diltiazem. The overall mean plasma concentration was 1850 (range <5 – 4340) ng/ml for this group.

Reviewer comments:
1. This was a placebo-controlled, 3-way crossover study of one week of ranolazine IR 400 mg TID vs. atenolol vs. placebo. No interim washout period was used.
2. Two different exercise methods were used and may have confounded some of the results.
3. In some of the efficacy variables, treatment differences were not consistent in all centers and there were significant treatment by investigator interactions.
4. Ranolazine significantly improved the time to angina at approximately 1 hour post-dosing (primary endpoint) compared to placebo. The first period analysis supported this finding and was also statistically significant.
5. Ranolazine did not demonstrate superiority over atenolol with respect to the primary endpoint. A significant improvement in exercise duration (all patients but not evaluable population) was seen with respect to ranolazine vs. atenolol.

RAN 1514:

Title: A Double-Blind, Placebo-Controlled, Extended-Period Crossover Study to Assess the Efficacy and Safety of Three Dosing Regimens of Ranolazine in Patients with Chronic Stable Angina Pectoris (Vol. 317-318) (Final Protocol May 1, 1992).

Primary Objective: Determine, in patients with chronic stable angina, the trough effect on treadmill time to onset of angina of the following doses of ranolazine: 267 mg tid, 400 mg bid, and 400 mg tid.

Secondary Objectives:
1. Compare, in patients with chronic stable angina, the peak effect (1 hour postdose) of these 3 dosing regimens on treadmill time to onset of angina, as well as effects at peak and trough on total duration of exercise and time to 1 mm ST depression or change;
2. In addition, analysis of the following parameters was planned: plasma ranolazine concentration at trough and peak (1 hour postdose); number of anginal attacks/week; number of sublingual nitroglycerin tablets consumed/week.

Patient population: Patients with stable effort angina pectoris responding to medical therapy.

Study Summary:
This multicenter, Latin square, crossover study consisted of two phases: a single-blind placebo qualifying phase (2-7 weeks) and a placebo-controlled double-blind treatment phase (total of 5 weeks). Each patient received single-blind placebo for 2-7 weeks; during this time, patients were taken off one or more of their antianginal medications, beginning with long-acting nitrates, and underwent treadmill testing at each visit. Only patients whose time to angina shortened by at least 1 minute upon discontinuation of one or more antianginal therapies was allowed into double-blind. When a third drug was discontinued to meet the time decrease criteria, one of the three drugs should have been associated with a reduction in time to onset of angina of a minimum of 30 seconds. During double-blind, three regimens of ranolazine (as noted above, under primary objective) and placebo will be administered, each for one week, with the fourth period repeated during a fifth period; trough and peak treadmill performance was assessed at each visit. Blood samples were collected for trough and peak ranolazine levels.
In addition, information regarding anginal episodes and nitroglycerin consumed/week was collected.

Table 1. RAN 1514: Schedule of Assessments

Phase	Single-blind placebo			Double-blind phase						
Visit	1	2¶	3	4	5	6	7	8		Early withdrawal***
Week	0	1-2	2-7	8	9	10	11	12		
Consent	X									
History	X									
Physical	X							X		X
Concom. Meds (chge)		X		X	X	X	X			
Concom. Meds (all)			X					X		X
AE		X	X	X	X	X	X	X		X
AP freq/NTG use		X	X	X	X	X	X	X		X
ETT screening	X									
ETT qualifying		X	X							
ETT peak										
ETT trough										X
Plasma peak/trough				X	X	X	X	X		X**
12-lead ECG	X	X	X	X	X	X	X	X		X
Lab tests*	X			X	X		X	X		X

*Liver panel at trough only at Visits 4, 5, 6, 7. Urinalysis at Visits 1 and 8 or upon early withdrawal.
**Plasma trough level only upon early withdrawal (only if patient has received double-blind medication).
¶ If the time to angina on the second stress test is not at least 1 minute shorter that that seen on the first stress test, Visit 2 can be repeated up to 2 additional times.
***Only if early withdrawal occurs after patient received double-blind medication.

Exercise Treadmill Test (ETT):
All patients underwent treadmill tests, done 7-10 am (prior to am dose for the trough study) and 1 hour post-dose (peak study). Each patient was to have ETT done at the same time of day throughout the study; patients were required to stop smoking 2 hours before testing, wear similar clothing for each test, and avoid sublingual nitroglycerin 60 minutes prior to testing. A light breakfast was allowed up to 1 hour prior to testing. A modified Bruce protocol was used.
Trough was defined as either 8 hours post-dose for the tid regimen and 12 hours post-dose for the bid regimen.[19]

Table 2. RAN 1514: Inclusion/Exclusion criteria

Inclusion criteria	Exclusion criteria

[19] The reviewer has questioned how the different regimens of exercise testing, including timing of trough ETT, affected "blinding." During double-blind, patients took 1 capsule four times a day. According to the sponsor (verbal communication), ETT at trough was done 8 hours after the last dose.

Single-blind placebo phase: 1. At least 21 years old; 2. At least 3 month history of chronic stable effort angina relieved by rest/sublingual nitroglycerin and improved (by symptoms or ECG signs of ischemia) with medical therapy (beta blocker, calcium channel blocker and/or long-acting nitrate); 3. Signed approved informed consent. Double-blind phase: 1. Time to angina during first exercise test (before any antianginal med is discontinued) is at least 3 and not more than 13 minutes; 2. After withdrawal of $\geq$ one antianginal medications, the patient shows a decrease in ETT time to angina of at least 1 minute; if a 3rd drug is discontinued, discontinuation of at least one of these drugs should have resulted in a reduction in time to angina of a minimum of 30 sec. 3. Definite ECG signs of ischemia (> 1 mm ST depression in one lead) during the ETT that meets the 1 minute time criteria as above; 4. ETT time to angina for the last 2 consecutive qualifying ETT(t2 and t3) does not differ by more than 15% of t2; 5. Reason for stopping ETT should be angina;	1. Factors interfering with ECG interpretation or causing false positive result; 2. NYHA Class III-IV CHF; 3. Significant valvular heart disease or septal defects; 4. Unstable angina; 5. 2nd or 3rd degree AV block or uncontrolled arrhythmia other than sinus or occas. extrasystoles; 6. MI within past 3 months; 7. Ongoing acute myocarditis/pericarditis; 8. Cardiomyopathy; 9. Condition likely to hinder or confuse follow-up; 10. Significant lab abnormality; 11. Cannot discontinue digoxin or long-acting nitrates; 12. Participation in another investigational drug study within 1 month of entering this study; 13. Pacemaker; 14. Labile diabetic or subject to hypoglycemia; 15. Childbearing potential.

Efficacy Parameters:
The primary efficacy parameter was time to onset of angina at trough. Duration of exercise will be used if angina is not attained.

Secondary efficacy parameters:
1. Time to onset of angina at peak; duration of exercise will be used if angina is not attained;
2. Duration of exercise at both peak and trough;
3. Time to 1 mm ST depression or change from rest, at both peak and trough; duration of exercise will be used if ST change is not attained;

Analysis Plan:
According to the protocol, the primary analysis was an analysis of all patients contained in complete (evaluable) squares. All continuous treadmill data was to be analyzed using an extended-period Latin Square ANOVA model with the following effects: investigator, patient within investigator, period, previous treatment, treatment, investigator by period interaction, investigator by previous treatment interaction, and investigator by treatment interaction. For the primary and secondary parameters at trough, a first period analysis, including only those patients with valid baseline and first period double-blind treatment data was planned. There were prespecified criteria for pooling.

Sample Size: 240 patients planned in order to obtain 192 evaluable patients.

Protocol Amendments:
1. June 5, 1992: Added as exclusions: supine DBP > 100 mm Hg or SBP < 100 mm Hg; labile diabetic or subject to hypoglycemia (otherwise, changes appeared to be minor);
2. April 28, 1993: 1) Analysis changed from "all patients with valid data during double-blind" to all patients who had any data during double-blind." 2) Added supplemental complete squares analysis of monotherapy patients (with different pooling criteria).

Results:

Patient Disposition: Forty nine sites (42 in US, 5 in Canada, 2 in Mexico) enrolled 318 patients. A total of 29 patients (9.1%) withdrew prematurely. The mostly common reason for withdrawal was adverse event/new or worsening illness/lab abnormality (total of 15 patients, 3 on placebo and 12 on a treatment of ranolazine).

Baseline characteristics: Majority (72%) male, 86% Caucasian (7% Black), about half were 65 years and older; mean age was 64.2 years, mean weight 82 kg, 83% were nonsmokers and 96% had a history of noncardiovascular disease at entry. Patients had a history of angina for a median of 5.8 years; 43% had a history of MI, 32% underwent prior CABG, and 100% had used cardiovascular medication during the last month. Forty-one patients in this study were taking concomitant diltiazem.

Of the 318 patients, 312 had both trough and peak ETT data and were included in the all-patients analysis. Of those 312 patients, 260 and 248 patients were considered evaluable for the ETT per-protocol analyses at trough and peak, respectively.

Efficacy:
Results of the primary efficacy parameter are shown below. While mean time to angina, duration of exercise and time to 1 mm ST depression trend in favor of ranolazine (ie, mean differences are positive vs. placebo), the primary endpoint does not achieve a statistically significant result and the null hypothesis is not rejected. The only statistically significant trough parameter is a pooled "all ranolazine regimens" vs. placebo for time to 1 mm ST depression. The per-protocol complete squares analysis was similar except the time to 1 mm ST depression did not make statistical significance for all ranolazine vs. placebo (p=0.06, trend in favor of ranolazine).

Table 3. RAN 1514: Trough Endpoint ETT Pairwise Treatment Comparisons (all patients analyses)

Parameter	Statistic	Ran 400 BID-DB placebo	Ran 267 TID-DB placebo	Ran 400 TID-DB placebo	All ranolazine regimens-DB placebo
Time to onset angina (min)	Mean difference (SE)	0.18 (0.12)	0.19 (0.12)	0.07 (0.12)	0.15 (0.10)
	95% CI	-0.06, 0.42	-0.05, 0.43	-0.17, 0.31	-0.05, 0.34
	p-value	NS	NS	NS	NS
Duration of exercise (min)	Mean difference (SE)	0.05 (0.09)	0.06 (0.09)	0.10 (0.09)	0.07 (0.07)
	95% CI	-0.13, 0.23	-0.11, 0.24	-0.08, 0.27	-0.07, 0.21
	p-value	NS	NS	NS	NS
Time to 1 mm ST depression (min)	Mean difference (SE)	0.19 (0.13)	0.18 (0.13)	0.27 (0.13)	0.21 (0.11)
	95% CI	-0.07, 0.45	-0.08, 0.44	0.01, 0.53	0.003, 0.42
	p-value	NS	NS	NS	0.047

Source: Sponsor, Table 18. All statistics based on ANOVA. Significant investigator and period effects were seen with regard to the primary endpoint and duration of exercise (all $p < 0.01$); significant investigator effects were seen with regard to time to 1 mm ST depression ($p<0.01$) (Source: Table 16) DB placebo= double-blind placebo

Results at peak levels of ranolazine are shown in the following table. The per-protocol complete squares analysis showed improvements in mean time to angina that were only significant for the "all ranolazine" regimen column vs. placebo.

Table 4. RAN 1514: Peak Endpoint ETT Pairwise Treatment Comparisons (all patients analyses)

Parameter	Statistic	Ran 400 BID-DB placebo	Ran 267 TID-DB placebo	Ran 400 TID-DB placebo	All ranolazine regimens-DB placebo
Time to onset angina (min)	Mean difference (SE)	0.32 (0.13)	0.39 (0.13)	0.32 (0.13)	0.34 (0.10)
	95% CI	0.07, 0.57	0.14, 0.64	0.07, 0.57	0.14, 0.55
	p-value	0.013	<0.01	0.012	<0.01
Duration of exercise (min)	Mean difference (SE)	0.07 (0.09)	0.20 (0.09)	0.17 (0.09)	0.18 (0.07)
	95% CI	-0.01, 0.34	0.03, 0.38	-0.002, 0.34	0.04, 0.32
	p-value	NS	NS	NS	0.013
Time to 1 mm ST depression (min)	Mean difference (SE)	0.28 (0.12)	0.41 (0.12)	0.36 (0.12)	0.35 (0.10)
	95% CI	0.05, 0.52	0.17, 0.65	0.13, 0.60	0.16, 0.55
	p-value	0.02	<0.01	<0.01	<0.01

Source: sponsor, Table 27. All statistics based on ANOVA. DB placebo= double-blind placebo. Significant period effects ($p< 0.01$) were seen with regard to duration of exercise and time to 1 mm ST depression; a significant investigator x treatment effect ($p=0.03$) was seen with regard to time to onset angina (Source: Table 28).

According to the sponsor, there were no significant carryover effects for either peak or trough analyses. Significant period effects, however, were seen for the duration of exercise and time to 1 mm ST depression at times of peak ranolazine concentration.

A first-period analysis showed no statistically significant difference for peak or trough time to angina, exercise duration or time to 1 mm ST depression. No treatment-by-period analysis was submitted. Hence, a treatment-by-period interaction cannot be excluded by the reviewer.

Table 5. RAN 1514: Peak exercise treatment change from baseline to endpoint pairwise treatment comparisons: First period per-protocol analyses (n=304)

		Ran 400 mg bid vs. DB placebo	Ran 267 mg tid vs. DB placebo	Ran 400 mg tid vs. DB placebo
Time to Onset of Angina (min)	Mean difference (SEM)	0.78 (0.43)	0.59 (0.43)	0.43 (0.42)
	95% CI	-0.07, 1.63	-0.25, 1.43	-0.40, 1.27
Duration of exercise (min)	Mean difference (SEM)	0.39 (0.3)	0.29 (0.3)	0.13 (0.29)
	95% CI	-.20, 0.98	-0.29, 0.88	-0.45, 0.71
Time to 1 mm ST depression (min)	Mean difference (SEM)	0.40 (0.38)	0.94 (0.38)	0.48 (0.38)
	95% CI	-.35, 1.15	0.19, 1.68	-.26, 1.22

Statistics were estimated by the sponsor from ANOVA. The overall test was not significant.

Other efficacy parameters:
Other analyses were presented as per-protocol complete squares analyses. Trough ETT reasons for cessation are presented below (for peak ETT, 72-79.4% of ranolazine patients stopped due to angina, vs. 83.2% on double-blind placebo). The median difference in weekly rate of anginal attacks and nitroglycerin consumption was 0.0 for ranolazine treatment comparisons vs. placebo (hence no meaningful difference).

Table 6. RAN 1514: Trough ETT cessation reasons by treatment (per-protocol complete squares analyses) (n (%) (reasons occurring > 3%)

	Placebo (single-blind)	Placebo (double-blind)	Ran 400 mg BID	Ran 267 mg Tid	Ran 400 mg TID
Total # ETT performed	260	325	325	325	325
Angina	260 (100%)	283 (87%)	274 (84%)	270 (83%)	274 (84%)
Fatigue	6 (2)	61 (19)	63 (19)	68 (21)	64 (20)
Dyspnea	6 (2)	28 (9)	29 (9)	24 (7)	27 (8)
ST deviation	9 (4)	13 (4)	11 (3)	10 (3)	11 (3)

Source: sponsor, table 31.

Hemodynamic data: For trough ETT results, investigator effects (ANOVA $p < 0.01$) were seen with regard to maximum workload double product and maximum workload heart rate. A significant treatment effect ($p < 0.01$) was seen for standing heart rate. The pattern of standing HR results was graphically similar to that seen with trough double-product (shown below); mean changes were <2 beats per minute and no significant changes in standing heart rate were seen. Otherwise, no hemodynamic patterns were seen by the reviewer. Hemodynamic data for peak ETT showed decreases in maximum workload double product, heart rate and SBP, as well as standing double product, heart rate and SBP for all ranolazine doses vs. double-blind placebo. Statistical significance was only achieved in the "all ranolazine regimens" vs. placebo for maximum workload double product and SBP.

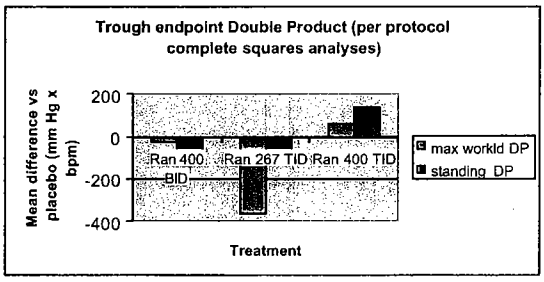
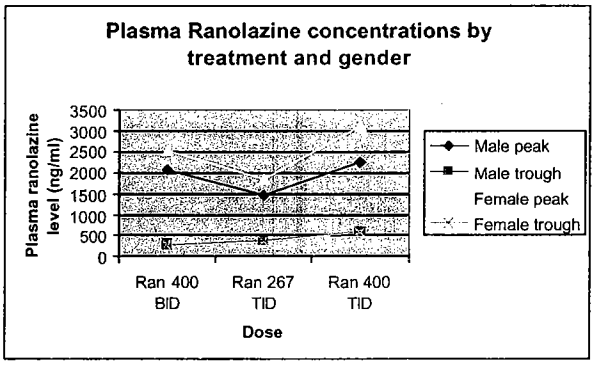


Figure 1. Trough ETT double product comparison vs. placebo (per protocol complete squares analysis)

Figure 2. Ranolazine peak/trough levels by treatment and gender



The sponsor's analysis of plasma ranolazine levels showed an increase in peak levels for females but not males (trough levels for both genders are superimposable per figure).

Additional analysis: A gender subgroup analysis showed statistically significant improvements in all ranolazine groups (peak ETT per protocol complete squares analyses) for males but confidence intervals that crossed zero for all ranolazine doses for females (parameters measured included: time to angina, duration of exercise and time to 1 mm ST depression).

Safety: please see the Safety Review.

Reviewer Comments:

1. This was a placebo-controlled crossover study comparing IR ranolazine 400 mg BID, 267 mg TID and 400 mg TID.
2. There was no statistically significant improvement in the primary efficacy parameter vs. placebo. Mean comparisons vs. placebo for the primary efficacy parameter trended toward improvement with ranolazine.
3. Significant period and investigator effects were seen ($p < 0.01$) with regard to the primary endpoint.
4. Statistically significant results were seen with respect to time to onset of angina and time to 1 mm ST depressions at peak. However, a significant period effect were seen with respect to the time of onset of angina at peak, and the first period analysis did not show a statistically significant treatment effect. No treatment-by-period analysis was submitted. A treatment-by-period interaction cannot be excluded.

RAN 081

Title: An Evaluation of the Long-Term Efficacy and Safety of Ranolazine in Patients with Angina Pectoris. (procotol date November 21, 1991; protocol amendments: December 19, 1991, January 22, 1992, May 14, 1992, March 8, 1993).

Objectives:

1. safety and tolerability of long-term ranolazine (IR) administration over a one year open-label phase. Appropriate patients may be continued for a second year;
2. assess effects of ranolazine withdrawal after stabilization of patients on their maximum tolerated dose of ranolazine on anginal symptoms and exercise capacity by performing a one-week, double-blind, withdrawal phase.

Study Summary: This study was designed as a follow-on from study RAN 080, which evaluated short-term ranolazine efficacy compared with atenolol. This study consisted of an open-label titration phase to achieve optimal medical control of anginal symptoms. One month after stabilization of therapy, an ETT at peak will be performed before and after a one week double-blind withdrawal period during which patients will be randomized to either ranolazine or placebo. All patients will then continue on ranolazine for up to 1 year. Hematology and chemistry labs will be done entry into RAN 080 and after 2 weeks in RAN 081, at 3, 6 and 9 months, at the end of the double-blind withdrawal phase and at one year. Twelve-lead ECGs were planned at both withdrawal visits as well as Week 2, and Months 3, 6, and 9.
All ETT were planned at 1 hour post-dose and, depending on the center, were either treadmill (modified Bruce protocol) or bicycle (starting load 20 watts, increasing by 20 watts every minute until ST depression and angina occur).
Allowed concomitant medications: short-acting nitrates were permitted as escape medication, long-acting nitrates and calcium channel blockers were allowed as background therapy if used during RAN 080. Beta blockers were not permitted.

Patient population: up to 108 eligible patients will be entered.

Inclusion criteria: successful completion of RAN 080 with reasonable compliance and without severe adverse events.

Notable Exclusion criteria: clinically significant arrhythmias or CHF, unstable angina/MI less than one month prior to RAN 080, pregnant/breastfeeding women, investigation drug use other than ranolazine in past 28 days, hepatic/renal dysfunction, significant lab abnormalities in RAN 080, cerebral hemorrhage/thrombosis/aneurysm, pulmonary hypertension, COPD, pacemaker.

Efficacy data: these data were obtained following the two exercise tests performed before and after the 1-week double-blind withdrawal period:
Time to angina, Exercise duration, Time to 1 mm ST depression, Maximum ST depression, Integrated ST depression.[20]
Hemodynamic data included: Heart rate, SBP and DBP, Rate pressure product[21].
Other: nitroglycerin consumption and number of angina attacks were collected from diary cards.

Analysis plan:
According to the protocol, the time to angina (peak) was listed as primary analysis. For this variable, those patients failing to reach angina pre- and post-withdrawal phase were to be excluded; for patients failing to reach angina on one of the two days, exercise duration may be substituted for time to angina. A planned secondary analysis would substitute exercise duration for all patients failing to reach angina on any study day; if there were a large number of protocol violators/noncompliant (< 70%) patients, this secondary analysis would be repeated, excluding those patients.

All statistical tests were two-tailed, with a 5% level of significance. The statistical significance of within-treatment group changes would be tested using the paired t-test. Between treatment group changes were compared using ANOVA with terms for center, treatment (during double-blind withdrawal) and treatment by center interaction..

Safety: adverse events, laboratory tests

Protocol amendments:
1. (Dec. 1991): added chemistry tests at months 3, 6, and 9.
2. (Jan. 1992): minor changes.
3. (June 1992): added hemodynamic data collection for each ETT; added hematology and chemistry testing prior to ETT;
4. (March 1993): added option to continue ranolazine for a second year.

Results:
According to a Syntex Interim Report dated December 1994 (study period March 1992-December 1993), 135 patients in 9 centers (119 males and 16 females), aged 41-77 years, received open-label ranolazine. Of this group, 66 received ranolazine and 60 placebo during the double-blind withdrawal period; nine patients were not randomized. Sixty-six ranolazine and 59 placebo patients were included in the safety analyses for double-blind withdrawal period and the "all patients" efficacy analyses. Fifty nine ranolazine and 50 placebo patients were evaluable for efficacy analysis of double-blind withdrawal period.
Six centers used treadmill for exercise testing. The other 3 centers used bicycle testing.

Baseline characteristics: Mean pulse was lower in the placebo (N=59, mean pulse 68 bpm) group compared to patients on ranolazine (N=66, mean pulse 72.5 bpm). Otherwise, no obvious differences in baseline characteristics (gender, mean age, weight, SBP, DBP) were noted across treatment groups. The population was 100% Caucasian. The median time on double-blind treatment was 7 days for both ranolazine and placebo.

Also of note, 22 patients (2/3) in one site (Dr. Rousseau) were started on background beta blocker during the titration phase. Of the 31 patients in this site, 20 had zero angina attacks during double-blind treatment, regardless of study medication. Four other sites had a majority of patients with zero angina attacks during double-blind.

[20] Integrated ST depression was calculated as the area under the ST depression/time curve.
[21] Rate pressure product was calculated as heart rate x SBP.

Primary efficacy parameter:
For the time to onset of angina at 'peak' (all patients), the pre- and post-withdrawal means for ranolazine were similar (391.7 and 390.7 seconds, respectively) while the pre-and post-withdrawal means for placebo were 428.9 and 364.7 seconds, respectively. The treatment by investigator interaction was significant (p=0.005) for between-treatment comparison of changes, indicating heterogeneity of results by site (interactions between investigator (p=0.0001) and treatment (p=0.002) were also significant). The calculated treatment difference (R minus P), adjusted for imbalance in the number of patients receiving each treatment in each center was 53.61 (SE 16.6) seconds with a 95% CI of 20.7 to 86.5 seconds (not crossing zero). Although the decrease on placebo was consistent, there were also differences in mean change according to exercise method used.
In the all patients analysis, it should be noted that 50% and 43% of the data points in the ranolazine and placebo groups respectively were substituted data (ie, exercise duration substituting for time to onset of angina).

Table 1. RAN 081: Time to onset angina at peak (sec) (all patients) by method of exercise (All patients)

	Ranolazine N=59	Placebo N=50
Treadmill N	43	36
Mean change (post-pre) (SEM)	-2.09 (12.96)	-82.64 (24.44)
Bicycle N	23	23
Mean change (post-pre) (SEM)	0.96 (7.12)	-35.43 (10.55)

Results were similar for the evaluable population (although mean change for treadmill (post-pre) was +3.12).

Dr. Cocco' site[22]: The mean treatment difference in favor of ranolazine seen in Dr. Cocco's center was much higher than thoses seen in other centers. In Dr. Cocco's center, a mean decrease of 9 seconds in time to onset of angina was seen for patients on ranolazine and a mean decrease of 210 seconds for patients on placebo (the adjusted mean across all centers was 48.41 sec). Large decreases were seen for 3 patients on placebo at that site (# 198: 545 seconds; # 199: 540 seconds; #204: 403 seconds). At baseline, all 3 patients did not reach angina during exercise lasting 10 minutes 5 seconds, 11 minutes, and 9 minutes 43 seconds respectively. During the post-withdrawal test, the onset of angina occurred after 1, 2 and 3 minutes respectively for the three patients.
An imbalance was also seen in Dr. Cocco's site regarding mean weekly angina attacks (3.3 in the placebo vs. 1.56 observed in the placebo group across all centers during the double-blind phase).

Exercise duration at peak:
Exercise duration decreased for both ranolazine (mean change = -10 sec) and placebo (mean change = -25.91 sec) during the withdrawal period (p=ns). There was a significant investigator interaction (p=0.001). The magnitude of effect varied by method of exercise.

Table 2. RAN 081: Exercise duration at peak (sec) (all patients)

	Ranolazine	Placebo
Treadmill N	43	36
Mean change (post-pre) (SEM)	-15.49 (12.99)	-40.72 (21.97)
Bicycle N	23	23
Mean change (post-pre) (SEM)	-5.96 (6.37)	-19.09 (4.72)

Time to 1 mm ST depression: As in the previous analyses, results varied by method of exercise. In addition, there was a significant treatment by investigator interaction (p=0.015). The mean change for

[22] Dr. Cocco's site is also mentioned in study RAN 080.

ranolazine was 13.96 sec (increase) while the mean change for placebo was –42.4 sec (decrease). Results for evaluable patients were similar.

Heart rate, SBP, DBP: Evaluation of heart rate, SBP and DBP at rest and at end of exercise did not reveal any clinically meaningful differences between ranolazine and placebo for either analysis population (all patients vs. evaluable population).

Rate Pressure Product (RPP): There was no statistically significant difference between ranolazine and placebo with regard to RPP at rest and at end of exercise. A statistically significant investigator effect was noted for RPP at rest (evaluable patients).

Table 3. RAN 081: Weekly rates of angina attacks and nitroglycerin consumption

	Whole study	Double-Blind withdrawal phase	
		Ranolazine	Placebo
Angina attacks			
N	135	66	59
Mean (SD)	0.8 (0.97)	1.22 (1.7)	1.56 (1.82)
NTG use			
N	135	66	59
Mean (SD)	0.39 (0.63)	0.6 (0.94)	1.15 (1.72)

The above table shows week rates of angina attacks and nitroglycerin consumption, with higher rates in both groups (higher on placebo) compared to rates for the whole study.

Safety: For a detailed safety discussion please see the safety review.

Reviewer Comments:

1. This was an open-label study with a one-week double-blind IR ranolazine vs. placebo period to assess withdrawal from therapy. The primary efficacy variable was time to angina. All exercise testing was performed at peak.
2. About 50% of the time to angina data represented substituted data (exercise duration).
3. Decreases in time to angina, total exercise duration, and time to 1 mm ST depression were seen in the placebo-treated patients; also seen were increases in angina attacks and nitroglycerin use.
4. There was a hetergeneous response with regard to the primary efficacy variable. In particular, one site showed large decreases in time to onset of angina (210 sec mean decrease in Dr. Cocco's site, compared to an adjusted mean of 48.41 seconds across all centers).
5. There was no statistically significant difference in exercise duration between ranolazine vs. placebo.
6. Imbalances were noted in terms of background therapy.
7. No meaningful changes were seen with regard to hemodynamic parameters (heart rate, BP, RPP).

RAN 015.

Title: A Crossover Study of Two Doses of Ranolazine 120 mg and 180 mg TID and Placebo in Coronary Artery Disease (Volume 245) (Protocol date: March 18, 1987)

Objective (listed as 'aim'): evaluate, using exercise tolerance, anginal frequency and nitrate consumption, whether 120 mg and 180 mg RS 43285 (ranolazine IR) administered three times daily are well tolerated, give effective antianginal control and whether there is a dose relationship.

Study Summary: This was a double-blind, 3-way crossover study using a Latin square design in patients with stable angina. After a 1 week washout period, where previous therapy was withdrawn, patients entered a 2-4 week placebo washout (Phase 1). On two days during the second week of Phase 1 (one of

these days being Day 14), patients underwent a treadmill ETT; if the time to onset of angina did not differ by more than 15% between these two tests, the patient proceed to Phase 2 (double-blind treatment) where either ranolazine 120 mg, 180 mg or placebo would be given tid. Efficacy was evaluated primarily by ETT 1.5 and 8 hours post-dose in each treatment phase; in addition, anginal frequency and nitroglycerin consumption would be evaluated.
Sublingual nitroglycerin was allowed if used as treatment for angina (not prophylactically).

Sample Size: The enrollment planned for 30 patients randomized in order to achieve 24 completed patients. The sample size was based on an assumed standard error of the difference of about 0.4 min, based on results with other antianginal drugs, with a 70% power to detect a 1 minute difference in exercise times, and a 95% power to detect a difference of 1.5 minutes.

Table 1. RAN 015. Inclusion/exclusion criteria

Inclusion criteria	Exclusion criteria
1. Males and females, 25-65 years old, incapable of conception; 2. At least 6 month history of stable effort angina relieved by rest/nitroglycerin; 3. If measured, resting EF $\geq$ 50%; 4. On standard ETT: a) difference in exercise time between last 2 ETT at baseline placebo must be less than 15% of the longer time; b) time to onset angina must be within 2-10 minutes; c) evidence of ischemia must be present in a standard ECG lead (J point depression $\geq$ 1 mm and ST depression $\geq$ 1 mm 80 msec after J point) with normal or interpretable resting ECG; 5. Patients under treatment for angina will be admitted only if their response to such treatment is inadequate or complicated by unwanted effects;[23] 6. Verbal informed consent.	1. Presence of factors associated with false positive stress test; 2. Uncompensated CHF; 3. Significant valvular heart disease/septal defects; 4. Unstable angina; 5. Second/third degree AV block/uncontrolled arrhythmia other than sinus arrhythmia or occas. extrasystoles; 6. MI within past 3 months; 7. Acute myocarditis/pericarditis; 8. High grade left main disease; 9. SBP < 95 mm Hg; 10. Condition likely to hinder/confuse follow-up; 11. Abnormal pretreatment renal/hepatic/thyroid/potassium tests or anemia; 12. Unable to discontinue long-acting nitrates/beta blockers/calcium blockers. Digitalis is not permitted; 13. Significant disease requiring medical therapy or supervision (other than angina); 14. Inability to undergo ETT; 15. IDDM; 16. Female subjects capable of conception.

Principal Efficacy variables: 1. Total exercise time; 2. Heart rate, BP, and rate-pressure product at end of exercise; 3. Workload at termination of treadmill. Additional variables: angina attacks/week; nitroglycerin consumption/week.

Pharmacokinetic samples will also be drawn.

Safety monitoring: adverse events, vital signs, ECG, laboratory tests.

Protocol amendments: no substantive changes.

Results:
Twelve (2 female, 10 male) patients, 41-64 years old, were enrolled and 11 patients completed all phases of the study. The study was terminated prematurely due to slow progress. One patient withdrew prematurely due to adverse event during placebo run-in and prior to randomization.

Baseline characteristics: The study population (n=12) was 100% Caucasian. Mean age was 53 years old, weight 79 kg, height 171 cm.

[23] Not further defined.

Efficacy: For the key endpoints, neither 120 or 180 mg ranolazine showed effects greater than placebo. For nitroglycerin consumption/week and anginal attacks/week, ranolazine showed no improvement compared to placebo.

Safety: Seven adverse events were reported in three patients (3/7 were reported while on placebo). One patient on ranolazine 120 mg complained of listlessness and intermittent nausea, dizziness and musculoskeletal pain (4 adverse events).

Reviewer comments:
1. This study does not support efficacy of ranolazine IR at the doses and regimen used.
2. The sample size was smaller than originally planned and this change in size may have impacted results.

RAN 020:

Title: A Double-Blind Crossover Study of Ranolazine (RS-43285) 60 and 120 mg tid versus Placebo in Patients with Angina Pectoris (Protocol date: March 1987) (Volume 246).

Primary Objective (listed as "aim"): evaluate, using exercise tolerance, anginal attack frequency and nitrate consumption, the relative efficacy and tolerance of two weeks dosing with RS 43285 60 and 120 mg tid.

Secondary Objective:
Patient population: Males and females with stable effort angina (see Inclusion criteria). The protocol called for 15 enrolled or 12 completed patients..

Study Summary: This was a double-blind, 3-phase crossover study in patients with stable angina. After a one week washout followed by a placebo run-in, patients were randomized to ranolazine 60 mg tid, ranolazine 120 mg tid or matching placebo for a period of 2 weeks each; there were no washout periods between the active phases.
Two ETT were planned at the end of placebo run-in; both tests were to be performed at the same time (either 1.5 or 7.5 hours post-dose). If the time to onset of angina did not differ by more than 20% they were to proceed directly into active treatment. Otherwise, patients were to continue on placebo for up to 2 more weeks during which time they further trained on the treadmill.
Patients not satisfactorily treated (this was not further defined) on long-acting nitrates and beta blockers were to have these drugs tapered off and discontinued before the end of the 1 week washout.

Concomitant medication: Sublingual nitroglycerin was allowed as treatment for anginal attacks.
Prophylactic use of any form of nitrate necessitated withdrawal from the study.

Table 1. RAN 020: Inclusion/Exclusion criteria:

Inclusion criteria:	Exclusion criteria:
1. Males and females, 21-70 years old, incapable of conception;	1. Presence of factors associated with false positive stress tests (e.g. IVCD, WPW, LBBB, etc);
2. At least 3 month history of classic stable effort angina pectoris relieved by rest/nitroglycerin;	2. Uncompensated CHF;
3. Difference in treadmill exercise time (last 2 ETT prior to active treatment) must be less than 20% of the longer time;	3. Clinically significant valvular disease;
4. Ischemia (J point depression $\geq$ 1mm and ST	4. Unstable angina;
	5. Second/third degree AVB/ uncontrolled arrhythmia other than sinus arrhythmia;
	6. MI within past 3 months;

[24] This point is not further defined in the protocol. It is not clear whether "inadequate reponse" means that the patient was given adequate or maximal doses of a particular medication.

depression ≥ 1mm at 80 msec after J point) resulting from the ETT must be present in a standard lead. Resting ECG should either be normal or not interfering with interpretation of ST changes. 5. Maximal exercise time at end of placebo phase must be 3-10 minutes; 6. If the patient has had a coronary angiogram, 50% or greater occlusion in a single view of a major coronary artery or one of its primary branches must be evident; 7. Patients who are currently under treatment for angina will be admitted to this study only if their response to treatment is inadequate or is complicated by unwanted effects;[24] 8. Must give consent.	7. Acute myocarditis/pericarditis; 8. High grade left main disease; 9. SBP < 95 mm Hg or sitting BP > 165/110 mm Hg; 10. Any condition likely to hinder/confuse follow-up; 11. Abnormal pretreatment renal, hepatic function, potassium levels, anemia; 12. Patients unable to discontinue therapy with long-acting nitrates, beta blockers, antihypertensive medication, calcium channel blockers or any investigational drug. Digitalis was not permitted during this study. Diuretics were permitted if continuous throughout study; 13. Inability to undergo ETT; 14. IDDM, systemic infection, female subjects capable of conception.

Efficacy assessments:
All patients were to have treadmill ETT under uniform conditions at the same time of day at each visit. Time to angina, 1 mm ST depression, 2 mm ST depression and maximal exercise capacity were noted for all ETT. No smoking or sublingual nitroglycerin was allowed on the morning of the clinic visit where ETT was scheduled.
Forms for 2 weekly diaries were given to patients in order to record time of angina attack, number of nitroglycerin tablets used and time of study medication.
No primary efficacy variable was specified in the protocol. Principal efficacy variables to be compared among dosage regimens included: angina attacks/week; nitroglycerin consumption/week; total treadmill time; heart rate/BP and rate-pressure products at the end of exercise; workload at termination of treadmill exercise.

Safety: Safety monitoring included vital signs, ECGs, laboratory testing, adverse reactions and withdrawals.

Analysis Plan: No analysis population was prespecified in the protocol.

Amendments/changes in the conduct of the Study: Amendment 1 (April, 1987) increased enrollment to 30 patients or until 24 have completed. Amendment 2 (June, 1987) included one day of 24 hour ECG monitoring at the end of each active treatment phase. Otherwise, there were no substantive changes to the study.

Results:
Patient Disposition:
Baseline characteristics: Mean age was about 63 years. The total patient population (n=36) was 86% male, 72% Caucasian, 22% Asian, 28% smokers and 78% admitted to alcohol consumption. The patients deemed "valid for efficacy" were divided into 6 groups (P/60/120 (n=4); P/120/60 (n=4); 60/P/120 (n=3); 60/120/P (n=4); 120/P/60 (n=4); 120/60/P (n=5). The baseline characteristics for each group are too small to permit comparisons. The total N deemed valid for efficacy was 24. Of the 12 patients excluded from analysis, 2 dropped out prior to active treatment (Phase 1), 5 were inappropriately enrolled, 2 did not meet ETT criteria for inclusion, 2 were protocol violators via noncompliance, and 2 patients required prohibited medication.
Of the 2 premature terminations during active treatment, one patient dropped out of ranolazine 120 mg tid due to unsatisfactory response (worsening of angina). The other patient on ranolazine 120 mg tid was withdrawn due to inappropriate enrollment.

Efficacy:

Nitroglycerin consumption/angina frequency: Mean angina attacks and nitroglycerin consumption for all patients valid for efficacy decreased from baseline for all groups but active treatment was either the same as or slightly worse (less of a decrease) than placebo. No statistical analysis was done by the sponsor.

ETT: There were no significant or meaningful differences in exercise time, time to angina, and time to 1 mm ST depressions between ranolazine 60 or 120 mg tid and placebo for the 1.5 or 7.5 hour exercise studies. Carryover effects were seen for: changes in mean SBP at onset of angina (pre-test values to post-test values); changes in double product at onset of angina from pretest to 1.5 hour ETT; ranked times to 2 mm ST depression for 1.5 hour ETT.
Several statistically significant findings were noted; according to the study report these finding were felt to result from the large number of analyses and no statistical corrections were made to accommodate this factor.

Safety: Please see the safety review for a detailed safety discussion.

Reviewer comments:
1. This was a 3-way crossover study of ranolazine 60 and 120 mg tid and placebo. Only sublingual (prn) nitroglycerin was allowed as a concomitant medication. There was no interim washout period between treatments.
2. This study does not support efficacy of ranolazine IR 60 or 120 mg po tid as given in this trial.

RAN 054.

Title: A Double-Blind Crossover Study of Ranolazine 120 and 240 mg TID Versus Placebo in Patients with Angina Pectoris. (Volume 248) (Protocol date: April 1988)

Primary Objective (listed as 'aim'): evaluate relative efficacy and tolerance of 4 weeks dosing with ranolazine 120 and 240 mg itd, using exercise testing, anginal attack frequency and nitrate consumption.

Study Summary: This was a double-blind, 3 phase crossover study in patients with stable angina. After a 1 week washout period (previous therapy withdrawn), patient entered a single-blind placebo phase (phase 0, 2-4 weeks). On 2 days, several days apart, during the second week of placebo (phase 0), patients were given ETT to determine eligibility (see Inclusion criteria for difference in ETT time).
Following the placebo phase, patients were given ranolazine 120 mg tid, 240 mg tid or placebo tid each—in random order--for a period of 4 weeks. There was no interim washout period between treatments. ETT were performed during the initial placebo period as well as 60 minutes and 7.5 hours post-dose between days 24 and 30 of each treatment phase.

Sample size: A total enrollment of 120 planned to achieve 100 completed patients. The sample size was based on an 80% power to detect a 10% difference in exercise time with a 95% level of significance.

Table 1. RAN 054 Inclusion/exclusion criteria

Inclusion criteria:	Exclusion criteria:
1. Males and females, 21-75 years old, incapable of conception; 2. At least 3 month history of stable effort angina relieved by rest/nitroglycerin; 3. Difference in ETT exercise time (last 2 ETT prior to active treatment) must be less than 20% of the longer time; 4. Evidence of ischemia during baseline ETT must be	1. Presence of factors which may cause false positive stress test; 2. CHF; 3. Unstable angina in the past 4 weeks; 4. Second/third degree AV block or uncontrolled arrhythmia other than sinus arrhythmia or occasional extrasystoles; 5. MI within psat 3 months;

present in a standard ECG lead (≥ 1 mm J point depression and ≥ 1 mm ST depression at 80 msec after the J point and occurring within 9 min of the start of the 2 tests). Resting ECG should be normal or of such pattern as not interfere with interpretation during angina; 5. Onset of angina within placebo phase must be within 9 min or less; 6. If the patient has had a coronary angiongram, 50% or greater occlusion in a single view of a major coronary artery or one of its primary branches must be evident; 7. Patients under treatment for angina will be admitted only if their response to treatment is inadequate or complicated by unwanted effects; 8. Written consent.	6. Acute myocarditis/periocarditis; 7. High grade left main disease; 8. SBP < 95 mm Hg or sitting BP > 165/110 mm Hg; 9. Any condition likely to hinder/confuse follow-up; 10. Abnormal pretreatment renal/hepatic/ potassium tests or anemia; 11. Inability to discontinue long-acting nitrates, beta blockers, ACE inhibitors, calcium channel blockers or investigational drug. Digitalis is not permitted. Diuretics are permitted if use is continuous throughout the study; 12. Systemic infection; 13. Inability to undergo ETT; 14. IDDM; 15. Females capable of conception.

Concomitant medication: Sublingual nitroglycerin was permitted only as treatment for anginal attacks. The use of prophylactic nitrates necessitated withdrawal from the study. Initiating or changing therapy with antihypertensive or antiarrhythmic therapy also necessitated study termination.

Efficacy analyses: No primary efficacy variable was prespecified in the protocol. The principal efficacy variables to be compared among dosage regimens were: anginal attacks/week; nitroglycerin consumption/week; total treadmill time plus time to exercise induced angina; heart rate, BP and rate-pressure product at end of exercise; workload at treadmill termination.
According to the Statistical Report (Appendix D, dated October 1992) the primary efficacy variable of interest was peak (1 hour) total exercise time. Pooling of centers, while not prespecified in the protocol, was done for thoses centers who did not recruit at least one patient into each of the 6 possible treatment centers.
All patients were to undergo treadmill testing under uniform conditions, at the same time of day at each visit.
For documentation of anginal attacks, forms for 2 weekly diaries were provided to all patients.

Protocol Amendments:
1. Nov. 1988: added inclusion criterion that patients needed at least 2 angina attacks/week, on average, during placebo run-in.
2. March 1989: added a review of results, without statistical analysis, once 60 patients completed the trial.
3. July 1989: changed exercise testing to Bruce (rather than modified Bruce) protocol;
4. Sept. 1989: excluded antiarrhythmics from this study;
5. May 1991: added calculation for workload (in order to define workload at treadmill termination).

Study Conduct:
A 3-page document entitled Practical Conduct of the Study was included in the submission (Appendix A-2). It was noted that the clinical phase of the study ran from October 1988-April 1990 and the process of data discrepancy resolution and reporting took until October 1992.
1. Methodologies for the various measured parameters varied across the centers. Recording equipment for treadmill ETT varied from fully automated systems to manual ones; this difference impacted most significantly (according to the sponsor) on measurement of ST depression. BP was recorded using a standard sphygmomanometer in all centers except one (Stephen) which used a Hawksley random zero recorder.
2. Pre-trial and end of phase ECG were recorded under varying conditions (supine, sitting, upright) according to center.
3. Clinical chemistry and hematology results were generated at 4 different laboratories.

Results:

Patient Disposition: A total of 144 patients were enrolled at 8 centers. Of those enrolled, 7 were not randomized (leaving 137 randomized patients) and another 8 patients never entered the active treatment phase. One patient was excluded due to poor compliance. Another 13 patients had less than 2 phases worth of data. According to the statistical report (although not found in the protocol) the criteria for inclusion in the analysis stipulated that each patient should have at least 2 phases worth of data. One patient failed to meet the requirement for 3 full phases and it was deemed appropriate (not clear how this decision was made) to increase the requirement to 3 phases and not include that patient in the efficacy analysis.
Therefore, 114 patients were included in the sponsor's Full patients analysis. Of these 114 patients, 90 patients were included in the Valid Patients analysis (24 were excluded because of noncompliance).

A total of 16 patients withdrew due to adverse events (4 during placebo run-in, another 4 on double-blind placebo); of the patients on ranolazine, 2 withdrew while receiving 120 mg tid (one with worsening angina associated with hypertension/headache, and another with sudden death) and 6 withdrew while on 240 mg tid (2 with headache/vasodilatation, 1 with chest burning/depression, 1 with hip pain, 1 with infection and 1 with chills/fever).

Baseline Characteristics: In the all patients group, mean age was 59 years; mean height 171 cm; mean weight 77 kg without imbalances across centers. History of MI ranged from 18% to 32% in one center (Raj); a similar imbalance was seen with respect to history of hypertension. Heterogeneity across centers was seen with respect to baseline aspirin use (13-64%) and diltiazem use (1-27%).

Efficacy:
According to the study report, "the exercise data presented some problems with data verification and analysis which meant that the analysis as planned in the protocol had to be considerably altered."

For ST depression, there was variation between centers in the method used to calculate ST depression, failure of some investigators to use a standardized lead, changing of the protocol from modified Bruce to Bruce (removing Phase) leading to inappropriateness of data to time periods, and loss of monitoring manpower due to sickness. Therefore, the sponsor has stated that this dataset (ST depression, time to 1 and 2 mm ST depression) could not be rendered sufficiently accurate and reliable.

Diary card data for nitrate use/number of angina attacks was suspect because patients/investigators were unclear about which point should be used to represent the end of one phase and beginning of the next.

Rate pressure product was not calculated and analyzed because measurements of BP and HR prior to ETT was not sufficiently controlled for posture, and it was concluded by the sponsor that the uncontrolled addition of this variable would make interpretation of baseline and change from baseline unreliable.

Table 2. RAN 054: Selected efficacy parameters (Full patients analysis- complete data in all 3 phases: N=114)

Parameter	Ran 120 mg – placebo	Ran 240 mg - placebo
Total exercise time (min) (peak: 1 hr) (treatment by center interaction p=0.002, period effect p=0.01)		
LSM difference from baseline (SEM)	0.09 (0.12)	0.22 (0.12)
95% CI	(-0.15, 0.33)	(-0.02, 0.45)
p-value	NS	NS
Total exercise time (min) (trough: 7.5 hr) (treatment by center interaction p=0.04; period effect p=0.06)		
LSM difference from baseline (SEM)	0.19 (0.13)	0.24 (0.13)
p-value	NS	NS
Time to angina (peak: 1 hr) (treatment by center interaction NS; period effect p=0.0001)		
LSM difference from baseline (SEM)	0.17 (0.19)	0.11 (0.19)
95% CI	(-0.2, 0.55)	(-0.26, 0.48)
p-value	NS	NS
Time to angina (trough: 7.5 hr) (treatment by center interaction NS; period effect p=0.08)		

LSM difference from baseline (SEM)	0.32 (0.18)	0.42 (0.17)
95% CI	(-0.03, 0.67)	(0.08, 0.76)
p-value	0.08 (NS)	0.02
Angina attacks per week (treatment by center NS)		
LSM difference from baseline (SEM)	-1.35 (0.46)	-0.73 (0.45)
95% CI	(-2.26, -0.43)	(-1.62, 0.17)
p-value	0.004	NS
NTG consumption per week (treatment by center NS)		
LSM difference from baseline (SEM)	-0.55 (0.35)	-0.66 (0.34)
p-value	NS	NS

Source: sponsor. P-values based on ANOVA model

For the valid patients analysis (N=75 for total exercise time, N=88 for time to angina) patients on ranolazine 240 mg experienced a statistically significant improvement in total exercise time vs. placebo (both peak and trough: statistically significant period effect $p< 0.05$). The time to angina was significantly longer for peak ranolazine 120 mg but not 240 mg (period effect p=0.0008) and for both doses at trough (period effect $p < 0.05$).

End of Exercise heart rate:
Slight increases in heart rate were seen pre-exercise (peak and trough) in ranolazine vs. placebo. Variations in heart rate were 3 beats per minute or less and were not statistically significant. Pre-exercise mean systolic and diastolic BP were not different between treatment groups. Post-exercise heart rate was significantly higher in the ranolazine 240 mg group (both peak and trough) vs. placebo

Safety: One death during the study (Patient 414, on ranolazine 120 mg) was noted. According to narratives, this was a 62 year old man, on ranolazine 120 mg tid for one month, who experienced intermittent chest pain, unresponsive to nitroglycerin over 7, and then developed severe chest pain (ambulance called)and found to be in asystole when the crew arrived.
Another death (DT 415) was noted 2 days post-completing the study. A 57 year old man who completed ranolazine 120 mg tid (last phase) collapsed and died.

Reviewer Comments:

1. This was a randomized, double-blind, 3 phase crossover study comparing patients with angina on ranolazine IR 120 mg tid, 240 mg tid and placebo. There were no interim washout periods.
2. Primary efficacy variable and analysis population was mentioned in the statistical report but not prespecified in the protocol.
3. There were difficulties in the conduct of this study, including changing the exercise protocol (modified Bruce to standard Bruce), heterogeneity in ST segment interpretation, issues regarding monitoring, and recording of ECGs and vital signs under different conditions.
4. For several efficacy variables, there were significant treatment by center and period interactions.
5. For total exercise time at peak, the results of "all patients analysis" and "valid patients analysis" were not consistent. In addition, there was a significant treatment by center interaction, with heterogeneity by center.
6. For all patients analyzed, there were slight improvements in total exercise time (favorable for ranolazine) that were not statistically significant.
7. Two deaths (one during the study and another 2 days post-study) in ranolazine-treated patients were noted.

RAN 1490.

Title: Double-Blind Parallel Dose-Scheduling Study of RS-43285 Versus Placebo in Patients with Chronic Stable Angina Pectoris. (Protocol volume 265) (Protocol date: May 28, 1987).

Objective: evaluate efficacy and safety of RS-43285 as treatment of chronic stable angina, and to provide an estimate of the optimal total daily dose and dosing interval.

Sample size: The study was originally planned for 48-72 patients, to be determined by the number of dose levels tested. However, due to slow enrollment, a decision was made to discontinue the study after 12 patients had enrolled. Eleven completed the study; one withdrew prematurely.

Study Summary: This was a double-blind, randomized, placebo-controlled, dose ranging and scheduling trial. Eligible patients would receive 2 weeks of single-blind placebo prior to double-blind randomization. Treadmill ETT was planned 2 days after starting placebo (Visit 3) and at end of run-in (Visit 4). If the duration of exercise from these 2 baseline tests did not differ by more than 15% of the duration of the longer test, then patients qualified for double-blind and were randomized to receive 5 days of study medication or placebo. ETT was performed on Day 5 at peak (2 hours following the first morning dose) and on Day 6 at trough (one full dosing interval post-dose).
Medication was taken every 12 hours (if bid), every 8 hours (if tid) and every 6 hours (if qid).
The study consisted of 4-6 substudies, each involving 12 patients (4 patients given placebo and 8 patients given active treatment). These substudies involved ascending doses of ranolazine. However, only the first dosing group, 60 mg po tid, was given prior to the study being stopped.

Notable Inclusion criteria: Patients, 21-70 years old, with at least 3 month history of chronic stable effort angina relieved by rest/nitroglycerin. The first ETT must not exceed 12 minutes and the reason for stopping must be angina, with ST depression ($\geq$ 1 mm) in a standard lead. Time to angina for the last 2 ETT prior to double-blind must not differ by more than 15% of the duration of the longer test. Resting ECG must be normal or not interfere with ETT interpretation.

Notable Exclusion criteria: pregnant/breastfeeding women, factors associated with false positive stress test, CHF, unstable angina, MI within past 2 months, myocarditis/pericarditis/cardiomyopathy, high-grade AV block, nonobstructive CAD, SBP < 95 mm Hg, significant lab abnormality, inability to discontinue anginal medication or undergo ETT, labile DM or IDDM.

Efficacy Parameters:
Primary efficacy parameter: duration of treadmill exercise to maximal tolerated angina or other limiting symptomatology.
Secondary efficacy parameters: exercise time to onset of angina, specified amounts of ST depression and changes in severity of angina and ST changes at maximal work loads.

Results:
Patient Disposition:
5 women and 7 men, mean age 62 years, were randomized; 8 were given ranolazine 60 mg tid and 4 were given placebo.

Efficacy: Because only 12 patients from the first group entered the study, no formal analysis was done by the sponsor.
The reviewer looked at the data listings (nitroglycerin consumption, anginal attacks, and treadmill data at peak/trough) and did not note any striking pattern.

Reviewer Comments: No efficacy conclusions can be drawn from this study.

RAN 1513.

Title: Double-blind Parallel Efficacy and Safety Study of Various doses of Ranolazine vs. Placebo in Patients with Chronic Stable Angina Pectoris (Protocol volume 265, dated April 19, 1989; six Amendments between June, 1989-February, 1990).

Objective: evaluate safety and cardiac anti-ischemic properties of ranolazine 30, 60, 120 mg tid or placebo tid as treatment for patients with chronic stable angina who may also have silent ischemia.

Primary Objective: assess change in duration of treadmill exercise to maximum tolerated angina or other limiting symptoms.

Secondary Objectives:
1. Change in number of angina attacks;
2. Change in number of episodes of ST depression as documented by 48 hour Holter monitors (at the end of 4 weeks treatment);
3. Change in duration of episodes of ST depression.

Sample Size: 284 planned (four groups of 71 patients).

Study Summary: This was a double-blind parallel-group randomized placebo-controlled dose-finding study. Eligible patients entered a 5 day washout period where antianginal medications were withdrawn (except prn sublingual nitroglycerin), followed by a one week single-blind placebo period prior to double-blind medication.
On Day 0 of the placebo run-in, patients underwent laboratory testing and 48 hour Holter monitoring; on Day 2, the Holter was removed and, following removal, a treadmill ETT was performed. A second treadmill ETT was done 2-10 days following the first ETT; in order to qualify for double-blind, there must be at least 36 hours of interpretable ECG on Holter (read by a central Holter lab), the duration of exercise of the two ETT must be 3-9 minutes and not differ by more than 15% of the duration of the longer test, and the reason for stopping must be angina. The final ETT during single-blind placebo will be considered to be the baseline test. The patient must also have reported at least one anginal episode in a diary the week before randomization. Eligible patients were then randomized to receive 30, 60, 120 mg ranolazine tid or placebo tid for 4 weeks; medication was to be taken q 8 hrs.
Two days before the end of week 4, the am dose was given in clinic; one hour post-dose, a serum sample was drawn and the patient underwent an ETT at peak. Following the ETT, another 48 hour Holter monitor was done. After 48 hours, the Holter monitor was removed, a trough plasma sample was drawn and the final ETT at trough was performed.

Table 1. RAN 1513. Inclusion/exclusion criteria

Inclusion criteria	Exclusion criteria
1. ≥ 21 years old; 2. At least 3 month history of chronic stable effort angina relieved by rest/nitroglycerin; 3. At least 1 subjective attack of angina recorded in their diary during the week prior to randomization; 4. Qualifying ETT: Primary reason for stopping must be angina, duration of test must be 3-9 minutes, exercise time (time to angina) for last 2 consecutive tests (prior to double-blind) must not differ by more than 15% of duration of the longer test. All ETT were planned according to Bruce protocol; all ETT must show evidence of ischemia, ≥ 1 mm ST depression, measured 80 msec from J point, in a standard lead ; 5. Resting ECG should not interfere with interpretation of ST changes during angina; 6. Patients with intermittent atrial fibrillation during the Holter must have 36 hours of readable tape without atrial fibrillation; 7. Patients must have telephone and sign informed consent.	1. Women of childbearing potential; 2. Presence of factors associated with false positive ETT; 3. Uncompensated CHF; 4. Valvular heart disease; septal defects; unstable angina; second/third degree AV block; uncontrolled arrhythmia; acute myocarditis/pericarditis; cardiomyopathy; pacemaker. 5. Nonobstructive CAD; 6. High grade left main coronary disease; 7. MI within the past 2 months; 8. Standing SBP < 95 mm Hg; 9. Any condition likely to hinder/confuse follow-up; 10. Clinically significant lab abnormality; 11. Inability to discontinue long-acting nitrates, calcium channel blockers, beta blockers, or any investigational drug. Digitalis is not permitted in this study; 12. Inability to undergo ETT; 13. Labile DM/subject to hypoglycemia; 14. Participation in investigational drug study within previous month.

Efficacy Analysis: Using Fisher's Least Significant Difference procedure, all ranolazine doses would be tested vs. placebo if the overall treatment effect was significant at the 0.05 level. All efficacy variables will be tested using ANOVA including effects of treatment, center, and treatment by center interaction.

Primary Efficacy variable: exercise duration at peak.

Secondary Efficacy variables: (2-6 measured at peak and trough).

1. Exercise duration at trough;
2. Time to onset of angina (duration of exercise will be used if angina does not occur);
3. Time to 1 mm ST depression from rest; duration of exercise will be used if 1 mm ST depression is not attained;
4. Standing heart rate on treadmill and at maximum workload;
5. Standing SBP on the treadmill and at maximum workload.

Diary card data:
1. Number of anginal attacks;
2. Number of nitroglycerin tablets taken.

Holter data:
Of the two ECG leads used per patient, the lead giving the maximum total duration of ischemia during the 48 hour qualifying phase will be chosen for statistical analysis. The same lead will be used for subsequent analyses. The following tertiary parameters were obtained from a 48-hour Holter monitor:
1. Number of silent ischemic attacks;
2. Number of subjective attacks;
3. Total duration of ischemic attacks over the 48-hour period (silent and subjective);
4. Median of the areas of the individual attacks, where the area is defined as the integral of ST shift over time.

Holter analysis will only include patients with documented ischemia on the baseline Holter. Documented ischemia is defined as at least 3 episodes of ST depression, $\geq$ 1 mm, lasting $\geq$ 30 seconds and separated from other events by at least one minute or at least one episode lasting 3 minutes or longer.

Results:
Patient Disposition:
A total of 319 patients were enrolled. The "all patients" analyses (N=299) included patients who had both baseline and endpoint data; the per-protocol analyses (N=258) included patients without protocol deviations who had both baseline and endpoint data. The safety analysis included all enrolled patients (N=319).
Thirty-one (9.7%) of 319 patients prematurely terminated the study. Adverse events, new illness or laboratory abnormalities led to premature terminations for 5 of 81 patients (6.2%) in the RAN 30 group, 2 of 81 patients (2.5%) in the RAN 60 group, 3 of 78 patients (3.8%) in the RAN 120 group, and 5 of 79 patients (6.3%) in placebo. Unsatisfactory response led to premature termination in 0-2 patients per treatment arm with no preponderance in any treatment group.

Baseline characteristics:
Regarding all randomized patients, as well as the patients included in the primary efficacy parameter analysis, an imbalance was seen with respect to congestive heart failure history (0 in the RAN 120 group versus 5 (or 7%) in the placebo group. Treatment by center imbalances (indicating heterogeneity by site) were seen with regard to history of rest angina, myocardial infarction, prior CABG as well as enrollment by gender. Otherwise, no imbalances were seen. The mean age (all randomized patients) was 64-66 years, and the study population was about 79% male and 86% Caucasian, with 13-19% reporting tobacco use and over 80% reporting at least 2 anginal attacks per week.

Efficacy:
Of the randomized patients, at least 74% achieved baseline and follow-up endpoints for duration of exercise, time to angina onset and time to 1 mm ST depression for peak and trough.
Key ETT parameters are shown in the table below. There were no statistically significant differences between the placebo group and the three ranolazine dose groups in any key ETT parameter. There also were no statistically significant treatment effects in the per-protocol analyses of key ETT parameters, diary and Holter data.

Table 2. RAN 1513: Efficacy results: Ranolazine vs placebo comparisons from ANOVA for the change from baseline (all patients analysis)**

Mean (SEM) change from baseline (vs. Placebo)	Ran 30 mg	Ran 60 mg	Ran 120 mg
Peak:			
Exercise duration (min)*	-0.15 (0.21)	0.13 (0.21)	-0.04 (0.17)
Time to angina onset (min)	0.08 (0.27)	0.39 (0.27)	0.15 (0.26)
Time to 1 mm ST depression (min)	0.03 (0.29)	0.36 (0.29)	0.09(0.29)
Trough:			
Exercise duration (min)	-0.23 (0.24)	-0.06 (0.24)	-0.13 (0.24)
Time to angina onset (min)	a	0.15 (0.29)	-0.20 (0.28)
Time to 1 mm ST depression (min)	0.16 (0.31)	0.43 (0.31)	0.26 (0.30)

*Primary efficacy parameter. **Mean, SEM and between treatment p-values were estimated from ANOVA models which include treatment, center and treatment by center factors. Source: sponsor.

Other parameters:
Reasons for stopping exercise: There were no significant differences between treatments in reasons for stopping exercise ("angina vs. not angina" or "all individual reasons") for either peak or trough ETT .
Hemodynamic data: There were no statistically significant changes from baseline (either peak or trough ETT) in resting heart rate, heart rate at maximum workload, SBP at rest and at maximum workload.

Pharmacokinetics:
In this study, trough plasma levels increased proportionally with dose; however, peak levels were not proportional with dose ($p < 0.05$). According to the sponsor, the spread in sample collection time for peak concentration ranged from 10 minutes to 1.5 hours, resulting in variability in concentration during the absorption phase. (?saturation of cytochrome P450 3A4 enzyme). No correlation between efficacy variables and plasma ranolazine levels were noted.

Safety: There were no deaths in this study. Most frequently reported adverse events (for ranolazine-treated patients) were headache, dizziness, and asthenia. For further discussion please see the safety review.

Reviewer comments:
1. There were no statistically significant treatment effects of ranolazine, compared to placebo, in any measured efficacy parameter, peak or trough, in this study.
2. In this trial, peak ranolazine levels were not proportional with dose.

RAN 2240.

Title: A Double-Blind, Placebo-Controlled, Parallel-Design Study of the Effect of Ranolazine SR 1000 mg bid on Utilization of Elective Revascularization Procedures in Patients with Refractory Chronic Stable Angina Pectoris Referred for Percutaneous Transluminal Coronary Angioplasty (PTCA). (volume 365).

Study period: February-October, 1994.

Primary Objective: Determine whether ranolazine SR 1000 mg bid prolonged time to revascularization (PTCA or CABG) compared to placebo in patients referred for elective PTCA to relieve refractory symptoms of chronic stable angina.

Secondary Objectives:
1. Determine whether ranolazine SR 1000 mg vs. placebo prolonged time to 1. First occurrence of revascularization or cardiovascular death; 2. First occurrence of revascularization, nonfatal MI or cardiovascular death.
2. Determine whether ranolazine SR decreased medical care utilization for which a diagnosis of angina was made.

Study Summary: Patients with angina refractory to maximal medical therapy, within 2 weeks post-coronary angiography resulting in recommendation for PTCA, were randomized to receive either ranolazine SR 1000 mg bid or placebo. Throughout the trial, background medications were kept constant. Patients were followed via clinic visits after 2 weeks, 1 month, 3 months, and every 3 months until revascularization with ECG monitoring, QOL questionnaired, laboratory tests, and assessments of medical care utilization. After revascularization, study medication was discontinued and limited follow-up continued for collection of data concerning concomitant anginal medications, medical care utilization and QOL.
Because of low enrollment at the study center, a decision was made in August, 1994 to discontinue the trial.

Patient population: Patients were at least 21 years old and had angina refractory to medical therapy; all had undergone coronary angiography within 2 weeks of randomization. Patients were excluded if they had left main or severe proximal triple-vessel disease, had a large amount of myocardium in jeopardy, or had unstable angina within 4 weeks of beginning the trial or had Class III-IV CHF.

Results: A total of 11 patients, aged 46-76, 10 males and 1 female, entered the trial. Nine patients were Caucasian and 2 were Hispanic. Seven patients received placebo and 4 received ranolazine SR 1000 mg bid. Two patients terminated double-blind because of adverse events (1 out of 2 underwent revascularization), 8 patients terminated the double-blind phase of the study because of unsatisfactory response and underwent revascularization, and one patient (on placebo) terminated the study because of sponsor termination of the study. Ten of 11 patients participated in the post-revascularization phase of the trial and terminated follow-up when the sponsor (Syntex) discontinued the trial. At the time that the trial was discontinued, 9 patients had taken double-blind medication for 4-92 days.

Safety: No deaths were reported during the trial. Thirteen adverse events were reported by 6 patients in the trial. For the 3 of 4 patients reported adverse events on ranolazine SR, the adverse events were: musculoskeletal should pain (#1002), dizziness, sweating and myocardial infarction (#1004), urinary incontinence, polyuria, headache, and nausea (#1008). Patients #1004 and 1008 withdrew prematurely due to adverse events.

Reviewer Comments: No conclusions can be drawn from this study.

Pharmacodynamic Studies:

RAN 003.

Title: A Single-Dose Tolerance Study to Investigate RS-43285 (ranolazine) in Subjects with Ischemic Heart Disease (Protocol date: June 20, 1985) (Study started September, 1985-completed February 1986).

Objective: determine safety, tolerance, invasive cardiac hemodynamic effects and pharmacokinetic features of single intravenous doses of RS 43285 over the dose range 25-200 μg/kg in patients with coronary artery disease undergoing diagnostic cardiac catheterization.

Study Summary: This was initially a single-dose, open-label, ascending dose study. After the first patient received 25 μg/kg and completed the study, the design was changed to become single-blind, placebo-controlled, ascending dose. The second patient received a dose of 25 μg/kg and two patients were dosed at each subsequent dose (50, 100, 150 and 200 μg/kg). Each dose of ranolazine was preceded by a single intravenous dose of placebo (saline); approximately 20 minutes was allowed between injections. Prior to and 10 minutes after both placebo and ranolazine administration, hemodynamic measurements were taken. Patients were to receive no cardioactive drugs except for: calcium blockers/beta blockers up to 48 hours prior to catheterization; long-acting nitrates up to 12 hours prior to study; and sublingual nitroglycerin up to 2 hours prior to procedure.
Symptoms and ECGs were monitored throughout the procedure. Blood was collected for pharmacokinetic analysis as well as routine screens.

Patient Population: Males and postmenopausal women, 21-75 years, with a clinical diagnosis of stable angina based on a positive exercise test or history of MI. The planned sample size was 10 patients, 2 at each dose level.

Notable Exclusions: congenital/valvular disease; LV dysfunction (PCWP > 18 mm or EF < 40%); Prinzmetal's or unstable angina; MI within 12 weeks; bradycardia/LBBB/high-grade AV block; SBP < 95 or DBP > 100 mm Hg; contraindications to cardiac catheterization.

Hemodynamic Measurements: Two catheters, one venous and one arterial, were used. The venous (Swan-Ganz) catheter, via femoral vein, was advanced to the pulmonary capillary wedge position and used to measure right heart and pulmonary pressures as well as cardiac output (via thermodilution). A double lumen arterial catheter, via femoral artery, was used for systemic arterial pressure as well as angiography. Hemodynamic parameters included: right atrial pressure, pulmonary artery pressures, cardiac output, heart rate, systemic arterial pressures, left ventricular pressures, left ventricular end diastolic pressure (LVEDP), pulmonary capillary wedge pressure, left ventricular dp/dt, left ventricular Vmax.

Results: Ten patients, mean age 53 years, mean weight 77 kg, entered and completed the study. The diagnosis of stable angina was based on a positive stress test in 9 patients, and a history of MI in one patient. Two patients received furosemide throughout the study.

Hemodynamics:
The data presented are limited by the fact that pre- and post-dose values exist only for placebo. For ranolazine-treated patients only post-dose values are represented. A review of the available hemodynamic data showed no appreciable effect of ranolazine (at these dose levels, compared to pre- and post-doses for saline) on mean right atrial pressure, mean pulmonary artery pressure, pulmonary artery systolic and diastolic pressures, PCWP, systemic/pulmonary vascular resistance, LV dp/dt, cardiac output, left ventricular Vmax.
There was a suggestion of increased LV EDP with ranolazine (from 6 mm Hg pre-saline to 11 mm Hg post-saline, to 16 mm Hg post-ranolazine), only seen at the 200 μg/kg dose level. Whether this represents a drug effect or (in the absence of a concurrent placebo group) some other effect is unclear.

Pharmacokinetics: Plasma profiles were obtained from only one subject at 150 and 200 μg/kg. Peak levels were seen at 5-10 minutes post-administration.
According to the sponsor, ranolazine plasma concentration declined in a biexponential manner following intravenous administration. The distribution phase half-life ranged from 1-8 minutes (mean 4 minutes) and the terminal elimination ranged from 1-6 hours (mean 2.4 hours).

The sponsor concluded that single intravenous doses of ranolazine had no effect on cardiac preload, afterload, cardiac output, and LV function including contractility.

Reviewer Comment:
1. With the data available, results did not show ranolazine effects on cardiac output, right-sided pressures, systemic/pulmonary vascular resistance, indices of LV contractility or systemic pressures. There was a suggestion of increased LVEDP at the highest dose level; however, the meaning of this single finding is unclear.
2. According to the sponsor, pharmacokinetic results are consistent with a two compartment model with elimination from the central compartment. No dose dependency was noted for either clearance or terminal half-life although there was significant intersubject variation.

RAN 003B.

Title: A Study to Investigate the Potential Anti-Anginal Efficacy of Intravenous Ranolazine (RS 43285) in Subjects with Ischemic Heart Disease
(Protocol date: February 12, 1986) (Study started: April, 1986-completed February 1987)

Objective: determine safety, tolerance, pharmacokinetic features, hemodynamic and cardiac metabolic effects of ranolazine 200 μg/kg in patients with ischemic heart disease undergoing atrial pacing.

Study Summary: This was a single-dose, single-blind study. Males with a clinical diagnosis of angina received a saline injection first, followed by administration of intravenous ranolazine 200 μg/kg. Prior to and 20 minutes after saline and 20 minutes after ranolazine dosing the patient was to undergo atrial pacing. The effect of the compound will be assessed by measurements of: coronary sinus blood flow, coronary sinus oxygen, lactate and pyruvate content, systemic arterial oxygen, lactate and pyruvate content, time to pacing-induced angina, BP/HR.
If a coronary sinus catheter could not be successfully inserted, then a pacing wire would be inserted and only BP, HR and time to pacing-induced angina would be measured.
In addition, symptoms/ECGs would be monitored, and blood for pharmacokinetic analysis/safety screened were to be obtained.

Study Population: Males, 21-75 years, undergoing cardiac catheterization or atrial pacing test, with a clinical diagnosis of angina based on a positive exercise test or history of MI. Patients must not have received cardiac drugs for one week prior to the study, except for: calcium blockers/beta blockers up to 48 hours prior to the study; long-acting nitrates up to 12 hours prior to the study; sublingual nitroglycerin up to 2 hours before the study.

Notable Exclusions: Please see Study RAN 003 (identical exclusions).

Procedures:
Atrial pacing: Atrial pacing was performed pre- and 20 minutes post-saline and 20 minutes post-active dosing. Starting at 100 beats per minute the rate was to be increased gradually by 10 beats/minute and each rate held for 3 minutes. The criteria for discontinuation was: chest pain or 1 mm ST depression below resting; upsloping ST depression was to be measured 0.08 seconds after the J point.

Coronary sinus (CS) blood flow: This parameter was measured using a continuous thermodilution technique. During the third minute at each level of pacing CS flow will be calculated 3-4 times over consecutive 10-15 second intervals. The recorded flow values on the case report form will represent the average of measurements obtained over the one minute interval. CS flow will also be measured in a similar manner before the start of pacing (baseline) and during the 5 minutes after pacing has stopped. Blood samples for myocardial oxygen uptake, pyruvate levels and % lactate extraction will be obtained from the arterial catheter and CS during the 3rd minute but 15-30 seconds after each flow measurement.

Hemodynamic data:
Blood pressure was obtained by sphygmomanometry. Duplicate recording was planned at each time point. Hemodynamic measurements were planned during the 3rd minute at each level of pacing; in addition, values would be obtained before pacing (baseline) and during the 5 minutes after pacing. Central hemodynamic measurements would be obtained by averaging the values from at least 5 consecutive heart beats.

ECG: Six-lead ECGs were obtained prior to, catheterization; at least two leads will be continuously monitored throught the study. A 6-lead ECG will be recorded within 1-4 hours after the procedure, and a 12-lead ECG will be obtained on the morning following catheterization.

Angiographic data:
Left ventriculography and coronary angiography were to be performed in the usual manner, if clinically indication, after the completion of post-dosing hemodynamic measurements. The findings were to be recorded in the case report form.

Pharmacokinetic sampling:
Venous blood samples were to be taken prior to ranolazine dosing, 2, 5, 10 and 20 minutes after dosing, and immediately and 5 minutes after completion of post-drug pacing measurements.

Other data:
The following will be analyzed if the data are available: cardiac index, stroke volume, systemic vascular resistance, left ventricular dP/dt, left ventricular Vmax, total coronary resistance (mean aortic pressure/coronary sinus flow), coronary arteriovenous (AV) oxygen content difference (coronary artery minus CS oxygen content), coronary AV lactate difference, coronary AV pyruvate difference, myocardial oxygen uptake index (coronary AV oxygen difference x CS flow).

Results:
Eleven males, mean age 57 years, entered and completed the study. One patient (#6) was excluded from some of the analyses because the point at which he experienced anginal pain was not well-defined. All patients had a clinical diagnosis of angina and documentation (via coronary angiography) of occlusion in at least one major coronary artery. Another patient (#7) completed the drug assessment (and was included in the analysis) but did not complete angiography due to multiple vessel spasm occurring with the injection of contrast. A third patient (#4) had an elevated LV EDP (38 mm) pre-saline; all other patients met eligibility criteria.
Four patients took concomitant medication (3 took nitrates) throughout the study.

Time to Pacing-Induced Angina:
The mean time to angina was 418 sec (pre-saline), 448 sec (post-saline), and 550 sec (post-ranolazine). The mean increase from baselines were: (pre/post saline) 30 sec (p=NS); and (post-ranolazine/post-saline) 102 sec (95% CI 1.03, 1.42, $p < 0.05$).

Hemodynamic results: There were no significant or meaningful changes in mean SBP, DBP, or mean BP post-ranolazine compared to pre- or post-saline. Slight increases post-ranolazine were seen. Mean resting HR was 3 bpm higher post-ranolazine compared to post-saline; HR at end of pacing was 133 bpm post-ranolazine compared to 124 bpm post-saline and 120 bpm pre-saline.

Table 1. RAN 003B: hemodynamic parameters (mean)

	Pre-saline	Post-saline	Post-ranolazine
Systolic BP (mm Hg)			
Rest	139	144	139
End of pacing	146	144	141
Heart rate (bpm)			
Rest	66	67	70
End of pacing	120	124	133
Change	54	57	63
Double Product (mm Hg.bpm)			
Rest	9276	9782	9765
End of pacing	17,668	18088	19029
Change	8392	8306	9264

Measurements of LV systolic pressures and contractility were taken only at rest. LV systolic and end-diastolic pressures were similar between pre-saline, post-saline and post-ranolazine values. LV dp/dt (minus Patient #10 post-ranolazine) showed a lower (1602 mm Hg/sec, p=NS) mean compared to pre-saline (1673 mmHg/sec) and post-saline (1645 mm Hg/sec); Vmax/sec post-ranolazine was calculated as 35.1 compared to 33.9 post-saline and 30.8 pre-saline.

Metabolic results:
There were no significant treatment effects of ranolazine on mean pH, mean hemoglobin levels, mean pCO_2 levels, mean % hemoglobin O_2 saturation, mean pO_2 levels, noradrenaline levels, mean lactate levels or mean free fatty acid levels. There was one statistically significant result, namely, the change over pacing in coronary sinus mean adrenaline (nm) levels post-ranolazine compared to post-saline, reflecting a greater increase in coronary sinus adrenaline during pacing in the post-ranolazine group compared to the post-saline group. Given the lack of consistency in other metabolic effects, the meaning of this finding (to this reviewer) is unclear.

Ranolazine plasma assay results:
The submitted data contain more than one data point for only 2 patients; therefore, the available data are limited for analysis. From the limited data available, there does not seem to be an apparent plasma relationship.

Reviewer Comments:
1. This was a small, single-blind, single dose study. The placebo control was not concurrent but always preceded ranolazine dosing.
2. There was a statistically significant increase in the time to pacing-induced angina in the post-ranolazine group; however, the contribution of a training or sequence effect cannot be excluded.
3. An increase in heart rate and double-product at the end of pacing was noted in the post-ranolazine group (p=NS).
4. An increase in adrenaline levels following pacing was seen in the ranolazine group; the meaning of this finding is unclear.
5. No ranolazine effects were seen with respect to BP, lactate, free fatty acids, O_2, CO_2.

RAN 004

Title: A Study to Investigate the Potential Anti-Anginal Efficacy of Intravenous Ranolazine (RS-43285) in Subjects with Ischemic Heart Disease
(Protocol date: November 13, 1985) (Study start date: April 1986; Study completed: April 1987)

Objective: determine safety, tolerance, pharmacokinetic features, invasive cardiac hemodynamic and metabolic effects at rest and after exercise of RS 43285 dosed at 200 μg/kg intravenously in patients with ischemic heart disease.

Study Summary: This was a randomized double-blind placebo-controlled single-dose study of 10 male patients with angina pectoris undergoing diagnostic cardiac catheterization. In this cohort, 4 subjects received placebo and 6 received ranolazaine 200 μg/kg. The following parameters were tested pre- and post-exercise prior to and 30 minutes after dosing: mean right atrial pressure (RAP), mean and phasic pulmonary artery pressure (PAP), mean pulmonary capillary wedge pressure (PCW), cardiac output (CO) by thermodilution, phasic and mean systemic arterial pressure (SAP), heart rate, left ventricular (LV) systolic pressure, LV end diastolic pressure (LVEDP), coronary sinus lactate content, time to exercise induced angina.
In addition, symptoms and EKGs were to be monitored. Blood for pharmacokinetic analysis and safety screens and urine for safety tests would be obtained.
After the pre-dose exercise schedule, hemodynamic parameters were allowed to return to baseline prior to dosing with active compound or placebo.

Inclusion criteria: Males, 21-75 years, with classic angina history, ischemic resting EKG, positive stress test, or remote ($\geq$ 3 month) history of MI. Patients must not have received cardiac drugs for one week prior to the study except for: calcium channel blockers and beta blockers up to 48 hours prior to catheterization; long acting nitrates up to 12 hours prior to the study; and sublingual nitroglycerin for up to 2 hours before the procedure.

Exclusions: congenital heart disease; significant valvular heart disease; LV dysfunction (PCW> 18 mm Hg or EF < 40%); left main coronary stenosis; Prinzmetal's/unstable angina; recent MI; bradycardia/LBBB/2nd or 3rd degree AV block; resting SBP < 95 mm Hg or supine DBP > 100 mm Hg, history of MAOI, tricyclic, reserpine or investigational drug use within one month prior to study; DM/hepatic/renal disease; any abnormal laboratory test that would preclude catheterization.

Exercise testing: Supine bicycle testing was planned pre- and 30 minutes post-dosing.

Results:
Patient Disposition: Ten males, 36-71 years old, undergoing diagnostic cardiac catheterization for clinically diagnosed angina, were enrolled. One patient developed a transient ischemic attack (presumed to be related to the catheterization procedure) and was withdrawn prior to receiving study medication. Nine patients (6 on ranolazine, 3 on placebo) completed the trial.

Efficacy:
After dosing, the mean time to exercise-induced angina increased by 5% in the ranolazine-treated group (n=5) and increased by 28% in the placebo-treated group (n=3). Overall exercise times were reduced by 6% in the ranolazine-treated group but increased after placebo.

Pharmacokinetic results: Ranolazine plasma levels fell from a mean (sd) of 675(17.2) ng/ml after 2 minutes to 197 (43.2) ng/ml after 20 minutes.

Hemodynamic measurements: A review of the central hemodynamic measurements, pre and post dosing, showed up to 4% increase in Cardiac Output (CO) with ranolazine, compared to a 10% increase with placebo; after exercise there was a 13% reduction in CO compared to a 51% increase with placebo.
Cardiac Index (at rest, after 60 watt exercise load and peak value during exercise) change after dosing by +8%, -22% and –13% with ranolazine, compared to +29%, +186% and +129% for placebo.
Heart rate, peak LV systolic pressures, and mean pulmonary artery pressure changes were similar between ranolazine and placebo.
There were slight decreases in SBP (mean decrease 12 mm Hg for ranolazine, 7 mm for placebo) in both ranolazine and placebo-treated groups.
Mean aortic lactate content before and after dosing increased by 148% (ranolazine) and 78% (placebo) at rest and fell by 18% at a work load of 60 watts (increased 11% after placebo); mean coronary sinus lactate levels increased after ranolazine dosing by over 5 fold at rest (49% increase after placebo) and fell by 9% after exercise (increased 14% after placebo).

Safety: No adverse events were reported after the time of study drug administration. Interpretation of laboratory results was confounded by the high level of missing data. For further safety discussion of ranolazine, please see the safety review.

Reviewer Comments:
1. The sponsor concluded that this study has not generated evidence for a hemodynamic or metabolic basis for ranolazine use as an antianginal agent.
2. The hemodynamic data suggest that ranolazine is associated with decreases in Cardiac Output/Index and decreases in coronary sinus/aortic lactate with exercise; however, this study is too small to generate definitive conclusions.

RAN 006A.

A Study to Investigate the Potential Anti-Anginal Efficacy of Intravenous RS 43285 in Subjects with Ischemic Heart Disease (protocol date: September 2, 1985)

Objective: establish a dose of ranolazine which demonstrates potential anti-anginal efficacy (defined as an increase in time to pacing induced angina of 10% or more).

Study Summary: This was an open-label, ascending dose study of subjects with clinically diagnosed angina who were undergoing diagnostic cardiac catheterization. Fifteen males were to be receive a single dose of intravenous ranolazine, 5 at each of the dose levels 50, 100 and 200 µg/kg. Prior to and 20 minutes after dosing the patient would undergo right atrial pacing; hemodynamic parameters, symptoms and ECGs would be monitored and blood/urine samples for pharmacokinetics/safety would be obtained.

Right atrial pacing was planned to start at 100 beats/minute, increasing by 10 beats/minute with each rate held for 3 minutes. The criteria for discontinuation was chest pain or ST depression (measured 0.08 after the J point) of 1 mV below the resting level.

Patient Population: Males, 21-75 years, with clinical diagnosis of angina (positive exercise test or history of MI), who have not received cardiac drugs for one week prior to study, except for: calcium channel blockers/beta blockers up to 48 hours prior to catheterization; long acting nitrates up to 12 hours prior to study; sublingual nitrates up to 2 hours prior to procedure.

Notable Exclusions:congenital/valvular heart disease, LV dysfunction (PCWP > 18 mm or EF < 40%), left main stenosis, Prinzmetal's/unstable angina, MI within 12 weeks, bradycardia/LBBB/greater than 1st degree AVB, SBP < 95 mm or DBP > 100 mm, DM/hepatic/renal disease.

Results:
Fourteen males, mean age 55 years, mean weight 85 kg, entered the study. Six had a history of MI, one had angina, one had intermittent claudication, one had a positive stress test and in 5 the diagnosis was not stated.
Four patients received ranolazine at 50 mcg/kg; 5 received ranolazine at 100 mcg/kg and 5 received 200 mcg/kg.
The duration of pacing in the pre-dose and post-dose assessments is shown below.

Table 1. RAN 006A: Duration of pacing prior to and after dosing

Dose	N	Mean duration (min:sec)	Range (min: sec)
Pre-Dose			
50 µg/kg	4	4:15	1:56-6:03
100 µg/kg	5	5:20	1:30-9:35
200 µg/kg	5	7:13	4:24-11:15
Post-Dose			
50 µg/kg	4	7:31	5:00-10:14
100 µg/kg	5	8:15	3:52-14:20
200 µg/kg	5	9:38	6:00-14:59

Hemodynamics:
Heart rates during pacing appear to reflect pacing rather than drug effect. Both diastolic and systolic mean BP increased during pacing except for the 200 mcg/kg dose, where mean SBP decreased by 0.8 mm Hg.

Pharmacokinetics: Mean plasma ranolazine levels are shown below. The 50 and 100 mcg/kg doses do not appear to be dose-proportional

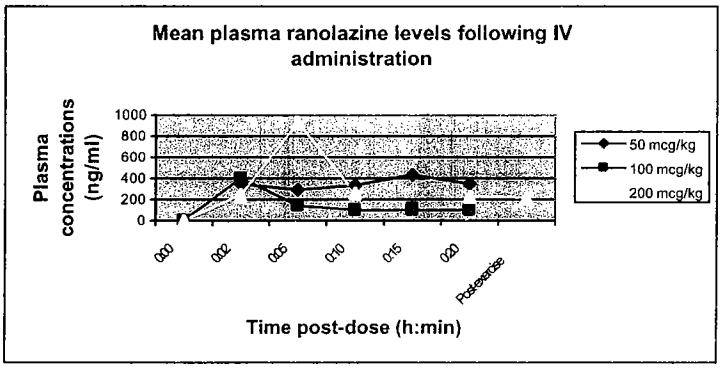


Figure 1. Mean plasma ranolazine levels following iv administration

Safety: For further safety discussion, please see the safety review.

Sponsor conclusions:
The sponsor concluded that the results are of uncertain clinical significance but might support that ranolazine has anti-anginal efficacy. The study failed to identify an appropriate dose for further examination. However, the doses utilized here were not associated with any significant adverse events or disturbances in laboratory parameters. There appeared to be no significant changes in ECG recording during the study period.

Reviewer Comments:
Without a concurrent placebo control it is difficult to interpret the post-dose increase in duration of pacing.

RAN 007.

Title: A Study to Investigate the Potential Anti-Anginal Efficacy of RS 43285 in Subjects with Ischemic Heart Disease (protocol date: December 23, 1985)
Note: Study Terminated Prematurely—see below

Objective: Establish a dose of RS 43285 which demonstrated potential anti-anginal efficacy. In addition, assess hemodynamic, pharmacokinetic, tolerance and safety features of the administered doses.

Study Summary: This was a double-blind placebo-controlled crossover trial of males with stable angina. Eligible patients with a stable baseline treadmill exercise test will receive, on 4 consecutive days, single doses of placebo and 10, 20 and 30 mg RS 43285 administered according to a double-blind crossover schedule. Prior to and ninety minutes after dosing a treadmill ETT will be performed. In addition, hemodynamic monitoring, ECGs, and blood for pharmacokinetic analysis/safety would be obtained.

Patient Population: Males, 21-75 years, with clinical diagnosis of stable angina and positive exercise test (limited by angina) or history of MI. Patients must be off cardiac drugs except for: calcium/beta blockers up to to 48 hours prior to any study day; long-acting nitrates up to 12 hours prior to any study day; and sublingual nitroglycerin up to 2 hours prior to dosing on any study day. Patients must be pretrained 2 days prior to the study, with 2 consecutive treadmill exercise tests showing exercise tolerance differences of less than 15%.

Notable Exclusions:congenital/valvular heart disease, LV dysfunction (PCWP > 18 mm or EF < 40%), left main stenosis, Prinzmetal's/unstable angina, MI within 12 weeks, bradycardia/LBBB/greater than 1st degree AVB, SBP < 95 mm or DBP > 100 mm, DM/hepatic/renal disease.

Exercise testing: A multistage exercise test according to the standard Bruce protocol was planned prior to and 90 minutes after dosing. Testing was to stop for: symptoms of angina, reduction in SBP $\geq$ 10 mm Hg, ECG effects/arrhythmia/high grade heart block.

ECGs: Twelve-lead ECGs were recorded prior to and within 1-4 hours after dosing on each day of the study.

Protocol Amendment: (November 4, 1986): Because 5 patients experienced minimal effects at 10 and 20 mg ranolazine, the doses were increased to 20, 40 and 60 mg.

Results:

A total of 12 patients, mean age 58 years, mean weight 85 kg, were enrolled. After 5 patients received the original doses, an additional 7 patients received doses of 20, 40 and 50 mg ranolazine and placebo in random order.
The study was apparently terminated prematurely because of slow progress or the lack of clear hemodynamic or antianginal effects. No efficacy data were provided and no details were given for the incompleteness of data.

Plasma Levels: Mean (sd) ranolazine concentrations after 90 minutes were: 28.1 (13.7), 60.5 (35.1) 88.1 (48.3), 179 (57.7) and 239 (41.1) ng/ml for 10, 20, 30, 40 and 60 mg, respectively.

Reviewer Comment: No efficacy data have been presented in this study. According to the study report, no evidence is available to support the pharmacologic activity of ranolazine in the 10-60 mg range.

RAN 010.

Title: A Pilot Dose-Finding Study of Oral RS 43285 (ranolazine) in Stable Angina Pectoris (Protocol date: September, 1986)

Objective: Determine efficacy and tolerability of 3 dose levels of RS 43285 compared with placebo.

Study Summary: This was a double-blind, randomized, placebo-controlled study. After a 7 day withdrawal period from cardiac medications, patients entered a 1 week placebo run-in period followed by randomization to placebo or 10, 30 and 50 mg RS 43285 tid. On the 7th day of each week the patient underwent a side effect questionnaire as well as treadmill exercise test. Patients who fail to produce at least 1 mm ST deviation within 15 minutes (or 240 watts) on treadmill exercise, or fail the compliance check on Day 7 of the placebo period, will be discharged, not included in the analysis, and replaced.

No concomitant medications were permitted in placebo or active treatment periods. Short-acting nitrates was allowed only for treatment of angina attacks.

A daily record of angina attacks and nitrate consumption was planned.

Patient Population: Males or females, 21-70 years old, with at least 3 month history of stable angina, > 50% stenosis in one or more major coronary arteries, normal LV function (EF > 50%) and sinus rhythm.

Notable Exclusions: MI within 3 months; CHF: hypertension (DBP > 95 mm Hg); cardiac arrhythmia; left main disease; pregnant/lactating women; significant laboratory abnormality.

Exercise Testing: The exercise test, done 60 minutes after the morning dose, followed the Bruce protocol to the maximum work tolerance. Reasons for stopping the test included: 1. More than 15 minutes; 2. Angina; 3. Dyspnea/fatigue without chest pain; 4. Other; 5. Arrhythmia or other contraindication to continuing.
Other measured parameters for 15 minutes after testing included: time to 1 mm ST change; time to 2 mm ST change; maximum ST change; time from end of exercise to ST segment returning to isoelectricity; summed ST change (ST deviation to nearest 0.5 mm from start of test to 15 minutes after end of exercise test).

Side Effect Questionnaire: This was completed in English by the physician and involved frequency, severity and relationship of side effect to study drug.

Results:
Twenty-five patients were enrolled in the study; one patient, a protocol violator (age 78), was still included in the analysis. The trough exercise test was not performed by a proportion of patients; in addition, some

safety laboratory tests were lost. Of the twenty-five patients, 6 patients were on no pre-study anginal therapy, had no anginal attack during placebo run-in, and therefore did not stop the exercise test due to angina.

Baseline characteristics: The study population was mostly male, mean age 55-61 years, mean weight 67-79 kg, 20-50% smokers.

Anginal attacks/NTG consumption: On placebo, anginal attacks fell from a mean 3.8/week during run-in to 2.3/week during the second week. Mean anginal attacks and NTG consumption increased, compared to placebo, in the 10 mg TID ranolazine group and decreased, compared to placebo, in the 50 mg tid group; missing data were noted in the ranolazine 30 and 50 mg tid groups.

Exercise times:
Mean exercise times at both peak and trough (Day 14) were higher in the placebo group (10.64 and 9.95 sec, respectively) than for ranolazine 50 mg tid (9.34 and 8.54 sec, respectively). The change from baseline for the peak study was also higher for placebo than ranolazine 50 mg tid. The other measured parameters (time to 1 mm ST deviation, time to 2 mm ST deviation, maximum ST depression, recovery time) did not show a consistent pattern.

Pharmacokinetic data:
Serum samples for ranolazine were apparently mishandled and therefore not available for analysis.

Reviewer Comments: No evidence of antianginal efficacy was seen in this study.

RAN 011.

Title: A Study of RS-43285 (Ranolazine) on Myocardial Metabolism. (Protocol date: June, 1987)

Objective: Study the metabolic changes induced by I.V. RS 43285 (ranolazine) in the human myocardium

Study Summary: This was an open-label, nonrandomized study comparing pre-treatment and post-ranolazine data without the use of a concurrent placebo control. Ten male patients with angina and at least 50% LAD stenosis will be selected; another 10 males with chest pain, and normal coronary arteries, exercise tests, hyperventilation responses and echocardiography will also be studied. A preliminary 12-lead ECG bicycle test (starting at 50 watts, increasing by 50 watts every 3 minutes) must show at least 1 mm ST depression in the patients with coronary artery disease.

All cardio-active medication, except for short-acting nitrates and diuretics, were to be withdrawn at least 7 days prior to study. On the day prior to study, patients will be admitted, receive screening laboratory tests, and undergo post-midnight fasting. Patients with normal screening laboratory tests will be sent to the catherization laboratory to undergo insertions of: femoral artery (BP/arterial samples) catheter, coronary sinus catheter (coronary sinus blood flow (CSBF) and sampling), pulmonary artery catheter via femoral vein (PAS, PAD, PAP), and venous cannula for drug administration. In addition, a single lead ECG would record heart rate and ST changes (ST segment deviation will be measured at 30 second intervals).

Two test sequences would then follow, with about 45 minutes between each sequence. The first would be without drug, and the second sequence would occur after a 2 minute bolus of RS 43285 (140 μg/kg) followed by a steady state infusion of 1.2 μg/kg/min RS 43285 by syringe pump. Ranolazine will be started 7 minutes before the test sequence and will continue throughout the test sequence.

Each test sequence would consist of a base phase (5 minutes at sinus rate) followed by a pacing phase (150 bpm), followed by a recovery phase (10 minutes at sinus rate). Each test sequence will be preceded by determination of HR and BP, and the sequence not started until 3 consecutive readings do not differ by more than 10%.

In the basal phase, and in the first and second minutes following onset of angina in the pacing phase, and in recovery, CSBF, CS and arterial samples will be obtained and HR, arterial BP and pulmonary artery pressures will be measured. If the CAD patient does not experience angina with ST changes in the first (control) test sequence they must be discharged from the study. Time to onset of angina in the first

sequence must be noted and the patient paced to the same time in the second (drug) sequence. Pacing should stop once angina is established and should not last longer than 10 minutes.

Patients with normal coronary arteries will be paced for a maximum of 10 minutes if no angina occurs.

Arterial and CS samples will be analyzed for: oxygen (for MV02), lactate, citrate, alanine, glutamic acid, free fatty acids, glucose, xanthine and hypoxanthine.

Patients will remain supine from admission until completion of study. Each pacing phase will be preceded by 0.5 mg atropine IV. Prior to pacing and after recovery a plasma sample for RS 43285 will be drawn.

Patient Population: 1. Males with CAD, effort angina and at least one 50% stenosis on coronary angiogram in 1 or 2 vessels, one of which must be the LAD.; 2. Males with atypical chest pain with normal exercise tests, echocardiography, angiograms, normal hypoventilation responses and metabolic profiles.

Notable Exclusions: left main disease, contraindications to exercise testing, clinical significant ECG/laboratory abnormality, contraindications to the procedure, single RCA disease.

Analysis Plan: According to the protocol, a 5% level of significance will be used in the study analysis; the statistical methods will be decided after preliminary review of the data. No primary efficacy variable was prespecified in the protocol.

Protocol Amendments: There were 4 protocol amendments that: specified basal phase hemodynamic/metabolic measurements at 2 time points (2 and 3 minutes) and measured hypoxanthine/xanthine at one time point during basal, pacing and recovery periods; doubled ranolazine loading dose and infusion; changed ranolazine dosing and regimen (200 μg/kg iv bolus + 20 μg/kg/min) so that loading and infusion doses would occur simultaneously (goal was plasma concentrations of 500 ng/ml occurring 20 minutes after the start of dosing).

Results:

Disposition and Baseline Characteristics:

Seventeen males (9 with CAD and 8 with normal coronaries) were enrolled and completed the study. Mean age in the CAD group was 55 years compared with 45 years in the group with normal coronaries. Mean weight in the group with CAD was 78 kg; mean weight in the normal group was 85 kg. Eight patients in the CAD group were Caucasian and one was Asian. In the normal group, half were Caucasian and half were Asian. There were no differences between the two groups in mean atrial/ventricular rate, PR, QRS and QT intervals.

The mean total (mg) IV ranolazine dose received was 32.7 mg in the CAD group and 31.7 mg in the normal group.

Metabolic Results: The following calculations were used:

MV(myocardial uptake or release) = (arterial minus coronary sinus) x CSBF

$$\text{Extraction} = \frac{\text{arterial minus coronary sinus}}{\text{arterial}} \times 100\%$$

ANOVA, including group (CAD or normal), phase (control or treatment) and stage (ie basal, pacing 1 and 2, recovery 1-4),, as factors and interaction terms, was used to compare control and treatment phases. Due to the large amount of missing xanthine and hypoxanthine data, calculated values were not analyzed.

Myocardial oxygen uptake during control and treatment phases is graphically depicted (Figure 1). It should be noted that results of the two groups, during the control (pre-drug) phase, are not superimposable. During the treatment phase, values consistently increased in the normal group and decreased in the CAD group. According to the sponsor, a statistically significant group by treatment interaction (p=0.043) was seen for MV oxygen.

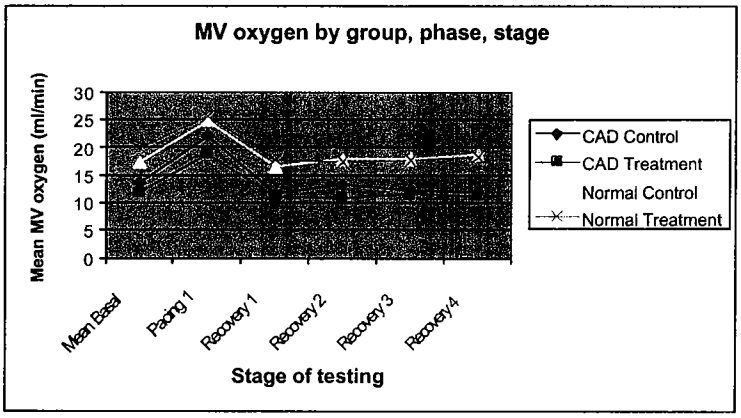
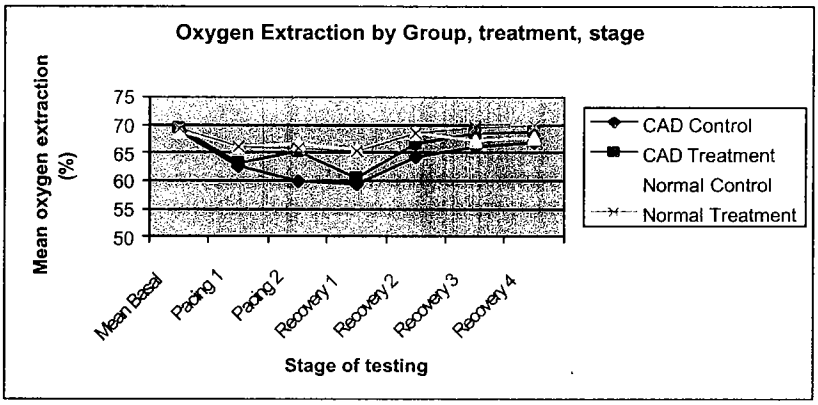


Figure 1. Myocardial oxygen uptake by group (CAD or normal), phase, sequence (control or drug).

Oxygen extraction is also depicted graphically (Figure 2). According to the sponsor, a statistically significant difference was seen between control and treatment (p=0.02).

Figure 2. Oxygen extraction by group, treatment, phase.



Other statistically significant differences were noted for glutamic acid extraction ($p < 0.001$) and free fatty acid uptake (MV FFA) (p=0.01). There was no statistically significant difference seen for MV citrate; for MV lactate, MV glucose and glucose extraction, values were lower with treatment with differences yielding p=0.06 to 0.07 range. Myocardial lactate production (negative arterio-coronary sinus difference in lactate concentration) was seen in 3 patients only during control pacing.

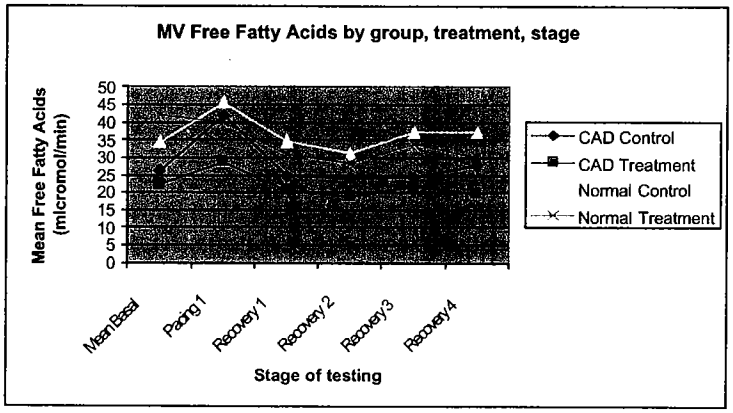


Figure 3. FFA Uptake (µmol/min).

Reviewer: The mean basal levels are not superimposable; there is about a 2-fold difference between normal treatment and CAD treatment. Except for the normal treatment group, MV FFA increased with pacing and decreased toward baseline during recovery 1 and 2. In both CAD and normal groups there is a decrease in MV FFA with treatment (see above, according to the sponsor this difference was statistically significant). According to the sponsor, the phase x stage interaction was not statistically significant.

Pacing Results: There were no statistically significant differences between control and treatment values for time to onset of angina, time to 1 mm ST depression, time to maximum ST depression and duration of pacing. Maximum ST depression was statistically significant between control (median =-2 mm, range –4 to –1 mm) and treatment (median =-1 mm, range –2 to –0.5 mm) phases (p=0.02).

Reviewer: Given the lack of other statistically significant differences (e.g., time to angina, duration of pacing) with pacing, the meaning of one positive finding, maximum ST depression, is unclear.

Hemodynamic Results:
Examination of heart rates and DBP did not reveal any meaningful differences between control and treatment groups.

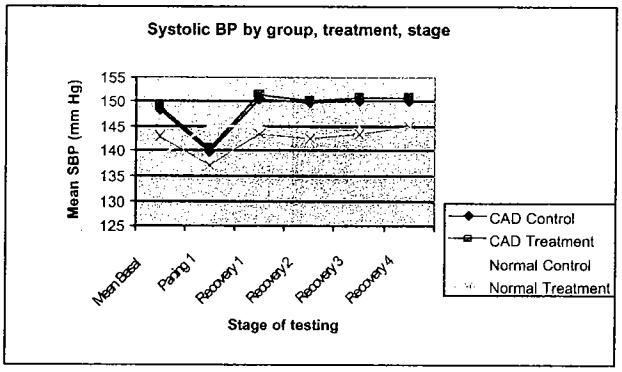


Figure 4. SBP

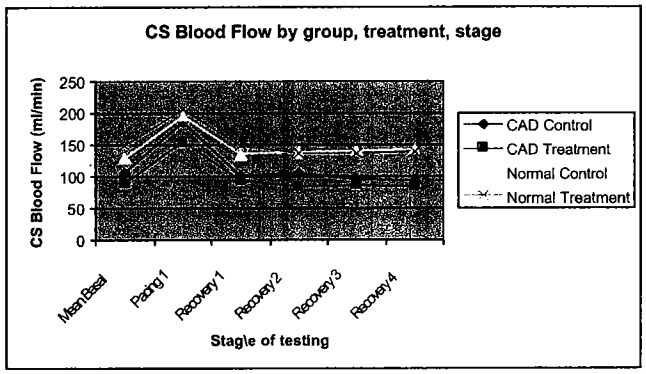


Figure 5. CS Blood Flow

Results of CS Blood flow and SBP are shown. Differences between "normals" and patients with CAD seem more apparent compared with differences between control and treatment.

Ranolazine plasma levels:

Table 1. RAN 011: Ranolazine plasma levels

Mean (SD)	140 mcg/kg iv bolus + 1.2 mcg/kg/min iv infusion	200 mcg/kg iv bolus + 2.0 mcg/kg/min iv infusion
Baseline	<10	<10
12 minutes	312 (112)	498 (374)
18 minutes	214 (63.9)	581 (243)

Safety: For safety discussion, please see the Safety Review

Reviewer Comments:

1. This was a small, open-label, nonrandomized study of males with either atypical chest pain and normal coronary arteries or effort angina and CAD. There was no placebo control group. No primary efficacy parameter or statistical method of analysis was prespecified. Method of analysis, according to the protocol, was decided after preliminary review of the data.
2. There appear to be differences between normal and CAD patients in myocardial oxygen uptake, coronary sinus blood flow and SBP. Differences between normal and CAD patients may confound several results in this study.
3. In this study without a concurrent placebo group, a reduced FFA uptake is noted with ranolazine treatment compared to baseline. Given the ranolazine plasma levels achieved, along with the lack of several anti-anginal treatment effects (ie, time to angina, pacing duration, time to 1 mm ST depressions), it is unclear whether what role this effect (reduction in FFA uptake) plays in anti-anginal treatment benefits of ranolazine.

RAN 012.

Title: A Single-Blind Study of Ranolazine (RS 43285) versus Placebo in Patients with Angina Pectoris (Protocol date: November 6, 1986) (Study dates: November 1986-May 1987)

Objective: evaluate, using anginal attack frequency, nitrate consumption and exercise tolerance, the 2 week efficacy of ranolazine 30 mg and 60 mg tid.

Study Summary: This was a single-blind single-site study of the safety and efficacy of ranolazine 30 mg po tid and then, if tolerated, 60 mg po tid; both doses would be administered for two weeks each. A one week washout period and 2 week placebo period preceded the active treatment phases. There was no washout period in between the two active treatment periods.

Efficacy of each treatment would be evaluated by comparing each patient's ETT performance during washout, at the end of placebo and each active treatment phase. Efficacy was also measured by assessment of daily patient recordings of anginal frequency and nitroglycerin consumption.
Safety evaluations consisted of AE monitoring and laboratory testing. In the event of an increase in frequency or severity of anginal symptoms during placebo run-in or active treatment periods, patients may be advanced into the next treatment period as an alternative to withdrawal. If the patient had continued medication to that point, the ETT must be done first.

Concomitant medication: Use of sublingual nitroglycerin was allowed only as treatment for anginal attacks.

Sample size: 15 enrolled or 12 completers.

Inclusion criteria:
1. Males and nonpregnant females, 21-75 years old, with at least 3 month history of stable effort angina relieved by rest/nitroglycerin;
2. The difference in exercise time (on ETT) between the first 2 exercise tests, prior to active treatment, must be less than 20% of the longer time; in addition, evidence of ischemic (i.e. ST depression $\geq$1mm at 80 msec after J point) must be present in a standard ECG lead;
3. Maximal ETT at the end of placebo phase must be 3-10 minutes;
4. If the patient has had a coronary angiogram, 50% or greater occlusion in a major coronary artery (or one of its primary branches) must be present;
5. Patients currently under treatment for angina will be admitted only if their response to such treatment is inadequate or complicated by unwanted effects;[25]

Notable Exclusions:
1. Presence of factors associated with false positive stress test;
2. Uncompensated CHF;
3. Valvular heart disease/septal defects;
4. Unstable angina within the last 4 weeks;
5. Second or third degree AVB or any uncontrolled arrhythmia other than sinus arrhythmia/occasional extrasystoles;
6. MI within the past 3 months;
7. Acute myocarditis/cardiomyopathy/acute pericarditis;
8. SBP < 95 mm Hg or sitting BP > 165/110 mm Hg;
9. Abnormal renal/hepatic tests/potassium/anemia/IDDM.
10. Inability to discontinue long-acting nitrates, beta blockers, antihypertensive medication, calcium channel blockers or any investigational drug. Digitalis was not permitted in this study. Diuretics were allowed if continuous throughout the study.

Exercise Tolerance Tests:
Time to angina, time to 1 mm ST depression, time to 2 mm ST depression and time to maximal exercise capacity were recorded for all ETT. All exercise tests were performed on a motor-driven treadmill under uniform conditions, at the same time of day at each visit. The exercise protocol will be determined by the principal investigator and would be the same for all patients in the study.
Each patient would undergo an ETT at the end of washout, 90 minutes post-dose on the first day of ranolazine 30 mg tid (phase 2), and the end of placebo, ranolazine 30 and 60 mg tid treatment periods (phases 1, 2, and 3) and 7 hours post-dose at the end of each phase.
Patients were to refrain from smoking or sublingual nitroglycerin use on the morning of the clinic visit or within 2 hours prior to ETT.

[25] It is not stated whether or not patients needed to be on maximal therapy.

Criteria for stopping ETT: signs of vasoconstriction (pale, clammy skin), atrial fibrillation/tachycardia, dyspnea, electrical alternans, SBP $\geq$ 230 mm Hg, fatigue, faintness, musculoskeletal pain/discomfort, progressive angina/ BP drop/ST changes/QRS widening/increase in PVCs/ventricular tachycardia.

Angina frequency/nitroglycerin consumption: These events were recorded by patients on weekly diary cards.

Results:
Patient Disposition: Sixteen patients were recruited; 15 patients received at least one dose of active treatment and 12 completed all phases of the study. One patient withdrew from active treatment due to chest pain requiring hospitalization. Another patient did not meet entry criteria. Two patients were withdrawn from placebo (because of ineligibility and noncompliance, respectively).

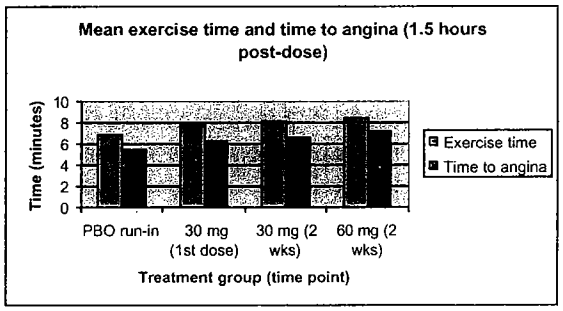
Baseline characteristics:
Twelve males and four females, mean age 59 (range 39-74) years, 100% Caucasian, were enrolled.

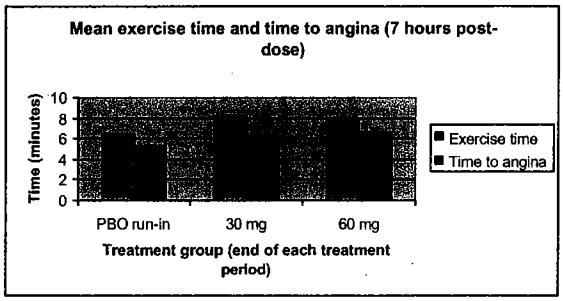
Ranolazine plasma levels:
According to the sponsor, a freezer malfunction damaged a number of stored samples (for example, for patients 2 and 3, only 2 plasma samples were available). The mean plasma level 1.5 hours following the first dose of ranolazine 30 mg was 113 ng/ml. On Day 28, the mean ranolazine concentration was 167 ng/ml (1.5 hours after the last dose of ranolazine 30 mg tid) and 29.2 ng/ml (7 hours after the same dose). On Day 42, the mean ranolazine concentration was 354 ng/ml (1.5 hours after the last dose of ranolazine 60 mg tid) and 73.3 ng/ml (7 hours after the same dose).

Drug Accountability:
According to the sponsor, it was discovered, during one of the monitoring visits, that written records were not being kept of drug dispensing and accounting for treatments issued to patients. Drug accountability records were then performed retrospectively from the returned drugs and tablet count data. No data were provided on the assessment of patient compliance.

Efficacy:
Anginal attacks/nitroglycerin consumption: During placebo, mean number of anginal attacks and nitroglycerin (ntg) consumption were 12 ± 4 and 11 ± 4, respectively. During the ranolazine 30 mg phase, mean number of anginal attacks and ntg consumption were 10 ± 4 and 10 ± 4, respectively; during the ranolazine 60 mg phase, mean number of anginal attacks and ntg consumption were 6 ± 2 and 3 ± 1, respectively. These differences were not statistically significant.

ETT:
Exercise time and time to angina are depicted graphically for the 1.5 and 7 hour post-dosing timepoints. It should be noted that the time to angina was similar between peak and trough times for placebo and the ranolazine 30 mg tid groups. Significant p-values were calculated for exercise time and time to angina (each active treatment vs. placebo) but not for 30 mg vs. 60 mg dosing.





Figures 1 and 2. Mean exercise time and time to angina at peak and trough

The sponsor has noted that methodology for ST depression measurement was not always uniform (ie, the first ETT for patient #13 was recorded manually; recordings for patient #15 were performed on the "Mark 12" equipment). On the [redacted] equipment, " peak" was defined at end of exercise (not necessarily the same time as maximum ST depression). In addition, the initial exercise tests did not always agree with the ± 20% entry criterion as defined in the protocol.

Hemodynamic measurements: Heart rate at rest and peak exercise, resting and peak SBP and rate pressure product (peak exercise) showed no appreciable changes at peak and trough times in this study.

Safety: For safety discussion, please see the safety review.

Reviewer Comments:

1. This was a small, single-blind, ascending dose pilot study. There was no concurrent placebo control.
2. Improvements in exercise time and time to angina may have been related to sequence effects rather than a true treatment effect.
3. The dose-concentration relationship appears to be linear in this dose range.
4. In this dose range and treatment period, there were no meaningful ranolazine effects on resting and peak heart rate and SBP as well as peak rate pressure product.

RAN 014.

A Study of the Effects of RS-43285 on Coronary Blood Flow, Myocardial Metabolism and Left Ventricular Function in Patients with Angina Pectoris (Protocol date: April, 1987)

Objectives (listed as aims): 1. Determine if RS 43285 improves myocardial biochemistry in patients with angina pectoris under resting conditions and during transient high demand ischemia; 2. Determine dose/response relationship of these effects in patients; 3. Determine effects of the compound on global coronary hemodynamics (coronary vascular resistance, coronary sinus flow) and left ventricular function.

Study Summary: This was an open-label, non-randomized, ascending dose study without a placebo control. Patients with angina underwent hemodynamic and metabolic measurements first in the basal state in sinus rhythm and then after increasing the heart rate up to an average of 115 bpm by atrial pacing. These measurements, at basal state and with pacing, were then repeated 20 minutes after intravenous ranolazine administration. The dose of ranolazine was 50 μg/kg in the first 5 patients, 100 μg/kg in the next 5 patients, and 150 μg/kg in the last 5 patients. In addition, C-14 lactate was infused continuously during the study at a rate of 12 μCi/hour after a primimg bolus of 10 μCi/hour, in order to determine the net transcardiac lactate production.

In order to account for "spontaneous variation," ten patients were to be restudied after 20 minutes in the absence of drug treatment.

Patients were instrumented as follows: left heart catheterization (Judkins) with LV pressure recording, arterial pressure, right heart catheterization (thermodilution) with pacing electrodes in the coronary sinus, arterial and coronary venous blood samples. Hemodynamic and ECG were digitized and processed off-line to derive heart rate, LV systolic and end-diastolic pressure, dP/dt max and min (dp/dt/DP40)[26], T1, T,[27] ejection time, systolic/diastolic/mean arterial pressures, coronary sinus blood flow (CSBF), coronary vascular resistance[28]. In addition, blood samples were analyzed for lactate-glucose plasma levels, alanine, glutamine, glutamic acid, free fatty acids and C-14 lactate. A venous blood sample for plasma ranolazine was taken after each drug phase.

Patient Population: Males and females, 35-75 years old, with angina pectoris and angiographic evidence of CAD.

Notable Exclusions: LV dysfunction, abnormal impulse generation/conduction, MI within 3 months of study, pregnant/lactating females, significant laboratory abnormality.

Analysis: No primary efficacy parameter was prespecified in the protocol. A 5% level of statistical significance was prespecified. The statistical method used will be decided after preliminary review of the data.

Concomitant medication: All cardioactive drugs except short-actingd nitrates were to be stopped at least 2 days before study and no premedication will be used.

Protocol Amendment: One protocol amendment (June, 1988) replaced exclusion criterion of "LV dysfunction" with "evidence of congestive heart failure," and added sampling for pyruvate and safety laboratory tests.

Results: Fifteen patients (14 males), 38-68 years (median 53 years) were enrolled and completed the study; no side effects were reported. Two patients had a history of MI within 3 months of study entry but were included in the analysis; another patient received isosorbide dinitrate on demand and did not stop other cardiovascular medication until the day prior to study entry.

Coronary hemodynamics, LV function, Systemic arterial pressure: Mean coronary sinus blood flow increased and coronary vascular resistance decreased with pacing in all groups; there were no statistically significant differences between treatment vs. control in either basal or pacing state. In addition, heart rate, diastolic pressure, mean arterial pressure increased and LV systolic pressure, arterial systolic pressure, decreased in all groups with no statistically significant difference between treatment vs. control in either basal or pacing state.
Mean LVEDP decreased with pacing in all groups; there was a statistically significant difference ($p=0.04$) between treatment vs. control in the basal state only (ie, the basal values in the treatment sequence were lower compared to basal values in the control group).
There were no statistically significant differences between treatment vs. control in dP/dT indices or in the indices of relaxation.
A statistically significant decrease was seen (treatment vs. control) in the basal state ($p=0.03$) with regard to mean transcardiac oxygen extraction (arterial minus coronary sinus oxygen); however, no statistically significant difference was seen (during basal or pacing states) with regard to mean myocardial oxygen consumption.

Reviewer: Other than a lower basal mean LVEDP, no statistically significant consistent changes were seen with regard to hemodynamics.

---

[26] As indices of inotropic state, maximum of the first derivative of LV pressure (dP/dt Max) and dP/dt measured at a developed pressure of 40 mm Hg and normalized for this pressure [(dP/dT)/DP40] were used.
[27] Time-constants of the early (0-40 ms) and late (0-80 ms) exponential of LV pressure decrease during relaxation and dP/dt Min were used as indices of relaxation.
[28] Coronary vascular resistance was defined in the study report as the ratio of mean aortic pressure/mean coronary blood flow.

Metabolic data: Median lactate uptake and percent lactate extraction showed no significant difference difference between treatment and control.
The median C-14 lactate uptake (μmol/min) showed a difference (treatment minus control) of –4.6 at the basal state ($p < 0.001$); no significant difference was seen with pacing.
The percentage C14 lactate extraction showed no statistically significant differences between treatment vs. control.
Median lactate production (μmol/min) also was significantly lower (treatment minus control) at the basal state (difference of 5.3, $p < 0.01$); no significant difference was seen with regard to pacing.
Median myocardial glutamic acid uptake (μmol/min) showed a statistically significant increase with pacing (but not at basal state); the median difference (treatment pacing minus control pacing) was 1.27 ($p=0.04$).
Myocardial metabolism of alanine, glutamine, free fatty acids or pyruvate showed no significant changes in the basal state or during pacing.

Plasma ranolazine levels:
Mean levels at 20 minutes post-dosing ranged from 51 $ng.ml^{-1}$ in the ranolazine 50 μg/kg group to 217 $ng.ml^{-1}$ in the ranolazine 150 μg/kg group.

Reviewer comments:
1. This was a small, open-label, nonrandomized study without a placebo control in a population with angina and CAD.
2. The only statistically significant finding during pacing was an increase in median myocardial glutamic acid uptake; the significance of this isolated finding, in the absence of other positive findings for pyruvate, free fatty acids, etc. is unclear.
3. Statistically significant differences at the basal state were seen (ranolazine vs. control) with regard to: mean LVEDP, median C-14 lactate uptake, median lactate production; the significance of these findings is unclear.

RAN 017.

A Single-Dose Placebo-Controlled Study of Ranolazine (RS-43285, 120 and 240 mg) on Ischemic Burden

Primary Objective: compare effect of two single doses of ranolazine (120 and 240 mg) with placebo on ST displacement profile during bicycle ergometry and during recovery.

Study Summary: This was a double-blind randomized placebo-controlled crossover study in patients with stable exertional angina receiving no anginal medication other than short-acting nitrates (which were prohibited for 2 hours prior to exercise testing). Each patient attended on two test days, at an interval of 3 + 1 days, where they performed bicycle ergonometric exercise testing at baseline (0), and 2 and 6 hours following a single dose of ranolazine or placebo. Plasma samples were obtained at 2 and 6 hours post-dose. Patients were excluded if there was not at least 0.1 mV ST depression in one of the standard ECG leads during the baseline exercise test.
The original protocol (March 17, 1987) specified only the 120 mg dose; this protocol was amended (February 16, 1988) after an "informal interim analysis" to allow 240 mg to be studies in a further series of patients. A minimum of 10 patients was required to be studied at each dose level.

Study Population: Males, 21-75 years, with stable, classic exertional angina, ischemic heart disease (confirmed by at least 75% stenosis in one coronary artery, history of MI, or history of angina with ST depression on exercise), normotensive, sinus rhythm with no evidence LV impairment, and at least 0.1 mV ST depression in one lead during the baseline test on day 1.
In an amendment (November 17, 1987) postmenopausal or surgically sterilized females, up to 75 years old, were allowed to enroll.

Notable exclusions: Patients on antianginals which could not be withdrawn prior to study; presence of clinically significant disease requiring continuing medical therapy or supervision; significant laboratory abnormality.

Sample size: 12 per dose level. Evaluable patients were required to complete the full test day protocol (6 hours) on both dose occasions.

Exercise testing: Symptom-limited bicycle testing was performed, starting at 25 watts, increasing by 25 watts every 2 minutes. ECG recordings were taken in the last 10 seconds of every minute; BP was measured every 2 minutes. After cessation of exercise, BP and ECG were monitored each minute for up to 10 minutes. The permitted difference in duration between the two baseline tests (test day 1 and 2) was $\pm$ 20%.

Analysis: Two dose groups were entered in this study, the second dose group beginning after completion of the first. A double-blind two-period crossover study was carried out within each dose group. No randomization was carried out between doses and the random code supplied for the 120 mg group was repeated for the 240 mg dose.

The primary variable of interest was ST depression during exercise and recovery. This was calculated as the summed ST depression during exercise, during recovery and for the total period. Summed ST depression at 2 and 6 hours was analyzed separately, and the difference from the pre-dose exercise test was analyzed as well.
An analysis of variance was used, with treatment sequence, between-patient error, treatment effect and the treatment by sequence interaction (period effect) included in the model.

Results:

Patient Disposition: Ten patients were entered in the 120 mg group and 12 in the 240 mg group. However, 3 of the patients in the 240 mg group had already entered and completed the study in the 120 mg group and were excluded from the efficacy analysis for the 240 mg group. Therefore, 9 patients were included in the efficacy analysis for 240 mg. One patient in the 120 mg group received ranolazine and placebo in the wrong order (as shown by plasma levels) and was therefore included in the placebo/ranolazine, rather than ranolazine/placebo group.

Study days were 2-5 days apart.

Baseline characteristics: Sixteen males and 3 females with median age 63-66 years (range 49-74 yrs). Median number of angina attacks were 3.5-4 per week. A majority (12 patients) had NYHA Class III symptoms.

Efficacy:
The comparison of summed ST depression during exercise showed no significant benefit of ranolazine over placebo. This was true for both doses and at both time points.

During recovery, no significant effect of ranolazine compared to placebo was seen at 2 hours. A significant improvement in the rate of ST segment return to baseline was seen in the 120 mg group ($p=0.04$) but not confirmed in the 240 mg dose group.

Plasma ranolazine levels ranged from mean (sd) 310 (78) ng/ml (2 hours after 120 mg) and 742 (240) ng/ml (2 hours after 240 mg) to 104 (68) ng/ml (6 hours after 120 mg) and 44 (84) ng/ml (6 hours after 240 mg) and did not correlate with ST changes.

Reviewer comments: Ranolazine did not affect ST depression in this study.

The majority of early withdrawals in CVT 3033 occurred within the first 6 weeks after randomization. There were no withdrawals during the rebound assessment phase.

In Study 3031, A total of 191 patients were randomized into 4 treatment sequences (ABCD, BDAC, CADB and CDBA where A=500 mg bid, B=1000 mg bid, C=1500 mg bid and D=placebo). There were 45-50 patients randomized to each treatment sequence; the numbers of patients receiving each treatment (ie, placebo, ranolazine SR 500 mg bid, 1000 mg bid or 1500 mg bid) were 179-187. A total of 175 patients (92%) were included in the near/all completer population, 185 patients (97%) in the ITT population, 184 (96%) in the first period population, 135 (71%) in the per-protocol population, and 191 (100%) in the safety population. Fifteen (8%) patients discontinued prematurely due to AE (11 of these were in the highest dose ranolazine group).

Baseline Characteristics:
In Study 3033 (ITT population), the mean age was 64 years, with half of the patients 65 years and older. About 75-80% were male, and 96-99% Caucasian. Mean vital signs and exercise test durations were similar across treatment groups (with lower heart rates in the group taking concomitant beta blocker). The treatment groups were also balanced with respect to stratified background medication, other concomitant medication, baseline weekly angina frequency, and weekly nitroglycerin consumption. Fewer patients on placebo had a history of prior CABG compared with those on ranolazine (see Individual Study Report, 3033); however, this difference was not statistically significant (p=0.06). About 55-60% had a prior MI and about 29-32% were classified as either Class I or II CHF. About 64% had a history of hypertension, 21-25% of patients were diabetic (most did not take insulin) and 5-10% had asthma/COPD.

In Study 3031, (all treated patients) baseline characteristics, except for gender (p=0.05, higher percentage males in the ABCD and BDAC sequences), appeared to be balanced among treatment sequences. Statistically significant differences were seen with regard to diabetics on insulin (p=0.02), history of unstable angina (p=0.037) and prior stroke (p=0.03); however the numerical differences between these groups were small.
Mean age was about 64 years and about half of the patients were 65 years and older.
The safety population was about 90% Caucasian and 4-8% Black. About half had a prior MI, about 13-20% had a history of CHF, and 28% had a prior CABG. About 60-70% had a history of hypertension.
No gross imbalances were seen with respect to concomitant medications. The most frequently used medications included antiplatelet agents (about 80%), ACE inhibitors (about 25-27%), nitrates (about 54%), HMG CoA reductase inhibitors (about 50%) and sulfonamides (about 10%).

Efficacy Results:
The primary efficacy endpoint for both pivotal studies was the change from baseline to endpoint in ETT duration at trough (12 hours post-dosing).

CVT 3033: efficacy variables:
The primary endpoint for Study 3033 is presented below:

Table 5. CVT 3033: Primary Efficacy analysis: Change from baseline in ETT (sec) at trough Week 12 (ITT LOCF)—comparison of treatment differences from ANCOVA Model 1*

	Ran SR 750 mg bid vs. placebo	Ran SR 1000 mg bid vs. placebo
LS Mean difference (SE)	23.7 (10.9)	24 (11)
95% CI	(2.3, 45.1)	(2.4, 45.7)
p-value	0.03	0.029

Source: Table 2.0.0. Study 3033
*Model 1: effects for treatment, baseline covariate, pooled site and background therapy using type III sum of squares. Baseline covariate is the average of visits 1 and 2 data.

When the primary endpoint was analyzed via the efficacy evaluable population, results were statistically significant only for the Ran SR 1000 mg bid group.
The primary efficacy endpoint was also analyzed by stratified medication, pooled/individual site, and other subgroups. Please see Subgroup Section and the Individual study review for further details.

Secondary efficacy variables, related to exercise testing, are presented below. The treatment effect in any variable does not increase when the dose is increased from 750 to 1000 mg bid. Treatment effects are greater at peak times compared to trough.

Table 6. CVT 3033: Exercise Efficacy Variables (primary and secondary): Change from baseline at Week 12 (ITT LOCF) at peak and trough

	Ranolazine SR 750 mg bid				Ranolazine SR 1000 mg bid			
	N	Trough	N	Peak	N	Trough	N	Peak
Exercise duration (sec)								
LS Mean (SE)	272	115.4 (8)	270	99.4 (7.8)	261	115.8 (8.2)	255	91.5 (8.1)
Mean difference vs. placebo (SE)		23.7 (10.9)		34 (10.7)		24 (11)		26.1 (10.8)
p-value		0.03		0.001		0.029		0.016
Time to Onset of Angina (sec)								
LS Mean (SE)	272	144 (8.9)	270	126.9 (9.1)	261	140.3 (9.1)	255	126.8 (9.4)
Mean difference vs. placebo (SE)		29.71 (12.07)		38.02 (12.38)		26.01 (12.2)		37.88 (12.56)
p-value		0.014		0.002		0.033		0.003
Time to 1 mm ST depression (sec)								
LS Mean (SE)	260	145.1 (9)	248	100 (8.7)	244	146.2 (9.3)	236	93.8 (8.9)
Mean difference vs. placebo (SE)		19.9 (12.2)		40.8 (11.8)		21.1 (12.4)		34.5 (11.9)
p-value		NS		<0.001		NS		0.004
Maximum ST depression (mm)								
LS Mean (SE)	266	0.37 (0.05)	254	0.10 (0.05)	251	0.21 (0.05)	240	0.03 (0.05)
Mean difference vs. placebo (SE)		0.18 (0.07)		0.10 (0.07)		0.02 (0.07)		0.03 (0.07)
p-value		0.006		NS		NS		NS
Primary Reason for Stopping ETT, n (%)								
Angina	254	178 (70.1)	249	143 (57.4)	239	168 (70.3)	237	136 (57.4)
p-value (vs. not angina)		NS		0.011		NS		0.011

CVT 3033: Angina/nitroglycerin consumption: Weekly anginal episodes and nitroglycerin consumption, as reported by patients in a weekly diary, were secondary efficacy variables in Study CVT 3033. Results (below) showed a significant improvement in patient-reported weekly anginal episodes and nitroglycerin consumption.

Table 7. CVT 3033: Weekly angina episodes and nitroglycerin consumption (ITT)

	Ranolazine SR				Placebo	
	750 mg bid		1000 mg bid			
Angina episodes/wk	N		N		N	
Mean (SE) baseline	272	4.4 (0.3)	261	4.4 (0.3)	258	4.6 (0.4)
Mean (SE) during double-blind	272	2.47 (0.23)	261	2.13 (0.24)	258	3.31 (0.3)
p-value vs. placebo		0.006		<0.001		

	Ranolazine SR				Placebo	
	750 mg bid		1000 mg bid			
Nitroglycerin use/wk	N		N		N	
Mean (SE) baseline	258	4 (0.5)	244	3.7 (0.5)	247	4.1 (0.4)
Mean (SE) during double-blind	262	2.11 (0.27)	244	1.76 (0.28)	252	3.14 (0.38)
p-value vs. placebo		0.016		<0.001		

Ranolazine vs. placebo calculated from ANOVA using ranked scores data adjusted for treatment, baseline covariate, pooled site and background. Also see Individual study review.

Efficacy Analysis: CVT 3031:

The individual study review of CVT. 3031 highlighted issues in study design (lack of interim washout periods, lack of baseline measurements for each period, etc) as well as the presence of treatment-by-period interaction and possible carryover effect. Because of these issues, analysis of the first period was taken as the double-blind portion not subject to bias. Results are presented below:

Table 8. CVT 3031: Comparison of Treatment Differences in ETT duration: First Period Population

	Ran SR 500 mg vs. placebo	Ran SR 1000 mg vs. placebo	Ran SR 1500 mg vs. placebo
ETT duration **(trough)**: LS Mean difference (SE)	11.7 (21.5)	12.7 (21)	4.5 (21.5)
95% CI	-30.4, 53.8	-28.4, 53.8	-37.6, 46.7
p-value	NS	NS	NS
ETT duration **(peak)**: LS Mean difference (SE)	37.8 (19.5)	56.8 (19)	38.7 (19.7)
95% CI	-0.4, 76.1	19.5, 94	0.1, 77.3
p-value	0.054	0.003	0.051

Source: CVT 3031. Table 2.3.2. ANCOVA model includes effects for baseline ETT duration, treatment, pooled site.

These results do not support a statistically significant effect at trough; however, there appears to be a treatment effect at peak (with marginally significant results at the lowest and highest doses). In this study, there does not appear to be further improvement in the primary efficacy variable above the ranolazine SR dose of 1000 mg bid.

Ranolazine IR studies demonstrating efficacy at peak:

The following three ranolazine IR crossover studies were cited by the sponsor to support efficacy at peak. Potentially confounding issues include: use of more than one testing method in the same study; lack of interim washout period/ variable interim period, and significant sequence effects. Significant sequence effects were seen in several efficacy variables in RAN 72 and RAN 1514.

Of the three studies, only RAN 80 also included a "first period" analysis that showed a statistically significant treatment effect, supporting efficacy at peak. The first period analysis of RAN 1514 did not support a significant treatment effect (at peak or trough).

It should also be noted that the definition of peak time differed across studies.

RAN 72: This was a single-dose crossover study of ranolazine IR 10, 60, 120 or 240 mg and placebo in CAD patients who were symptomatic despite medical therapy and admitted for coronary angiography. Background medication included either beta blocker or diltiazem. Each patient would receive a dose of ranolazine on one study day and placebo on the other study day. Bicycle exercise testing was performed at peak only, at a median interval of 5-7 days (range 1-17 days) between study days. The primary efficacy variable was not explicitly prespecified in the protocol, but the main exercise-related test variable was exercise duration at peak (2.5-3 hours post-dosing).

Results: Significant improvements compared to placebo are only seen in the 240 mg group (combined and on beta blocker). The percentage increase in exercise duration, time to 1 mm ST depression and time to angina were all consistent in that statistically significant improvements,

compared to placebo, were seen at the 240 mg dose and in the group receiving beta blocker (but not calcium channel blocker) as background therapy. Sequence effects were seen with respect to "time to angina" and ST depression and treatment-by-period interactions cannot be excluded.

Table 9. RAN 072: Exercise duration (sec) at peak

	N	Adjusted difference (R minus P)* (SE)	p-value
Beta blocker group:			
Ran 10 mg	14	7.21 (16.24)	NS
Ran 60 mg	15	21.28 (15.73)	NS
Ran 120 mg	17	5.11 (14.98)	NS
Ran 240 mg	15	39.42 (16.02)	0.02
Calcium channel blocker group			
Ran 10 mg	10	11.9 (19.22)	NS
Ran 60 mg	11	6.2 (18.4)	NS
Ran 120 mg	12	-8.82 (17.79)	NS
Ran 240 mg	10	33.8 (19.22)	0.08
Combined			
Ran 10 mg	24	9.56 (12.58)	NS
Ran 60 mg	26	13.74 (12.1)	NS
Ran 120 mg	29	-1.86 (11.63)	NS
Ran 240 mg	25	36.6 (12.51)	0.004

Source: RAN072 Table 5. *Ranolazine minus placebo. Differences were adjusted to account for [illegible] each group on each sequence.

RAN 80: This was a double-blind, crossover study of ranolazine IR 400 mg tid, atenolol 100 mg qd (double dummy) and placebo tid in stable angina patients responding to medical therapy. Each double-blind treatment was administered for one week. No interim washout period was planned between treatments. Exercise testing (either bicycle or treadmill, depending on the site) was done 1 hour post-dose. The primary efficacy variable was the time to onset of angina at peak (1 hour post-dose).

Results: Significant treatment effects were seen for both ranolazine and atenolol (without superiority) in the primary efficacy variable; in addition, a significant treatment effect was seen in the first period analysis. Similar results were seen in the evaluable population (please see the Individual Study review for further details). Overall analyses of the time to onset of angina showed significant treatment by investigator interaction, suggesting heterogeneity across centers. In the "all patients" analysis of time to onset of angina, there was also a significant treatment by method interaction (p=0.01).

Table 10. Study RAN 080: Time to Onset of Angina: First Period Analysis

	Baseline	Ranolazine	Atenolol	Placebo
N	158	53	51	51
Mean time to angina (SEM) (sec)		62.5 (11.9)	59.6 (12.2)	23.2 (12.2)
		Ranolazine vs. placebo	Atenolol vs. placebo	Ranolazine vs. atenolol
Mean difference		39.3	36.4	2.9
95% CI		6.7, 72.1	3.4, 69.4	-29.8, 35.6
p-value		0.02	0.03	NS

Source: RAN 080, Table 12. Means are adjusted. Statistics calculated from ANOVA.

RAN 1514: This was a double-blind, Latin square crossover study of placebo and ranolazine IR: 267 mg tid, 400 mg bid, and 400 mg tid for one week treatment periods with no interim washout period between treatments. The double-blind treatment phase lasted a total of 5 weeks, with one of the treatments repeated during a fifth period. Exercise testing was performed at trough (8 or 12 hours post-dosing) or peak (1 hour post-dosing). The primary efficacy variable was time to onset of angina at trough.

Results: No statistically significant treatment effects were demonstrated for the primary endpoint, or for other trough exercise variables (exercise duration, time to 1 mm ST depression). For peak results (secondary efficacy variables), analysis of time to angina, exercise duration and time to 1 mm ST depression showed statistically significant differences vs. placebo. Significant period effects ($p< 0.01$) were seen with regard to duration of exercise and time to 1 mm ST depression. A first-period analysis showed no statistically significant treatment effects for either peak or trough exercise variables. No treatment-by-period analysis was submitted, and a treatment-by-period interaction therefore cannot be excluded.

Table 11. RAN 1514: Peak exercise treatment change from baseline to endpoint pairwise treatment comparisons: First period per-protocol analyses (n=304)

		Ran 400 mg bid vs. DB placebo	Ran 267 mg tid vs. DB placebo	Ran 400 mg tid vs. DB placebo
Time to Onset of Angina (min)	Mean difference (SEM)	0.78 (0.43)	0.59 (0.43)	0.43 (0.42)
	95% CI	-0.07, 1.63	-0.25, 1.43	-0.40, 1.27
Duration of exercise (min)	Mean difference (SEM)	0.39 (0.3)	0.29 (0.3)	0.13 (0.29)
	95% CI	-.20, 0.98	-0.29, 0.88	-0.45, 0.71
Time to 1 mm ST depression (min)	Mean difference (SEM)	0.40 (0.38)	0.94 (0.38)	0.48 (0.38)
	95% CI	-.35, 1.15	0.19, 1.68	-.26, 1.22

Statistics were estimated by the sponsor from ANOVA. The overall test was not significant.

Ranolazine IR studies that did not demonstrate efficacy:
Most of these studies (see table 3) either used Ran IR $\leq$ 240 mg, or were stopped/discontinued.

Reviewer:

1. Three ranolazine studies, CVT 3033, CVT 3031 (first period) and RAN 80 (first period) support efficacy at peak, where peak is defined as 4 hours post-dose (am) in studies CVT 3033 and 3031, and 1 hour post-dose in RAN 80.
2. Study CVT 3033 supports efficacy at trough, where trough is defined as 12 hours after the p.m. dose.
3. The statistically significant (patient-reported) decreases in angina episodes and nitroglycerin use also support efficacy, but were only demonstrated in CVT 3033.

Dose-response/Drug concentration-response Relationship:

According to the sponsor, the IR formulation used the dihydrochloride salt of ranolazine, in contrast to the SR formulation, in which ranolazine base is the active ingredient. The conversion factor for ranolazine dihydrochloride salt to ranolazine base is 0.854.

Ranolazine dihydrochlorride and free base equivalent plasma concentrations (ng/ml) at Trough and Peak doses for the three ranolazine IR efficacy studies (RAN 072, RAN 080 and RAN 1514) are presented below:

Table 12. Ranolazine Dihydrochloride and Free Base Equivalent Plasma Concentrations (ng/mL at Trough and Peak doses for the three ranolazine IR studies

Ranolazine IR dose	Ranolazine dihydrochloride mean (SD)				Ranolazine free base mean (SD)**			
	N	Trough	N	Peak	N	Trough	N	peak
*RAN 072**								
10 mg		NA	20	46 (35)		NA	20	39 (30)
60 mg		NA	18	249 (225)		NA	18	213 (192)
120 mg		NA	23	589 (406)		NA	23	503 (347)

240 mg		NA	21	1,030 (556)		NA	21	880 (475)
RAN 080								
400 mg tid		NA	143	2,039 (1201)		NA	143	1,741 (1026)
RAN 1514								
267 mg tid	292	371 (394)	298	1,576 (965)	292	317 (336)	298	1,346 (824)
400 mg bid	302	275 (338)	304	2,204 (1,281)	302	235 (289)	304	1,882 (1094)
400 mg tid	311	602 (585)	308	2,492 (1403)	311	514 (500)	308	2,128 (1198)

Source: ISE * Single dose study. **Conversion: 1 mg ranolazine dihydrochloride = 0.854 mg ranolazine free base. Ranolazine free base is the active ingredient in the ranolazine SR formulation.

Using the preceding table, coupled with the sponsor's claim of efficacy at peak of the ranolazine doses in RAN 072, RAN 080 and RAN 1514, the sponsor concludes that concentrations of $\geq$ 880 ng/ml, corresponding to the ranolazine IR dose of 240 mg (from RAN 072), are effective.

Table 13. CVT 3033: Ranolazine Plasma Concentrations (ng/ml) at Week 12 at Trough and Peak during the Double-blind phase: safety population

	Ranolazine SR 750 mg	Ranolazine SR 1000 mg
Trough Mean (SE)	1577.6 (71)	2164.7 (89.2)
Peak Mean (SE)	2031.1 (78.8)	2607.1 (90)

Source: Table 11P, Table 3.5.0, 3.6.0.

Table 14. CVT 3033: Trough and Peak mean (SD) exercise duration at baseline and Change from baseline at Week 12 (ITT LOCF)

	Ran SR 750 mg bid				Ran SR 1000 mg bid				Placebo			
	Trough		Peak		Trough		Peak		Trough		Peak	
	N	Value	N	Value	N	Value	N	Value	N	Value	N	Value
Baseline mean (SE), sec	272	416.4 (6.2)	270	464.8 (8.1)	261	414.7 (6.3)	255	470.4 (7.9)	258	418.3 (6.3)	256	466.5 (8.2)
Mean difference (SE), sec		23.7 (10.9)		34 (10.7)		24 (11)		26.1 (10.8)		--		--
p-value		0.03		0.001		0.029		0.016		--		--

In the above table, the sponsor has made the point that baseline mean exercise duration was about 50 seconds longer at peak than at trough. According to the sponsor, these differences may have occurred, at least in part, because levels of background anti-anginal medications were lower at trough and higher at peak. The sponsor also claims that the difference in baseline exercise duration between peak and trough may explain why changes from baseline are smaller at peak than trough.

Table 15. CVT 3031: Ranolazine SR concentration measurements—Safety population (N=191)

Parameter	Placebo (N=179)	Ran SR 500 mg (N=181)	Ran SR 1000 mg (N=180)	Ran St 1500 mg (N=187)
	N=175	N=173	N=175	N=170
Trough plasma concentration (ng/ml) mean (SE)	16 (11.3)	848.9 (55)	1959.2 (107.5)	3241 (150.9)
	N=173	N=169	N=174	N=166
Peak plasma concentration (ng/ml) mean (SE)	35.2 (19.5)	1122.6 (55.9)	2476 (115.1)	3930.5 (161.3)

Source: Panel 11E, Table 1.14.0, (CVT 3031)

In study CVT 3031, the sponsor has noted that ranolazine levels at trough for ranolazine SR 500 mg are close to 880 ng/ml. If one accepted the sponsor's claim of efficacy at a serum concentration of 880 ng/ml, then, following this line of argument, it would mean that ranolazine SR 500 should be an effective dose.

Onset of effect:

According to the sponsor, the anti-anginal effect of ranolazine occurs with the first dose and is maintained for the duration of treatment with ranolazine.

In CVT 3033, statistically significant treatment effects at trough were noted as early as Week 2 (see Individual study review) for both ranolazine doses.

A statistically significant increase in exercise duration was seen at *peak* (2.5-3 hours post-dose) with a single dose of ranolazine IR 240 mg (Study RAN 072). However, sequence effects were seen with other exercise measurements in RAN 072.

Two other studies did not demonstrate significant treatment effects with ranolazine IR 240 mg (see Table 3: RAN 017, RAN 054); however, it might be argued that these other studies suffered from either small numbers or administrative problems.

Study RAN 080 showed a significant treatment effect at *peak* (1 hour post-dose) time to angina) after one week dosing with ranolazine IR 400 mg tid (see Individual study review).

However, RAN 1514 (n=72 to 72 per ranolazine treatment group and n=84 for placebo) showed no statistically significant treatment effect at peak (1 hour post-dosing) in the first-period analysis (after one week of dosing) using doses (ranolazine IR 400 mg bid, 267 mg bid, or 400 mg tid) larger than 240 mg.

Reviewer:

1. A statistically significant treatment effect at trough is demonstrated as early as Week 2 in a single study (CVT 3033).
2. Results of RAN 1514 (first period) is inconsistent with the sponsor's claim of significant treatment effect at peak after a single dose.

Maintenance of anti-anginal effect/Testing for Rebound effects:

Study CVT 3033 included, as part of the study design, a 48 hour rebound assessment period. During this period, patients on ranolazine at the end of the 12 week treatment period were randomized, in a double-blind procedure, to either continue their blinded ranolazine treatment or receive matching placebo for a 48 hour period. At the end of 48 hours, these patients would undergo an ETT at trough.

As noted in the CVT 3033 individual study review, large differences in the change from baseline in ETT duration at trough were seen between Ran 1000/placebo vs. Ran 1000/Ran 1000 group (ITT and evaluable populations). However, no significant differences were seen between either Ran/placebo group vs. placebo/placebo. In addition, there were no reports of worsening angina or nitroglycerin consumption.

These results appear consistent with a marginally significant treatment effect in the Ran 1000/placebo vs. Ran 1000/Ran 1000 group and support the sponsor's claim of maintenance of efficacy after 12 weeks of treatment, lack of tolerance and lack of demonstrated rebound effects.

Table 16. CVT 3033: Mean Difference in Change from Baseline in ETT duration at Trough at the End of the Rebound Assessment Phase (ITT)

	Ran 750/placebo vs. Placebo/placebo	Ran 1000/placebo vs. Placebo/placebo	Ran 750/placebo vs. Ran 750/Ran 750	Ran 1000/Placebo vs. Ran 1000/Ran 1000
Mean Difference (SE)	4.7 (14.7)	-1.5 (15.1)	-21.8 (17.1)	-33.9 (17.5)
95% CI	-24.2, 33.5	-31, 28.1	-55.3, 11.7	-68.2, 0.5
p-value	NS	NS	NS	0.053

Source: ISE, CVT 3033

Effects on heart rate and systolic blood pressure:

From the sponsor's analyses, there appear to be small decreases in heart rate and SBP compared to placebo. These decreases appear to be largely consistent across studies. Decreases in heart rate and SBP were also seen in Study CVT 3031, with statistically significant decreases in standing pre-exercise heart rate (both trough and peak) in the ranolazine SR 1500 mg bid group (LSM difference from placebo of –2.8 and –2.6 bpm, p <0.001 and p=0.001, respectively). Also in CVT 3031, the standing pre-exercise SBP was significantly decreased vs. placebo (LSM difference from placebo = -2.3 mm Hg, p=0.039).

Table 17. CVT 3033: HR and SBP data (All patients with ETT data at Week 12 (N=737))

	LSM Difference from placebo p-values < 0.05			
Ranolazine dose	750 mg bid		1000 mg bid	
	Trough	Peak	Trough	peak
Standing pre-exercise HR (bpm)	-1.5 NS	-1.4 NS	-1.5 NS	-1.3 NS
Standing pre-exercise SBP (mm Hg)	-1.8 NS	-1.6 NS	-2.8 NS	-2.8 NS

Source: ISE. Differences from placebo are from ANCOVA model with effects for baseline, pooled site, background therapy and treatment.

Table 18. Heart rate and BP data in Studyes RAN 072, RAN 080 and RAN 1514.

	LSM Difference from placebo p-values ≤ 0.05				
Ranolazine dose	240 mg (RAN 072)*	267 mg tid (RAN 1514)	400 mg bid (RAN 1514)	400 mg tid (RAN 1514)	400 mg tid (RAN 080)*
	Peak	Peak	Peak	Peak	Peak
Standing pre-exercise HR (bpm)	-2.2 NS	-0.3 NS	-0.6 NS	-0.2 NS	1.5 NS
Standing pre-exercise SBP (mm Hg)	-0.1 NS	-1.4 NS	-0.6 NS	-2.7 NS	-0.5 NS

* All patients analysis. In study RAN 1514, a per-protocol complete squares analysis was performed (HR/BP analysis was not performed on all patients).

Interaction with Background Therapy:

Study CVT 3033: Study CVT stratified patients to background therapy of amlodipine, atenolol and diltiazem. The primary efficacy endpoint by background therapy is shown below. There appears to be an interaction with diltiazem with greater differences vs. placebo in the diltiazem group, especially at the higher doses and at peak (where the treatment effect at peak, on ranolazine SR 750 mg bid, is two-fold higher in the diltiazem group, compared to other subgroups).

Table 19. CVT 3033. Change from baseline in Exercise duration at Trough and Peak at Week 12 (ITT LOCF) by stratified background therapy

	Treatment and Background Therapy					
	Ranolazine 750 mg bid vs. placebo			Ranolazine 1000 mg bid vs. placebo		
	Diltiazem 180 mg qd	Atenolol 50 mg qd	Amlodipine 5 mg qd	Diltiazem 180 mg qd	Atenolol 50 mg qd	Amlodipine 5 mg qd
Trough (sec)						
LSM difference (SE) vs. placebo	20.6 (21.5)	23.2 (16.5)	27.4 (19.7)	42.9 (22.1)	7.5 (16.7)	32.3 (19.7)
95% CI	-21.7, 62.9	-9.2, 55.6	-11.2, 66.1	-0.6, 86.4	-25.2, 40.2	-6.4, 70.9
p-value	NS	NS	NS	0.053	NS	NS
Peak (sec)						
LSM difference (SE) vs. placebo	56.4 (21.1)	24.4 (16.1)	29.7 (19.2)	66.6 (21.8)	4.4 (16.4)	24.5 (19.3)
95% CI	14.9, 97.9	-7.2, 56	-8, 67.5	23.8, 109.4	-27.7, 36.5	-13.4, 62.5
p-value	0.008	NS	NS	0.002	NS	NS

According to the sponsor, the treatment by background therapy was not statistically significant.

In study RAN 72, a single-dose crossover study, increased serum concentrations at peak (2.5-3 hours post-dosing) for ranolazine are seen in diltiazem-treated patients compared to patients on beta-blocker. Statistically significant treatment effects are seen in the overall group and the group [illegible]-blocker (effects that are not consistent with CVT 3033).

Efficacy in Subgroups:

Efficacy by Geographic Region

Please see CVT 3033 (individual study review). According to the sponsor, there was no significant difference by pooled site (based on geographic region) for the effect of ranolazine SR on exercise parameters. However, heterogeneity by pooled sites was noted by the reviewers. Exclusion of one outlier site (710) resulted in a statistically "non-significant" treatment effect.

Efficacy by Gender, Race, Age

Gender:

Subgroup analyses by gender are presented below. Sample size imbalances are noted between genders and other imbalances between the subgroups cannot be excluded. Results appear consistently significant for males and not significant for females. Note the trends in opposite directions at the time of peak ranolazine concentrations. The sponsor found no statistically significant differences in the response to ranolazine between male and female patients for any of the peak/trough ETT variables.

Table 20. CVT 3033. Change from baseline ETT duration (sec) *peak* and *trough* by Gender (ITT LOCF)

	Ran SR 750 (N=272)		Ran SR 1000 (N=261)	
peak	*Female (N=59)*	*Male (N=211)*	*Female (N=47)*	*Male (N=208)*
LS Mean Difference (SE) vs. placebo	-1.9 (22)	44.3 (12.2)	-12.7 (23.5)	35.3 (12.2)
95% CI	-45.1, 41.3	20.4, 68.2	-58.7, 33.4	11.3, 59.3
p-value	NS	<0.001	NS	0.004
trough				
	Female (N=59)	*Male (N=213)*	*Female (N=51)*	*Male (N=210)*
LS Mean Difference (SE) vs. placebo	1.3 (22.5)	28.9 (12.4)	8.6 (23.4)	26.1 (12.5)
95% CI	(-42.9, 45.5)	(4.5, 53.2)	(-37.4, 54.6)	(1.6, 50.6)
p-value	NS	0.02	NS	0.037

Race: Because 98% of patients in CVT 3033 were Caucasian, a subgroup analysis by race was not done. There are insufficient numbers of non-Caucasians in this submission to allow reasonable interpretation of efficacy.

Age:
In study CVT 3033, no consistently significant treatment effects are seen at trough when analyzed by the age subgroup and no definitive patterns at peak or trough can be concluded.

Table 21. Change from baseline ETT duration (sec) at *trough* and *peak* by Age (ITT LOCF)

	Ran SR 750 (N=272)		Ran SR 1000 (N=261)	
trough				
Age (years)	*< 65 (N=140)*	*≥ 65 (N=132)*	*< 65 (N=134)*	*≥ 65 (N=127)*
LS Mean Difference (SE) vs. placebo	27.9 (15.5)	16.9 (15.3)	25.8 (15.6)	19.2 (15.4)
95% CI	(-2.5, 58.3)	(-13.1, 46.9)	(-4.9, 56.5)	(-11, 49.4)
p-value	0.07	NS	NS	NS
peak				
Age (years)	*< 65 (N=139)*	*≥ 65 (N=131)*	*< 65 (N=133)*	*≥ 65 (N=122)*
LS Mean Difference (SE) vs. placebo	39.7 (15.2)	26.2 (15)	27.8 (15.3)	21.9 (15.2)
95% CI	10, 69.5	-3.3, 55.6	-2.3, 57.9	-8, 51.8
p-value	0.009	0.08	0.07	NS

Source: Tables 2.1.7, 2.1.7.1, 2.1.8, 2.1.8.1, 2.1.9, 2.1.9.1, 2.1.10, 2.1.10.1. LSM, SE and p-values from ANCOVA Model 6 with effects for treatment, baseline covariate, pooled site, background therapy, subgroup and treatment by subgroup interaction. Baseline covariate is the visit 2 data.

According to the sponsor, treatment by subgroup interaction terms (above) were non-significant.

Efficacy in "Intolerant" Populations:
The sponsor has presented efficacy data in groups of patients who may be intolerant to currently available anti-anginal medication. Included were those patients with low BP, low heart rate/prolonged PR interval and co-morbid conditions including reactive airway disease, CHF or diabetes.

Low heart rate, low BP and/or prolonged PR interval: The sponsor selected a threshold of standing SBP ≤ 100 mm Hg or standing HR ≤ 60 bpm as a lower limit to define a *post-hoc* subgroup of patients with low blood pressure and/or low heart rate. Prolonged PR interval was defined as PR ≥ 200 msec.
Analysis of this subgroup using data from CVT 3033 is presented in the following table:

Table 22. CVT 3033. Exercise Performance by Subgroup: Patients with baseline SBP ≤ 100 mm Hg, HR ≤ 60 bpm, or PR interval ≥ 200 msec (ITT)

	Placebo		Ran 750 mg bid		Ran 1000 mg bid	
	Yes	No	Yes	No	Yes	No
Change from baseline ETT duration at Trough (sec)						
N	79	179	88	184	82	179
LSM (SE)	90.8 (14.5)	92.2 (9.8)	125 (13.8)	110.8 (9.6)	125.6 (14.1)	111.2 (9.8)
Treatment difference vs. placebo (SE)	--	--	34.2 (19.5)	18.6 (13.2)	34.8 (19.9)	19 (13.3)
p-value	--	--	0.080	NS	0.080	NS
Change from baseline in Time to onset of Angina at Trough (sec)						
N	79	179	88	184	82	179
LSM (SE)	116.6 (16.1)	113.3 (10.8)	152.7 (15.4)	139.8 (10.7)	149.6 (15.7)	135.9 (10.9)
Treatment difference vs. placebo (SE)	--	--	36.1 (21.6)	26.5 (14.7)	33 (22)	22.6 (14.7)
p-value	--	--	0.095	0.072	NS	NS

Change from baseline in Time to 1 mm ST depression at Trough (sec)						
N	76	171	84	176	79	165
LSM (SE)	128.3 (16.2)	123.6 (10.9)	137.6 (15.6)	148.6 (10.8)	148.6 (15.8)	145 (11.2)
Treatment difference vs. placebo (SE)	--	--	9.4 (21.9)	24.9 (14.9)	20.3 (22.2)	21.4 (15.1)
p-value	--	--	NS	0.094	NS	NS
Change from baseline in ETT duration at Peak (sec)						
N	79	177	88	182	81	174
LSM (SE)	43.9 (14.1)	74.8 (9.6)	95.7 (13.5)	101.2 (9.4)	84.2 (13.8)	94.9 (9.7)
Treatment difference vs. placebo (SE)	--	--	51.8 (19)	26.3 (13)	40.3 (19.4)	20.1 (13.1)
p-value	--	--	0.007	0.043	0.038	NS
Change from baseline in time to onset of Angina at Peak (sec)						
N	79	177	88	182	81	174
LSM (SE)	73.1 (16.4)	95.9 (11.1)	119.7 (15.7)	130.4 (11)	116.7 (16.1)	131.7 (11.3)
Treatment difference vs. placebo (SE)	--	--	46.6 (22.1)	34.6 (15.1)	43.5 (22.5)	35.8 (15.2)
p-value	--	--	0.035	0.022	0.054	0.019
Change from baseline in time to 1 mm ST depression at Peak (sec)						
N	70	164	77	171	76	160
LSM (SE)	31.5 (15.8)	71 (10.5)	112.2 (15.2)	94.3 (10.3)	87.1 (15.1)	96.9 (10.7)
Treatment difference vs. placebo (SE)	--	--	80.7 (21.4)	23.4 (14.2)	55.6 (21.4)	25.9 (14.4)
p-value	--	--	0.0002	.10	0.01	0.073

Source: Table 36, ISE. Includes patients in the ITT population with baseline SBP $\leq$ 100 mm Hg (N=26), HR $\leq$ 60 bpm (N=163) or PR $\geq$ 200 msec (N=102). According to the sponsor, the patients in this table were already on stratified background medication when these baseline measurements were done. Treatment by subgroup interactions, according to the sponsor, were not statistically significant for above peak or trough variables.

Reviewer:

1. Only 26 patients (total) in this subgroup analysis were noted to have a baseline SBP $\leq$ 100 mm Hg. Most patients in this pooled subgroup analysis were noted to have baseline bradycardia or PR interval prolongation.
2. Since CVT 3033 stratified patients to background therapy of atenolol, diltiazem or amlodipine, it is not clear from this table what number (or percent) of patients with bradycardia were already on diltiazem or atenolol, medications associated with bradycardia.[2] Diltiazem is also associated with AV block. The three stratified medications are indicated for both angina and hypertension. Drug effects related to concomitant medications (and imbalances across groups due to concomitant medications) cannot be excluded.
3. Effects related to diltiazem or interactions with diltiazem (especially at peak) on the above exercise performance parameters cannot be excluded.
4. Significant treatment effects are <u>not</u> seen with respect to trough parameters. One might then conclude that ranolazine does not show a significant benefit at trough in this subgroup. However, study 3033 was also not "powered" to show a treatment difference in this subgroup. The lack of statistically significant subgroups at trough may be consistent with a modest overall treatment effect at trough, where subgroup analyses "wipe out" any significant p-values.

The sponsor presented subgroup analyses for patients with low SBP, slow HR and prolonged PR interval (as defined above) in CVT 3031, and in pooled studies RAN 72, 80 and 1514. From RAN 72, RAN 80 and

[2] Bradycardia has been reported in placebo-controlled angina and hypertension trials in patients receiving diltiazem up to 360 mg daily (Source: Cardizem CD labeling, Physician's Desk Reference).

RAN 1514, pooling included patients with baseline SBP $\leq$ 100 mm Hg (N=9), HR $\leq$ 60 bpm (N=56) or patients with PR $\geq$ 200 msec (N= 40).

Reviewer:

1. Because of the difficulty in interpreting the primary efficacy results of Study 3031, subgroup analyses related to Study 3031 will not be further interpreted.
2. A small number of patients relative to the submitted database (only 9 in the pooled IR studies) were noted to have baseline SBP $\leq$ 100 mm Hg.
3. It is not clear whether RAN 72, 80 and 1514 are appropriate for pooling given the different concomitant medications, exercise testing methods, length of treatment and primary efficacy variables.
4. RAN 80 included patients on diltiazem and atenolol. Drug effects (bradycardia, first degree AV block) related to concomitant medications cannot be excluded.

Co-Existing Medical Conditions:

Reactive Airway Disease: Studies CVT 3033 included 58 patients (total) and CVT 3031 included 13 patients with reactive airway disease.

Analysis of results by the subgroup with and without reactive airway disease are presented below:

Table 23. CVT 3033: Selected Exercise Performance parameters by Presence of Reactive Airway Disease

	Placebo		Ran 750 mg bid		Ran 1000 mg bid	
	Yes	No	Yes	No	Yes	No
Change from baseline in ETT duration at Trough (sec)						
N	14	244	26	246	17	244
LSM (SE)	14.7 (33.7)	96.2 (8.4)	101 (24.9)	117 (8.3)	90.6 (30.7)	117.5 (8.4)
Treatment difference vs. placebo (SE)	--	--	86.3 (41.6)	20.8 (11.3)	75.9 (45.3)	21.3 (11.3)
p-value	--	--	0.038	0.066	0.094	0.060
Change from baseline in time to onset of Angina at Trough (sec)						
N	14	244	26	246	17	244
LSM (SE)	30.6 (37.3)	119.2 (9.4)	149.9 (27.5)	143.5 (9.2)	87.7 (34)	144 (9.3)
Treatment difference vs. placebo (SE)	--	--	119.3 (46)	24.3 (12.5)	57.1 (50.1)	24.8 (12.5)
p-value	--	--	0.0097	0.053	NS	0.048
Change from baseline in time to 1 mm ST depression at Trough (sec)						
N	14	233	25	235	16	228
LSM (SE)	83.5 (37.1)	127.6 (9.5)	152.1 (28)	144.3 (9.4)	135.2 (34.8)	146.9 (9.6)
Treatment difference vs. placebo (SE)	--	--	68.6 (46.1)	16.7 (12.7)	51.7 (50.6)	19.3 (12.8)
p-value	--	--	NS	NS	NS	NS
Change from baseline in ETT duration at Peak (sec)						
N	14	242	26	244	17	238
LSM (SE)	27.3 (32.9)	67.6 (8.3)	90.5 (24.3)	100.4 (8.2)	60.7 (30)	93.7 (8.4)
Treatment difference vs. placebo (SE)	--	--	63.2 (40.7)	32.8 (11.1)	33.4 (44.3)	26.1 (11.2)
p-value	--	--	NS	0.003	NS	0.02

Includes patients in the ITT populations with baseline reactive airway disease. According to the sponsor, the diagnosis of reactive airway disease was made by a review of the patient's medical history by CVT clinicians. Patients were included in this subgroup (under "yes") if a diagnosis of asthma, COPD or chronic bronchitis was recorded. This analysis was performed after the code was broken; according to the sponsor, the patient history was considered without attention paid to treatment group.

Exercise performance parameters (change in ETT duration, time to onset of angina, time to 1 mm ST depression) at Peak showed treatment differences vs. placebo that trended in a direction favorable for ranolazine (p=NS for subgroups with reactive airway disease); the subgroup without reactive airway disease included a greater sample size and showed statistically significant improvements vs. placebo. For purposes of brevity, only the change in ETT duration at peak is shown in the above table.

Reviewer:
1. The sample size of patients with baseline reactive airway disease is small and numerically imbalanced relative to the subgroup without reactive airway disease. Other imbalances across subgroups (for example, related to concomitant therapy or other conditions) cannot be excluded.
2. The standard errors are also larger in the subgroup with baseline reactive airway disease.
3. It does not seem reasonable that ranolazine is effective at trough (with significant treatment effects vs. placebo) and not effective at peak.

There are insufficient data to permit definitive conclusions regarding effectiveness in this subgroup.

Congestive Heart Failure:
In CVT 3033, 242 patients with NYHA Class I or II CHF and 581 non-CHF patients were randomized. At trough, there was minimal improvement vs. placebo in patients treated with ranolazine SR 750 mg bid for the 3 measured exercise parameters (ETT duration, time to onset of angina, time to 1 mm ST depression). For patients treated with ranolazine SR 1000 mg bid, there were improvements, compared to placebo, in the measured exercise parameters at trough (p=NS for all). Subgroup analyses at peak are presented below:

Table 24. CVT 3033: Exercise Performance parameters at Peak by CHF

	Placebo		Ran 750 mg bid		Ran 1000 mg bid	
	Yes	No	Yes	No	Yes	No
Change from baseline in ETT duration at Peak (sec)						
N	74	182	86	184	76	179
LSM (SE)	56.5 (15.8)	68.4 (9.6)	79.9 (14.9)	107.8 (9.4)	99.5 (15.9)	87.2 (9.6)
Treatment difference vs. placebo (SE)	--	--	23.4 (19.4)	39.4 (12.8)	43 (20)	18.9 (12.9)
p-value	--	--	NS	0.002	0.032	NS
Change from baseline in time to onset of Angina at Peak (sec)						
N	74	182	86	184	76	179
LSM (SE)	87.7 (18.3)	88.9 (11.1)	109.2 (17.3)	134.6 (10.9)	131.2 (18.5)	124.2 (11.1)
Treatment difference vs. placebo (SE)	--	--	21.5 (22.5)	45.7 (14.9)	43.5 (23.2)	35.3 (15)
p-value	--	--	NS	0.002	0.061	0.02
Change from baseline in time to 1 mm ST depression at Peak (sec)						
N	66	168	75	173	65	171
LSM (SE)	51 (17.7)	62.6 (10.6)	101.5 (16.9)	99.5 (10.3)	101.3 (18.1)	91 (10.4)
Treatment difference vs. placebo (SE)	--	--	50.5 (21.9)	36.8 (14.1)	50.3 (22.6)	28.4 (14.1)
p-value	--	--	0.021	0.009	0.027	0.045

According to the sponsor, treatment by subgroup interaction p = NS

Reviewer: At peak, treatment differences vs. placebo trend in favor of ranolazine with statistically significant results in the Ran SR 1000 mg bid group.
Because of the numerical imbalances between subgroups (CHF vs. non-CHF), lack of appropriate power and post-hoc nature of these subgroup analyses, this reviewer is wary of forming definitive conclusions based on these data. Also, imbalances between subgroup due to factors other than CHF cannot be excluded.

Diabetes:

In CVT 3033, 189 diabetic and 634 non-diabetic patients were randomized. At trough, the change from baseline in exercise performance parameters (ETT duration, time to onset of angina, time to 1 mm ST depression) in diabetic patients showed an improvement with ranolazine compared to placebo that was statistically significant only with respect to the time to onset of angina in the Ran SR 750 mg bid group (p=0.044). Treatment by diabetes interaction p-values, according to the sponsor, were not significant for any measured exercise performance parameter.

At peak, results of exercise performance in diabetics also showed improvement in ranolazine-treated groups vs. placebo with statistically significant results obtained for the time to onset of angina at peak (Ran SR 1000 mg bid group), and time to 1 mm ST depression (both ranolazine groups); there was a trend toward treatment by subgroup interaction (p=0.09) in the time to 1 mm ST depression at peak. (please see individual study review for further details).

Anti-Anginal efficacy of Ranolazine in Patients Taking Maximal Therapy:

The sponsor has noted patients taking ranolazine against a background of atenolol 50 mg qd (CVT 3033) and claims that the maximum effect of atenolol on exercise tolerance is achieved 3 hours after a steady-state dose of 50 mg qd.

Reviewer: It is not clear from labeling that the maximum effect of atenolol is achieved with 50 mg once daily[3].

1. The two pivotal trials, CVT 3033 or CVT 3031, as well as RAN 72, RAN 80 or RAN 1514 did not specifically study patients on maximal anginal therapy, whether by maximal dosing or via maximal concomitant medications (including long-acting nitrate, calcium channel blocker, and beta-blocker).
2. According to the sponsor, a small number of patients in the database were on 3 concomitant anti-anginal medications. No specific analyses on these patients were performed.
3. It is not known, from the available data, whether ranolazine provides a benefit to patients on maximal anti-anginal therapy.

The sponsor has defined "adequacy of background anti-anginal dose" by using criteria of clinical response to the anti-anginal drug, projected plasma concentration of the background anti-anginal drug, and labeled dosage strength of the anti-anginal drug. According to the sponsor, efficacy of ranolazine in patients with low BP, low heart rate and/or prolonged PR interval is evidence of ranolazine effectiveness in patients receiving adequate doses of other anti-anginal medication.

Reviewer: Ranolazine treatment effects are statistically significant at peak but not at trough in a post-hoc pooled subgroup of patients with low BP, low heart rate and /or prolonged PR interval. These results, however, may merely reflect a more robust result at peak and a marginal result at trough.

The sponsor also used a pharmacokinetic/pharmacodynamic rationale to evaluate adequacy of dosing of background anti-anginal medication. With diltiazem pharmacokinetic modeling, the sponsor has predicted plasma diltiazem levels of about 105-115 ng/ml at about 2.5-3 hours after steady-state dosing with diltiazem IR 60 mg tid. The plasma diltiazem level at trough (24 hours after dosing) with steady-state diltiazem CD 360 mg qd was predicted at 128 ng/ml. Since plasma diltiazem concentrations are generally dose-proportional, therefore, trough plasma diltiazem levels at steady state dosing with diltiazem CD 300 mg qd were predicted to be about 5/6 of that predicted with 360 mg qd, or about 107 ng/ml, or similar to the levels expected with diltiazem IR 60 mg tid at 2.5-3 hours post-dose (the time of exercise testing in RAN 072).

[3] From current TENORMIN labeling: Under Dosing and Administration in Angina, "The initial dose of TENORMIN is 50 mg given as one tablet a day. If an optimal response is not achieved within one week, the dosage should be increased to TENORMIN 100 mg given as one tablet a day. Some patients may require a dosage of 200 mg once a day for optimal effect. Twenty-four hour control with once daily dosing is achieved by giving doses larger than necessary to achieve an immediate maximum effect. The maximum early effect on exercise tolerance occurs with doses of 50 to 100 mg, but at these doses the effect at 24 hours is attenuated, averaging about 50% to 75% of that observed with once a day oral doses of 200 mg." (Source: electronic Physician's Desk Reference).

The sponsor has also stated that "any dose of an anti-anginal drug greater than the labeled starting daily dose for chronic angina is considered to be an adequate dose of that background anti-anginal therapy in this ISE."
Reviewer: This definition of "adequate dosing" is generated by the sponsor. This reviewer does not necessarily agree with the sponsor's definition. In addition, the design of the major studies in this submission did not allow for up-titration to maximally tolerated doses; thus, it cannot be known whether patients were given therapeutic doses of background anti-anginal medication.

Since CVT 3033 utilized stratified background medication, the table of the primary endpoint by background stratified medication (from the Individual study review) is presented in the section on Stratified medication (table 19). Results show trends in a direction favorable to ranolazine (less so for patients on high doses with concomitant atenolol) with wide confidence intervals. Statistically significant treatment effects are only seen at the time of peak ranolazine in patients receiving concomitant diltiazem therapy.

Individual Study Reviews:

Phase 3 Studies:

CVT 3033:

Title: A Double-Blind, Randomized, Stratified, Placebo-Controlled, Parallel Study of Ranolazine SR at Doses of 750 mg Twice a Day and 1000 mg Twice a Day in Combination with Other Anti-Anginal Medications in Patients with Chronic Stable Angina Pectoris
(Protocol date: March 26, 1999)

Primary Objective: Effect of ranolazine SR 750 mg bid and 1000 mg bid, compared to placebo, on symptom-limited treadmill exercise duration at trough ranolazine concentrations (12 hours postdose) after 12 weeks of treatment in patients with chronic stable angina receiving a stable dose of a single concomitant antianginal medication.

Secondary Objectives:
1. Effect of ranolazine, during exercise treadmill testing (ETT) (at trough: 12 hours post dose), on time to onset of angina, time to 1 mm ST depression, maximum ST depression, and reason for stopping exercise;
2. Effect of ranolazine, during ETT (at peak: 4 hours post-dose) on exercise duration, time to onset of angina, time to 1 mm ST depression, maximum ST depression, reason for stopping exercise;
3. Effect of ranolazine on angina frequency, severity and duration, as well as nitroglycerin consumption;
4. Determine if there are any rebound increases in angina, as measured by exercise duration, following discontinuation of ranolazine SR compared to patients maintained on ranolazine SR.

Study Summary:
This was a multicenter, double-blind, randomized, stratified, placebo-controlled, parallel study. The study was comprised of three phases: a single-blind placebo qualifying phase lasting approximately 1-2 weeks, a double-blind treatment phase lasting 12 weeks, and a rebound assessment phase lasting 2 days. A safety follow-up visit was scheduled 2 weeks after study completion. The allowed concomitant anti-anginal medications were: diltiazem 180 mg PO QD in a once-daily formulation, atenolol 50 mg PO QD, or amlodipine 5 mg PO QD. Sublingual nitroglycerin was permitted only as treatment for anginal attacks.

Patients were treated with one of the 3 allowed anti-anginal medications at the specified dose for a minimum of 5 days prior to Visit 1. Those meeting study inclusion/exclusion criteria entered the single-

blind placebo qualifying phase. Single-blind qualifying visits consisted of physical examinations, laboratory tests, and ECGs, with one trough exercise treadmill test (ETT) at Visit 1, and two (trough and peak) ETT at Visit 2.

Qualifying patients at Visit 2 were stratified according to background antianginal therapy and randomized to either ranolazine SR 750 mg BID, ranolazine SR 1000 mg BID or placebo (BID) for the 12 week double-blind treatment phase. At each of 3 double-blind visits (Visits 3, 4, and 5, corresponding to Weeks 2, 6 and 12 of double-blind), patients underwent trough ETT (12 hours after the previous dose taken the evening before). At Visits 3 and 5 (Weeks 2 and 12), patients remained in the clinic and underwent peak ETT 4 hours after the in-clinic dose. Plasma levels for trough and peak ranolazine levels were drawn with the corresponding ETT. At the end of the 12 week double-blind treatment phase, patients entered a 2-day Rebound Assessment Phase where they received, in a double-blind manner, either the same treatment as during the 12 week double-blind phase or placebo. After 2 days, patients returned to the clinic (Visit 6) for a final trough ETT. Two weeks after completing the study, patients returned for a safety follow-up Visit (Visit 7) comprising a history (concomitant medication, adverse events) and physical examination.

Table 1. CVT 3033: Schedule of events

Visit	1	2	3	4	5	6	7
Procedure	Screening	Screen/Qualifying	Double-blind		Double-blind/early withdrawal	Rebound Assessment	Safety follow-up
Time		1-2 weeks	2 weeks	6 weeks	12 weeks	2 days	2 weeks later
History	X						
Physical exam	X				X		X
Body weight	X				X		
Inclusion/exclusion criteria	X	X					
Vital signs	X	X	X	X	X	X	
Angina/ntg use diary review		X	X	X	X	X	
Lab tests*	X				X		
Trough levels			X	X	X	X	
Peak levels			X				
Trough ECG and ETT	X	X	X	X	X	X	
Peak ECG and ETT		X	X		X		
Adverse events		X	X	X	X	X	X
Concomitant meds	X	X	X	X	X	X	X

* TSH and T4 were only done at screening. Serum HCG in females was planned at Visits 1,3, 4, and 5.

Table 2. CVT 3033: inclusion/exclusion criteria

Inclusion criteria	Exclusion criteria (similar to CVT 3031)
1. ≥ 21 years old; 2. at least 3 month history of stable effort angina relieved by rest/sublingual nitroglycerin; 3. diagnosis of coronary disease via at least one: ≥ 60 % stenosis of ≥ 1 major coronary artery on angiogram, past MI documented by enzymes/ECG changes, exercise/pharmacologic stress/echo study; 4. minimum of 5 days treatment prior to Visit 1 with either diltiazem 180 mg QD, atenolol 50 mg QD or amlodipine 5 mg QD; 5. willing to discontinue other antianginals 5 days prior	1. ECG/other factors interfering with ECG interpretation or associated with false + ETT; 2. NYHA Class III-IV CHF; 3. Significant valvular/congenital heart disease; 4. MI/unstable angina/CABG or PCI within past 2 months; 5. 2nd/3rd degree AVB, uncontrolled arrhythmias or life-threatening ventricular arrhythmias unassociated with MI; 6. QTc > 500 msec at Visit 1; 7. Requiring medications known to prolong QTc or

to Visit 1 and throughout study; 6. stable tobacco habits throughout study; 7. if female of childbearing potential, then not pregnant/breastfeeding, using contraceptives and not intending to become pregnant; 8. sign approved consent form;	inducing/inhibiting cytochrome P450 3A4; 8. Unwilling to refrain from grapefruit (juice) consumption; 9. Requiring digoxin; 10. Active acute myocarditis/pericarditis; 11. Hypertrophic cardiomyopathy; 12. Uncontrolled hypertension or SBP < 100 mm Hg; 13. Chronic illness likely to alter f/u evaluation; 14. Significant laboratory abnormality; 15. Participation in another study w/investigational agent within 1 month of this study;

Qualifying for Double-Blind Phase:

1. Symptom-limited exercise duration during trough ETT at Visits 1 and 2 was 3-9 minutes (incl.) of exercise on a modified Bruce protocol.
2. Exercise duration for the two trough ETT at Visits 1 and 2 did not differ by more that 20% of the longer of the two times and did not differ by more than 60 seconds.
3. The primary reason for stopping the two trough ETT at Visits 1 and 2 must be moderately severe angina.
4. Definite ECG signs of ischemia during the ETT at both Visits 1 and 2 (ie, one additional mm of horizontal or downsloping ST depression beyond baseline and at least 1mm below the isoelectric line) were present in at least one standard ECG leading during ETT with a modified Bruce protocol.
5. At Visit 1, the 1 mm ST depression must be verified by the ECG Core laboratory prior to the patient being allowed to continue on to Visit 2.
6. For the Visit 2 ETT, the Investigator should determine if the patient has met study entry criteria and enter this information on the CRF. Patients will enter the double-blind portion based on this determination. The ETT will be sent to the ECG Core laboratory.[4]

Concomitant medications:

Besides the stratified background antianginal medication and sublingual nitroglycerin (as needed), aspirin and stable doses of antihypertensive medications (diuretic or ACE inhibitor) were allowed. Ophthalmic beta blockers were allowed if their use was constant throughout study. Diltiazem, atenolol or amlodipine were allowed only if used as the single concomitant antianginal medication.

Efficacy Evaluations:

1. Exercise treadmill tests: (ETT). A modified Bruce protocol was used. Testing was planned under uniform conditions, optimally by the same technician and supervising physician each time[5]. Trough ETTs were done between 7 am and 12 noon, prior to scheduled morning dose of study medication. If patients did not take their medication 12 hours (± ½ hour) prior to the trough ETT, then the ETT was rescheduled within 3 days. ETT at the time of peak plasma concentrations were planned at 4 hours (± ½ hour) after the in-clinic dose of study medication. The following efficacy variables were recorded: time to onset of angina, symptom-limited exercise duration, primary reason for stopping exercise. In addition, BP/HR were recorded at rest (supine/standing), during the last minute of each stage, at end of exercise, and during recovery (every minute for the first 5 minutes and then q5 minutes until values return to baseline). Standard supine and standing 12-lead ECG recordings were taken at rest.
    1. Efficacy variables recorded by the Investigator during each ETT: time to onset of angina, symptom-limited exercise duration, primary reason for stopping exercise.
    2. ECG variables measured by the Core Laboratory included: time to 1 mm ST depression, maximum ST depression during exercise. The ST depression recorded was to be the average of at least 3 consecutive ST segments.
    3. According to the protocol the ECG lead that best reflected exercise-induced ischemia during Visit 1 was identified and used throughout the study to monitor the patient.

[4] According to the sponsor, the ECG Core Lab was blinded to treatment.

[5] According to the protocol, patients were not to be pushed, coached or encouraged to tolerate symptoms during ETT which are more sever than symptoms which would typically cause them to stop exercise.

4. At Visits 1 and 2, patients stopping exercise for any reason other than moderately severe angina failed to qualify for study entry and were considered screening failures. During double-blind and rebound assessment visits, reasons for stopping ETT could include: unacceptable angina, shortness of breath or fatigue, excessive BP rise, fall in BP during exercise, feeling of faintness, musculoskeletal pain/discomfort, completion of the modified Bruce protocol.

2. Angina and Nitroglycerin use diary: Patients were to maintain a diary of angina attacks and nitroglycerin use for review at each visit.

Pharmacokinetic Evaluations: Plasma concentrations were collected immediately before 12-lead ECG and ETT during Visits 3-6. Trough sampling was planned 12 hours (± ½ hour) after the last dose of study medication. The peak sample was drawn 4 hours (± ½ hour) after the in-clinic dose.

Safety Evaluation: physical examination, vital sign measurements, ECG data, laboratory tests, adverse events and concomitant medications. Official ECG reading was performed by the ECG Core Laboratory.

Analysis populations:

1. The Intent-to-Treat (ITT) population, all randomized patients who have taken at least one dose of study medication and have at least one ETT performed during double-blind, was the population for the primary efficacy analysis.
2. The efficacy-evaluable (EFF) population, all randomized patients with 67-125% compliance during double-blind and rebound assessment phases, with Visit 5 ETT within the stated window and have not violated key protocol criteria, inclusion/exclusion criteria, or have not taken prohibited medications (defined prior to database lock).
3. The general safety population (GSP), all randomized patients who have taken at least one dose of double-blind study medication.
4. The ECG safety population (ECG-SP), all randomized patients who have taken at least one dose of double-blind study medication and have at least one ECG performed during double-blind or rebound assessment phases.

Timepoints:
Baseline was defined as the average from the two ETT during single-blind placebo or, if only one ETT was done, then the single measurement from this phase.

Endpoint was defined as the last post-randomization visit carried forward (LOCF).

Primary Efficacy Variable: Change from baseline in ETT duration at 12 hours post-dose (trough ranolazine concentration) using LOCF.

Secondary Efficacy Variables:

1. Time to onset of angina and change from baseline during trough ETT;
2. Time to 1 mm ST-depression and change from baseline during trough ETT;
3. Change from baseline in maximum ST depression during trough ETT;
4. Primary reason for stopping trough ETT;
5. Change from baseline in exercise duration during peak ETT (4 hours post-dose);
6. Time to onset of angina and change from baseline during peak ETT;
7. Time to 1 mm ST depression and change from baseline during peak ETT;
8. Change from baseline in maximum ST depression during peak ETT;
9. Primary reason for stopping ETT at peak;
10. Exercise duration during ETT comparing patients who were discontinued 48 hours previously from ranolazine after 12 weeks of treatment to placebo-treated patients;
11. Self-reported frequency, severity and duration of anginal episodes during 12 weeks of double-blind treatment;
12. Self-reported nitroglycerin consumption during 12 weeks of double-blind treatment.

Statistics:
Unless otherwise indicated, all statistical tests were to be two-sided. An alpha of 0.05 determined statistical significance.

The primary efficacy parameter, change from baseline in ETT duration at trough ranolazine levels, was prespecified for the ITT population at endpoint using analysis of variance (ANOVA). Terms for treatment, pooled site, and baseline ETT duration would be included. Treatment by site interaction would be tested and included if significant. Type III sums of squares would be used to produce the test statistics.
The assumption of normality would be investigated; if this assumption did not hold, then the primary efficacy parameter was to be analyzed non-parametrically, with baseline ETT duration and primary efficacy parameter ranked across all sites and background therapies. Residuals were to be computed from fitting an ANOVA model with ranked primary efficacy parameter as the response variable, and with effects for pooled site, background therapy and ranked baseline ETT duration.
In addition, the primary efficacy parameter would be analyzed for the ITT and EFF populations to explore possible interaction of treatment with pooled site, background therapy, or baseline ETT separately. If a statistically significant (level of 0.05) interaction was found, the treatment effect would be described for the different levels of the factor. The primary efficacy parameter (ITT) would also be analyzed using Generalized Estimating Equations (GEE) incorporating change from baseline in ETT duration at 2^{nd}, 6^{th}, and 12^{th} week visits.
For the primary efficacy parameter, the multiple comparisons issue was to be addressed by a two-stage step-down procedure.

Interim Assessment: An interim assessment was planned when one-half of the planned completed study patients (N=231) were followed for 12 weeks. Randomized patients who took at least one dose double-blind medication and performed at least one ETT during double-blind were included in this assessment. The purpose of this interim look at the data was to recalculate the standard deviation of change from baseline in ETT duration at trough. Based on these results, the sponsor planned to increase the sample size by no more than 186 additional evaluable patients. (please see Protocol Amendment 2, below).

Reviewer's note: According to the sponsor, the interim assessment was performed, without unblinding, by the Contract Research Organization carrying out the study and the sponsor had no direct involvement.[6]

Sample Size Calculation: The sample size was based on the change from baseline in ETT duration at trough plasma ranolazine levels. Assuming a normal distribution and standard deviation of 80 seconds, a sample size of 462 evaluable patients was projected, with 90% power, to detect a minimum difference of 30 seconds between ranolazine (750 mg, 1000 mg) and placebo. Adjusting for a 20% potential dropout rate increased the sample size to 577 randomized patients to provide 462 completed, evaluable patients. The sample size could be reevaluated based on the interim assessment of the standard deviation.

Amendments to the Protocol:
1. (August 13, 1999): Ensured that no more than 50% of randomized patients will be on one of the three antianginal medication strata; changed qualifying ECG lead (Visit 2) to any lead with 1 mm ST depressions; for ECG analysis, the ECG Core laboratory will identify one lead, best reflecting ischemia, to be used throughout the study for maximum ST depression and time to 1 mm ST depression.

1A. (January 27, 2002): Added 2D-echocardiographic parameters from Italian centers.

2. (January 3, 2001): Clarified steps/logistics of adjusting sample size, specified a maximum enrollment, incorporated a non-parametric analysis as potential primary efficacy analysis if the assumption of normality was not satisfied; included background therapy as covariate in the analyses of primary and secondary efficacy variables.

[6] According to the Study Report (Section 9.7.3, paragraph 2), the procedure followed an Interim Procedure Document, dated January 2, 2001, and listed in Appendix 16.1.13; a copy of this document has been requested by the reviewer.

Amendments to the Statistical Analysis Plan (SAP): The Statistical Analysis Plan was issued on May 24, 2001. Four amendments (7/31/01, 9/26/02, 10/12/01 and, following unblinding, 2/27/02) were submitted. These amendments do not impact the primary analysis.

Results:

Patient disposition:
This study was performed at 118 sites in 15 countries.
A total of 823 patients were randomized (269 to placebo, 279 to ranolazine 750 mg QD, and 275 to ranolazine 1000 mg QD). The highest percentage of randomized patients came from the Czech Republic (24.4 %), followed by Russia (23.8 %), with 6.9% of patients from the U.S. (Source: sponsor, Table 1.1.1). Of those randomized, 90% of those treated with placebo and ranolazine 750 mg QD, and 87% of those treated with ranolazine 1000 mg QD, completed the trial (including double-blind and rebound phases). Background therapy (diltiazem, atenolol, or amlodipine) was well balanced between the three treatment arms; about 117-119 were on atenolol, 81-89 on amlodipine, and 69-74 on diltiazem per treatment arm.[7] Percentages of dropouts are shown below. In the safety population, increased percentages of dropouts due to unacceptable AE are seen in ranolazine patients given diltiazem as background therapy; this difference was not seen in the ITT analysis.
A majority of the withdrawals occurred by Week 6 (the second visit after randomization), regardless of background therapy.

Table 3. CVT 3033. Patient populations

	Placebo	Ran 750	Ran 1000	Total
All randomized patients	269	279	275	823
Safety population	269	279	275	823
ECG Safety population	262	273	269	804
ITT	258	272	261	791
Efficacy-evaluable	176	184	177	537
Efficacy rebound	174	181	171	526

Reviewer: Patients were most commonly excluded from the ITT population for not performing an ETT after the start of study medication, with the largest number of exclusions in the ranolazine 1000 group (14 patients) and the smallest in the ranolazine 750 group (7 patients). Also note that the efficacy-evaluable population is about 65% of the safety population. The most common reason for exclusion was ST depression < 0.9 mm at either visit 1 or 2 (135 patients). The second most common reason was < 67% or > 125% compliance during entire study period. Thirty-five patients were excluded from the efficacy-evaluable analysis due to early withdrawal and lack of Visit 5 trough ETT data. Please note that patients may have been excluded because of more than one factor.

Echocardiogram Sub-Study Note: According to the sponsor, only one patient (on placebo) was enrolled and withdrew prior to study completion. Therefore, no echocardiographic analyses were performed.

Table 4. CVT 3033. Patient Disposition (All Randomized patients).

N (%)	Placebo	Ran 750	Ran 1000
#Randomized	269	279	275
#Completed*	243 (90)	250 (90)	238 (87)
Early w/d	26 (10)	29 (10)	37 (14)
Unacceptable AE	13 (5)	20 (7)	24 (9)
Noncompliance	2 (0.7)	2 (0.7)	0

[7] According to the sponsor, nearly all patients in CVT 3033 took the protocol-specified dose of background therapy for the duration of the study (5 days prior to Visit 1 through Visit 6). In the ITT population, there were 10 protocol violations related to background therapy. Of these, six took an incorrect dose of background medication (4 related to atenolol doses; in addition, one patient took amlodipine 10 mg qd and 1 patient took diltiazem 120 mg qd for the duration of the study).

Elective withdrawals	4 (2)	1 (0.4)	5 (2)
Lost to follow-up	0	0	1 (0.4)
Death	2 (0.7)	2 (0.7)	1 (0.4)
Other	5 (2)	4 (1)	6 (2)

Source: sponsor: Table 1.4.1. Percentages ≥ 1 are rounded off to the nearest integer.
* Completed = patient completed both double-blind and rebound phases.

Baseline characteristics: The study population (ITT) was about 97-98% Caucasian and majority (75-81%) male; mean age about 64 yrs (about half were 65 and older); weight, height were balanced between groups. The safety and efficacy-evaluable population yielded similar results.

Cardiac history (ITT): In terms of cardiac history, fewer patients in placebo (34 patients, or 13%) had a prior CABG performed > 2 months (before randomization) compared to ranolazine 750 mg (53 patients, 20%) and 1000 mg (52 patients, 20%) (p=0.06 for ITT population).
Otherwise, no obvious imbalances were seen between groups. In the ITT group, about 21-23% of patients experienced unstable angina > 2 months before randomization, and a majority (55-60%) had a prior MI. About 17-20% underwent a prior PTCA, 7-9% also had intermittent claudication, 4-6% had a history of prior stroke, and about 29-32% were classified as either Class I or II CHF (percentages of each were balanced between groups). About 7-8% had a history of atrial arrhythmias, 7-10% had a history of ventricular arrhythmias, and 0.4-2% had a prior cardiac arrest. About 4-8% had a history of clinically significant valvular disease. About 64% had a history of hypertension, 21-25% of patients were diabetic (most did not take insulin) and 5-10% had asthma/COPD. Results for the safety population were similar.

Baseline background medications (Visit 2 and continuing): Most common medications included: antiplatelet agents (excl. heparin), 78-95%; ACE inhibitors (37-44%), HMG CoA reductase inhibitors (40-54%), nitrates (81-90%). No gross imbalances were seen between treatment arms.
Background therapy prior to Visit 2 also appeared balanced between treatment arms. Most commonly prescribed anti-anginal agents included nitrates (about 50%) and beta blockers (about 40%).

Baseline vital signs/ETT: In general, peak values were lower than trough values. Heart rates and rate pressure products were consistently lower in the atenolol subgroup; end of exercise heart rates and rate pressure products were consistently higher in the amlodipine subgroup. Mean BP and HR appeared slightly, but consistently higher in the ranolazine SR 1000 mg bid group, with correspondingly higher RPP in that group. No gross imbalances were seen across treatment groups.
The baseline (average of visits 1 and 2) ETT duration at trough was 415-418 sec.

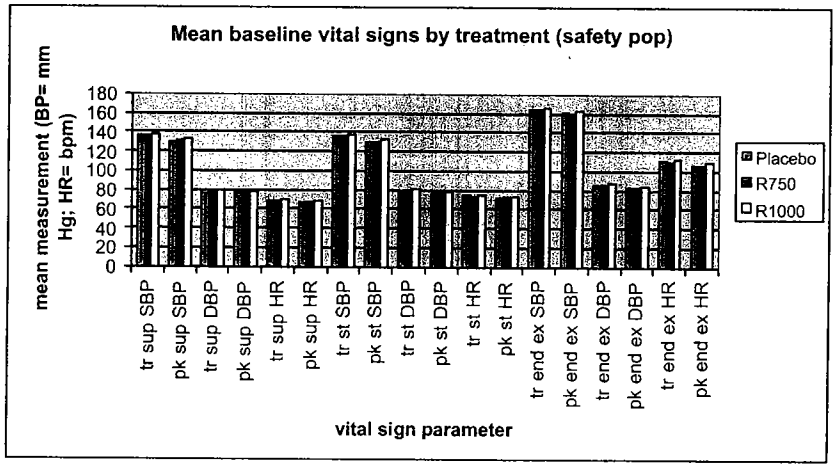


Figure 1. CVT 3033: Baseline vital signs by treatment (safety population)

Pk=peak; tr=trough, sup=supine; st=standing; end ex=end exercise

Number/discontinuations due to angina: A total of 4 patients (1 each from placebo and Ran 1000, and 2 from Ran 750) discontinued due to myocardial ischemia. There were no imbalances noted across groups.

Efficacy Results:

The primary efficacy analysis shows a significant improvement from baseline in the ranolazine groups vs. placebo. Results for the two ranolazine groups appear almost indistinguishable (see Table 4, Figure 3) and there does not appear to be further improvement with the higher dose.

Table 5. CVT 3033: Primary Efficacy analysis: Change from baseline in ETT (sec) *trough* Week 12 (ITT LOCF)—comparison of treatment differences from ANCOVA Model 1*

	Ran SR 750 mg vs. placebo	Ran SR 1000 mg vs. placebo
LS Mean difference (SE)	23.7 (10.9)	24 (11)
95% CI	(2.3, 45.1)	(2.4, 45.7)
p-value	0.03	0.029

Source: Table 2.0.0.*Model 1: effects for treatment, baseline covariate, pooled site and background therapy using type III sum of squares. Baseline covariate is the average of visits 1 and 2 data.

The change from baseline in ETT duration at Trough (ITT LOCF) was statistically significant (ANCOVA) for both ranolazine groups vs. placebo at Weeks 2, 6, and 12.

Table 6. CVT 3033: Change from baseline in ETT duration (sec) at Trough at Weeks 2, 6 and 12 (ITT)

	Ran SR 750 mg vs. placebo	Ran SR 1000 mg vs. placebo
Week 2 LSM difference (SE)	34.1 (8.8)	38.5 (8.9)
95% CI	16.8, 51.4	21, 55.9
p-value	<0.001	<0.001
Week 6 LSM difference (SE)	28.2 (10.6)	31.3 (10.8)
95% CI	7.4, 49	10.1, 52.5
p-value	0.008	0.004
Week 12 LSM difference (SE)	27.1 (11.3)	26.8 (11.5)
95% CI	4.9, 49.4	4.2, 49.3
p-value	0.017	0.020

Source: CVT 3033, Table 2.0.11. LSM difference, SE, p-values calculated from ANCOVA Model 1, including effects for treatment, baseline covariate, pooled site and background therapy using type III sum of squares. Baseline covariate was the average of Visits 1 and 2 data.

Results for the efficacy-evaluable population showed a smaller change from baseline for the 750 mg BID group, but trended in the same direction.

Table 7. CVT 3033: Change from baseline in ETT (sec) *trough* Week 12 (Efficacy evaluable population)—comparison of treatment differences from ANCOVA Model 1*

	Ran SR 750 mg vs. placebo	Ran SR 1000 mg vs. placebo
LS Mean difference (SE)	18.9 (13.3)	32.5 (13.4)
95% CI	(-7.3, 45.0)	(6.1, 58.9)
p-value	NS	0.016

Source: Table 2.0.1. See Table 4 for Model 1 adjustments. P-values obtained from ANCOVA model adjusted for stated effects.

Increases with dose are seen for the change from baseline ETT in the efficacy evaluable population. However, the treatment effect is smaller and not statistically significant in the 750 mg bid treatment arm.

Subgroup analyses:

Effect of background therapy:

Analysis by background therapy is shown in the next table:

Table 8. CVT 3033: Change from baseline ETT (sec) trough Week 12 (ITT LOCF) ANCOVA Model 2*

	Ran 750 mg vs. placebo			Ran 1000 mg vs. placebo		
	Diltiazem	Atenolol	Amlodipine	Diltiazem	Atenolol	Amlodipine
LS Mean difference (SEM)	20.6 (21.5)	23.2 (16.5)	27.4 (19.7)	42.9 (22.1)	7.5 (16.7)	32.3 (19.7)
95% CI	-21.7, 62.9	-9.2, 55.6	-11.2, 66.1	-0.6, 86.4	-25.2, 40.2	-6.4, 70.9
p-value	NS	NS	NS	0.053	NS	NS

Source: Table 2.0.3. *Model 2 includes effects for treatment, baseline covariate, pooled site, background therapy , and treatment by background therapy interaction using type III sum of squares. P-values obtained from ANCOVA model adjusted for stated effects.

The change from baseline (vs. placebo) is most pronounced in the high-dose group with diltiazem as background therapy, compared to background therapy with atenolol and amlodipine. The change from baseline in this diltiazem subgroup was not statistically significant in the efficacy-evaluable population.

Effect of Site/Pooled site:

The following table shows heterogeneity of the treatment effect over the pooled sites:

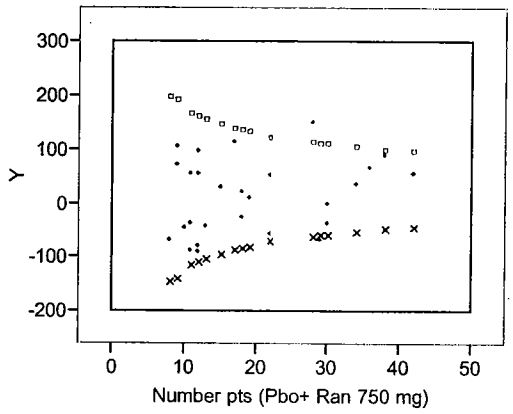
Table 9. Change from baseline ETT (sec) trough Week 12 (ITT LOCF) ANCOVA Model 3*

Ran SR 750 mg vs. placebo								
Pooled site	1	2	3	4	5	6	7	8
LS Mean difference (SEM)	69.1 (43.6)	12.2 (26.1)	12.4 (27)	67.2 (32.9)	-18.5 (21.3)	16.9 (33.9)	90.9 (40.6)	81.8 (46.5)
95% CI	-16.5, 154.7	-39.1, 63.4	-40.6, 65.4	2.5, 131.8	-60.4, 23.3	-49.7, 83.4	11.1, 170.6	-9.4, 173.1
p-value	NS	NS	NS	0.042	NS	NS	0.026	0.079
Ran SR 1000 mg vs. placebo								
Pooled site	1	2	3	4	5	6	7	8
LS Mean difference (SEM)	52.6 (44.2)	28.4 (25.9)	21.7 (28.1)	86.8 (33.4)	0.5 (21.1)	-20.3 (35.7)	26.7 (40.1)	50 (47.3)
95% CI	-34.1, 139.3	-22.5, 79.3	-33.5, 76.9	21.1, 152.5	-40.9, 41.9	-90.4, 49.9	-52, 105.4	-42.9, 142.9
p-value	NS	NS	NS	0.01	NS	NS	NS	NS

Source: Table 2.0.5. *Model 3 includes effects for treatment (p=0.003), baseline covariate (NS), pooled site (p=0.007), background therapy (NS) and treatment by pooled site interaction (NS) using type III sum of squares. . P-values obtained from ANCOVA model adjusted for stated effects. Pooled site 4 =Russia; site 7 = Spain, New Zealand, UK, Australia, Greece, Ireland, Italy.

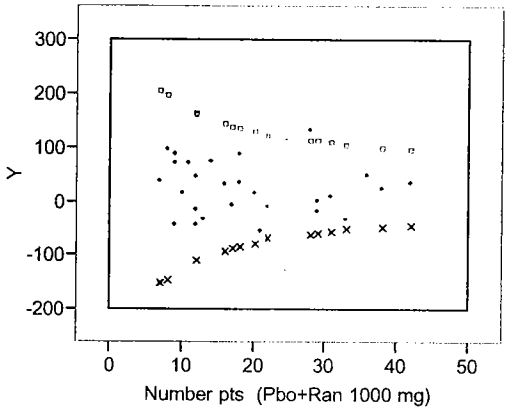
Funnel plot Analyses: To further explore the heterogeneity of the treatment effect by individual centers, the following funnel plot analyses were performed:

Funnel Plot 1.
Ranolazine Study CVT 3033, ITT Population,
Change from Baseline to Week 12 in ETT Duration at Trough
Ran SR 750 mg minus Placebo (Centers with less than 10 patients are pooled into 8 pooled sites in which they fell in the original sponsor's analysis). The outlier above the 95% CI is Siberian center #710.



Y × Lower 95% CI, 750mg
▫ Upper 95% CI, 750mg
◆ Ran 750 mg minus Placebo

Funnel Plot 2.
Ranolazine Study CVT 3033, ITT Population,
Change from Baseline to Week 12 in ETT Duration at Trough
Ran SR 1000 mg minus Placebo (Centers with less than 10 patients are pooled into 8 pooled sites in which they fell in the original sponsor's analysis). The outlier above the 95% CI is Siberian center #710.



Y × 1000 mg, Lower 95% CI
▫ 1000mg, Upper 95% CI
◆ Ran 1000 mg minus Placebo

Of the 118 individual centers, 19 centers had 10 or more patients[8]. In this analysis, Siberian site #710 in Barnaul with 42 (5%) patients seemed to be a possible outlier with highly statistically significant (placebo adjusted) treatment effects: 152 seconds ($p<0.001$) for Ran 750 mg and 136 seconds ($p=0.003$) for Ran 1000 mg. All other 18 centers had non-significant treatment effects.

In a subsequent sensitivity analysis excluding Site #710, the following results were obtained:

Table 10. CVT 3033: Change from Baseline in ETT duration (sec) at Trough and Peak at Week 12 (ITT LOCF) Excluding Site 710

Statistic	Ran SR 750 mg vs. Placebo	Ran SR 1000 mg vs. Placebo
Trough		
LS Mean Difference (SEM)	16.5 (11.1)	17.6 (11.2)
95% Confidence Interval	(-5.3, 38.3)	(-4.4, 39.7)
p-value	NS	NS
Peak		
LS Mean Difference (SEM)	29.4 (10.9)	24.1 (11.1)
95% Confidence Interval	(8, 50.8)	(2.3, 45.9)
p-value	0.007	0.03

Treatment Differences were compared using ANCOVA Model 1, including effects for treatment, baseline covariate, pooled site and background therapy using Type III sum of squares. Baseline covariate for trough measurement was the average of visits 1 and 2 data; baseline covariate for peak measurement was visit 2 data.

Reviewer: The trough effect size, excluding site 710, is smaller and comparison vs. placebo is not statistically significant. While the peak effects in this analysis remain statistically significant, note that site 710 was excluded based on effects at trough, not peak. One implication of the data is that trough effects are not robust; hence, excluding an "outlier" site will take away statistical significance.

To support the robustness of the primary efficacy analysis, the sponsor performed an alternative analysis of exercise time with individual center and treatment-by-center interaction as random effects (Table 4, August 6, 2003 submission) that shows results similar to the primary efficacy results in Study CVT 3033. If the exercise time in this center is randomly high, then the sponsor's analysis can support the robustness. However, if there is a systematic bias in Center #710, no statistical analysis can uncover it. The question remains whether there is a systematic or unquantifiable bias with this center.

Subgroups by gender, age, and presence of diabetes or heart failure:

Table 11: Change from baseline ETT (sec) *trough* Week 12 (ITT LOCF) ANCOVA Model 6*

	Ran SR 750 (N=272)		Ran SR 1000 (N=261)	
CHF	*Yes (N=87)*	*No (N=185)*	*Yes (N=76)*	*No (N=185)*
LS Mean Difference (SE) vs. placebo	2.1 (19.7)	34.7 (13)	26.9 (20.4)	22.2 (13)
95% CI	(-36.6, 40.8)	(9.2, 60.2)	(-13, 66.9)	(-3.3, 47.8)
p-value	NS	0.008	NS	0.087
Gender	*Female (N=59)*	*Male (N=213)*	*Female (N=51)*	*Male (N=210)*
LS Mean Difference (SE) vs. placebo	1.3 (22.5)	28.9 (12.4)	8.6 (23.4)	26.1 (12.5)
95% CI	(-42.9, 45.5)	(4.5, 53.2)	(-37.4, 54.6)	(1.6, 50.6)
p-value	NS	0.02	NS	0.037
Diabetes	*Yes (N=68)*	*No (N=204)*	*Yes (N=60)*	*No (N=201)*
LS Mean Difference (SE) vs. placebo	28.6 (22.8)	22.4 (12.5)	34.1 (23.5)	21.2 (12.5)
95% CI	(-16.1, 73.4)	(-2, 46.9)	(-12, 80.2)	(-3.4, 45.8)
p-value	NS	0.07	NS	0.09

[8] In this analysis, centers with less than 10 patients were combined into 8 pooled sites. The pooling was performed in such a way that centers with less than 10 patients fell in the pooled sites used in the original analysis. Source: Sponsor's Table ETT-7 in the June 6, 2003, submission.

Age (years)	*< 65 (N=140)*	*≥ 65 (N=132)*	*< 65 (N=134)*	*≥ 65 (N=127)*
LS Mean Difference (SE) vs. placebo	27.9 (15.5)	16.9 (15.3)	25.8 (15.6)	19.2 (15.4)
95% CI	(-2.5, 58.3)	(-13.1, 46.9)	(-4.9, 56.5)	(-11, 49.4)
p-value	0.07	NS	NS	NS

Source: Tables 2.0.13 -16. ANCOVA Model 6 includes effects for treatment, baseline covariate, pooled site, background therapy , subgoup, and treatment by subgroup interaction using type III sum of squares. P-values obtained from ANCOVA model adjusted for stated effects. Treatment by CHF, treatment by gender, treatment by diabetes and treatment by age interactions were not statistically significant.

Reviewer: The results of subgroup analyses by gender, race, age, show trends in the same direction (less so for females) with non-significant results for females, elderly and patients with CHF that are consistent across doses. It is entirely possible that ranolazine is less effective in females, the elderly, and patients with CHF. Given the wide confidence intervals and degree of confidence interval overlap between the various subgroups, the medical reviewer is wary of drawing definitive subgroup conclusions from the above analyses. It may be that the primary endpoint effect is modest, with statistically significant results rendered "nonsignificant" when the data are "sliced" in various ways.
The change from baseline in ETT duration was smaller for females compared to males in all groups, including placebo.

Secondary endpoints:
ETT Duration at peak plasma concentration:

Table 12. CVT 3033: Change from baseline in ETT duration (sec) *peak* Week 12 ITT LOCF (ANCOVA Model 1)

	Ran SR 750 mg vs. placebo	Ran SR 1000 mg vs. placebo
LS Mean difference (SE)	34 (10.7)	26.1 (10.8)
95% CI	(13.1, 55)	(4.9, 47.4)
p-value	0.001	0.016

Source: Table 2.1.0. Model 1 includes effects for treatment, baseline covariate, pooled site and background therapy using type III sum of squares. P-values obtained from ANCOVA model adjusted for stated effects. Baseline covariate is the Visit 2 data.

The Week 12 change from baseline in ETT duration at peak, in the efficacy-evaluable population, showed mean differences of 28.5 sec (95% CI: 2.7, 54.2) for ranolazine SR 750 mg vs. placebo, and 14.8 sec (95% CI: –11.3, 40.9, p=NS) for ranolazine SR 1000 mg vs. placebo (source: Table 2.1.1).

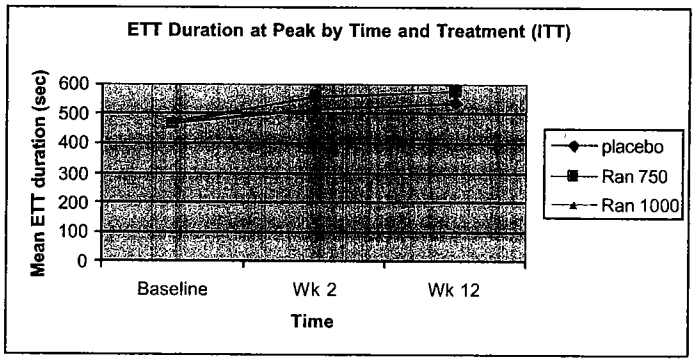
Change from baseline at Weeks 2 and 12 are presented below. For a given time point, the change from baseline at peak (vs. placebo) appeared larger in the 750 compared to the 1000 mg group.

Table 13. CVT 3033: Change from baseline in ETT duration at *peak* at Weeks 2 and 12 (ITT)

	R 750 vs. placebo		R1000 vs. placebo	
	Week 2	Week 12	Week 2	Week 12
LSM difference (SE)	51.2 (8.8)	34.2 (11.1)	41.7 (8.9)	24.3 (11.2)
95 % CI	34, 68.5	12.5, 55.9	24.2, 59.2	2.2, 46.3
p-value	<0.001	0.002	<0.001	0.03

Source: Table 2.1.5. LSM, SE and p-values calculated from ANCOVA model 1, including effects for treatment, baseline covariate, pooled site and background therapy using type III sum of squares. Baseline covariate was the Visit 2 data.

Figure 2. ETT duration at Peak by time and treatment (ITT). Source: Table 2.1.0.3. Baseline was the Visit 1 data. Means are unadjusted.



ETT duration at Peak: Subgroup analyses:
Background therapy:
There was a statistically significant difference between ranolazine and placebo for patients on diltiazem background therapy. P-values for ranolazine 750 and 1000 mg dose groups (on diltiazem) were 0.008 and 0.002, respectively.

Table 14. CVT 3033: Change from baseline to Week 12 in ETT duration at *Peak* (sec) by Treatment and Background Therapy (ITT LOCF)

	Ran 750 vs. placebo			Ran 1000 vs. placebo		
	Diltiazem	Atenolol	Amlodipine	Diltiazem	Atenolol	Amlodipine
LS mean difference (SE)	56.4 (21.1)	24.4 (16.1)	29.7 (19.2)	66.6 (21.8)	4.4 (16.4)	24.5 (19.3)
95% CI	14.9, 97.9	-7.2, 56	-8, 67.5	23.8, 109.4	-27.7, 36.5	-13.4, 62.5

Source: Table 2.1.2. LSM differences, SE, and p-values calculated from ANCOVA Model 2, including effects for treatment, baseline covariate, pooled site, background therapy and treatment by background therapy interaction using type III sum of squares.

When the change from baseline at Weeks 2 and 12 were analyzed by stratified background therapy, significant differences vs. placebo were seen at week 2 for diltiazem ($p < 0.001$) and amlodipine (both doses of ranolazine) and a borderline significant result ($p=0.054$) for atenolol only in the ranolazine 750 mg dose group; at Week 12, a statistically significant difference vs. placebo was seen at both doses only for the group on diltiazem.

Reviewer: The interaction with diltiazem appears to be consistent with findings seen in other studies, including pharmacokinetic studies.

Subgroup analysis: Interaction with Pooled Site: For the ranolazine 750 mg group, there were statistically significant differences vs. placebo for pooled sites 1, 7 and 8 ($p=0.04$, 0.02 and 0.03, respectively). For the ranolazine 1000 mg group, there were no statistically significant differences vs. placebo per pooled site.

Subgroups by gender, age, and presence of diabetes or heart failure:

Table 15. CVT 3033: Change from baseline ETT duration (sec) *peak* by CHF, Diabetes, Gender, Age (ITT LOCF)

	Ran SR 750 (N=272)		Ran SR 1000 (N=261)	
CHF	*Yes (N=86)*	*No (N=184)*	*Yes (N=76)*	*No (N=179)*
LS Mean Difference (SE) vs. placebo	23.4 (19.4)	39.4 (12.8)	43 (20)	18.9 (12.9)
95% CI	-14.7, 61.4	14.3, 64.6	3.8, 82.2	-6.5, 44.2

p-value	NS	0.002	0.032	NS
Gender	*Female (N=59)*	*Male (N=211)*	*Female (N=47)*	*Male (N=208)*
LS Mean Difference (SE) vs. placebo	-1.9 (22)	44.3 (12.2)	-12.7 (23.5)	35.3 (12.2)
95% CI	-45.1, 41.3	20.4, 68.2	-58.7, 33.4	11.3, 59.3
p-value	NS	<0.001	NS	0.004
Diabetes	*Yes (N=67)*	*No (N=203)*	*Yes (N=59)*	*No (N=196)*
LS Mean Difference (SE) vs. placebo	33.6 (22.3)	34.7 (12.2)	43.7 (23)	21.1 (12.3)
95% CI	-10.2, 77.4	10.8, 58.7	-1.5, 88.8	-3.1, 45.3
p-value	NS	0.005	0.058	0.087
Age (years)	*< 65 (N=139)*	*≥ 65 (N=131)*	*< 65 (N=133)*	*≥ 65 (N=122)*
LS Mean Difference (SE) vs. placebo	39.7 (15.2)	26.2 (15)	27.8 (15.3)	21.9 (15.2)
95% CI	10, 69.5	-3.3, 55.6	-2.3, 57.9	-8, 51.8
p-value	0.009	0.08	0.07	NS

Source: Tables 2.1.7, 2.1.7.1, 2.1.8, 2.1.8.1, 2.1.9, 2.1.9.1, 2.1.10, 2.1.10.1. LSM, SE and p-values from ANCOVA Model 6 with effects for treatment, baseline covariate, pooled site, background therapy, subgroup and treatment by subgroup interaction. Baseline covariate is the visit 2 data._According to the sponsor, treatment by subgroup interaction terms (above) were non-significant.

Reviewer: Subgroup analyses at the time of peak show trends in the opposite direction by gender. This finding, coupled with clinical pharmacology results, suggests a differential effect by gender. Trends favorable for ranolazine appear with respect to the elderly and CHF subgroups.

Time to Angina (trough and peak):

Results are presented below. At both trough and peak, the time to onset of angina showed statistically significant differences vs. placebo in favor of ranolazine. The effect size is larger for peak than trough. With the increase in dose from 750 to 1000 mg bid, the mean difference in the ITT population appears unchanged.

Table 16. CVT 3033: Change from baseline in Time to Onset of Angina (sec) at *Trough* Week 12 ITT LOCF (ANCOVA Model 1)

	Ran SR 750 mg vs. placebo	Ran SR 1000 mg vs. placebo
LS Mean difference (SE)	29.71 (12.07)	26.01 (12.2)
95% CI	(6, 53.4)	(2.1, 49.9)
p-value	0.014	0.033

Source: Table 2.4.0. Baseline covariate is the average of Visits 1 and 2 data. ANCOVA Model 1 inlcudes effects for treatment, baseline covariate, pooled site and background therapy using type III sum of squares.

The same analysis for the efficacy-evaluable population showed mean differences of 27.8 (p=0.06) and 42.99 (p=0.004) for the ranolazine SR 750 mg and 1000 mg (vs. placebo), respectively.

Table 17. CVT 3033: Change from baseline in Time to Onset of angina (sec) at *Peak* Week 12 ITT LOCF (ANCOVA Model 1)

	Ran SR 750 mg vs. placebo	Ran SR 1000 mg vs. placebo
Mean difference (LSM) (SE)	38.02 (12.38)	37.88 (12.56)
95% CI	(13.7, 62,3)	(13.2, 62.5)
p-value	0.002	0.003

Source: Table 2.5.0. See Table 17 for ANCOVA Model 1.

The same analysis for the efficacy-evaluable population showed mean differences (vs. placebo) of 30.09 (p=0.47) and 29.18 sec (p=0.57) for the ranolazine SR 750 and 1000 mg groups, respectively.

A survival analysis of the time to onset of angina (trough and peak levels of ranolazine) using a log rank test did not support the primary analysis and was only significant between ranolazine 750 mg and placebo at peak.

Time to 1 mm ST depression (trough and peak):
For this analysis, greater effect sizes, with statistically significant results, are seen at the time of peak but not trough.

Table 18. CVT 3033: Change from baseline in Time to Onset 1 mm ST depression (sec) *trough* and *peak* Week 12 ITT LOCF (ANCOVA Model 1)

	Ran SR 750 mg vs. placebo	Ran SR 1000 mg vs. placebo
Trough		
Mean difference (LSM) (SE)	19.9 (12.2)	21.1 (12.4)
95% CI	(-4.1, 43.9)	(-3.3, 45.5)
p-value	NS	0.09
Peak		
Mean difference (LSM) (SE)	40.8 (11.8)	34.5 (11.9)
95% CI	(17.6, 63.9)	(11.1, 58)
p-value	<0.001	0.004

Source: Tables 2.8.0, 2.9.0

Change in maximum ST depression (trough and peak):
Statistically significant results are not consistently seen (only noted at trough for the 750 mg bid group) in the change from baseline in maximum ST depression.

Table 19. CVT 3033: Change from baseline Maximum ST-Depression (mm) *trough* Week 12 ITT LOCF (ANCOVA Model 1)

	Ran SR 750 mg vs. placebo	Ran SR 1000 mg vs. placebo
Mean difference (LSM) (SE)	0.18 (0.07)	0.02 (0.07)
95% CI	(0.05, 0.31)	(-0.11, 0.15)
p-value	0.006	NS

Source: Table 2.2.0. Model 1 includes effects for treatment, baseline covariate, pooled site and background therapy using Type III sum of squares. P-values obtained from ANCOVA model adjusted for stated effects. Baseline covariate is the average of Visits 1 and 2 data.

Consistent 95% CI and p-values were seen in the same analysis for the efficacy-evaluable population (although the mean difference was –0.01 for the ran SR 1000 mg vs placebo group).

Table 20. CVT 3033: Change from baseline in Maximum ST depression (mm) *peak* Week 12 ITT LOCF (ANCOVA Model 1)

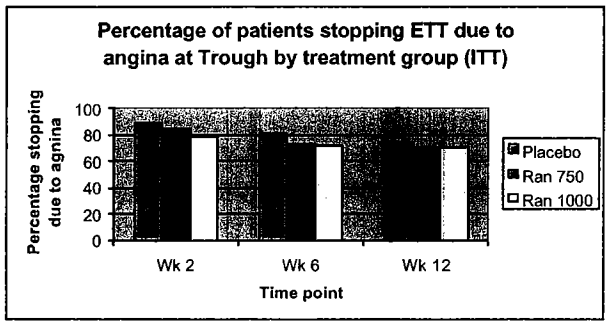
	Ran SR 750 mg vs. placebo	Ran SR 1000 mg vs. placebo
Mean difference (LSM) (SE)	0.1 (0.07)	0.03 (0.07)
95% CI	(-0.03, 0.23)	(-0.10, 0.16)
p-value	NS	NS

Source: Table 2.3.0. Please see preceding Table for further explanation of ANCOVA Model 1, p-values, baseline covariate.

The same analysis was consistent for the efficacy-evaluable population.

Reasons for Stopping ETT:
Percentage of patients stopping due to angina are presented below. For all 3 groups, including placebo, the percentage stopping due to angina decreased (and the percentage stopping due to overall fatigue increased) over time, especially (with regard to the ranolazine groups) from Weeks 2-6. The difference between Ran 750 and Ran 1000 at Week 12 was negligible.

Figure 3. Percentage of patients stopping ETT due to angina (trough, ITT)



Source: Table 2.12.0.

Pairwise comparisons (via CMH stratified for background therapy) at Weeks 2 and 6 were statistically significant for Ran 1000 vs. placebo; however, the same analysis was not statistically significant at 12 weeks. The same analysis was marginally significant (p=0.059) for Ran 750 only at the Week 6 timepoint. For the efficacy-evaluable population, statistically significant results, using the same CMH analysis, were only seen for the Ran 1000 vs. placebo group only at Week 2.

The percentage of patients stopping peak ETT due to angina also showed a decrease over time for all 3 groups, including placebo (with an increase in percentage, in all 3 groups) of patients stopping due to overall fatigue. The percentage stopping due to angina at Week 12 was the same for Ran 750 and 1000. Pairwise comparisons showed statistically significant differences for both dosage groups compared to placebo.

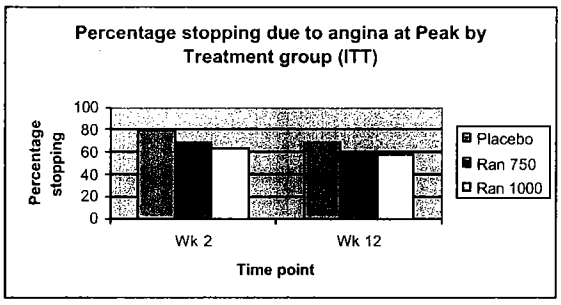


Figure 4. Percentage of patients stopping ETT due to angina at peak (ITT).

Source: Table 2.13.0. Pairwise comparisons of ranolazine 750 mg vs. placebo (via CMH stratified by background therapy) were statistically significant (p values of 0.006 and 0.011 at Weeks 2 and 12, respectively). Not surprisingly (judging from the graph), pairwise comparisons of ranolazine 1000mg vs. placebo (same analysis as above) were also statistically significant (p values of <0.001 and 0.011 at Weeks 2 and 12, respectively).

Other analyses:

Normalized frequency of angina during 12 Weeks by treatment (ITT): According to the sponsor, the normalized frequency of angina (i.e., total number of angina episodes during double-blind normalized by the number of weeks on double-blind) during the 12 week treatment period (ITT) showed a mean (SE) of 3.31 (0.3) events on placebo, 2.47 (0.23) on Ran 750 and 2.13 (0.24) events on Ran 1000. Using ranked scores data and non-parameteric ANCOVA (model fitted with ranked data for treatment (p=<0.001), baseline covariate (p= < 0.001), pooled site (p=0.015), and background therapy (p=0.88) using type III sum of squares), the sponsor calculated significant differences (p=0.006: Ran 750 vs. placebo; p=<0.001: Ran 1000 vs. placebo) for the two active treatment groups vs. placebo. A similar evaluation of the efficacy-evaluable population was consistent.

Maximum and Average Duration of angina during 12 weeks by treatment (ITT, evaluable): The sponsor performed a similar analysis using ranked scores data and non-parametric ANCOVA. No statistically significant difference for ranolazine vs. placebo was demonstrated.

Maximum Severity of angina during 12 weeks by treatment (ITT): The percentage of moderate angina was slightly higher in the placebo group (50.4%) compared to Ran 750 (47.6%) and Ran 1000 (44.4%). However, the incidence of severe angina was similar in all 3 treatment groups (32%). Pairwise comparisons (van Elteren test stratified by background therapy) did not show any statistically significant differences.

Median Severity of angina during 12 weeks compared to baseline (ITT) (Source: Table 2.17.25): Based on patients with at least one episode of angina at baseline and double-blind, 14-15% of patients worsened (essentially no change across treatment groups); 66% on placebo, 57% on Ran 750 and 59% on Ran 1000 were unchanged; and 19% on placebo, 30% on Ran 750 and 26% on Ran 1000 were improved from baseline.

Nitroglycerin consumption: Analysis of normalized nitroglycerin consumption (number of uses of nitroglycerin during double-blind normalized by the number of weeks on double-blind) for the ITT population showed a mean (SE) of 3.14 on placebo, 2.11 (0.27) on Ran 7509 and 1.76 (0.28) on Ran 1000. Statistics using ranked scored data and non-parameteric ANCOVA (model fitted with ranked data for treatment (p=0.002), baseline covariate (p=<0.001), pooled site (p=0.16) and background therapy (p=0.58) using type III sum of squares) showed statistically significant differences vs. placebo for Ran 750 (p=0.016) and 1000 (p <0.001) (Source: Tables 2.18.2, 2.18.2.1)

Rebound Effects:

The prespecified secondary efficacy parameter was the change from baseline in ETT duration at trough. This parameter is graphically depicted for both ITT and evaluable (eff) populations. For the ITT population, the change from baseline for Ran 1000/Placebo vs. Ran 1000 staying on therapy was borderline statistically significant (p=0.053,[9] mean change –33.9 sec). For the evaluable population, the same parameter resulted in a mean change of –55.5 sec (p=0.007). The other comparisons did not show statistically significant effects.

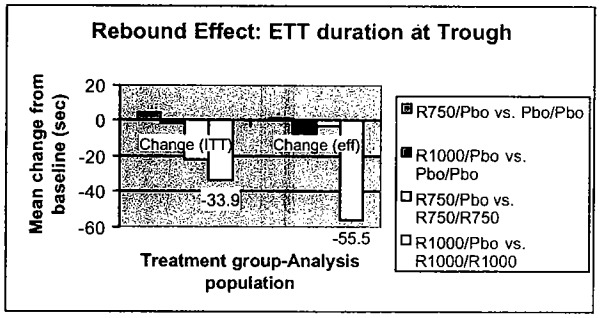


Figure 5. Rebound Assessment: ETT duration at trough.

Source: Table 2.19.0. Means are LSM estimates from ANCOVA model.

Reviewer: The results of withdrawal from therapy appear consistent with a treatment effect which appears attentuated after 48 hours of withdrawal from drug. Note that the R1000/placebo group (below) is similar to, and not worse than, placebo/placebo.

[9] P-values obtained from ANCOVA model adjusted for effects for treatment sequence (p=0.11), baseline covariate (p=0.003), pooled site (p < 0.001) and background therapy (p=0.70) using Type III sum of squares.

Table 21. CVT 3033: Mean change in ETT duration at Trough at the end of the Rebound Assessment Phase (ITT)

	Placebo/placebo	R 750/placebo	R1000/placebo	R750/R750	R1000/R1000
N	243	128	118	120	118
LS Mean (SE)	98.6 (9.2)	103.3 (12.1)	97.2 (12.8)	125.1 (12.7)	131.1 (12.7)

Source: Table 2.19.0.1.

Also, there were no meaningful changes in frequency or severity of angina during the rebound assessment period. Nor were increases in nitroglycerin consumption seen (Source: Tables 2.19.7 (frequency), 2.19.8 (duration), 2.19.9 and 2.19.10 (maximum severity), 2.19.11 and 2.19.12 (median severity), 2.19.13 (nitroglycerin consumption)).

Table 22. CVT 3033: Change in Angina during the Rebound Assessment by Treatment (ITT)

N (%)	Placebo/placebo	R750/Placebo	R1000/placebo	R750/R750	R1000/R1000
Maximum severity of angina-- worsened	9 (11)	0	2 (6)	1 (4)	1 (3)
Maximum severity of angina—no change or improved	72 (89)	31 (100)	34 (94)	27(96)	30 (97)
Median severity of angina--worsened	16 (20)	6 (19)	7 (19)	4 (14)	8 (26)
Median severity of angina—no change or improved	65 (80)	26 (81)	29 (81)	24 (86)	23 (74)

Source: Tables 2.19.10, 2.19.12

Ranolazine Plasma Concentrations:

Table 23. CVT 3033: Ranolazine Plasma Concentrations (ng/ml) at Week 12 at Trough and Peak during the Double-blind phase: safety population

	Ranolazine SR 750 mg	Ranolazine SR 1000 mg	Ranolazine SR 1000 vs. 750 mg (mean difference)
Trough			
Mean (SE)	1577.6 (71)	2164.7 (89.2)	592.0 (110.1)
p-value			<0.001
Peak			
Mean (SE)	2031.1 (78.8)	2607.1 (90)	567 (118)
p-value			<0.001

Source: Table 11P, Table 3.5.0., 3.6.0. P-values obtained from ANOVA Model 7 including effects for treatment, pooled site and background therapy using type III sum of squares. Mean difference (SE) are LS mean estimates from ANOVA.

For the Rebound Assessment, plasma concentrations are shown below. Comparisons of R1000/1000 vs. R750/R750, R1000/placebo vs. R1000/R1000, and R750/placebo vs. R750/R750 were statistically significant ($p < 0.001$). The comparison of R1000/placebo vs. R750/placebo was not statistically significant.

Table 24. CVT 3033: Ranolazine Plasma Concentrations (ng/ml) at Trough at Rebound: safety population

	R750/R750	SR750/Placebo	R1000/R1000	R1000/Placebo
N	119	124	118	117
LS Mean (SE)	1413.2 (76)	108.2 (72.9)	2080.1 (75.7)	128.3 (76.8)

Source: Table 3.5.1.1. LS mean and SE obtained from ANOVA Model 7 (see Table 18). Note: two placebo/placebo patients at Rebound had nonzero ranolazine plasma concentrations.

Safety: For a detailed safety discussion, please see the Safety Review.

Reviewer Comments:

1. This was an 823 patient, multicenter, randomized placebo-controlled parallel-group study including 12 weeks active treatment and a 2 week rebound assessment period. The study evaluated ranolazine SR in doses of 750 and 1000 mg po bid in a patient population with stable angina and symptom-limited ETT.
2. Patients were stratified to submaximal doses[10] of background therapy of amlodipine, atenolol or diltiazem.
3. Analysis of the primary endpoint, ETT duration at trough, showed a statistically significant improvement in ranolazine groups compared to placebo. However, this effect was modest and differences were not consistently significant when the data were further examined by subgroup. In addition, when a single site outlier with highly significant results was excluded, the treatment differences were small and not statistically significant.
4. The prespecified primary analysis (ETT duration at trough, ITT) did not demonstrate a dose-response relationship at these dosages in this study.
5. Statistically significant changes from baseline to endpoint at *trough*, compared to placebo, were also seen with respect to time to onset of angina and time to onset of 1 mm ST depression.
6. Statistically significant effects at *peak* were seen with respect to: ETT duration, time to onset of angina, time to onset of 1 mm ST depression, and percentage of patients stopping ETT due to angina. The change in maximum ST depression did not show consistently significant results.
7. A statistically significant interaction with diltiazem was seen with respect to ETT duration at peak.
8. Results of the normalized frequency of angina during 12 weeks showed a significant decrease in mean events in ranolazine groups compared to placebo. Analyses of nitroglycerin consumption were also consistent with these findings.
9. Subgroup analyses (trough and peak) by gender showed differential effects. Results at peak, in the female subgroup, were favorable toward placebo.
10. A clinically significant 48 hour rebound effect was not seen when ranolazine, at these doses, was withdrawn.
11. Withdrawal of ranolazine after 12 weeks of treatment show a marginally statistically significant treatment effect (at trough) in the ranolazine 1000 bid group.

CVT 3031:

Title: A Double-Blind, Placebo-Controlled, 4-Period Cross-Over, Multiple-Dose Study of Ranolazine SR as Monotherapy for Chronic Stable Angina Pectoris at Doses of 500 mg bid, 1000 mg bid and 1500 mg bid. (Protocol date: September 3, 1997; amended October 29, 1997; April 8, 1998; August 25, 1998; December 28, 1998)

Primary objective: Determine effect of ranolazine SR monotherapy at doses of 500 mg bid, 1000 mg bid and 1500 mg bid, compared to placebo, on treadmill exercise duration at the time of trough ranolazine plasma levels (12 hours postdose).

Secondary objectives:
1. Determine effect of the three doses of ranolazine SR, compared to placebo, on time to onset of angina and time to 1 mm ST depression during exercise treadmill testing (ETT) at trough ;
2. Determine effect of the three doses of ranolazine SR, compared to placebo, on exercise duration, time to onset of angina, and time to 1 mm ST depression during ETT at the approximate time of peak ranolazine plasma levels (4 hours postdose; "peak").

Patient population: An enrollment of 203 patients with chronic stable angina, responding to antianginal therapy, was planned in order to yield 152 evaluable patients.

[10] Source: respective labeling of these medications.

Study summary: This was a double-blind, randomized, placebo-controlled, multiple-dose, 4-period crossover study. The study was composed of two phases: a single-blind placebo qualifying phase (2 screening visits) of about 1 week duration and a placebo-controlled double-blind phase lasting about 4 weeks, with a follow-up visit scheduled 2 weeks after completion of the double-blind phase.

Patients meeting inclusion/exclusion criteria entered the single-blind qualifying phase, where they underwent physical examination, laboratory testing, ECGs and ETTs. Those qualifying at Visit 2 entered the 4 week double-blind phase (Visits 3, 4, 5 and 6). Each week the patient received one of three active ranolazine treatments (500, 100 and 1500 mg bid) or placebo (given bid). Patients received one week treatment with each of the three dose regimens and placebo in random order. At each of the 4 double-blind visits, patients underwent trough ETT (12 hours after their previous drug dose from the evening before). Following the trough ETT, patients were given the final dose of that week's double-blind treatment (in clinic) and then underwent peak ETT 4 fours after that in-clinic dose.

Blood samples for peak/trough plasma levels, vital signs, hemodynamic measurements, and ECGs were collected at each of the 4 double-blind visits. Laboratory tests were performed at the first screening visit and at the end of the double-blind phase. Where available, ACTH challenge testing was performed at the second screening visit and at the final double-blind visit.

Table 1. CVT 3031: Inclusion/exclusion criteria (single-blind phase)

Inclusion criteria	Exclusion criteria
1. $\geq$ 21 years old 2. at least 3 month history of chronic stable effort angina, relieved by rest and/or sublingual nitroglycerin 3. coronary disease documented by any one or more of: angiographic evidence of $\geq$ 60% stenosis of one or more major coronary arteries; history of MI documented by positive enzymes or ECG changes 4. improvement/control of angina/ischemia with at least one of the following: beta blockers, calcium channel blockers, long-acting nitrates 5. willingness to discontinue antianginal therapy 48 hours before Visit 1 and for the duration of the study 6. willingness to maintain stable tobacco usage habits throughout the study 7. signed an approved consent form. 8. female patients of childbearing potential who are not breastfeeding, who have a negative pregnancy test and have no intention to become pregnant during the study, and who use contraception.	1. ECG abnormality interfering with ETT interpretation or associated with false positive results 2. NYHA Class III-IV CHF 3. Clinically significant valvular or congenital heart disease 4. Unstable angina, MI, CABG, PCI within the past 2 months 5. 2^{nd} /3^{rd} degree AV block or uncontrolled cardiac arrhythmia or life-threatening ventricular arrhythmias unassociated with MI 6. QTc > .50 seconds at Visit 1 7. Requiring medications known to prolong QTc 8. Requiring medications which affect cytochrome P450 3A4 9. Unwillingness to refrain from grapefruit juice 10. Requiring digoxin 11. Acute myocarditis/pericarditis 12. Hypertrophic cardiomyopathy 13. Uncontrolled hypertension or SBP < 100 mm Hg 14. Chronic illness, clinically significant laboratory abnormality, participation in another study $\leq$ 1 month before this trial.

Qualifying criteria for double-blind:
See qualifying criteria for double-blind in CVT 3033 as these were the same in CVT 3031.

Concomitant antianginal medications:
Concomitant beta-blockers, calcium channel-blockers and long-acting nitrates were not allowed during the study. Aspirin was permitted during the study. Sublingual nitroglycerin was allowed for the treatment of acute anginal episodes, but was not to be used within 60 minutes of the ETT. Opthalmic beta-blockers were allowed if their use was constant throughout the study.
Patients who elected not to enroll in the long-term, follow-up study (CV 3032) were allowed to resume their prior antianginal medications after completion of procedures at Visit 6 (Termination/Early Withdrawal).

Efficacy evaluations:
All patients were to have ETT under uniform conditions, optimally by the same personnel each time. Trough ETT were planned between 7:00 am and noon, 12 ± ½ hour after their prior evening dose. Patients were to stop smoking at least 2 hours before testing.
Efficacy variables were: time to onset of angina, exercise duration, primary reason for stopping exercise; ECG variables from each ETT were: time to 1 mm ST depression, maximum ST depression during exercise.

Criteria for Patient Removal:
1. Serious adverse event
2. Grossly noncompliant
3. Continued participation would jeopardize patient health
4. QTc widens to 130% of baseline duration and longer than 500 msec;
5. Unsatisfactory therapeutic response/investigator judgment
6. Patient wishes to withdraw
7. Sponsor elects to end the study.

Patients who withdrew early from the study were to be replaced by another patient who was randomized to the same sequence as the patient who prematurely withdrew.

Safety evaluation: adverse event monitoring, vital signs, ECG, and routine laboratory tests. An ACTH stimulation test, with collection of cortisol levels was planned at Visits 2 and 6.

Statistics:
According to the sponsor, carryover effects on treadmill efficacy parameters were not expected in CVT 3031; this expectation was based on two previous crossover studies of IR ranolazine, which suggested that drug effect on treadmill efficacy was influenced primarily by plasma level at the time of ETT and no first-order carryover effects were seen.

Efficacy analyses were planned on:
1. Near/all Completers, all randomized patients with evaluable efficacy measurements at baseline and for at least 3 our of the 4 double-blind periods, irrespective of protocol violations. If this population included 75% or more of the randomized patients, then it will be the primary analysis population (otherwise the primary analysis population will be ITT, below (#2));
2. Intent to treat (ITT), consisting of all randomized patients with evaluable efficacy measurements at baseline and for at least one double-blind period, irrespective of protocol violations.
3. First-period population, consisting of all randomized patients with evaluable efficacy measurement at baseline and from the first double-blind treatment period, irrespective of protocol violations;
4. Per-protocol population, consisting of all randomized patients with evaluable efficacy measurement at baseline and with at least 3 out of 4 treatment periods completed in accordance with the protocol.

The baseline efficacy measurement was defined as the average from the two ETT performed during the single-blind placebo phase, or if only one ETT was done, the single measurement from this phase.

The primary efficacy variable was ETT duration at the time of trough ranolazine plasma levels (12 hours postdose).

Secondary efficacy variables:
1. Time to onset of angina during ETT at trough;
2. Time to 1 mm ST depression during ETT at trough;
3. Exercise duration during ETT at time of peak levels;
4. Time to onset of angina during ETT at the time of peak levels;
5. Time to 1 mm ST depression during ETT at the time of peak levels;
6. Maximum ST depression during exercise;
7. Primary reason for stopping test.

Efficacy analyses (as outlined in the protocol):
If 75% or more randomized patients complete at least three of four periods, then the primary efficacy analysis population will be the all/near completers, and the analysis will be a standard crossover ANCOVA with treatment, period and patient as factors. A secondary efficacy analysis based on ITT will be done. The first period population will be analyzed using ANCOVA with terms for treatment and baseline value. The per-protocol population will be analyzed using ANCOVA for crossover design.

Safety variables: history, physical examination, vital signs, AE, laboratory tests, concomitant medications.

Sample Size calculation:
The sample size estimate was based on ETT duration at trough plasma levels of ranolazine. Based on a previous study, a standard deviation of difference in exercise duration of 95 seconds was selected to use in the sample size calculation. A sample of 152 patients would be sufficient for declaring a statistically significant mean difference of 25 seconds between a result on active treatment vs placebo at the 5% level with a power greater than 90%.

Reviewer: No washout period was done between treatment periods during double-blind phase.

Protocol Amendments:
1. Amendment 1: Oct. 29, 1997: Sample size increased to 203 enrolled in order to discriminate a smaller change in exercise duration; number of antianginal medications required to show response decreased from two to one; blood draw amount corrected in informed consent; added analysis population to include near completers and ITT; defined baseline variable; added secondary efficacy variables: primary reason for stopping test and maximum ST depression during exercise; defined ANCOVA factors on the all/near-completers population (ITT planned as secondary analysis). Other secondary analyses: ANCOVA to investigate and rule out carryover effects and center by treatment interaction; GEE (including effects for baseline period, treatment and poassibly other patient level covariates) to fit linear models to primary efficacy outcome.
2. Amendment 2: April 8, 1998: Defined serious adverse events per ICH guidelines; added information regarding ranolazine metabolism (cytochrome P450); clarified prohibited medications.
3. Amendment 3: August 25, 1998: Added prohibited medications (cytochrome P450 inducers); altered language to permit international sites; changed primary analysis population to all/near completers if this population included 75% or more of randomized patients; added that patients must discontinue antianginals for at least 48 hours prior to Visit 1.
4. Amendment 4: December 28, 1998: Added list of substances modifying CYP 3A4 activity (including grapefruit juice).

Interim Analyses: None performed in this study.

Results: Fifty-two sites recruited patients in the US (113 patients), Canada (15), Czech Republic (36) and Poland (27).

Patient Disposition: A total of 191 patients were randomized into 4 treatment sequences (ABCD, BDAC, CADB and CDBA where A = 500 mg bid, B = 1000 mg bid, C = 1500 mg bid and D = placebo).

A B C D
B D A C
C A D B
D C B A

There were 45-50 patients randomized to each treatment sequence; the numbers of patients receiving each treatment (i.e., placebo, ranolazine 500 mg bid, 1000 mg bid or 1500 mg bid) were 179-187. A total of 175 patients (92%) were included in the near/all completer population, 185 patients (97%) in the ITT population, 184 (96%) in the first period population, 135 (71%) in the per-protocol population, and 191 (100%) in the safety population. Twenty-three patients (12%) discontinued the study before completing all

trough and peak assessments at all treatment periods. Fifteen (8%) patients discontinued prematurely due to AE (11 of these were in the highest dose ranolazine group). One hundred forty-six patients (76%) enrolled in a long-term follow-up study (CVT 3032). There was one death in the Ran SR 500 mg group.

Baseline characteristics:

Except for gender (p=0.05, higher percentage males in the ABCD and BDAC sequences), baseline characteristics appeared to be balanced among treatment sequences. Statistically significant differences were seen with regard to diabetics on insulin (p=0.02), history of unstable angina (p=0.037) and prior stroke (p=0.03); however the numerical differences between groups was small.

Mean age was about 64 years (39-85 range) and about half of the patients were 65 years and older. The safety population was about 90% Caucasian and 4-8% Black. Mean weight was about 83 kg and height about 171 cm. About half had a prior MI, about 13-20% had a history of CHF, and 28% had a prior CABG. About 60-70% had a history of hypertension.

Efficacy:

The sponsor's analysis of the primary efficacy variable is presented below. According to the sponsor, a large effect size, statistically significant at all 3 doses, was seen. The results were consistent between ITT and all/near completers. An increase in mean difference was seen with increasing dose. The sponsor did not find statistically significant treatment by pooled site interaction, treatment-by-period interaction, or carryover effect[11]. Results for the per-protocol population (both peak and trough) were consistent. A supportive GEE analysis showed that addition of gender, unstable angina and history of stroke as variables into the GEE model did not alter results for treatment differences.

Mean Difference Compared to Placebo in ETT Duration at Trough Levels of Study Drug (sec) All/Near Completers Population and ITT Population

Population	Statistic	Avg of (Ran SR 1500 mg and Ran SR 1000 mg)	Ran SR 1500 mg vs Placebo	Ran SR 1000 mg vs Placebo	Ran SR 500 mg vs Placebo
All/Near Completers[1]	Mean Difference	39.8	45.9	33.7	23.8
	S. E. of Mean Difference	6.9	8.6	8.0	7.9
	95% Confidence Interval	[26.3,53.3]	[30.2,62.7]	[18.1,49.2]	[8.2,39.4]
	P-value	< 0.001**	< 0.001**	< 0.001**	0.003**
ITT[2]	Mean Difference	40.5	45.9	35.1	22.8
	S. E. of Mean Difference	7.9	8.5	8.8	8.4
	95% Confidence Interval	[25.1,55.8]	[29.2,62.6]	[17.9,52.3]	[6.3,39.4]
	P-value	< 0.001	< 0.001**	< 0.001**	0.007**

* 0.010 < p-value ≤ 0.050; ** p-value ≤ 0.010
[1] All/NC Population analyzed using ANOVA for cross-over study design with effects for pooled site, patient within pooled site, period, and treatment.
[2] ITT Population analyzed using GEE with effects for baseline ETT duration, pooled site, period, and treatments.
Note: Multiple comparisons adjusted for using closed testing and union intersection principles.
Note: Ran SR = Ranolazine SR
Data Source: Tables 2.1.2 and 2.2.2

Table 2. Primary Efficacy variable (analysis by sponsor).

The sponsor's results for peak values also showed statistically significant increases vs. placebo and increasing difference (vs. placebo) with increased dose.

[11] In the Statistical Methods section, the sponsor's reference # 14 is a monograph by Stephen Senn, Crossover Trials in Clinical Research. In the introductory chapter, and in Sections 3.8 - 3.11, the author states that tests for carryover are virtually impossible to interpret: "I do not carry out tests for carryover and do not advise the reader to do so". In Section 10.3, "Five Reasons for Believing that the Simple Carryover Model is not Useful", and also in Section 1.8, Stephen Senn explains that including carryover in the model requires restrictive assumptions about the nature of the possible carryover. If slightly different forms of carryover hold, then the model is useless. Instead, the author recommends to carry out many studies with different designs to support the results of the crossover studies.

Best Possible Copy

As with the trough results, the sponsor found no significant treatment by pooled site interaction, treatment-by-period interaction or carryover effect. The GEE analysis was consistent with the above ANOVA.

Mean Difference Compared to Placebo in ETT Duration at Peak Levels of Ranolazine (sec) All/Near Completers Population and ITT Population

Population	Statistic	Ran SR 1500 mg vs Placebo	Ran SR 1000 mg vs Placebo	Ran SR 500 mg vs Placebo
All/Near Completers [1]	Mean Difference	55.5	50.1	29.3
	S. E. of Mean Difference	7.3	7.2	7.2
	95% Confidence Interval	[41.2, 69.8]	[36.0, 64.2]	[15.2, 43.4]
	P-value	<0.001**	<0.001**	<0.001**
ITT [2]	Mean Difference	55.6	51.5	28.7
	S. E. of Mean Difference	7.8	7.3	7.2
	95% Confidence Interval	[40.4, 70.8]	[37.3, 65.8]	[14.6, 42.9]
	P-value	<0.001**	<0.001**	<0.001**

* 0.010 < p-value ≤ 0.050; ** p-value ≤ 0.010.
[1] A/NC Population analyzed using ANOVA for cross-over study design with effects for pooled site, patient within pooled site, period, and treatment
[2] ITT Population analyzed using GEE with effects for baseline ETT duration, pooled site, period, and treatment.
Note: Multiple comparisons adjusted for using closed testing and union intersection principles.
Note: Ran SR = Ranolazine SR
Data Source: Tables 2.1.2 and 2.2.2

Table 3. ETT duration at Peak (sponsor's analysis)

Reviewer's findings:

In study 3031, the sponsor's results for exercise duration were troubling to the reviewers and difficult to interpret:

1. There were very large differences in numerical increases of exercise time with ranolazine between periods. The first period was very different from the later periods. In the first period, each ranolazine dose had a small increase in exercise time as compared to placebo and there was no clear dose response. In the second and later periods, there was a very large increase in exercise time in favor of ranolazine.

Table 4. Exercise duration at Trough by Period (study 3031).

Period	Statistic	Ran 500 mg vs. placebo (A vs. D)	Ran 1000 mg vs. placebo (B vs. D)	Ran 1500 mg vs. placebo (C vs. D)
1	Mean difference	*11.7*	*12.7*	*4.5*
	p-value	0.59	0.55	0.83
2	Mean difference	*7*	*42*	*41*
	p-value	0.77	0.071	0.084
3	Mean difference	*34*	*57*	*68*
	p-value	0.17	0.026	0.008
4	Mean difference	*37*	*34*	*67*
	p-value	0.16	0.20	0.013

Source: Sponsor's Table 2.10.0, Vol. 146

As shown in Table 4, Ran 1500 mg had a very small effect of 4.5 seconds in the first period and a 9 - 15 fold increase in later periods. Likewise, ranolazine 1000 mg had a small effect of 12.7 seconds in the first period and 3 - 4 fold increase in later periods. A small effect of 34 seconds in the fourth period was observed probably because ranolazine 1000 mg immediately followed placebo in the sequence CADB. Ranolazine 500 mg also showed the tendency of a large increase in the third and fourth periods.

These observations seem to suggest the possible presence of treatment-by-period interactions that make it very difficult to interpret the results of the sponsor's crossover analysis pooling all periods.

The sponsor presented a non-significant p-value, p=0.62, for treatment-by-period interaction and stated that there was no treatment-by-period interactions. However, the study was planned to detect a treatment effect based on the crossover analysis pooling all the periods and assuming no treatment-by-period interactions. Thus, it can be expected that the sample size may not be sufficient to test treatment-by-period interactions. In the reviewer's view, the sponsor's non-significant p-values in the tests for these effects have no practical value.

2. The study showed a large period effect as shown in Table 5 (this is also the sponsor's Table ET13A). In the April 30, 2003 submission, the sponsor stated that the strong period effect in the study represented a training effect or learning effect.[12] If we accept the sponsor's explanation, then Table 5 implies that the learning effects for ranolazine doses seemed to be numerically much larger than the learning effect for placebo. For placebo, learning effect was smaller and not significant (p=0.50). In contrast, for ranolazine doses, learning effect was much stronger (nominal p-value ranged from p=0.027 for Ran 1000 mg to p<0.001 for Ran 1500 mg). Does this mean that ranolazine promotes learning effect? If so, should the promotion of learning effect be counted as a clinical benefit of ranolazine?

Table 5. Differential Period (Learning) Effects in Study CVT 3031

Comparison	Statistics	Treatment			
		Placebo	Ran SR 500 mg	Ran SR 1000 mg	Ran SR 1500 mg
Exercise Duration at Trough (sec.), Period 4 minus Period 1	Mean Difference	36	70	54	97
	P-value	0.15	0.005	0.024	<0.001
Exercise Duration at Trough (sec.), Period 3 minus Period 1	Mean Difference	25	42	64	82
	P-value	0.32	0.046	0.004	<0.001
Exercise Duration at Trough (sec.), Period 2 minus Period 1	Mean Difference	12	4	29	45
	P-value	0.62	0.84	0.16	0.044
Test for Period Effect Over All Four Periods	P-value	0.50	0.018	0.027	<0.001

Source: Sponsor's Table ET 13A, 17 June 2003 Submission.

3. The numerical pattern in Table 5A seems to suggest possible differential carryover effects for the two higher ranolazine doses. Longer exercise duration was observed when following a ranolazine dose, compared with following placebo, though the sponsor argued that there was no carryover effect (p=0.51). However, the sponsor's further analysis adjusting for 1st order carryover effect (Table 2, August 06, 2003 submission) seems to show little impact of possible carryover effect, if any (see footnote 11).

Table 5A. Placebo-Subtracted Exercise Duration by Preceding Treatment

Ranolazine treatment (Period)	Treatment effect in the First period	Preceding Treatment			
		Placebo	Ran SR 500 mg	Ran SR 1000 mg	Ran SR 1500 mg
Ran SR 500 mg	11.7 (1)	34 (3)	--	37 (4)	7 (2)
Ran SR 1000 mg	12.7 (1)	34 (4)	42 (2)	--	57 (3)
Ran SR 1500 mg	4.5 (1)	41 (2)	67 (4)	68 (3)	--

First Period Population:

Because of the strong suggestion of treatment-by-period interaction, the first period data were analyzed to obtain unbiased estimates of ranolazine effects (Table 6). Of 184 patients in the first period population who underwent ETT at trough levels of study drug in Period 1, forty-five received placebo, 45 received ranolazine SR 500 mg, 49 received ranolazine SR 1000 mg, and 45 received ranolazine SR 1500 mg. The same numbers of patients underwent ETT at peak levels of study drug. For trough, the first period data at

[12] " Carryover effects can have a variety of forms. They can be learning effects or fatigue effects, having, respectively, a positive or negative effect on the response, or they can be of a psychological, rather than of a physical, form." (From: Ratkowsky DA et. al. Crossover Experiments. Marcel Dekker, Inc. 1993).

Comparison of AUC Values

The comparisons of interspecies area-under-the-curve values is summarized below in the sponsor's tables.

Comparison of Ranolazine $AUC_{0\text{-}24h}$ from Humans, Rats and Mice

Species	Daily Dose	$AUC_{0\text{-}24h}$ (ng/mL·hr)	Multiple of Human Exposure
Human	30 mg/kg	55000	1X
Rat	150 mg/kg	210000	4X
Mouse	50 mg/kg	13000	0.24X

Area-under-the-curve that includes the metabolites, as determined from radiolabelled studies, is summarized in the sponsor's table below.

Comparison of Ranolazine Total $AUC_{0\text{-}\infty}$ from Humans, Rats and Mice

Species	Daily Dose	Approx $AUC_{0\text{-}\infty}$* (ng/mL·hr)	Multiple of Human Exposure
Human†	30 mg/kg	332000	1X
Rat††	150 mg/kg	245000	0.7X
Mouse	50 mg/kg	48714	0.15X

* Sum of ranolazine and all plasma metabolites from studies with ^{14}C-ranolazine.
† Data extrapolated from 0.5 mg/kg to 28.6 mg/kg daily human dose.
†† Intrapolated value between 5 mg/kg and 250 mg/kg.

Plasma exposure for the rats was determined at the end of the 91-week study using 2 rats per sex per dose. Concentration was determined using an HPLC method with fluorescence detection.

Conclusion: The highest dose in the rat study provides 0.7 – 4X the projected human exposure. The mouse plasma exposure gives 0.15- 0.24X the therapeutic human plasma level.

II. GENOTOXICITY/MUTAGENICITY ASSAYS

Ames Salmonella Assay
No specifics are given as to the strains used. Eight doses were used, from 1.0 µg to 10, 000 µg/plate, ±S9. Appropriate positive controls were used. Results were stated as no evidence for mutagenic potential.

Mutagenicity Evaluation by the Ames Salmonella/Microsome and Mitotic Conversion Assay with Yeast Strain D4 Plate Test

The test system was described as the Salmonella strains used in the above assay. Saccharomyces cerevisiae, D4 strain, heteroallelic at the adenine 2 and tryptophan 5 loci, thus resulting in nutritional deficiencies which prohibit the cells from growing on minimal or single supplemental media. Mitotic gene conversion results in the expression of a functional gene and the loss of nutritional requirement. Eight concentrations from 1.0 µg/plate to 10,000 µg/plate were tested ±S9. Appropriate positive controls were used. The results were reported as no evidence for mutagenic potential.

E.coli Reverse Mutation Preincubation Assay
Strain WP2*uvr*A was used ±S9 activation. Concentrations 0.3-5000 µg/plate were used. No ranolazine toxicity was observed in the absence of microsomal enzymes, but greater than 50% reduction in colonies per plate occurred in the presence of S9 activation at 3,333 and 5000 µg/plate. There was no increase in the number of trp+ revertants in the presence of ranolazine either ±S9 activation.

Mutagenic Evaluation by the In Vivo Micronucleus Assay
Strain ICR mice were orally dosed with 30, 100, 94 or 300 mg/kg. After 24 hours of exposure, the mice were euthanized and bone marrow smears prepared. It was reported that ranolazine (RS-43285) did not induce a significant increase in micronuclei.

CHO/HGPRT Mutation Assay
CHO-K1-BH4 cells were used. Concentrations of 200-1000µg/ml were tested ±S9 activation. Appropriate positive controls were used. Ranolazine did not produce genotoxic effects under the conditions of the assay.

III. STUDIES IN MICE

A. Three Month Oral Dose-Ranging Study in Mice

Study Dates: 2/17/1987 to 2/24/87
Report dated 9/7/93

Ten mice CD-1(ICR)BR) per sex per group were dosed at levels of 5,50, 100 and 200 mg/kg/day. Death was seen in the first week of the study in the 50, 100 and 200 mg/kg/day groups. This is summarized in the table below.

Summary of Animals Dying in First Week of Dosing

Dose Group (mg/kg/day)	Animals Dying In first Week	
	Males	Females
50	2/10	
100	1/10	2/10
200	2/10	4/10

The sponsor terminated the study at the end of the first week. During this short time period, there were no marked changes reported for body weight, food consumption or histopathology.

B. RS-43285: Two-Year Oral Carcinogenicity Study in Mice

CD-1(ICR)BR mice were given 5, 15 and 50 mg/kg of ranolazine as an oral gavage. The vehicle used for the control group was not specified. Fifty mice/sex/group were used.

Survival: Twenty-six animals died in the first two weeks of the study and were replaced. The deaths were reported to be evenly distributed throughout the groups. The sponsor's analysis showed no effect on mortality. CDER analysis indicated a marginally statistically significant effect on survival in the male mice.

Body weight and Food Consumption: The original review states that overall there was no drug-related effect on body weight or food consumption.

Non-Neoplastic Findings
No findings of significance reported.

Neoplastic Findings

There was an increased incidence of interstitial testicular tumors. The distribution of the tumor is shown below:

Incidence of testicular tumors in ranolazine-treated mice

Incidence of interstitial testicular tumors in mice	C1	C2	LD	MD	HD
	2/50	3/50	0/50	0/50	6/50

The sponsor's tables are attached as Appendix II.

IV. STUDIES IN RATS

A. 91/93 Day Oral Toxicity Study in Rats

Study start and completion dates: September 1984 and December 1984
Sprague-Dawley rats of the CD strain were gavaged with either 0, 5, 50, 250 or 500 mg/kg/day. Neither the dosing vehicle nor vehicle control group was specified.

Signs and Mortality
The HD group had total unscheduled mortality of 3/12m and 4/12f. Clinical signs included prostration, convulsions and hyperventilation, described as most prominent at the highest dose. There was no indication as to the specific incidence, timing and duration of the signs. It was not specified as to the specific doses associated with the signs. The sponsors considered this consistent with a central nervous system site of pharmacological effect with the adverse effects indicating a disturbance in the balance of fat/carbohydrate utilization and storage.

Body Weight and Food Consumption
Body weights in the HD group were listed as significantly lower than the control but percentage was not specified. The HD f were reported as having decreased food consumption (amount unspecified).

Hematology
A decrease in RBC was reported for 250 and 500 mg/kg groups of both sexes, peaking at 12 weeks. In females, the effect was seen earlier (4-8 weeks). There were corresponding decreases in hematocrit ($p \leq 0.05$) and increases in MCH and MCHC ($p \leq 0.05$).

Clinical Chemistry
The summary of clinical chemistry changes is shown in the table below. Quantitative details of the alterations were not provided.

Summary of clinical chemistry changes (MD = 250 mg/kg)

MD f, HD m + HD F	↑Alk phos
HD m + HD f	↓Serum albumin, ↓total protein
HD m + HD f	↓Blood urea nitrogen
MD, HD	↓Na, ↑glucose

Pathology

No gross alterations were reported.

The summary of pathological findings is shown in the table below.

Summary of pathological findings (MD=250 mg/kg)

MD, HD	Adrenal glands enlarged
All dose groups	↑absolute and relative adrenal weights, significant in the MD and HD groups
Dose-dependent MD, HD males (3/12 + 12/12), HD f 7/12	Vacuolation of adrenals
HD m+f	↑absolute and relative liver weight
1/12 HD m and 7/12 HD f	Centrilobular hepatocyte enlargement
MD and HD m	↑kidney weights
HD m (2/12 vs 1/12 controls)+ f (6/12 vs 0/12 controls)	Lungs:alveolar foamy cell proliferation
MD + HD m+ f	↑thyroid weight and ↑spleen weight

B. Other Rat Studies

A 6-month study and a 1-year were conducted at doses of 5, 50 and 200 mg/kg and 2,20,50 and 200 mg/kg/day respectively. The previous reviewer considered that the MTD of ranolazine was not clearly identified. There were no treatment-related effects on body weight, food consumption, ophthalmoscopy or urinalysis. There were dose-related changes in adrenal size, weight and histopath (foamy cytoplasm). Other findings were clinical chemistry changes in liver function indicators and changes in RBC morphology generally associated with altered liver function.

C. Rat Oral Carcinogenicity Study

Sprague-Dawley rats were orally gavaged with 5, 50, 150 mg/kg/day of ranolazine. The intended duration of the study was 104 weeks. It was terminated at 89-91 weeks because the sponsor felt that there was a decreased overall survival that warranted such action.

Survival in the HD male rats was significantly lower (p=0.036) than in the control group. Differences in female survival did not reach statistical significance. Survival and mortality is summarized in the two tables from the original reviewer (E. Barry).

Rat Mortalities and Approximate Time of Occurrence

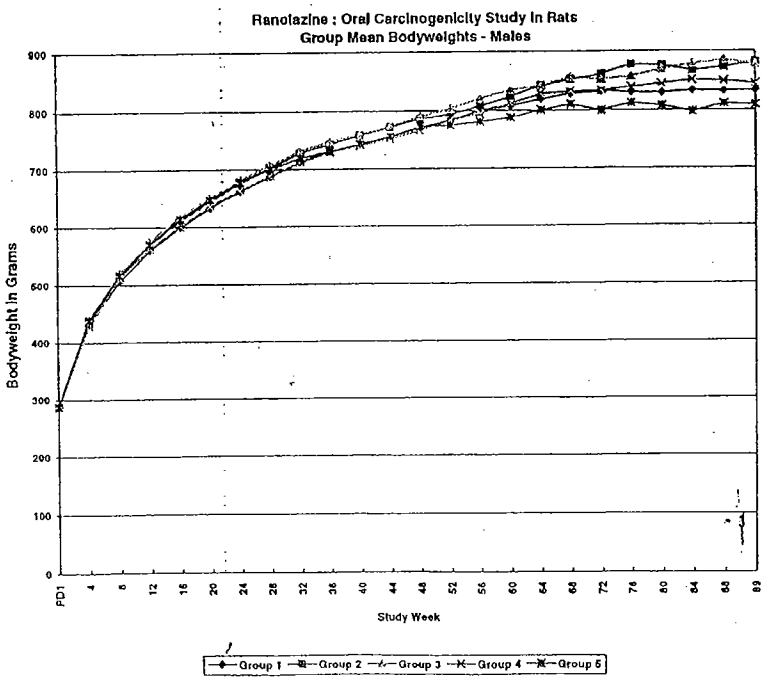
Dose (mg/kg)	Males (% dead by week on study)	Females (% dead by week on study)
0	26/60 43% by wk 81	33/60 55% by wk 78
0	33/60 55% by wk 81	38/60 63% by wk78
5	23/60 38% by wk 82	31/60 52% by wk 80
50	33/60 * 55% by wk 79	43/60 72% by wk 79
150	36/60 60% by wk 75	42/60** 70% by wk 76
	*Includes 1 m found dead wk90	**Includes 2 f accidentally killed wk 90

Percentage of Rats Surviving to Terminal Sacrifice

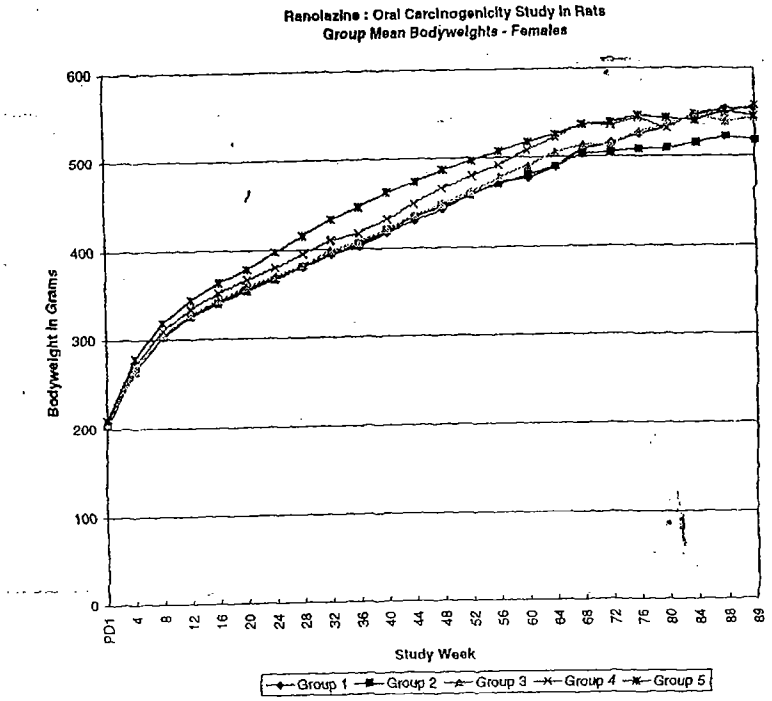
Dose (mg/kg)	Males	Females
0	34/60 57%	27/60 45%
0	27/60 45%	22/60 37%
5	37/60 62%	29/60 48%
50	28/60 47%	17/60 28%
150	24/60 40%	18/60 30%
	*Includes 1 m found dead wk90	**Includes 2 f accidentally killed wk 90

Weight Gain and Food Consumption

There were no significant differences in weight gain. Determination of food consumption was not required by the protocol. The sponsor's graphs of



body weight are shown below.



Clinical Signs in Rats

At the HD, rats showed gasping and dyspnea. Convulsions were also reported to occur before and after dosing. The sponsor considered the convulsions to be unrelated to drug treatment.

Hematology and Clinical Chemistry

Not required by protocol. Therefore not determined.

Non-Neoplastic Findings

- Increased incidence of adrenal medullary hyperplasia, cortical vacuolation and hypertrophy of the zona reticularis. Dose dependent increases reported for both males and females in MD and HD. No effect reported in controls or LD.
- Increased incidence of cytoplasmic pigment in hepatocytes
- Pulmonary foam cell
- Follicular hyperplasia in thyroids

Neoplastic Findings

Significant increases in the trends were noted for the tumor types summarized in the following table. The statistics are based upon the sponsor's analysis in which the control groups were combined for comparison.

Summary of Tumor Types With Significantly Increased Incidence in Drug-Treated Rats

Males		Females	
Neoplasia	**P value**	**Neoplasia**	**P value**
Benign follicular adenoma	<0.001	Benign pheochromocytoma	= 0.013
Benign pheochromocytoma	= 0.024	Benign adrenal cortical adenoma	= 0.032
Benign interstitial cell testicular tumor	= 0.001	Benign pituitary gland adenoma	= 0.020
Malignant histiocytic sarcoma	= 0.037	Malignant sarcoma	= 0.040

The sponsor's summary tables are attached as Appendix II.

Plasma Level Exposure

Blood concentration was reported to have been determined over a 24-hour period at the end of the study. The toxicokinetic summary provided by the sponsor is shown below.

Ranolazine: Oral Carcinogenicity Study in Rats
Mean Cmax and AUC values at End of Study

Dose (mg/kg/day)	Male		Female	
	Cmax (µg/ml)	AUC (µg.h/ml)	Cmax (µg/ml)	AUC (µg.h/ml)
5	1.10±0.646	3.76	1.08±0.148	5.08
50	12.7±4.75	88.2	13.1±0	72.5
150	19.7*	222	18.5±3.23	199

Rat historical control data was not collected from the Syntex Laboratories where the carcinogenicity studies were conducted.

The sponsor was asked in a telephone conversation with the present reviewer to provide historical control data. The sponsor replied that this could not be done because 1) at the time that the protocols were discussed with the FDA historical data was not specified and 2) no other chronic studies were ever conducted in the Syntex facility. The sponsor provided published data from rats and mice used as controls in 2-year studies. The animals in the published studies were from breeding colonies in this country as compared to the animals in the present studies that were from colonies in the UK.

SUMMARY

Given the sensitivity of the two rodent species for the CNS effects of the drug, the studies done were adequate. The mouse had on average <0.25X the human exposure while the rat had <4X the human exposure, yet higher doses were probably not feasible. The lack of metabolism data for the mouse is of some concern. Based upon the presence in murine urine of several metabolites, the assumption appears to have been made that there was adequate representation of the human metabolites. One may consider the question from the other direction and ask if there were murine-specific metabolites, perhaps that were genotoxic.

The appearance of the same tumor type (benign interstitial cell tumor of the testes) in both rats and mice at low multiples of human exposure is suggestive of carcinogenic potential. This material will be submitted to the Executive CAC for their deliberation.

Elizabeth A. Hausner, D.V.M.
Pharmacology

Al F. DeFelice, Ph.D.
Supervisory Pharmacologist

APPENDIX I. Summary of Mouse Neoplasias

RS-43285 : Two Year Oral Carcinogenicity Study in Mice

Summary of Neoplastic Lesions by Site - Females

	Dose Group (mg/kg/day)				
	0	5	15	50	p-value
Adrenal, Pheochromocytoma					
Number of Animals with tumour	0	1	2	1	0.207[4]
Number of Animals without tumour	99	49	48	49	
% with tumour	0%	2%	4%	2%	
Harderian Gland, Adenoma					
Number of Animals with tumour	8	3	3	4	0.459[2]
Number of Animals without tumour	92	47	47	46	
% with tumour	8%	6%	6%	8%	
Pituitary, Adenoma					
Number of Animals with tumour	3	3	2	2	>0.500[3]
Number of Animals without tumour	95	46	47	46	
% with tumour	3%	6%	4%	4%	
Mammary Gland, Adenocarcinoma					
Number of Animals with tumour	2	5	3	0	>0.500[2]
Number of Animals without tumour	98	45	47	50	
% with tumour	2%	10%	6%	0%	
Ovary, Tubular Adenoma					
Number of Animals with tumour	1	0	0	2	0.101[4]
Number of Animals without tumour	97	50	50	47	
% with tumour	1%	0%	0%	4%	
Uterus, Polyp					
Number of Animals with tumour	6	0	3	2	>0.500[3]
Number of Animals without tumour	93	50	47	48	
% with tumour	6%	0%	6%	4%	

1 - One tailed Prevalence trend p-value
2 - One tailed Time-to-Tumour p-value
3 - One tailed Combined trend p-value
4 - One tailed Exact Trend Test p-value

APPENDIX I continued

RS-43285 : Two Year Oral Carcinogenicity Study in Mice

Summary of Neoplastic Lesions by Site - Females

	Dose Group (mg/kg/day)				
	0	5	15	50	p-value
Lung, Alveolar/Bronchiolar Adenoma					
Number of Animals with tumour	10	4	5	3	>0.500[1]
Number of Animals without tumour	89	46	45	47	
% with tumour	10%	8%	10%	6%	
Lung, Alveolar/Bronchiolar Carcinoma					
Number of Animals with tumour	8	2	1	5	0.356[3]
Number of Animals without tumour	91	48	49	45	
% with tumour	8%	4%	2%	10%	
Haemopoietic System, Histiocytic Sarcoma					
Number of Animals with tumour	8	6	2	4	>0.500[3]
Number of Animals without tumour	92	44	48	46	
% with tumour	8%	12%	4%	8%	
Haemopoietic System, Lymphoma					
Number of Animals with tumour	13	5	7	7	0.489[3]
Number of Animals without tumour	87	45	43	43	
% with tumour	13%	10%	14%	14%	

1 - One tailed Prevalence trend p-value
2 - One tailed Time-to-Tumour p-value
3 - One tailed Combined trend p-value
4 - One tailed Exact Trend Test p-value

APPENDIX I continued

RS-43285 : Two Year Oral Carcinogenicity Study in Mice

Summary of Neoplastic Lesions by Site - Males

	Dose Group (mg/kg/day)				
	0	5	15	50	p-value
Adrenal, Cortical Adenoma					
Number of Animals with tumour	6	6	1	2	>0.500[1]
Number of Animals without tumour	92	43	49	48	
% with tumour	6%	12%	2%	4%	
Harderian Gland, Adenoma					
Number of Animals with tumour	10	4	8	3	>0.500[3]
Number of Animals without tumour	90	46	42	47	
% with tumour	10%	8%	16%	6%	
Liver, Hepatocellular Carcinoma					
Number of Animals with tumour	10	3	3	6	0.269[3]
Number of Animals without tumour	90	47	47	44	
% with tumour	10%	6%	6%	12%	
Lung, Alveolar/Bronchiolar Adenoma					
Number of Animals with tumour	12	8	6	8	0.166[1]
Number of Animals without tumour	88	42	44	42	
% with tumour	12%	16%	12%	16%	
Testes, Interstitial Cell Tumour					
Number of Animals with tumour	5	1	2	5	0.019[1]
Number of Animals without tumour	95	49	48	44	
% with tumour	5%	2%	4%	10%	
Haemopoietic System, Lymphoma					
Number of Animals with tumour	5	3	4	1	>0.500[2]
Number of Animals without tumour	95	47	46	49	
% with tumour	5%	6%	8%	2%	

1 - One-tailed Prevalence trend p-value
2 - One-tailed Time-to-Tumour p-value
3 - One tailed Combined trend p-value

RS-43285 : Two Year Oral Carcinogenicity Study in Mice

Summary of Neoplastic Lesions - Males

	Dose Group (mg/kg/day)				
	0	5	15	50	p-value
Primary Tumours					
Number of Animals with tumour	66	31	27	29	0.496
Number of Animals without tumour	34	19	23	21	
% with tumour	66%	62%	54%	58%	
Benign Tumours					
Number of Animals with tumour	43	19	21	22	0.100
Number of Animals without tumour	57	31	29	28	
% with tumour	43%	38%	42%	44%	
Malignant Tumours					
Number of Animals with tumour	36	16	14	16	>0.500
Number of Animals without tumour	64	34	36	34	
% with tumour	36%	32%	28%	32%	

APPENDIX I continued

RS-43285 : Two Year Oral Carcinogenicity Study in Mice

Summary of Neoplastic Lesions - Females

	Dose Group (mg/kg/day)				
	0	5	15	50	p-value
Primary Tumours					
Number of Animals with tumour	61	31	27	26	>0.500
Number of Animals without tumour	39	19	23	24	
% with tumour	61%	62%	54%	52%	
Benign Tumours					
Number of Animals with tumour	32	15	15	16	>0.500
Number of Animals without tumour	68	35	35	34	
% with tumour	32%	30%	30%	32%	
Malignant Tumours					
Number of Animals with tumour	34	18	14	18	>0.500
Number of Animals without tumour	66	32	36	32	
% with tumour	34%	36%	28%	36%	

Appears This Way
On Original

APPENDIX II Rat Neoplasias

Appendix F
Ranolazine : Oral Carcinogenicity Study in Rats

Table 9
Summary of Statistically Analysed Neoplastic Lesions by Site - Males

	Dose Group (mg/kg/day)				
	0	5	50	150	p-value
Liver, [M] hepatocellular carcinoma					
Number of animals with tumour	3	0	3	1	
Number of animals without tumour	117	60	57	59	0.399[3]
% with tumour	3%	0%	5%	2%	
Pancreas, [B] islet adenoma					
Number of animals with tumour	6	5	4	5	
Number of animals without tumour	114	55	56	55	0.142[1]
% with tumour	5%	8%	7%	8%	
Pancreas, [B] exocrine adenoma					
Number of animals with tumour	2	0	1	2	
Number of animals without tumour	118	60	59	58	0.177[4]
% with tumour	2%	0%	2%	3%	
Thyroid, [B] C cell adenoma					
Number of animals with tumour	6	4	2	2	
Number of animals without tumour	114	56	56	58	>0.500[1]
% with tumour	5%	7%	3%	3%	
Thyroid, [B] follicular adenoma					
Number of animals with tumour	3	1	2	6	
Number of animals without tumour	117	59	56	54	<0.001[1,*]
% with tumour	3%	2%	3%	10%	
Parathyroid, [B] adenoma					
Number of animals with tumour	1	3	0	0	
Number of animals without tumour	101	42	54	52	>0.500[4]
% with tumour	1%	7%	0%	0%	
Skin, [B] papilloma					
Number of animals with tumour	3	2	0	0	
Number of animals without tumour	117	58	60	60	>0.500[4]
% with tumour	3%	3%	0%	0%	

[1] - One-tailed Prevalence trend p-value
[2] - One-tailed Time-to-Tumour trend p-value
[3] - One-tailed Combined trend p-value
[4] - One-tailed Exact trend test
[*] - Statistically significant result
[B] - Benign Neoplasm
[M] - Malignant Neoplasm

Appendix F
Ranolazine : Oral Carcinogenicity Study in Rats

Table 9 (continued)
Summary of Statistically Analysed Neoplastic Lesions by Site - Males

	Dose Group (mg/kg/day)				p-value
	0	5	50	150	
L.N., [B] mesenteric, haemangioma					
Number of animals with tumour	3	5	0	0	
Number of animals without tumour	117	55	60	60	>0.500[1]
% with tumour	3%	8%	0%	0%	
Adrenal, [B] phaeochromocytoma					
Number of animals with tumour	13	5	9	10	
Number of animals without tumour	107	55	51	50	0.024[3]
% with tumour	11%	8%	15%	17%	
Adrenal, [B] cortical adenoma (TA)					
Number of animals with tumour	1	2	1	1	
Number of animals without tumour	119	58	59	59	0.415[4]
% with tumour	1%	3%	2%	2%	
Pituitary, [B] adenoma (TA)					
Number of animals with tumour	55	28	34	22	
Number of animals without tumour	65	32	25	38	>0.500[3]
% with tumour	46%	47%	58%	37%	
Testes, [B] interstitial cell tumour					
Number of animals with tumour	5	3	4	8	
Number of animals without tumour	115	57	56	52	0.001[3*]
% with tumour	4%	5%	7%	13%	
Subcutaneous tissue, [B] fibroma (TA)					
Number of animals with tumour	4	4	3	4	
Number of animals without tumour	116	56	57	56	0.125[2]
% with tumour	3%	7%	5%	7%	
Subcutaneous tissue, [B] lipoma					
Number of animals with tumour	9	5	4	0	
Number of animals without tumour	111	55	56	60	>0.500[3]
% with tumour	8%	8%	7%	0%	

[1] - One-tailed Prevalence trend p-value
[2] - One-tailed Time-to-Tumour trend p-value
[3] - One-tailed Combined trend p-value
[4] - One-tailed Exact trend test
[*] - Statistically significant result
[B] - Benign Neoplasm
[M] - Malignant Neoplasm

Appendix F
Ranolazine : Oral Carcinogenicity Study in Rats

Table 9 (continued)
Summary of Statisitically Analysed Neoplastic Lesions by Site - Males

	Dose Group (mg/kg/day)				
	0	5	50	150	p-value
Lymph./haemop. system, [M] histiocytic sarcoma					
Number of animals with tumour	2	1	1	3	
Number of animals without tumour	118	59	59	57	0.037[2]
% with tumour	2%	2%	2%	5%	
Lymph./haemop. system, [M] lymphoma					
Number of animals with tumour	8	2	5	1	
Number of animals without tumour	112	58	55	59	>0.500[3]
% with tumour	7%	3%	8%	2%	
Bone, [M] osteosarcoma					
Number of animals with tumour	0	0	2	0	
Number of animals without tumour	120	60	58	60	0.400[4]
% with tumour	0%	0%	3%	0%	

[1] - One-tailed Prevalence trend p-value
[2] - One-tailed Time-to-Tumour trend p-value
[3] - One-tailed Combined trend p-value
[4] - One-tailed Exact trend test
* - Statistically significant result
[B] - Benign Neoplasm
[M] - Malignant Neoplasm

Appendix F
Ranolazine : Oral Carcinogenicity Study in Rats

Table 10
Summary of Statistically Analysed Neoplastic Lesions - Females

	Dose Group (mg/kg/day)				
	0	5	50	150	p-value
Any Tumour (Benign or Malignant)					
Number of animals with tumour	116	58	58	57	
Number of animals without tumour	4	2	2	3	0.253[3]
% with tumour	97%	97%	97%	95%	
Benign Tumours					
Number of animals with tumour	113	55	57	56	
Number of animals without tumour	7	5	3	4	0.172[1]
% with tumour	94%	92%	95%	93%	
Malignant Tumours					
Number of animals with tumour	24	11	11	9	
Number of animals without tumour	96	49	49	51	>0.500[3]
% with tumour	20%	18%	18%	15%	

[1] - One-tailed Prevalence trend p-value
[2] - One-tailed Time-to-Tumour trend p-value
[3] - One-tailed Combined trend p-value
[4] - One-tailed Exact trend test
* - Statistically significant result

Appendix F
Ranolazine : Oral Carcinogenicity Study in Rats

Table 11
Summary of Statistically Analysed Neoplastic Lesions by Site - Females

	Dose Group (mg/kg/day)				
	0	5	50	150	p-value
Liver, [B] hepatocellular adenoma (TA)					
Number of animals with tumour	3	2	0	1	
Number of animals without tumour	117	58	60	59	>0.500[1]
% with tumour	3%	3%	0%	2%	
Pancreas, [B] islet adenoma					
Number of animals with tumour	1	0	2	0	
Number of animals without tumour	118	60	58	60	>0.500[4]
% with tumour	1%	0%	3%	0%	
Thyroid, [B] C cell adenoma					
Number of animals with tumour	3	3	4	3	
Number of animals without tumour	116	57	56	56	0.208[1]
% with tumour	3%	5%	7%	5%	
Mammary, [M] adenocarcinoma (TA)					
Number of animals with tumour	12	8	8	2	
Number of animals without tumour	108	52	52	58	>0.500[3]
% with tumour	10%	13%	13%	3%	
Mammary, [B] adenoma (TA)					
Number of animals with tumour	13	7	8	4	
Number of animals without tumour	107	53	52	56	>0.500[3]
% with tumour	11%	12%	13%	7%	
Adrenal, [B] phaeochromocytoma					
Number of animals with tumour	1	1	1	3	
Number of animals without tumour	119	59	59	57	0.013[1]*
% with tumour	1%	2%	2%	5%	
Adrenal, [B] cortical adenoma (TA)					
Number of animals with tumour	0	0	1	2	
Number of animals without tumour	120	60	59	58	0.032[4]*
% with tumour	0%	0%	2%	3%	

[1] - One-tailed Prevalence trend p-value
[2] - One-tailed Time-to-Tumour trend p-value
[3] - One-tailed Combined trend p-value
[4] - One-tailed Exact trend test
* - Statistically significant result
[B] - Benign Neoplasm
[M] - Malignant Neoplasm

0084SwICF

Appendix F
Ranolazine : Oral Carcinogenicity Study in Rats

Table 11 (continued)
Summary of Statistically Analysed Neoplastic Lesions by Site - Females

	Dose Group (mg/kg/day)				
	0	5	50	150	p-value
Pituitary, [M] adenocarcinoma					
Number of animals with tumour	4	3	0	2	
Number of animals without tumour	116	57	60	58	>0.500[3]
% with tumour	3%	5%	0%	3%	
Pituitary, [B] adenoma					
Number of animals with tumour	86	40	47	48	
Number of animals without tumour	34	20	13	12	0.020[3]
% with tumour	72%	67%	78%	80%	
Uterus, [M] stromal sarcoma					
Number of animals with tumour	0	1	0	2	
Number of animals without tumour	120	59	60	58	0.055[4]
% with tumour	0%	2%	0%	3%	
Uterus, [B] polyp					
Number of animals with tumour	3	1	5	0	
Number of animals without tumour	117	59	55	60	>0.500[1]
% with tumour	3%	2%	8%	0%	
Subcutaneous tissue, [M] sarcoma					
Number of animals with tumour	0	0	0	2	
Number of animals without tumour	120	60	60	58	0.040[4*]
% with tumour	0%	0%	0%	3%	

[1] - One-tailed Prevalence trend p-value
[2] - One-tailed Time-to-Tumour trend p-value
[3] - One-tailed Combined trend p-value
[4] - One-tailed Exact trend test
[B] - Benign Neoplasm
[M] - Malignant Neoplasm

Appendix F
Ranolazine : Oral Carcinogenicity Study in Rats

Table 12
Summary of Statistically Analysed Combined Neoplastic and Hyperplastic Lesions by Site - Males

	Dose (mg/kg/day)					p-value	p-value1
	0	0	5	50	₡50		
Thyroid, [B] follicular adenoma/hyperplasia							
Number of animals with tumour	5	2	3	3	11		
Number of animals without tumour	55	58	57	55	49	<0.001[1]*	0.001[1]*
% with tumour	8%	3%	5%	5%	18%		
Testes, [B] interstitial cell tumour/hyperplasia							
Number of animals with tumour	6	12	9	12	14		
Number of animals without tumour	54	48	51	48	46	0.012[3]	0.053[3]
% with tumour	10%	20%	15%	20%	23%		

p-value - p-value obtained using combined control group incidence
p-value1 - p-value obtained using the control group with the highest incidence
[1] - One-tailed Prevalence trend p-value
[2] - One-tailed Time-to-Tumour trend p-value
[3] - One-tailed Combined trend p-value
[4] - One-tailed Exact trend test
* - Statistically significant result
[B] - Benign Neoplasm
[M] - Malignant Neoplasm

Appears This Way On Original

Appendix F
Ranolazine : Oral Carcinogenicity Study in Rats

Table 13
Summary of Statistically Analysed Combined Malignant and Benign Neoplastic Lesions by Site - Females

	Dose Group (mg/kg/day)				
	0	5	50	150	p-value
Adrenal, phaeochromocytoma (Benign and Malignant)					
Number of animals with tumour	2	1	1	3	
Number of animals without tumour	118	59	59	57	0.034[1]
% with tumour	2%	2%	2%	5%	

[1] - One-tailed Prevalence trend p-value
[2] - One-tailed Time-to-Tumour trend p-value
[3] - One-tailed Combined trend p-value
[4] - One-tailed Exact trend test
* - Statistically significant result

TABLE 4

Ranolazine : Oral Carcinogenicity Study In Rats

Group Summary of Microscopic Findings - Tumour Data Summary

SYNTEX RESEARCH CENTRE
EDINBURGH

PAGE: 4
PATH/TOX SYSTEM

PRINTED: 26-JAN-95

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;FIND=B,M,N,I;SUBSET=ALL

--- N U M B E R - O F - A N I M A L S - A F F E C T E D ---

	SEX:	MALE				FEMALE				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
NEOPLASM CLASSIFICATION SUMMARY	NUMBER:	60	60	60	60	60	60	60	60	60	60
TOTAL PRIMARY NEOPLASMS		82	93	96	86	86	111	119	110	120	112
ANIMALS WITH ONE OR MORE		47	52	48	50	43	58	58	58	58	57
PERCENT WITH ONE OR MORE		78%	87%	80%	83%	72%	97%	97%	97%	97%	95%
TOTAL BENIGN NEOPLASMS		67	80	84	70	69	97	108	96	109	103
ANIMALS WITH ONE OR MORE		40	47	44	42	40	56	57	55	57	56
PERCENT WITH ONE OR MORE		67%	78%	73%	70%	67%	93%	95%	92%	95%	93%
TOTAL MALIGNANT NEOPLASMS		15	13	12	16	17	14	11	14	11	9
ANIMALS WITH ONE OR MORE		15	13	11	14	14	13	11	11	11	9
PERCENT WITH ONE OR MORE		25%	22%	18%	23%	23%	22%	18%	18%	18%	15%
TOTAL METASTATIC NEOPLASMS		7	3	5	0	1	5	1	3	2	2
ANIMALS WITH ONE OR MORE		2	2	4	0	1	5	1	3	2	2
PERCENT WITH ONE OR MORE		3%	3%	7%	0%	2%	8%	2%	5%	3%	3%
TOTAL LOCALLY INVASIVE NEOPLASMS		25	45	18	67	19	6	31	2	5	3
ANIMALS WITH ONE OR MORE		5	5	5	7	5	1	4	2	1	2
PERCENT WITH ONE OR MORE		8%	8%	8%	12%	8%	2%	7%	3%	2%	3%

Best Possible Copy

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Elizabeth Hausner
1/31/02 09:54:44 AM
PHARMACOLOGIST
ELizabeth Hausner

Albert Defelice
1/31/02 10:20:56 AM
PHARMACOLOGIST