CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER
21-290

Clinical Pharmacology and Biopharmaceutics Review

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA: 21-290, BB

Submission Dates: August 16, 2001
October 4, 2001

Bosentan (Tracleer®)
Actelion Pharmaceuticals

Reviewer: Gabriel J. Robbie

Type of Submission: Comparative dissolution of bosentan tablets in [redacted]% and 1% sodium lauryl sulfate in water dissolution media.

INTRODUCTION:

Bosentan (Tracleer™), an endothelin receptor antagonist, is to be marketed as 62.5 mg and 125 mg tablets for the long-term treatment of pulmonary arterial hypertension. In the original NDA submission the sponsor proposed to use 1% sodium lauryl sulfate in water as the dissolution specification medium. However, the Office of Clinical Pharmacology and Biopharmaceutics suggested the use of [redacted]% sodium lauryl sulfate in water because of good ([redacted]% dissolved in 30 minutes) dissolution. After a series of teleconference calls, the Sponsor was requested to provide data supporting the lack of "sink conditions" with [redacted]% sodium lauryl sulfate in water. The sponsor has therefore performed comparative dissolution of bosentan tablets in [redacted]% and 1% sodium lauryl sulfate in water and submitted the results in the present submission.

The batches used for comparative dissolution testing were 125 mg tablets:

1. Validation batch FAR003 manufactured at commercial site
2. Process challenge exercise batch (F0710A001)
3. Registration batch exposed to accelerated and inappropriate storage conditions (F0272B001)

METHODS & RESULTS:

The dissolution performance of bosentan tablets were evaluated USP apparatus 2 at a paddle speed of 50 rpm.

The results of comparative dissolution testing, mean (range), are presented in the following table.

Batch #	Time (min)	Mean (Range) %Dissolved in % SLS in Water	Mean (Range) %Dissolved in 1% SLS in Water
FAR003 (Commercial)	5	14 (11-19)	15 (9-22)
	10	36 (29-42)	37 (29-47)
	15	56 (48-60)	56 (45-67)
	30	**79 (76-81)**	**88 (84-93)**
	45	87 (86-89)	95 (89-101)
	60	91 (90-92)	96 (91-101)
F0710A001 (Process Challenge)	5	14 (11-15)	12 (9-15)
	10	34 (31-36)	30 (27-33)
	15	53 (50-55)	51 (49-55)
	30	**77 (77-78)**	**82 (78-84)**
	45	86 (83-89)	93 (90-94)
	60	91 (89-97)	95 (94-98)
F0272B001 (Accelerated Stability)	5	16 (12-26)	20 (15-26)
	10	32 (31-33)	40 (36-44)
	15	44 (43-45)	54 (52-59)
	30	**64 (60-66)**	**74 (72-75)**
	45	75 (71-78)	84 (83-86)
	60	81 (77-83)	89 (87-91)

The rate of dissolution up to 30 minutes in % SLS in water was slower than in 1% SLS in water. Q of % was not achieved in 30 minutes in % SLS in water dissolution medium. At 30 minutes, mean dissolution of 79%, 77% and 64% were observed for the 3 batches in % SLS in water. While in the dissolution medium of 1% SLS in water, at 30 minutes mean dissolution was 88%, 82% and 74% for the same 3 batches. The lower dissolution of 74% is expected for the accelerated stability batch, which indicates that the dissolution medium is sensitive to product changes.

Based on the new information provided by the sponsor, the proposed dissolution specification for bosentan should be Q not less than % dissolved in 30 minutes in 1% sodium lauryl sulfate in water at 50 rpm.

RECOMMENDATION:

The Office of Clinical Pharmacology and Biopharmaceutics recommends a dissolution specification of not less than % (Q) dissolved in 30 min in 1% sodium lauryl sulfate in water at a paddle speed of 50 rpm. The above recommendation is to be conveyed to the sponsor.

/S/ 11/1/01

Gabriel J. Robbie, Ph. D.

RD/FT by Patrick J. Marroum, Ph. D. 11/1/01

Cc: NDA 21-290, HFD 110, HFD 860 (Mehta, Robbie), CDER document room: Attn: Biopharm (CDER)

Clinical Pharmacology/Biopharmaceutics Review

NDA: 21-290 Submission Date: September 10, 2001
Tracleer™ (Bosentan)
Actelion, Ltd.

Reviewer: Gabriel J. Robbie

Type of Submission: Changes to bosentan markup label incorporating changes from clinical pharmacology.

Recommended changes to bosentan label from Office of Clinical Pharmacology and Biopharmaceutics are listed below.

Clinical Pharmacokinetics

Absorption

Draft

Special Populations

Draft

Drug Interactions

Glyburide:

Draft

Ketoconazole:

RECOMMENDATIONS:

The Office of Clinical Pharmacology and Biopharmaceutics has forwarded the suggested changes to Project Manager, Division of Cardiorenal Drug Products, via e-mail on September 10, 2001.

9/10/01

Gabriel J. Robbie, Ph. D.

RD/FT by Patrick J. Marroum, Ph. D. 9/10/01

Cc: IND[ ]HFD 110, HFD 860 (Mehta,Robbie), CDER document room: Attn: Biopharm (CDER)

21 pages redacted from this section of the approval package consisted of draft labeling

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA:	21-290	**SUBMISSION DATES**
IND:	[ ]	Original NDA: 11/17/00
TYPE:	1-S	10/27/98
BRAND NAME:	TRACLEER™	4/12/00
GENERIC NAME:	Bosentan	6/21/01
DOSAGE STRENGTH:	62.5-mg and 125-mg tablets	
SPONSOR:	Actelion Ltd.	

DIVISION OF PHARMACEUTICAL EVALUATION: I

PRIMARY REVIEWER: Gabriel J. Robbie, Ph.D.

TEAM LEADER: Patrick J. Marroum, Ph.D.

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RECOMMENDATION

The Office of Clinical Pharmacology and Biopharmaceutics has reviewed NDA 21-290 and finds the clinical pharmacology and biopharmaceutics section acceptable provided labeling comments #1 - 10 are adequately addressed. Moreover, the sponsor is requested to change the proposed dissolution medium from 1% sodium lauryl sulfate in water to [redacted] % sodium lauryl sulfate in water with a dissolution specification of Q not less than [redacted] % in 30 minutes.

COMMENTS:

1. A major deficiency in this NDA submission is the lack of information regarding pharmacokinetics of oral bosentan in PPH patients. Information such as single dose and steady-state concentrations expected following 62.5 mg and 125 mg doses, half-life, extent of enzyme induction, protein binding etc. are not known. The only study in which PPH patients received bosentan was an intravenous study in 7 patients only. In that study, different intravenous doses of bosentan were administered in series with no washout period. This confounds the true time course of pharmacodynamic effect and the identification of key pharmacodynamic information such time to onset and offset and slope of concentration-effect relationship.

2. The magnitude of change in bosentan concentrations observed in the special population study and drug interaction studies are difficult to extrapolate to PPH patients because of differences in bosentan pharmacokinetics between healthy and PPH patients.

OCPB briefing held on April 26, 2001. Attendees were Larry Lesko, Henry Malinowski, Arzu Selen, Mehul Mehta, Chandra Sahajwalla, Sang Chung, Maryann Gordon, Rajendra Uppoor and Patrick Marroum.

S

Gabriel J. Robbie, Ph.D.
Division of Pharmaceutical Evaluation I

S

Maryann Gordon, MD.
Division of Cardio-renal Drug Products

FT Initialed by Patrick J. Marroum, Ph.D. S

cc list: HFD-110 (Gordon): NDA 21-290; HFD-860: (Robbie, Marroum, Mehta); CDER Central Document Room

EXECUTIVE SUMMARY

Actelion Ltd. is seeking approval of Tracleer™ for the long-term treatment of pulmonary arterial hypertension. Tracleer™ tablets contain the active ingredient bosentan monohydrate, an endothelin receptor antagonist, which decreases both pulmonary and systemic vascular resistance resulting in increased cardiac output. The proposed dosing regimen is 62.5 mg twice daily for 4 weeks, increased to the target dose of 125 mg twice daily.

Section 6 of NDA 21-272 includes 24 studies. Fourteen studies were conducted with healthy volunteers, 2 in pulmonary arterial hypertension patients,

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Bosentan is highly bound to plasma proteins, about 98%, especially albumin. In vitro, plasma protein binding was saturable above 20 µg/ml. Absorption of bosentan is relatively rapid with an absolute bioavailability of 45 to 50%, estimated at a higher dose of 500 mg. Bosentan is extensively metabolized by the liver and subsequently eliminated in the bile. Following a single oral dose, mean recovery of radioactivity in feces was 94%, of which 30% was unchanged bosentan. Only 5% of an intravenous dose was excreted unchanged in the urine. The major enzymes responsible for bosentan metabolism are CYP 3A4 and 2C9. Three metabolites of bosentan have been identified, of which Ro 48-5033 is active. The concentrations and total exposure of the active metabolite Ro 48-5033 is less than 12% of the parent. Also, in vitro activity of Ro 48-5033 was approximately 2-fold less potent than bosentan. It should be noted, however, that Ro 48-5033 is less tightly bound to plasma proteins than bosentan and has a free fraction 3 times higher than that of the parent drug. Therefore, Ro 48-5033 may contribute to the pharmacological effects of bosentan probably up to 20%.

Upon multiple dosing, bosentan induces CYP 3A4 and 2C9, therefore, bosentan is likely to decrease the plasma concentrations of drugs and oral contraceptives which are metabolized by these isoenzymes. *In-vitro* human hepatic cytochrome P450 studies indicate that bosentan inhibits 2C9, 2C19 and 3A4, but clinically significant inhibition of these isoenzymes by bosentan are not expected at the proposed dose of 125 mg twice daily, since, the plasma concentrations are expected to be below the IC_{50} values observed in vitro.

In healthy volunteers, administration of ascending intravenous doses of bosentan increased AUC of bosentan more than proportionally especially after 500 mg because of decreasing clearance. On the contrary, administration of ascending oral doses of bosentan from 100 – 2400 mg resulted in less than proportional increases in Cmax and AUC, probably due to dissolution/solubility limitations. Plasma concentrations of bosentan declined in a bi-phasic manner after both intravenous and oral administration. Following a single 250-mg intravenous dose of bosentan in healthy volunteers, the clearance, volume of distribution at steady-state (Vss) and half-life of bosentan were 9 L/h, 18 L and 5 h, respectively. Following a single 125 mg oral dose of bosentan in healthy volunteers, the T_{max}, C_{max} and half-life of bosentan were 4 h, 1500 ng/ml and 6 h, respectively.

Upon multiple dosing in healthy volunteers, steady-state plasma concentrations of bosentan declined to 50% of Day 1 values due to enzyme induction. The clearance of bosentan following an intravenous dose on Day 11 was 2-fold higher compared to Day1.

Clearance of bosentan in primary pulmonary hypertension (PPH) patients was significantly lower than healthy volunteers (3.8 L/h vs. 9 L/h), following intravenous administration. This reduction is probably related to lower cardiac index in these patients. Both Vss (approx. 20 L) and half life (about 5 h) were similar in PPH patients and healthy volunteers.

The pharmacokinetics of bosentan and its metabolites in PPH patients following oral administration is not known since plasma concentrations were not measured.

Use of bosentan in PPH patients with mild and moderate hepatic insufficiency is contraindicated. In subjects with severe renal impairment, bosentan concentrations decreased slightly, mean C_{max} and AUC of bosentan were 37% and 11% lower, respectively, which could result in reduced effectiveness. Metabolite concentrations however, increased in severe renal impairment; the effect of the increased metabolite concentrations on safety is unknown at present.

Bosentan concentrations increased 30-fold after the first dose of cyclosporine and decreased to a 2-fold increase at steady-state. Similar increases in bosentan concentrations were observed at steady-state with ketoconazole. The significantly decreased clearance in PPH patients compared to healthy volunteers led to the reviewer's recommendation to contraindicate concomitant administration of bosentan and CYP 3A4 inhibitors. Coadministration of bosentan and warfarin, simvastatin or glyburide could result in decreased effectiveness because of lower plasma concentrations. Drug interactions between bosentan and digoxin or losartan were not observed in healthy volunteers. Coadministration of single intravenous dose of bosentan in SAH patients in the presence of steady-state nimodipine did not alter nimodipine concentrations. Bosentan pharmacokinetics in SAH patients was similar to healthy volunteers. It is difficult to extrapolate magnitude of change in bosentan concentrations observed in the special population study and drug interaction studies to PPH patients because of differences in bosentan pharmacokinetics between healthy and PPH patients.

The proposed dissolution method for bosentan tablets is USP method II (paddle) at a paddle speed of 50 rpm. The proposed medium for dissolution testing is 1% sodium lauryl sulfate in water at 37°C with a specification of Q not less than [redacted]% in 30 minutes. The concentration of sodium lauryl sulfate is too high. A more appropriate medium with similar performance would be [redacted]% sodium lauryl sulfate in water with a specification of Q not less than [redacted]% in 30 minutes. The intended to be marketed tablet formulations were used in the Phase III clinical trial (Study AC 052-351).

Two assay methods were used to quantify bosentan and its metabolites, [redacted] and [redacted] Both these methods were cross-validated and were found to be sensitive, specific, precise and accurate. The limit of quantification of bosentan in plasma using [redacted] was [redacted] ng/ml.

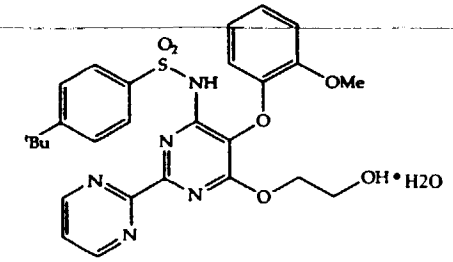
QUESTION BASED REVIEW

I. INTRODUCTION

A. WHAT ARE THE HIGHLIGHTS OF THE CHEMISTRY, FORMULATION AND PHYSICAL-CHEMICAL PROPERTIES OF THE DRUG AND DRUG PRODUCT?

STRUCTURE

Bosentan is 4-tert-butyl-N-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-[2,2']bipyrimidin-4-yl]-benzenesulfonamide monohydrate.



molecular formula: $C_{27}H_{29}N_5O_6S.\ H_2O$
molecular weight: 569.64 (monohydrate) and 551.62 (anhydrous)

FORMULATION AND MANUFACTURING

Tracleer™ contains the active ingredient bosentan monohydrate. It is a non-hygroscopic, white to yellowish powder. It is to be marketed as 62.5 mg and 125 mg film coated tablets for oral administration. The compositions of commercial bosentan tablets are listed in the following table.

	Quantity (mg)/Tablet	
	62.5 mg	**125 mg**
Bosentan monohydrate	64.541	129.082
Corn Starch		
Pre-gelatinized Starch		
Sodium starch glycolate		
Povidone		
Glyceryl behenate		
Magnesium stearate		
Film Coat		
Hydroxypropylmethyl cellulose		
Triacetin		
Talc		
Titanium Dioxide		
Iron Oxide Yellow		
Iron Oxide Red		
Ethylcellulose		

Bosentan drug substance is manufactured by Bosentan for commercial distribution will be manufactured, packaged, and labeled by Patheon Inc. Mississauga, Ontario. , Canada.

SOLUBILITY AND PARTITION COEFFICIENT

Bosentan is insoluble in water and in aqueous buffer solutions of pH 1 to 5, the solubility increases to 43 mg/100 ml at pH 7.5. Bosentan is freely soluble in acetone, acetonitrile, chloroform and is soluble in ethanol. The apparent pKa value of bosentan is 5.46. The octanol/water partition coefficients of bosentan at pH 4.0 is 3.1 and at pH 7.4 is 1.3.

B. WHAT IS THE PROPOSED MECHANISM OF ACTION AND THERAPEUTIC INDICATION?

The neurohormone endothelin-1 (ET-1) is a potent vasoconstrictor that acts via binding to ET_A and ET_B receptors. Bosentan is an endothelin receptor antagonist with affinity for both ET_A and ET_B receptors. Bosentan blocks the action of endogenous ET-1 thereby decreasing both pulmonary and systemic vascular resistance resulting in increased cardiac output. Actelion Ltd. intends to use bosentan for the long-term treatment of primary pulmonary arterial hypertension patients.

C. WHAT IS THE PROPOSED DOSAGE AND ADMINISTRATION?

The proposed dosing regimen is 62.5 mg twice daily for 4 weeks, increased to the target dose of 125 mg twice daily.

II. CLINICAL PHARMACOLOGY

A. WAS THERE REASONABLE BASIS FOR THE SELECTION OF THE CLINICAL ENDPOINTS, SURROGATE ENDPOINTS OR BIOMARKERS AND WERE THEY MEASURED PROPERLY TO ASSESS EFFICACY AND SAFETY IN CLINICAL PHARMACOLOGY STUDIES?

The clinical endpoint measured was distance walked in 6 minutes. This endpoint is used clinically to assess exercise capacity in patients with PAH.

The biomarkers and measurements used to assess improvement and deterioration in PPH patients only in Study AC-052-351 include the following:

mean pulmonary artery pressure (PAPm)
mean right arterial pressure (RAP)
Pulmonary capillary wedge pressure (PCWP)
pulmonary vascular resistance (PVR)
cardiac index (CI)
WHO functional class of pulmonary hypertension
BORG dyspnea index

B. WERE THE CORRECT MOIETIES IDENTIFIED AND PROPERLY MEASURED TO ASSESS CLINICAL PHARMACOLOGY?

Bosentan and its metabolites, Ro 48-5033, Ro 47-8634 and Ro 64-1056, were quantified in plasma.

ASSAY VALIDATION

Two assay methods were used to quantify bosentan and its metabolites[ ]and [ ]Both these methods were sensitive, specific, precise and accurate. The limit of quantification of bosentan in plasma using[ ]was[ ]ng/ml. The limit of quantification for all 3 metabolites, Ro 48-5033, Ro 47-8634 and Ro 64-1056, in plasma was[ ]ng/ml using [ ]Cross validation of the two methods using . samples was performed in Study 1 1 which indicated a mean difference of only 3.9%.

C. WHAT ARE THE EXPOSURE-RESPONSE RELATIONSHIPS FOR EFFICACY AND SAFETY?

The pharmacokinetics of bosentan in PPH patients following oral administration is not known since plasma concentrations were not measured. Sponsor's analysis of Study 352 indicates that the distance in the 6-minute walk test at Week 16 increased by a mean of 34.6 m with 125 mg b.i.d. and by 54.3 m with 250 mg b.i.d., while a slight decrease of 7.8 m was seen in the placebo group.

- *DO PK PARAMETERS CHANGE WITH TIME?*

Yes, upon multiple dosing, steady-state plasma concentrations of bosentan declined to 50% of Day 1 values due to enzyme induction. Mean Cmax and AUC of the active metabolite Ro 48-5033 and the inactive metabolite Ro 47-8634 decreased by a similar magnitude (about 40%) as bosentan. Cytochrome 3A4 and 2C9 were the primary isoenzymes induced by bosentan. In healthy volunteers, following an intravenous dose the clearance of bosentan on Day 11 was 2-fold higher than that observed on Day 1.

D. ARE THE PHARMACOKINETICS IN HEALTHY VOLUNTEERS SIMILAR TO PPH PATIENTS?

No, the clearance of bosentan in patients with primary pulmonary hypertension was significantly lower compared to healthy volunteers (3.8 L/h vs. 9 L/h), which is probably related to the lower cardiac index in PPH patients. The volume of distribution at steady-state was slightly higher in patients (21 L vs. 18 L) and the half life was unchanged between 4 and 5 hours. The pharmacokinetics of the bosentan and its metabolites in PPH patients following oral administration is not known.

ABSORPTION

In healthy volunteers, absorption of bosentan is relatively rapid with an absolute bioavailability of 45 to 50%, estimated at a higher dose of 500 mg. In an ascending oral dose study, bosentan concentrations increase in a less than dose-proportional manner over a dose range of 100 mg - 2400 mg. Following single oral administration of 125 mg bosentan in healthy volunteers, the T_{max}, C_{max} and half-life of bosentan were 4 h, 1500 ng/ml and 6 h, respectively. A higher Cmax and AUC is expected in PPH patients because of slower clearance.

DISTRIBUTION

In patients and healthy volunteers, the volume of distribution at steady-state was similar, 18 L to 21 L. *In-vitro* studies indicate that bosentan is highly bound, about 98%, to plasma proteins, mainly albumin. In vitro, plasma protein binding was saturable above 20 µg/ml.

METABOLISM

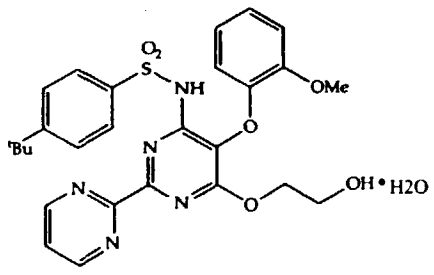
Bosentan is extensively metabolized in the liver and subsequently eliminated in the bile. In an ADME study, following a single oral dose mean recovery of radioactivity in feces was 94% of which 30% was unchanged bosentan. Only 5% of an intravenous dose was excreted unchanged in the urine. The major enzymes responsible for bosentan metabolism are CYP 3A4 and 2C9. Three metabolites of bosentan have been identified, Ro 48-5033, Ro 47-8634, and Ro 64-1056, of which Ro 48-5033 is active. The concentrations and total exposure of the active metabolite Ro 48-5033 is less than 12% of the parent and, based on in vitro activity Ro 48-5033 is approximately 2-fold less potent than bosentan.

COMPOUND	IC_{50} on ET_A (µM)	IC_{50} on ET_B (µM)	pA_2
Bosentan	0.08	0.16	7.4
Ro 48-5033	0.18	0.39	7.1
Ro 47-8634	26.1	5.5	n.m.
Ro 64-1056	2.5	5.3	n.m.

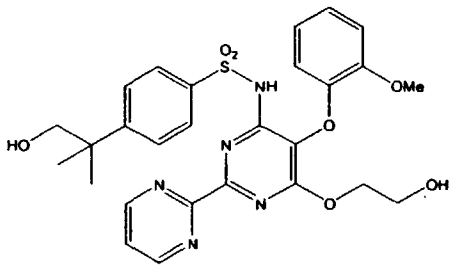
n.m.=not measured

It should be noted, however, that Ro 48-5033 is less tightly bound to plasma proteins than bosentan and has a free fraction 3 times higher than that of the parent drug. Therefore, Ro 48-5033 may contribute to the pharmacological effects of bosentan probably up to 20%.

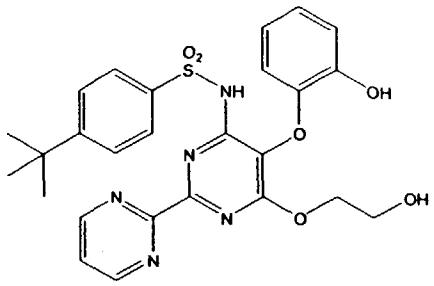
The binding of Ro 47-8634 to ET_A receptor was >300-fold less potent and its binding to ET_B receptor was approximately 30-fold less potent than bosentan. Ro 64-1056 was approximately 30-fold less potent than bosentan.



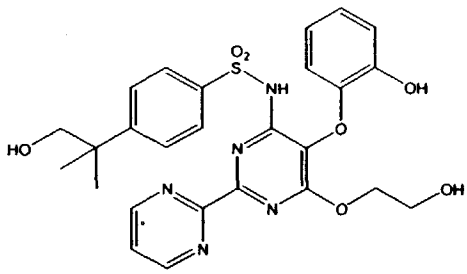
Bosentan



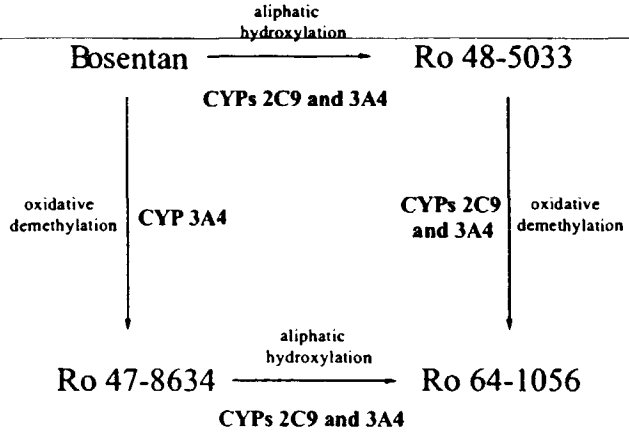
Ro 48-5033



Ro 47-8634



Ro 64-1056

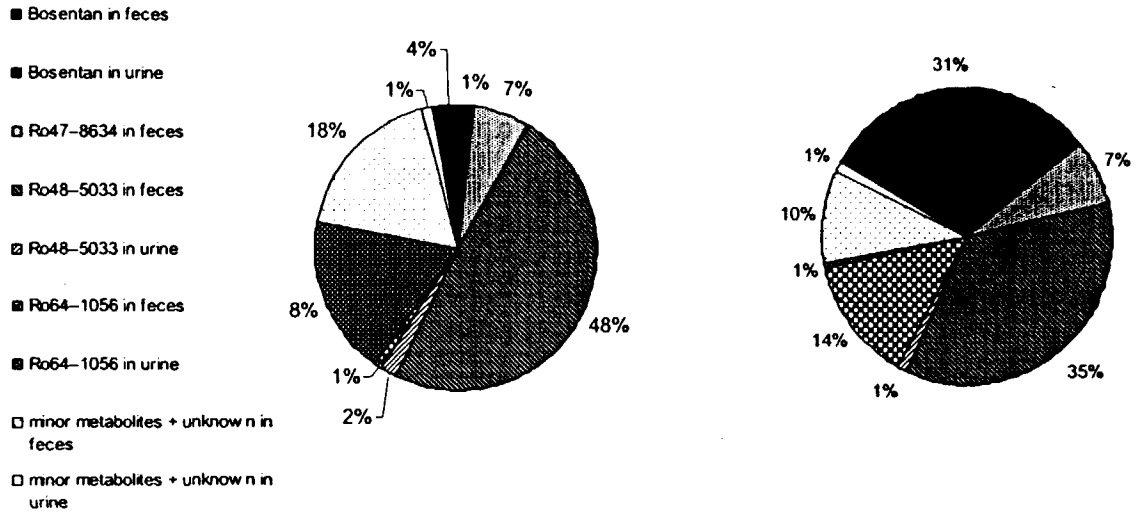


EXCRETION

After oral administration of radiolabeled bosentan, 94.5% of the radioactivity was excreted in the feces and only 2.8% in the urine. Total recovery at the end of the collection period was 97.3% (range[illegible]% to[illegible]%). Overall, 95% of total radioactivity was recovered within 3.5 days. After oral administration, a much higher percentage (30.2%) was excreted unchanged in the feces, which probably represents unabsorbed parent drug.

Recovery of bosentan and its metabolites from urine and feces over 9 days after intravenous (250 mg, A) or oral administration (500 mg, B) to healthy male subjects

A **B**



After intravenous administration of radiolabeled bosentan, 92.9% of the radioactivity was excreted in the feces and 5.2% in the urine. Total recovery at the end of the collection period was 98% (range % to %). Overall, 95% of total radioactivity was recovered within 3.5 days after intravenous administration. Most of the drug-related material in feces was the metabolite Ro 48-5033. Unchanged bosentan represented only 3.7% of material recovered in the feces after intravenous administration.

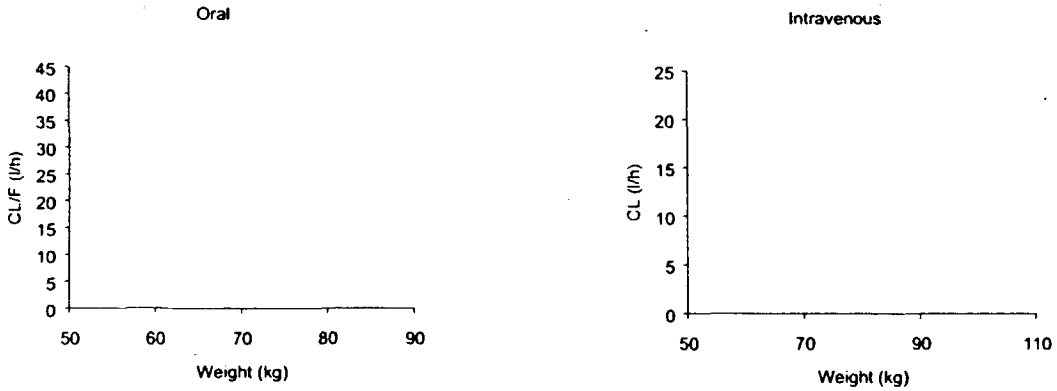
- ***WHAT ARE THE VARIABILITIES OF PK PARAMETERS IN VOLUNTEERS AND PATIENTS?***

The pharmacokinetics of bosentan is characterized by moderate to high variability, between 25% to 50%. The inter-individual variability between volunteers and patients was similar.

E. WHAT DOSAGE REGIMEN ADJUSTMENTS, IF ANY, ARE RECOMMENDED FOR EACH OF THESE GROUPS?

- ***BODY WEIGHT***

The sponsor has not conducted any studies evaluating the effect of body weight on the pharmacokinetics of bosentan. The sponsor has performed an analysis using pooled data from bosentan doses of less than 300 mg i.v. or doses between 300 mg and 600 mg p.o. (oral suspension) to investigate the effect of body weight of subjects on the pharmacokinetics of bosentan. However, the body weight of most individuals were between 65 kg and 90 kg, a range not wide enough to identify a trend. In fact, there seemed to be a trend toward increasing CL/F with body weight.



- ***GENDER***

No specific study was conducted to evaluate the effects of gender on the pharmacokinetics, safety, or tolerability of bosentan in women. Most of the early clinical pharmacology studies were conducted in healthy, young, male subjects. However, later studies also included healthy women. By pooling data from several studies, the sponsor contends that there are no significant difference in the pharmacokinetics of bosentan between men and women. However, it is not clear whether the data shown below is body weight corrected. The following Table shows a clear increase in Cmax and AUC in females compared to males on Day 5 after oral administration of bosentan 125 mg b.i.d. for 4 days and a single 125 mg dose on Day 5. Data are geometric means (and 95% CI) of data from all male (n = 24) and female (n = 16) subjects combined from 3 drug-drug interaction studies.

Pharmacokinetic parameters of bosentan after multiple oral doses in healthy male and female subjects

Gender	C_{max} (ng/ml)	t_{max} (h)	AUC_τ (ng·h/ml)	CL/F (l/h)
Male	733	2.75	3426	34.8
	(610, 881)	(1.5, 5)	(2931, 4006)	(31.2, 42.6)
Female	1043	3.75	4322	28.9
	(871, 1251)	(1, 5)	(3641, 5130)	(24.4, 34.3)

- ***RACE***

No specific study was conducted to evaluate the effects of race on the pharmacokinetics, safety, or tolerability of bosentan. Most of the pharmacokinetic studies were conducted in healthy Caucasian males. The sponsor has performed a pooled analysis comparing CL/F from 8 healthy black subjects enrolled in a bioavailability study to CL/F obtained from Caucasians at the same dose level of the same formulation. The mean CL/F in blacks and Caucasians were similar.

Apparent clearance of bosentan after multiple oral doses in Caucasian and black subjects

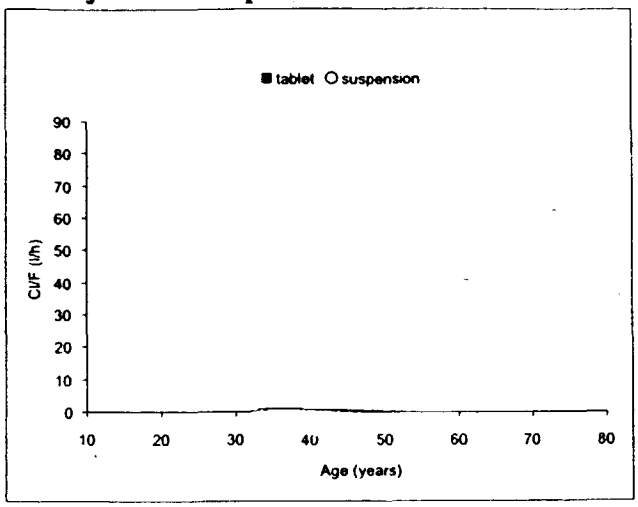
Ethnic group	N	CL/F (l/h)
Black	8	15.4
		(9.1, 26.0)
Caucasian	58	15.8
		(14.0, 15.9)

Data are geometric means (and their 95% CI)

- ***ELDERLY***

The sponsor did not conduct a study to evaluate the effects of age on the pharmacokinetics, safety, or tolerability of bosentan in the elderly. However, data from older patients enrolled in studies in and were compared with other studies and concluded that there was no evidence of an effect of age on the pharmacokinetics of bosentan. However, this might not be accurate because elderly subjects with and were compared to healthy young subjects. In fact visual inspection of the tablet data indicates a trend toward decreasing CL/F with age.

Effect of age of subjects on the pharmacokinetics of bosentan



- *PEDIATRIC PATIENTS*

The sponsor has not conducted any studies evaluating the pharmacokinetics of bosentan in pediatric patients.

- *RENAL INSUFFICIENCY*

In subjects with severe renal impairment, bosentan concentrations decreased slightly, mean C_{max} and AUC of bosentan were 37% and 11% lower, respectively. This could result in reduced effectiveness of bosentan. Metabolite concentrations, however, increased in severe renal impairment; the effect of the increased metabolite concentrations on safety is unknown at present. Dosage adjustment is not recommended by the sponsor.

- *HEPATIC INSUFFICIENCY (HI)*

Use of bosentan in PPH patients with moderate to severe hepatic insufficiency is contra-indicated.

E. WHAT ARE THE EXTRINSIC FACTORS THAT INFLUENCE EXPOSURE OR RESPONSE?

- *DRUG-DRUG INTERACTIONS*

In-vitro

Bosentan induces CYP 3A4 and 2C9 isoenzymes, therefore, bosentan is likely to decrease the plasma concentrations of drugs and oral contraceptives metabolized by these isoenzymes.

In-vitro human hepatic cytochrome P450 studies indicate that bosentan inhibits 2C9, 2C19 and 3A4, but clinically significant inhibition of these isoenzymes are not expected at the proposed dose of 125 mg twice daily, since, the plasma concentrations are expected to be below the IC_{50} values observed in vitro.

In-vivo

Cyclosporin: Concomitant administration of bosentan and cyclosporin A affects the pharmacokinetics of both drugs. Concomitant administration of cyclosporin increased bosentan concentrations by 30-fold after the first dose. However, upon multiple dosing the magnitude of increase in bosentan trough concentrations decreased and reached steady-state by Day 5. At steady-state, bosentan trough concentrations, C_{max} and AUC, were higher by % and , %, respectively, compared to single dose trough concentration in the absence of CsA. The magnitude of increase in steady-state bosentan Cmax and AUC is actually higher than the reported increase of 100%, which was obtained by incorrectly comparing steady-state bosentan C_{max} and AUC in the presence of CsA to single dose bosentan Cmax and AUC in the absence of CsA. This is because bosentan concentrations decline upon multiple dosing to 50% of their single dose concentrations because of enzyme auto-induction. Bosentan decreased cyclosporin steady-state Cmax, AUC and trough concentration values by ., %, % and %, respectively, probably by inducing metabolizing enzymes. **The concomitant use of bosentan and cyclosporin should be contraindicated.** This study was conducted using 500-mg bosentan, a

dose that is higher than the intended maximum dose of 125-mg. At therapeutic doses of bosentan the extent of interaction with CsA could be lower than what was observed in the present study.

Digoxin: Bosentan 500-mg BID administered for 7 days slightly decreased the C_{max} and AUC of digoxin by 9% and 12%, respectively. Day 14 C_{min} of digoxin decreased by 30% in the presence of bosentan. Comparison of the pharmacokinetics of 500-mg BID bosentan from the present study with another study in healthy individuals indicated no effect of concomitant administration of digoxin on bosentan pharmacokinetics. Since the dose of bosentan (500-mg/BID) used in the present study is higher than the intended maintenance dose (125-mg/BID), it is anticipated that lower doses of bosentan will not significantly affect the pharmacokinetics of digoxin.

Warfarin: Steady-state bosentan increased the elimination of both R- and S-warfarin, consequently, reducing the anticoagulation effect of warfarin as measured by prothrombin time and factor VII activity. The CL/F of R-warfarin and S-warfarin increased by 59% and 40%, respectively, and the half-life of R-warfarin and S-warfarin decreased by 37% and 33%, respectively, in the presence of bosentan. The increased elimination of warfarin is hypothesized to be due to induction of both CYP2C9 and CYP3A4 enzymes. Single-dose warfarin decreased the mean steady-state trough concentration of bosentan by 63%. The cause of this interaction is not known at present. Concomitant use of warfarin and bosentan requires more intense monitoring of prothrombin time. An increase in warfarin and bosentan dose should be considered when administered concomintantly. The 500-mg BID dose of bosentan used in the present study is much higher than the 125 mg BID dose proposed in the label. Therefore, a lower magnitude of interaction at the therapeutic dose of 125-mg BID is expected.

Ketoconazole: Concomitant administration of 200-mg QD ketoconazole significantly increased the steady-state Cmax and AUC of bosentan by 62% and 83%, respectively. The increase in bosentan Cmax and AUC in the presence of ketoconazole can be attributed to inhibition of bosentan metabolism via CYP3A4. This was evident from the decreased concentration of metabolites of bosentan, except Ro 47-5033, in the presence of ketoconazole. The Cmax and AUC of the active metabolite, Ro 47-8634, were lower by 33% and 12%, respectively, in the presence of ketoconazole. A greater magnitude of interaction is anticipated after the first co-administered dose compared to steady-state. Ketoconazole concentrations were not measured in the present study. **Concomitant administration of bosentan and ketoconazole should be contraindicated.**

Simvastatin: Coadministration of bosentan and simvastatin significantly decreased steady-state C_{max} and AUC of both simvastatin (31% and 49%, respectively) and its active metabolite, β-hydroxy simvastatin, by (33% and 60%, respectively). The metabolite to parent AUC ratio for β-hydroxy simvastatin decreased by 25% only, compared to the 60% reduction in β-hydroxy simvastatin AUC in the presence of bosentan indicating increased metabolism of β-hydroxy simvastatin. The metabolism pathway of β-hydroxy simvastatin is not known at present. Concomitant use of bosentan and statins, which are predominantly metabolized by CYP 3A4 such as, simvastatin, lovastatin, cerivastatin and atorvastatin, could result in decreased effectiveness of the coadministered statin. The possibility of reduced statin efficacy should be considered. An increase in statin dose should be considered when bosentan is started.

Glibenclamide: Coadministration of bosentan and glibenclamide significantly decreased steady-state C_{max} and AUC of both bosentan and glibenclamide. Steady-state Cmax and AUC of bosentan decreased by 24% and 29%, respectively, while that of glibenclamide decreased by 22% and 40%, respectively. This interaction is probably due to induction of liver enzymes/p-glycoprotein transport and/or increase in bile flow. This effect may be observed with other sulfonylurea hypoglycemic agents that are also metabolized by CYP2C9. Concomitant use of bosentan and other oral hypoglycemics could result in reduced hypoglycemic response at therapeutic doses. Concomitant use of glyburide and bosentan requires more intense monitoring of blood glucose levels. Alternative hypoglycemic agents should be considered, since, an increase in glyburide dose to offset reduced hypoglycemic response could increase the risk of elevated liver enzymes.

Nimodipine: The pharmacokinetic parameters of bosentan in patients obtained following a single 500-mg intravenous dose of bosentan in the presence of steady-state nimodipine was similar to those obtained in healthy volunteers in other studies. Except for one patient, single intravenous dose of bosentan did not alter steady-state nimodipine concentrations in patients. Upon multiple dosing of bosentan, however, nimodipine concentrations could decrease because of enzyme induction.

III. BIOPHARMACEUTICS

A. WAS AN ADEQUATE LINK ESTABLISHED BETWEEN THE CLINICAL AND TO-BE MARKETED FORMULATIONS OF BOSENTAN?

The intended to be marketed tablet formulations were used in the Phase III clinical trial (Study AC 052-351).

B. ARE THE SPONSOR PROPOSED DISSOLUTION MEDIUM AND SPECIFICATIONS ACCEPTABLE?

No, the proposed medium for dissolution testing is 1% sodium lauryl sulfate in water at 37°C with a specification of Q not less than % in 30 minutes. The concentration of surfactant used is relatively high. Similar dissolution performance is obtained with % sodium lauryl sulfate in water. Therefore, the biopharmaceutics reviewer proposes the following dissolution method, medium and specification; dissolution not less than % (Q) dissolved in 30 min in % sodium lauryl sulfate in water at 50 rpm using USP Apparatus II (paddle).

APPEARS THIS WAY
ON ORIGINAL

3 pages redacted from this section of the approval package consisted of draft labeling

APPENDIX I

APPENDIX II

STUDY B-158760 – IN VITRO BINDING OF THE ENDOTHELIN RECEPTOR ANTAGONIST Ro 47-0203 TO PLASMA PROTEINS IN MAN AND ANIMALS, AND RED BLOOD CELL/PLASMA PARTITIONING.

Study ID: B-158760
Volume: 2.23

OBJECTIVES:

1. To determine bosentan protein binding in human, marmoset, dog, rabbit, rat and mouse plasma
1. To determine bosentan red blood cell (RBC) binding in human, marmoset, dog, rabbit, rat and mouse blood

METHODS:

Plasma Protein Binding: ^{14}C-bosentan (0.086 to 348 µg/ml) was separated from plasma, α_1-acid glycoprotein, albumin by in a dialysis cell. Equilibrium dialysis was conducted for 3 hours at 37^{0}C and the concentration of parent drug was measured by concentration of radioactivity. Fraction unbound was calculated after dialysis using the following equation.

$$fu = \frac{Cbuffer}{Cplasma}$$

Red Blood Cell Binding: Whole blood was spiked with ^{14}C-bosentan to give concentrations over a range of to µg/ml and incubated at 37^{0}C. The RBC/plasma ratio (R/P) was determined using the following equation:

$$\frac{R}{P} = \frac{B - P(1-H)}{H \cdot P}$$

where, R = bosentan concentration in RBC; P = bosentan concentration in plasma; B = bosentan concentration in whole blood; H = hematocrit

RESULTS:

Bosentan is highly bound to plasma proteins in all species tested. Bosentan is 98.1% bound in human plasma. Binding in human plasma occurs almost exclusively to albumin and is saturable above 20 µg/ml. The following table summarizes the plasma protein binding and RBC/plasma ratios of bosentan in various species.

	Human	Marmoset	Dog	Rabbit	Rat	Mouse
% Bound in Plasma	98.1	93.6	95.9	96.0	98.5	98.5
RBC/plasma ratio	0.62	NP	0.79	NP	0.68	NP

NP=not performed

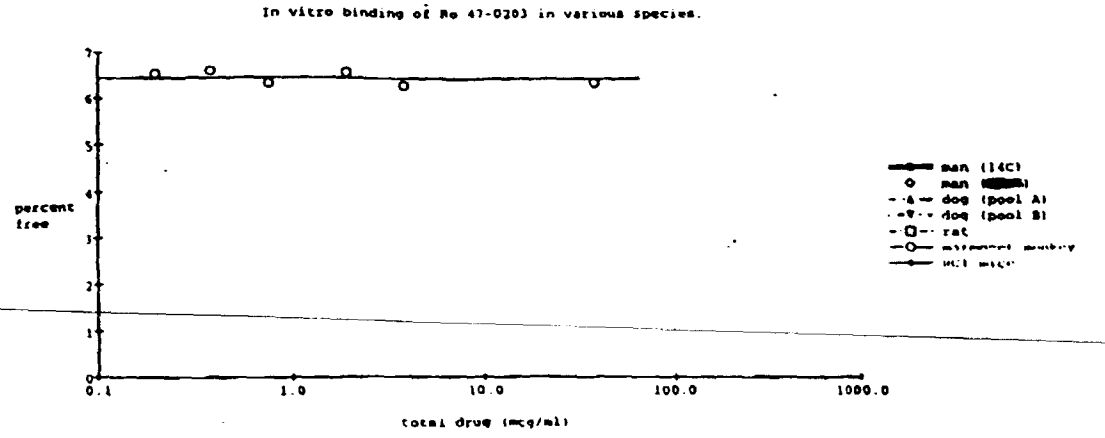
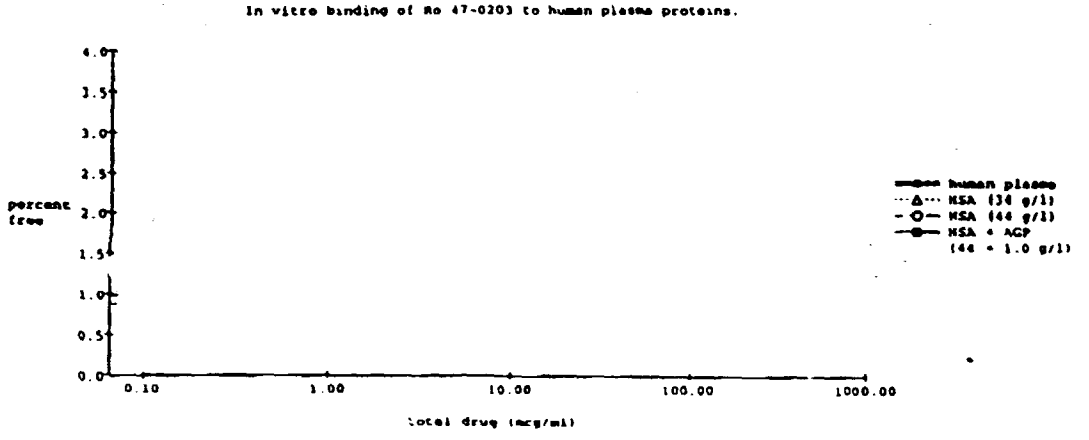

In vitro binding of Ro 47-0203 in various species.
percent free
total drug (mcg/ml)
man (14C)
dog (pool A)
dog (pool B)
rat

Figure 2


In vitro binding of Ro 47-0203 to human plasma proteins.
percent free
total drug (mcg/ml)
human plasma
HSA (34 g/l)
HSA (44 g/l)
HSA + AGP
(44 + 1.0 g/l)

STUDY B-157614 – IN VITRO PROTEIN BINDING OF Ro 48-5033 (MAJOR METABOLITE OF BOSENTAN) AND BINDING INTERACTION WITH BOSENTAN IN HUMAN, DOG AND RAT PLASMA

Study ID: B-157614
Volume: 2.23

OBJECTIVES:

1. To determine protein binding of major metabolite of bosentan, Ro 48-5033, in human, dog and rat plasma
2. To determine binding interaction between Ro 48-5033 and bosentan in human, dog and rat plasma.

RESULTS:

Ro 48-5033 is less highly bound to plasma proteins (93.4%) in humans compared to bosentan (98.1%). There were large interspecies differences in plasma protein binding of Ro 48-5033. The binding was independent of the concentration in dog, rat and man up to 6 μg/ml. Binding of Ro 48-5033 was not affected by the addition of bosentan in human and rat plasma up to 10 and 20 μg/ml, respectively. A slight increase by a factor of 1.2 was observed in dog at the highest bosentan concentration of 100 μg/ml. The following table summarizes the plasma protein binding of Ro 48-5033 in various species.

Ro 48-5033	Human	Dog	Rat
% Unbound in Plasma	6.6	27	12

STUDY B-168929 – IN VITRO PROTEIN BINDING OF Ro 47-8634 AND Ro 64-1056 (METABOLITES OF BOSENTAN) AND BINDING INTERACTION WITH BOSENTAN IN HUMAN, DOG, RAT AND MOUSE PLASMA

Study ID: B-168929
Volume: 2.23

OBJECTIVES:

1. To determine protein binding of Ro 47-8634 and Ro 64-1056, metabolites of bosentan, in human, dog, rat and mouse plasma.
2. To determine binding interaction between Ro 47-8634 and bosentan, and Ro 64-1056 and bosentan in human, dog, rat and mouse plasma.

RESULTS:

Ro 47-8634 was more highly bound to plasma proteins (99.64%) in humans compared to bosentan (98.1%). The extent of binding of Ro 47-8634 was similar across species. Binding of Ro 47-8634 was affected by the addition of 100 µg/ml bosentan in human and dog plasma; the free fraction increased by a factor of 1.7.

Ro 64-1056 and bosentan exhibited similar plasma protein binding in humans. However, the extent of binding of Ro 64-1056 was less than bosentan in other species. Also, plasma protein binding of Ro 64-1056 was dissimilar across species. Binding of Ro 64-1056 was affected by a factor of 1.9 in man and by 1.2 in dog plasma in the presence of 100 µg/ml bosentan.

The following table summarizes the plasma protein binding of Ro 47-8634 and Ro 64-1056 in various species.

	Human	Dog	Rat	Mouse
Ro 47-8634				
% Unbound in Plasma	0.36	1.9	0.58	0.49
Ro 64-1056				
% Unbound in Plasma	1.2	20.4	5.1	5.7

STUDY B-165967 – BOSENTAN, Ro 47-0203: IN VITRO PROTEIN BINDING INTERACTION STUDIES WITH DIGITOXIN, GLIBENCLAMIDE, PHENYTOIN, TOLBUTAMIDE AND WARFARIN

Study ID: B-165967
Volume: 2.21

OBJECTIVES:

1. To investigate the effect of bosentan on protein binding of digitoxin, glibenclamide, phenytoin, tolbutamide and warfarin in human serum.
2. To determine the effect of digitoxin, glibenclamide, phenytoin, tolbutamide and warfarin on bosentan in human serum.

METHODS:

Serum was obtained from blood of healthy male and female volunteers. The total serum protein concentration was 73.7 g/L and serum albumin concentration was 46.5 g/L. Equilibrium dialysis was conducted for 0.5, 1, 2, 3 or 4 h depending on compound at 37°C.

RESULTS:

Digitoxin at concentrations of 10 and 30 ng/ml were 98.8% and 98.6% bound, respectively. Bosentan at concentrations of 1.5, 5 and 10 µg/ml did not affect protein binding of digitoxin. Digitoxin concentrations of 10 and 30 ng/ml increased the unbound fraction of bosentan slightly, about 10-20%.

Glibenclamide at concentrations of 0.05 and 0.5 µg/ml were 99.6% and 99.4% bound, respectively. Bosentan at concentrations of 1.5, 5 and 10 µg/ml did not affect protein binding of glibenclamide. Glibenclamide at concentrations of 0.05 and 0.5 µg/ml did not affect bosentan protein binding at 1.5, 5 and 10 µg/ml.

Phenytoin at concentrations of 0.4 and 40 µg/ml were 87.5% and 84.9% bound, respectively. Bosentan at concentrations of 1.5, 5 and 10 µg/ml did not affect protein binding of phenytoin. Phenytoin at concentrations of 0.4 and 40 µg/ml decreased bosentan protein binding at 1.5, 5 and 10 µg/ml. The % unbound value for bosentan increased by 30%.

Tolbutamide at concentrations of 25 and 250 µg/ml were 98.1% and 95.7% bound, respectively. Bosentan at concentrations of 1.5 and 5 µg/ml did not affect protein binding of tolbutamide, however, bosentan at 10 µg/ml slightly decreased protein binding of tolbutamide to 97.8%. Tolbutamide at 250 µg/ml decreased bosentan protein binding slightly from 98% to about 96-97%.

Warfarin at concentrations of 0.5 and 5 μg/ml were 99.3% and 99.2% bound, respectively. Bosentan at concentrations of 1.5, 5 and 10 μg/ml did not affect protein binding of warfarin. Likewise, warfarin at both 0.5 and 5 μg/ml did not affect bosentan protein binding.

CONCLUSIONS:

At the proposed dose of 125 mg b.i.d., bosentan is not expected to alter plasma protein binding of digitoxin, glibenclamide, phenytoin, tolbutamide and warfarin. Coadministration of digitoxin phenytoin and tolbutamide, are expected to increase the free fraction of bosentan by 20%, 30% and 80%, respectively. Extrapolation of results from in vitro protein binding to in vivo significance is confounded by, lack of protein binding data in patients, effect of bosentan on liver function, lack of concentration-effect relationship, and, absence of correlation between in vivo free fraction and pharmacodynamic response.

APPEARS THIS WAY
ON ORIGINAL

STUDY B-166140 – DRUG-DRUG INTERACTIONS WITH BOSENTAN (Ro 47-0203) IN VITRO STUDIES OF THE INHIBITION POTENTIAL OF BOSENTAN ON THE MAIN HUMAN CYTOCHROME P450 ISOENZYMES.

Study ID: 44599
Volume: 2.21

OBJECTIVES:

1. To assess the in vitro inhibitory properties of bosentan toward the human cytochrome P450's 1A2, 2D6, 2C9, and 3A4

METHODS:

Human liver microsomes from 10 normal human livers were used in this study. The reactions were conducted at 37^0C in a buffer containing 0.05 M potassium phosphate (pH 7.4), 0.1 mM EDTA, 3 mM $MgCl_2$, microsomal protein, and substrate. .

The following table lists the details and results of the inhibition study.

CYP P450	Substrate	Bosentan Conc. Range (μM)	Amt. m'som (pmoles)	Reac.time (min)	Assay	IC_{50} Bosentan (μM)
1A2	Tacrine 1- & 7-hydroxylation		208	17.5		No Effect
2D6	Dextromethorphan O-demethylation		136	30		No Effect
2C19	Hydroxylation of S-mephenytoin		1000	30		203
2C9	4-hydroxylation of diclofenac		33	5		22
3A4/5	Hydroxylation of Midazolam		66	10		67

RESULTS:

In the present in vitro metabolism study bosentan did not have any inhibitory effect on CYP 1A2 and 2D6 at the concentration range studied,. Bosentan had a weak inhibitory effect on CYP 2C19 and 3A4/5 with IC_{50} of 203 μM and 67 μM, respectively. Bosentan significantly inhibited CYP 2C9 with a Ki of 22 μM. The expected C_{max} of 1500 to 2000 ng/ml following a single oral dose of 125-mg of bosentan is about 4 μM, this concentration is lower than the Ki value of 22 μM for CYP 2C9 inhibition. This probably explains the absence of in vivo interaction with CYP 2C9 substrate warfarin.

COMMENTS:

1. The methodology used in the present in vitro study should have been validated with model inhibitors of the various cytochrome P450 enzymes.

2. The concentration range studied in the inhibition of CYP 2C19 and 3A4/5 was not appropriate. The suggested IC_{50} values are larger than the highest concentration employed in the experiment.

3. The inhibitory activities of bosentan metabolites were not studied.

APPEARS THIS WAY
ON ORIGINAL

STUDY B-159245 – IN VITRO METABOLISM OF ENDOTHELIN RECEPTOR ANTAGONIST Ro 47-0203 (BOSENTAN) IN VARIOUS SPECIES, INCLUDING MAN

Study ID: B-159245
Volume: 2.23

OBJECTIVES:

1. To compare the metabolic profiles obtained in vitro by incubations of Ro 47-0203 with liver preparations of different species.

METHODS:

Bosentan was incubated with the S9 supernatant of rat, dog and human. Bosentan was also incubated with rat, dog, marmoset and human hepatocytes. Bosentan was also incubated with rat, dog, marmoset and human liver microsomes.

RESULTS:

The main metabolic processes were oxidative reactions. The metabolite profile in human was similar to those seen in dog, rat and marmoset. Biotransformation of bosentan was mediated enzymatically. Incubation with denatured liver fractions or in the absence of NADPH did not result in the formation of any metabolites. In the liver preparations from the different species formation of a major metabolite M1 was observed, which was later identified as Ro 48-5033.

COMMENTS:

1. The relative amounts of parent and metabolite generated after incubation with hepatic microsomes and liver slices in the various species was not presented.

STUDY B-163952 – BOSENTAN: METABOLISM OF Ro 47-0203 IN HUMAN LIVER MICROSOMES

Study ID: B-163952
Volume: 2.23

OBJECTIVES:

To characterize the pathways of metabolism of bosentan in vitro using human liver microsomes.

METHODS:

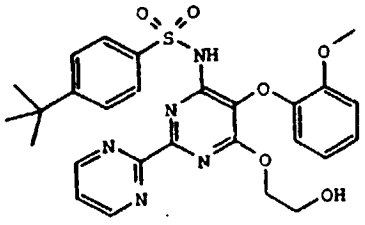
Bosentan (3.6 µM to 108 µM) was incubated with the human liver microsomes (0.5 mg/ml or 1 mg/ml) with a P450 content of 0.354 nmoles/mg protein. Specific inhibitors and competitors were used to inhibit metabolism of bosentan such as: 10 µM methoxypsoralen (CYP 2A6 inhibitor), 7 µM quinidine (CYP 2D6 inhibitor), 64 or 200 µM midazolam (CYP 3A4 substrate), 200 µM sulfaphenazole (CYP 2C9 inhibitor) or 50 µM furafylline (CYP 1A2 inhibitor).

RESULTS:

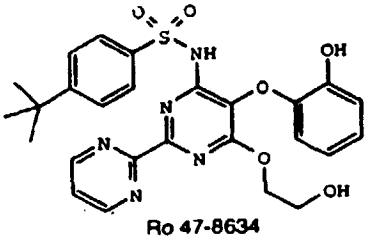
The in vitro human liver microsomal studies indicated that,

a) Hydroxylation of bosentan to the active metabolite Ro 48-5033 was mediated by CYP 3A4 and 2C9 in humans.
b) Demethylation of bosentan to Ro 47-8634 is mediated predominantly by CYP 3A4. This is a minor pathway.
c) CYP 3A4 and CYP 2C9 convert Ro 48-5033 and Ro 47-8634 to a common secondary metabolite.

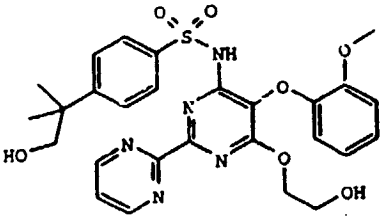
The metabolic pathway of bosentan and metabolites Ro 48-5033 and Ro 47-8634 is presented in the following page.



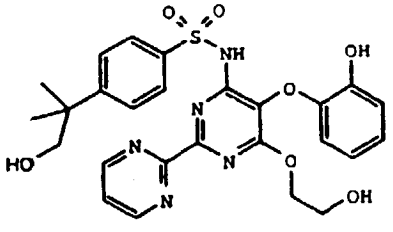
Ro 47-0203/009



Ro 47-8634



Ro 48-5033



I42

STUDY B-159041 – EXCRETION BALANCE, PHARMACOKINETIC AND METABOLISM STUDY AFTER A SINGLE P.O. AND A SINGLE I.V. DOSE OF [14C]-LABELED Ro 47-0203 IN HEALTHY MALE VOLUNTEERS.

STUDY INVESTIGATOR AND SITE:.

Report No.: B-159041
Volume No.: 8

OBJECTIVES:

1. To measure plasma concentrations, fecal and urinary recoveries of total radioactivity
2. To investigate the metabolic profile in plasma, feces and urine
3. To measure plasma, feces and urine concentrations of main metabolite(s) and related pharmacokinetic parameters
4. To measure plasma, feces and urine concentrations of unchanged bosentan and related pharmacokinetic parameters.

FORMULATIONS:

Bosentan intravenous solution – 300 mg, 4.4 MBq [^{14}C]-Ro 47-0203 (Batch #: GSU 0080)
Bosentan oral suspension – 50 mg/ml, 3.7 MBq [^{14}C]-Ro 47-0203 (Batch #: GFR 0072)

STUDY DESIGN:

This was a single-center, open-label, single dose, parallel group study in 8 healthy adult male volunteers between the ages of 21 to 33 years. Four subjects received a single intravenous dose of 250 mg [^{14}C]-labeled bosentan (3.7 MBq) as a 15 minute infusion, within 10 minutes of a standard breakfast. Four subjects received a single oral dose of 500 mg [^{14}C]-labeled bosentan (3.7 MBq) within 10 min of a standard breakfast.

ASSAY:

All samples were analyzed at .

Compound	*Matrix*	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
Bosentan	Plasma			NP			13.4	

Ro 48-5033	Plasma	[redacted]	NP	NP
Ro 47-0634	Plasma	[redacted]	NP	NP

NP=Not Provided

^{14}C-radioactivity was measured using a [redacted] analyzer. [redacted] was used for analysis of plasma bosentan concentrations below [redacted] ng/ml and [redacted] method was used for all other samples.

Sample Collection:
Blood samples (8-ml) for measurement of plasma concentrations of bosentan were collected in each of the 4 periods at 0 (predose), 15 min and 30 min and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 hours post dose.

RESULTS:

Total radioactivity and the relative amounts of bosentan and other metabolites recovered from urine and feces are listed in the table below.

Table 2: Relative Amounts of Substances Identified in Urine and Feces

	Radioactivity Excreted (% of Dose)			
	Intravenous		Oral	
Substance	Urine	Feces	Urine	Feces
Total Radioactivity	5.2	92.9	2.8	94.5
Bosentan	0.9	3.7	0.1	30.2

Ro 48-5033	1.9	47.5	1.1	34.6
Hydroxyphenol-Metabolite	1.0	17.7	0.5	13.2
Ro 47-8634	0.2	6.4	0.1	6.7
Minor Metabolites	0.5	8.0	0.3	5.1
Unknown	0.6	9.6	0.7	4.7

Mean recovery following a single intravenous radioactive dose of bosentan was 98%. Of the total radioactivity recovered, 93% and 5% were recovered from feces and urine, respectively. Excretion was complete between 2.5 days and 5 days. The major metabolites in the feces were Ro 48-5033, hydroxyphenol-metabolite, Ro 47-8634 and bosentan, which contributed 47.5%, 17.7%, 6.4% and 3.7%, respectively. Bosentan and its metabolites are negligibly eliminated in the urine. Hepatic metabolism and biliary excretion seemed to be the major pathway of elimination of bosentan.

Mean recovery following a single oral radioactive dose of bosentan was 97%. Of the total radioactivity recovered, 94% and 3% were recovered from feces and urine, respectively. Excretion was complete in 3.5 days. The major metabolites in the feces were Ro 48-5033, bosentan, hydroxyphenol-metabolite and Ro 47-8634, which contributed 34.6%, 30.2%, 13.2% and 6.7%, respectively. Unchanged bosentan recovery in the feces was greater following oral dosing compared to intravenous dosing (30 vs 4) probably due to radioactivity from unabsorbed bosentan.

Plasma radioactivity following both intravenous and oral dosing was almost entirely composed of bosentan and the 2 major metabolites Ro 47-5033 (active metabolite) and Ro 47-8634 up to 24 hours post dose (see figures below).

Figure 2. Mean Plasma Concentration Time Profiles of Bosentan (Ro 47-0203) and its Two Major Metabolites Ro 48-5033 and Ro 47-8634

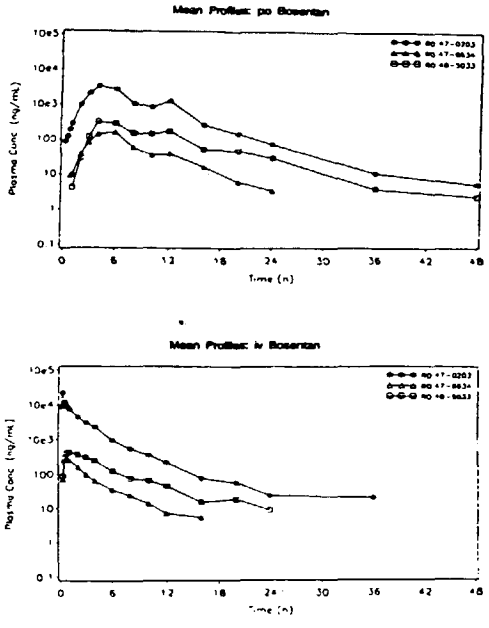
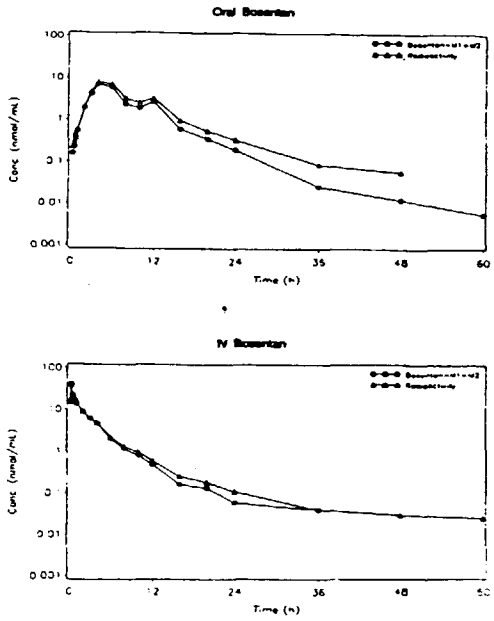


Figure 3. Mean Plasma Concentration Time Profiles of Radioactivity (nmol-equivalents) and the Sum of Bosentan and its two Major Metabolites Ro 48-5033 and Ro 47-8634



M1 = Ro 48-5033
M2 = Ro 47-8634

Plasma radioactivity and bosentan + metabolite levels deviated after 24 hours, with plasma radioactivity declining with a long half-life which was attributed to other metabolites. The terminal $T_{1/2}$ of total radioactivity in plasma was longer, 12 and 16 hours after oral and intravenous dosing, respectively, compared to the terminal $T_{1/2}$ of bosentan, 7 hours and 6 hours after oral and intravenous dosing, respectively.

The pharmacokinetic parameters of bosentan obtained following both intravenous and oral dosing are listed in the following table.

Table 3: Mean (%CV) Pharmacokinetic Parameters of Total Radioactivity, Bosentan and Metabolites

Treatment	C_{max} (ng/ml)	T_{max} (h)	$T_{0.5}$ (h)	AUC (ng.h/ml)	CL/F (L/h)	CL (L/h)	Vss (L)
Bosentan - Oral	3724 (21)	6.5 (58)	7.3 (52)	24290 (37)	22.6 (31)	-	-
Bosentan - Intravenous	-	-	5.6 (41)	31700 (63)	-	9.3 (45)	23.9 (49)
Ro 48-5033 - Oral	420.5 (32)	7.5 (46)	10.3 (46)	3089 (41)	-	-	-
Ro 48-5033- Intravenous	459.3 (63)	1.3 (40)	6.1 (13)	2414 (77)	-	-	-
Ro 47-8634 - Oral	171.7 (18)	5.5 (18)	3.6 (44)	1087 (30)	-	-	-
Ro 47-8634- Intravenous	286.0 (39)	0.8 (27)	2.9 (30)	844.9 (65)	-	-	-
Total Radioactivity-Oral			12.0 (55)		15.1 (29)		
Total Radioactivity – i.v.			16.3 (52)			19.4 (46)	86.3 (41)

Double peaks in bosentan concentration was observed approximately 12 hours after dosing. The double peaks could be due to biliary excretion of bosentan and subsequent reabsorption of bosentan from the gut resulting in entero-hepatic recirculation. The clearance of bosentan was 9.3 L/h following a single 250-mg intravenous dose. The absolute bioavailability of 500-mg suspension was about 40%.

Following oral administration of bosentan, the Cmax and AUC ratio of metabolite Ro 48-5033 to bosentan were 11% and 13%, respectively. The metabolite to parent ratio of both Cmax and AUC of Ro 47-8634 was 5%.

Following intravenous administration of bosentan, AUC ratio of metabolite Ro 48-5033 to bosentan and Ro 47-8634 were 7% and 3%, respectively.

CONCLUSIONS:

Hepatic metabolism and biliary excretion seemed to be the major pathway of elimination of bosentan. Mean recovery following a single intravenous radioactive dose of bosentan was 98%, of which, 93% and 5% were recovered from feces and urine, respectively. The major substances

in the feces were Ro 48-5033, hydroxyphenol-metabolite, Ro 47-8634 and bosentan, which contributed 47.5%, 17.7%, 6.4% and 3.7%, respectively.

Mean recovery following a single oral radioactive dose of bosentan was 97%, of which, 94% and 3% were recovered from feces and urine, respectively. The major substances in the feces were Ro 48-5033, bosentan, hydroxyphenol-metabolite and Ro 47-8634, which contributed 34.6%, 30.2%, 13.2% and 6.7%, respectively. Unchanged bosentan recovery in the feces was greater following oral dosing compared to intravenous dosing (30 vs 4) probably due to radioactivity from unabsorbed bosentan.

Plasma radioactivity following both intravenous and oral dosing was almost entirely composed of bosentan and the 2 major metabolites Ro 47-5033 and Ro 47-8634 up to 24 hours post dose.

COMMENTS:

1. The concentration units were incorrectly listed as micrograms/ml instead of ng/ml.

STUDY B-162282 – A SINGLE ASCENDING ORAL DOSE STUDY OF THE TOLERABILITY, SAFETY, PHARMACODYNAMICS AND PHARMACOKINETICS OF THE ENDOTHELIN RECEPTOR ANTAGONIST RO 47-0203 IN YOUNG HEALTHY MALE VOLUNTEERS

STUDY INVESTIGATOR AND SITE:.

Report No.: B-162282
Volume No.: 3

OBJECTIVES:

1. To investigate the tolerability and safety and to determine the maximal tolerated dose if less than 2400 mg.
2. To investigate pharmacodynamic effects:
   a) the skin reaction to intradermal endothelin-1 (30 pmol)
   b) the changes in endothelin plasma concentrations

FORMULATIONS:

Bosentan – as suspension in water (Batch #: GPM 0016)
Placebo – as solution (batch # GFR 0029)

STUDY DESIGN:

This was a single center, randomized, single-dose, double-blind, placebo controlled, dose escalation study in 64 healthy adult male volunteers between the ages of 18 to 34 years, weighing between 67 and 94 kg. Based on the safety and tolerance of the preceding dose, bosentan doses were to be escalated in the following scheme – 3, 10, 30, 100, 300, 600, 1200 and 2400 mg. In each dose group, 6 subjects were randomized to bosentan suspension and 2 were randomized to matching placebo.

The pharmacodynamic effect of bosentan was determined as % change of the skin reaction and skin blood flow following intradermal injections of a fixed endothelin-1 dose (30 pmol).

ASSAY:

All samples were analyzed at

Compound	Matrix	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%		Accuracy (% Bias)
							Intra	Inter	
Bosentan	Plasma						NP		
							NP		
							NP		
Bosentan	Plasma						NP		
							NP		
Bosentan	Urine						NP		
							NP		
							NP		

NP=Not Provided

~~method was used for all subjects in the 1200 and 2400 mg dose groups. Five subjects from 600~~ mg, 2 subjects from 300 mg and 3 subjects from 100 mg were also analyzed using For all other subjects the analytical results of the . method were used.

Sample Collection:
Blood samples (10-ml) for measurement of plasma concentrations of bosentan were collected at 0 (predose), 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18 (for doses 300, 600, 1200 and 2400 mg only) and 24 hours post-dose.

Urine samples were collected for the time-intervals, -12 to 0, 0-4, 4-8, 8-12 and 12-24 hours post dose at dose levels 300, 600, 1200 and 2400 mg.

Intradermal injections of 30 pmol ET-1 were administered at –1 (pre-dose) and at 0.5, 1.5, 3.5 and 7.5 hours post dose. Two additional injections of ET-1 (0.3 pmol) were given on the same arm at –1.5 (pre-dose) and 3.5 hours post dose for dose levels 1200 mg and 2400 mg.

Measurement of flare, pallor and skin blood flow were performed at –0.5 (pre-dose) and at 1, 2, 4 and 8 hours post dose. For the 1200 and 2400 mg groups, who received 0.3 pmol ET-1, measurements of skin flare, pallor and blood flow were performed pre-dose (-1.5 hour) and at 15, 30, 45 and 60 min post dose and at 3.5 hour post dose.

RESULTS:

The pharmacokinetic parameters of bosentan obtained following administration of a single dose of 3, 10, 30, 100, 300, 600, 1200 and 2400 mg as an oral solution in healthy males are listed in the following table.

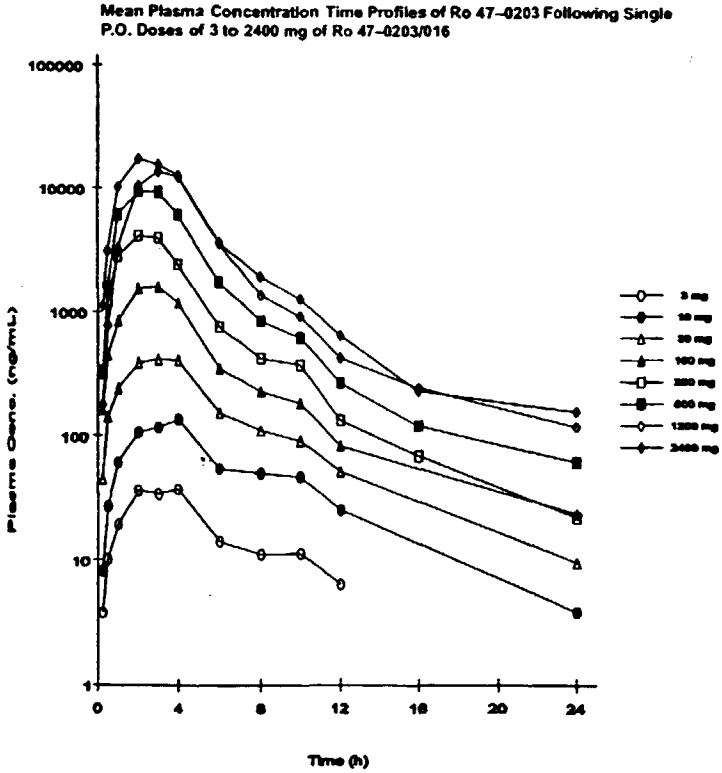
Table 2: Mean (%CV) Pharmacokinetic Parameters of Oral Bosentan

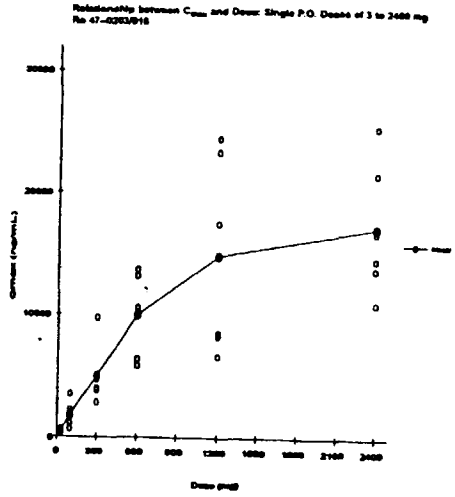
	C_{max} (ng/ml)	T_{max} (h)	$T_{0.5}$ (h)	AUC (ng.h/ml)	CL/F (L/h)
3 mg	38.7 (51)	2.8 (47)	3.7 (22)	267.8 (30)	12.2 (32)
10 mg	139.1 (53)	2.7 (45)	4.0 (10)	1009 (35)	11.0 (35)

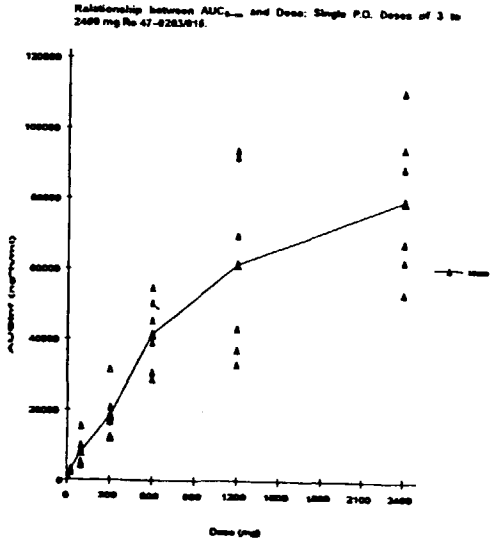
30 mg	477.7 (31)	2.5 (42)	4.5 (29)	2840 (16)	10.8 (17)
100 mg	1786 (57)	2.2 (19)	5.0 (32)	8180 (52)	14.9 (44)
300 mg	5000 (49)	2.3 (35)	5.3 (30)	18450 (39)	15.3 (27)
600 mg	9987 (33)	2.3 (35)	5.3 (30)	41480 (25)	15.3 (27)
1200 mg	14830 (54)	2.8 (27)	7.5 (34)	61420 (45)	23.3 (44)
2400 mg	17220 (31)	2.0 (0.4)	7.1 (33)	79810 (28)	32.1 (28)

Upon oral dosing, bosentan concentrations increased with a T_{max} between 2 and 3 hours. Secondary peaks were observed in all subjects at the higher dose levels around 10 hours post dose. After attaining C_{max} plasma concentrations of bosentan declined with a $T_{1/2}$, which increased with dose, that ranged between 4 and 7 hours. The terminal $T_{1/2}$ could be underestimated because the last sample was collected at 24 hours and not followed until LOQ at the high doses.

Both C_{max} and AUC of bosentan increased less than proportionally with dose, after 600 mg. The less than







proportional increase in AUC and Cmax at higher doses could be due to limited absorption because of low solubility of bosentan. The mean dose-normalized AUC and Cmax are presented in the table below.

Dose (mg)	AUC/Dose	Cmax/Dose
3	85.82	11.46
10	95.87	12.01
30	93.63	15.31
100	73.72	15.59
300	58.15	15.41
600	67.22	15.82
1200	46.89	10.82
2400	32.20	6.89

The mean dose-normalized AUC and Cmax decreased by approximately 50% at the highest dose of 2400 mg. Decreases in dose-normalized AUC are observed above the 100 mg dose.

The amount of bosentan excreted within 24 hours in urine was between 0.4% and 1.3% of the dose. The sponsor cautions that this might be underestimated by as much as 25% because of adsorption of bosentan to the wall of the plastic containers used for sampling and storage of urine.

PHARMACODYNAMICS:

Intradermal injection of the low concentration of ET-1 (0.3 pmol) did not produce the typical skin response (area of flare and pallor). Intradermal injection of the high concentration of ET-1 (3 pmol) did produce the typical skin response (area of flare and pallor). The area of pallor and flare were characterized by high inter-subject variation. Bosentan had a small effect on the skin responses produced by intra-dermal injections of ET-1 30 pmol. The area of pallor and the Doppler blood flow measured in the area of the flare were not different from placebo at the lower doses. At the 2 highest doses, the area of flare was reduced for over 8 hours with maximal inhibition of 57% (2-h) and 52% (4-h) after 1200 mg and 2400 mg, respectively, compared to baseline values (see Figure below).

There was a tendency toward lower blood pressure between 1 and 8 hours after dosing. The blood pressure lowering effect was more pronounced on standing blood pressure, but the decrease was not dose-dependent.

Endothelin-1 (ET-1) Concentrations:
Lower doses of bosentan, 100 and 300 mg, produced little or no effect on ET-1 concentrations. The highest dose of bosentan, 2400 mg, increased mean ET-1 levels 1.8 fold from baseline. The increase in ET-1 concentrations was dose-dependent (see Figure below).

SAFETY

There were no deaths. One subject (#17) had a syncopal episode with asystole and urine loss about 1 hour after dosing with 30 mg. This was described as vasovagal collapse. About 2 hours

after dosing, this subject had an episode of orthostatic hypotension with dizziness. He recovered without sequelae.

Routine adverse events reported by at least 2 subjects who received at least 600 mg were headache and head discomfort.

CONCLUSIONS:

Upon oral dosing, bosentan concentrations increased with a T_{max} between 2 and 3 hours. After attaining C_{max} plasma concentrations of bosentan declined with a $T_{1/2}$, which increased with dose, that ranged between 4 and 7 hours. Both C_{max} and AUC of bosentan increased less than proportionally with dose. The less than proportional increase in AUC and Cmax at higher doses could be due to limited absorption because of low solubility of bosentan. The mean dose-normalized AUC and Cmax decreased by approximately 50% at the highest dose of 2400 mg. Decreases in dose-normalized AUC are observed above the 100 mg dose.

The most common adverse event was headache/head discomfort. There was a tendency toward lower blood pressure between 1 and 8 hours after dosing. The blood pressure lowering effect was more pronounced on standing blood pressure, but the decrease was not dose-dependent. The highest dose of bosentan, 2400 mg, increased mean ET-1 levels 1.8 fold from baseline. The increase in ET-1 concentrations was dose-dependent

COMMENTS:

1. The analytical report was incomplete. Details of the standard curve, quality control samples, intra- and inter-day variability in assay of urine samples was not provided. The sponsor was requested to provide the missing analytical information via a teleconference call on April 24, 2001. Data submitted by the sponsor in the submission dated June 21, 2001 was subsequently incorporated into the review.

2. The sponsor should have measured the concentrations of the major metabolites of bosentan in this study. This would shed some light on the linearity and dose proportionality of metabolite concentrations with increasing bosentan doses.

STUDY B-162287 – A SINGLE ASCENDING INTRAVENOUS DOSE STUDY OF THE TOLERABILITY, SAFETY, PHARMACODYNAMICS, PHARMACOKINETICS AND THE ABSOLUTE BIOAVAILABILITY OF THE ENDOTHELIN RECEPTOR ANTAGONIST RO 47-0203 IN YOUNG HEALTHY MALE VOLUNTEERS.

STUDY INVESTIGATOR AND SITE:

Report No.: B-162287
Volume No.: 4

OBJECTIVES:

1. To investigate the tolerability and safety, pharmacokinetics and pharmacodynamic effects:
   a) the skin reaction to intradermal endothelin-1 (10 pmol)
   b) the changes in endothelin plasma and urine concentrations
2. To investigate the absolute bioavailability based on a high and a well tolerated intravenous/oral dose.

FORMULATIONS:

Bosentan intravenous – lyophilisate reconstituted with 10.5 ml of water for injection and further diluted with saline as necessary (Batch #: GSU0032 and GSU0033)
Bosentan oral – suspension (Batch #: GFR0030)
Placebo for bosentan intravenous – 5% dextrose solution (Batch GFR0029)

STUDY DESIGN:

This was a single center, randomized, two-part, single-dose, dose escalation study in 64 healthy adult male volunteers between the ages of 19 to 31 years. The study consisted of 2 parts:
Part I: Double-blind, placebo controlled, parallel group, single ascending intravenous dose study of bosentan and placebo in 64 subjects (48 bosentan/16 placebo). The different ascending doses and duration of infusion were, 10 mg/5 min, 50 mg/5 min, 250 mg/5 min, 500 mg/5 min, 750 mg/5 min, 500 mg/30 min, 500 mg/7.5 min, 750 mg (350 mg /21 min followed by 400 mg/219 min). In each dose group, 6 subjects were randomized to bosentan suspension and 2 were randomized to matching placebo
Part II: Two single doses 600 mg bosentan given orally and 250 mg given intravenously to 7 subjects in a open-label, crossover fashion with a randomized sequence with a washout period of at least 4 days but less than 14 days between the 2 treatments.

The pharmacodynamic effect of bosentan was determined as % change of the skin reaction and skin blood flow following intradermal injections of a fixed endothelin-1 dose (10 pmol).

ASSAY:

All samples were analyzed at

Compound	*Matrix*	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%		Accuracy (% Bias)
							Intra	Inter	
Bosentan	Plasma		NP	NP			NP		
							NP		
							NP		
Bosentan	Plasma		NP	NP			NP		
							NP		
Bosentan	Urine		NP	NP		NP	NP	NP	NP
						NP	NP	NP	NP

NP=Not Provided

was used for the lower dose groups of 10 and 50 mg and for all samples below ng/ml. method was used for all other samples.

Sample Collection:
Part I: Blood samples (10-ml) for measurement of plasma concentrations of bosentan were collected at 0 (predose), 5 min, 10 min, 20 min, 35 min, 1, 1.5, 2.5, 4, 6, 8, 10, 12 and 24 hours for Groups I, II, III, IV and V. Additional samples were obtained at 7.5 min, 15 min, 30 min and 45 min in addition to the regular schedule in Groups VI, VII and IX.
Part II: Intravenous: Blood samples (10-ml) for measurement of plasma concentrations of bosentan were collected at 0 (predose), 7.5 min, 15 min, 20 min, 30 min, 45 min, 1, 1.5, 2.5, 4, 6, 8, 10, 12 and 24 hours post dose.
Part II: Oral: pre-dose and 35 min, 1, 1.5, 2.5, 4, 6, 8, 10, 12, 16 and 24 hours post-dose.

Urine samples were collected in Part I for the time-intervals, -12 to 0, 0-4, 4-8, 8-12 and 12-24 hours post dose in all dose groups.

Part I: Intradermal injections of 10 pmol ET-1 were administered at –1 (pre-dose), end of iv (5-min), 2 and 5.5 hours post dose to subjects in Groups I through V.

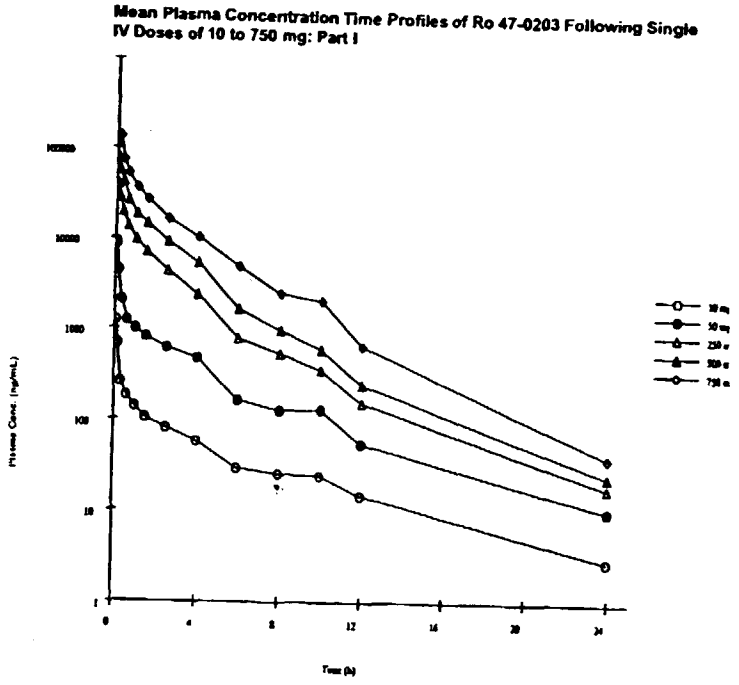
RESULTS:

Part I:
The pharmacokinetic parameters of bosentan obtained following administration of a single dose of 5, 50, 250, 500 and 750 mg bosentan as an intravenous infusion in healthy males are listed in the following table.

Table 2: Mean (%CV) Pharmacokinetic Parameters of Intravenous Bosentan

Dose	Inf. Duration (min)	$T_{0.5}$ (h)	AUC (ng.h/ml)	CL (L/h)	Vss (L)	Furine (%)
10 mg	5.0	4.3 (23)	949 (9)	10.8 (25)	47.7 (25)	
50 mg	5.0	3.9 (18)	6191 (38)	12.3 (40)	40.1 (40)	
250 mg	5.0	3.3 (12)	39680 (29)	8.2 (26)	17.9 (16)	
500 mg	5.0	3.1 (13)	80460 (25)	6.6 (27)	13.4 (23)	
750 mg	5.0	2.8 (14)	161400 (25)	5.7 (22)	13.1 (37)	0.78 (54)
500 mg	30.0	3.4 (18)	83080 (37)	8.8 (29)	19.8 (16)	
500 mg	7.5	2.9 (14)	72130 (19)	7.2 (21)	13.1 (22)	
750 mg	240	2.7 (7)	130000 (15)	6.8 (17)	22.7 (12)	

AUC of bosentan increased more than proportionally with dose after 500 mg. The more than proportional increase in AUC at higher doses implies slower clearance with increasing doses of bosentan. Administration of the same dose at a faster rate yielded a smaller CL compared to slower infusion rate. Example: 500 mg dose administered in 5 min, 7.5 min and 30 min yielded CL values of 6.6 L/h, 7.2 L/h and 8.8 L/h, respectively. However, when a model dependent approach was used and data was analyzed using NONMEM, the decrease in CL was not related to dose. The mean CL estimate obtained using NONMEM (by the sponsor) was 7.6 L/h with an intersubject variability of 25%. The more than proportional increase in AUC in the present intravenous study is in contrast to the less than proportional increase in AUC observed following oral dosing, which was attributed to limited absorption.



Secondary peaks were observed in most subjects at 4 hours and between 8 and 10 hours post dose. The $T_{1/2}$ of about 4 hours did not increase with dose as was seen in the previous oral ascending dose study.

The amount of bosentan excreted within 24 hours in urine was between 0.2% and 1.2% of the dose.

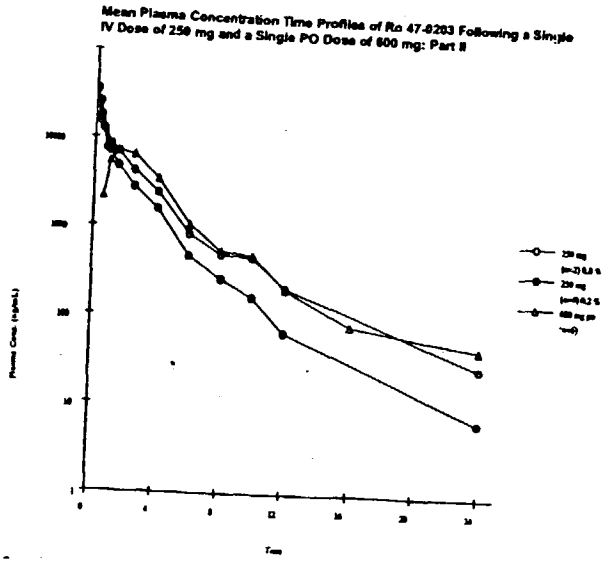
Part II:

The pharmacokinetic parameters obtained following administration of 250 mg intravenous bosentan and 600 mg oral bosentan in healthy males are listed in the following table.

Table 3: Mean (%CV) Pharmacokinetic Parameters of Intravenous and Oral Bosentan

Dose	Mode	T_{max} (h)	$T_{0.5}$ (h)	CL (L/h)	CL/F (L/h)	Vss (L)	F (%)
250 mg	Intravenous	-	3.4 (14)	10.4 (37)	-	21 (28)	-
600 mg	Oral	1.8 (28)	6.6 (21)	-	21.9 (29)	-	49.8 (44)

The absolute bioavailability of 600 mg bosentan was 50%, with a high interindividual variability between 30 and 78%. T_{max} was achieved within 2 hours of oral dosing. The $T_{1/2}$ of oral bosentan was higher than intravenous bosentan.

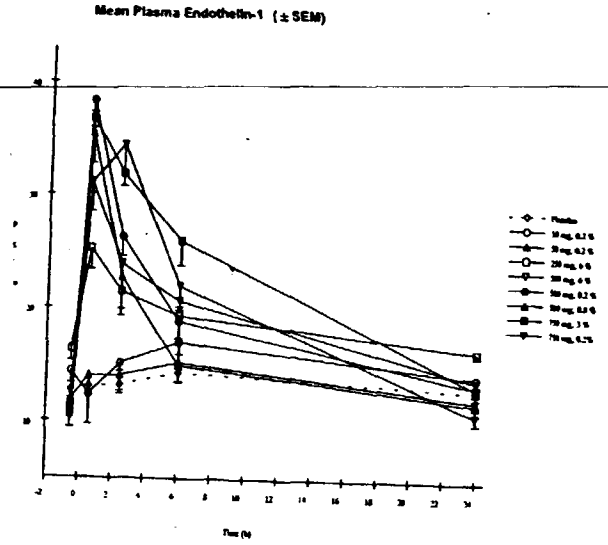


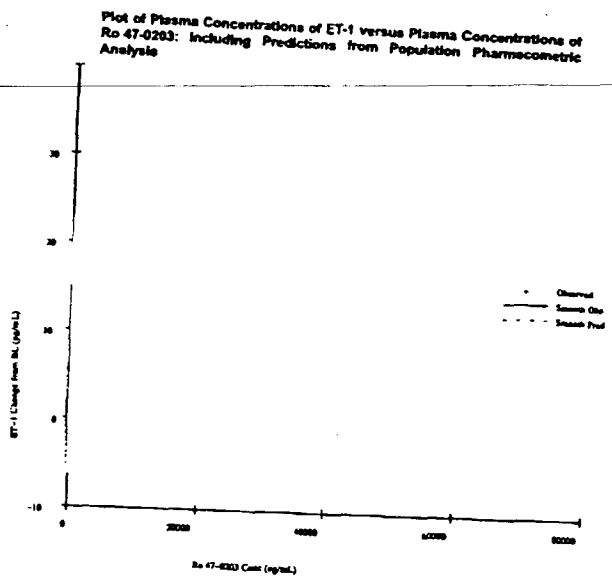
PHARMACODYNAMICS:

Endothelin-1 (ET-1) Concentrations:

Lower doses of bosentan, 10 and 50 mg, produced little or no effect on ET-1 concentrations. At doses >250 mg bosentan, ET-1 concentrations increased and remained elevated for 6 hours. The elevated ET-1 concentration returned to baseline values in 24 hours. Following 500 mg and 750 mg doses, ET-1 concentrations increased 3-fold, to about 25 pg/ml.

Modeling of the bosentan concentration effect on ET-1 concentrations using an E_{max} model predicted an ED50 of 12.5 µg/ml with a intersubject variability of 64%. The predicted E_{max} was 31 pg/ml and a baseline effect (E_0) of 0.7 pg/ml (see figure below).





The amount of ET-1 excreted per hour in the urine was similar to placebo and no dose-dependent increase in ET-1 excretion in urine was observed.

SAFETY:

Headache and head discomfort were commonly reported. Numerous reports of infusion site reaction were reported for the higher bosentan doses. Nausea with and without vomiting also started to be reported and was considered to be dose limiting. There were no serious events.

CONCLUSIONS:

Administration of ascending intravenous doses of bosentan increased AUC of bosentan more than proportionally especially after 500 mg. The more than proportional increase in AUC at higher doses implies slower clearance with increasing doses of bosentan. However, when a model dependent approach was used and data was analyzed using NONMEM, the decrease in CL was not related to dose. The mean CL estimate obtained using NONMEM was 7.6 L/h with

an intersubject variability of 25%. The more than proportional increase in AUC in the present intravenous study is in contrast to the less than proportional increase in AUC observed following oral dosing, which was attributed to limited absorption.

The absolute bioavailability of 600-mg bosentan was 50%. T_{max} was achieved within 2 hours of oral dosing. The $T_{1/2}$ of oral bosentan was higher, 6.6 h, than intravenous bosentan (3.4 h).

Modeling of the bosentan concentration effect on ET-1 concentrations using an E_{max} model predicted an ED50 of 12.5 µg/ml with an intersubject variability of 64%. The predicted E_{max} was 31 pg/ml and a baseline effect (E_0) of 0.7 pg/ml. The most common adverse event was headache/head discomfort that was probably related to bosentan.

COMMENTS:

1. The analytical report was incomplete. Details of the standard curve used in assay of plasma samples using either( ) or( ) were not provided. Also, details of the standard curve, quality control samples, intra- and inter-day variability in assay of urine samples was not provided.

2. The sponsor should have measured the concentrations of the major metabolites of bosentan in this study. This would shed some light on the linearity and dose proportionality of metabolite concentrations with increasing bosentan doses.

3. The sponsor was requested to provide the missing analytical information via a teleconference call on April 24, 2001.

STUDY B-159037 – MULTIPLE ASCENDING ORAL DOSE STUDY OF THE TOLERABILITY, SAFETY, PHARMACODYNAMICS AND PHARMACOKINETICS OF THE ENDOTHELIN RECEPTOR ANTAGONIST RO 47-0203 IN YOUNG HEALTHY MALE VOLUNTEERS.

STUDY INVESTIGATOR AND SITE:

Report No.: B-159037
Volume No.: 6

OBJECTIVES:

1. To investigate the multiple dose tolerability and safety, pharmacokinetics and the following pharmacodynamic effects:
   a) the skin reaction to intradermal endothelin-1 (10 pmol)
   b) the changes in endothelin plasma concentrations

FORMULATIONS:

Bosentan tablets – 100 mg (Batch #: G Lu 007)
Bosentan tablets – 500 mg (Batch #: G Lu 006)
Placebo for bosentan tablets – (Batch GFR0029)

STUDY DESIGN:

This was a single-center, randomized, double-blind, placebo-controlled, multiple dose escalation study in 32 healthy adult male volunteers between the ages of 18 to 32 years. Based on the safety and tolerance of the preceding dose, once-a-day doses of bosentan were to be escalated in the following scheme – 100, 200, 500 and 1000 mg. In each dose group, 6 subjects were randomized to bosentan and 2 were randomized to matching placebo. All doses were administered in the morning following an overnight fast.

The pharmacodynamic effect of bosentan was determined as % change of the skin reaction and skin blood flow following intradermal injections of a fixed endothelin-1 dose (10 pmol) in the 100 mg and 200 mg dose groups only.

ASSAY:

All samples were analyzed at .

Compound	Matrix	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
Bosentan	Plasma			NP				
Bosentan	Plasma			NP				
Bosentan	Urine			NP				

NP=Not Provided

[ ]was used for analysis of plasma bosentan concentration at the lower dose groups of 100 and 200 mg.[ ]method was used for all other samples.

Sample Collection:
Blood samples (6-ml) for measurement of plasma concentrations of bosentan were collected on Days 1 and 8 at 0 (predose), 15 min and 30 min and 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours post dose in all dose groups.

Urine samples were collected on Days 1 and 8 for the time-intervals, 0-12 and 12-24 hours post dose in all dose groups.

Intradermal injections of 10 pmol ET-1 were administered on Days 1 and 8 at –1 (pre-dose), 0.5, 1.5, 3.5 and 7.5 hours post dose in subjects receiving 100 and 200 mg doses.

RESULTS:

The pharmacokinetic parameters of bosentan obtained following once daily administration of 100, 200, 500 and 1000 mg bosentan tablets for 8 days in healthy males are listed in the following table.

Table 2: Mean (%CV) Pharmacokinetic Parameters of Oral Bosentan

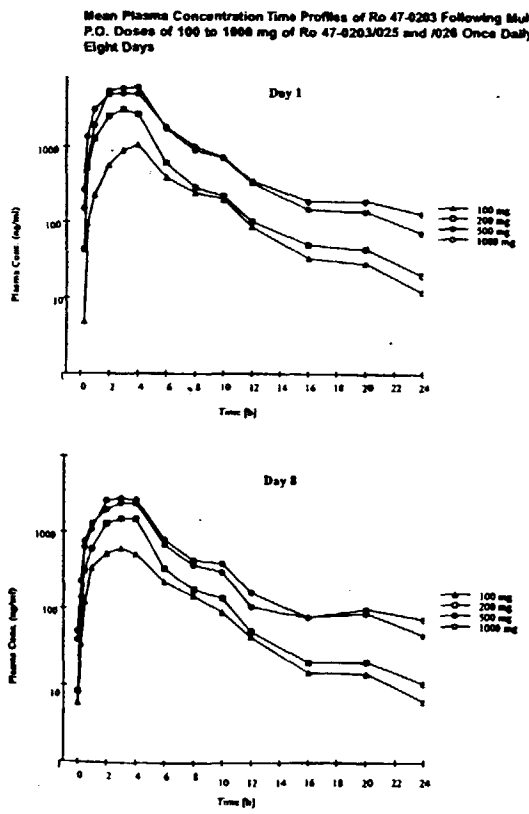
Dose	Day	T_{max} (h)	C_{max} (ng/ml)	$T_{0.5}$ (h)	AUC (ng.h/ml)	CL/F (L/h)	Furine (%)
100 mg	1	3.5 (24)	1105 (50)	4.8 (17)	5469 (44)	21.6 (46)	0.25 (33)
	8	2.8 (27)	695.8 (19)	4.7 (15)	3426 (18)	30.0 (17)	0.31 (15)
200 mg	1	3.3 (16)	3229 (43)	6.7 (57)	13800 (35)	16.5 (43)	0.10 (52)
	8	3.2 (24)	1884 (32)	7.3 (70)	7309 (32)	30.1 (35)	0.13 (61)
500 mg	1	3.3 (24)	6453 (56)	8.3 (63)	31640 (53)	19.9 (48)	0.14 (107)
	8	3.0 (30)	3491 (39)	7.1 (33)	15030 (39)	39.7 (55)	0.33 (56)

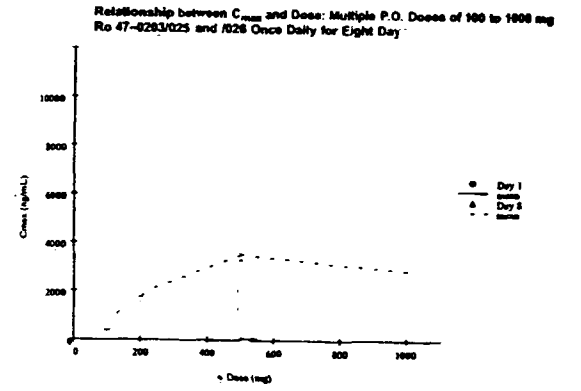
1000 mg	1	3.0 (30)	5441 (58)	13.4 (77)	32880 (54)	39.7 (58)	0.17 (45)
	8	3.2 (24)	2859 (38)	19.6 (32)	13310 (26)	79.7 (26)	0.15 (35)

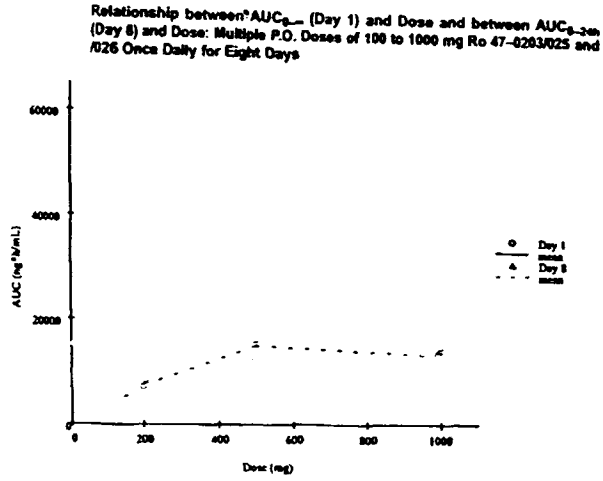
Cmax and AUC of bosentan on Day 8 was lower by approximately 50% compared to Day 1. The reduction in Cmax and AUC seen upon multiple dosing might be due to induction of metabolizing enzymes.

The Tmax of bosentan occurred between 2 and 3 hours. Secondary peaks were observed in most subjects approximately 10 hours post dose. The $T_{1/2}$ of about 5-8 hours increased to 14-20 hours at the high dose of 1000 mg. Fraction of the administered dose excreted unchanged in the urine, about 0.10% to 0.33% of dose.

Cmax and AUC of bosentan increased less than proportionally with dose especially after 500 mg. The Cmax and AUC of bosentan on both Days 1 and 8 following administration of 500 mg were higher than the respective values following the 1000 mg dose. The less than proportional increase in Cmax and AUC was also observed previously in a single ascending oral dose study and was attributed to limited absorption due to poor solubility of bosentan.







Bioavailability of bosentan from the suspension formulation (previous single ascending dose study) was higher than the present tablet formulation. This was very evident at higher doses of bosentan. Example: Day 1 Cmax and AUC following administration of 1200 mg suspension was 14830 ng/ml and 61420 ng.h/ml, respectively, which was significantly higher than the Cmax and AUC of 5441 ng/ml and 32880 ng.h/ml, respectively, obtained following administration of 1000 mg tablet. This implies that the bioavailability of bosentan especially at high doses is limited by solubility/dissolution when formulated as a tablet.

PHARMACODYNAMICS:

Vital signs:
Bosentan had little effect on supine and standing blood pressure. Subjects taking bosentan 1000 mg tended to have a mean increase in supine heart rate of about 10 bpm compared to those taking placebo. Mean standing heart rate also tended to be higher in the bosentan groups. However, no dose response was identified.

Endothelin-1 (ET-1) Concentrations:
Lower doses of bosentan, 100 and 200 mg, produced little or no effect on ET-1 concentrations.

SAFETY:

There were no deaths or serious adverse events or discontinuations because of adverse events. Routine adverse events reported by 2 or more subjects who received at least 500 mg bosentan included headache, somnolence, nasopharynx irritation, and fatigue. No event was dose related. Placebo subjects also reported headache.

Abnormal laboratory values included 2 bosentan subjects (200 mg and 500 mg) with mildly elevated and 1 bosentan subject (500 mg) with moderately elevated ALAT values. The latter subject (#20) had a baseline value of 8 U/L, which rose to 74 U/L after 9 days of dosing and then decreased to 33 U/L with continued dosing.

CONCLUSIONS:

Cmax and AUC of bosentan on Day 8 were lower by approximately 50% compared to Day 1. The reduction in Cmax and AUC seen upon multiple dosing might be due to induction of metabolizing enzymes.

Cmax and AUC of bosentan increased less than proportionally with dose especially after 500 mg. The Cmax and AUC of bosentan on both Days 1 and 8 following administration of 500 mg were higher than their respective values following the 1000 mg dose. A less than proportional increase in Cmax and AUC was also observed previously in a single ascending oral dose study and was attributed to limited absorption due to poor solubility of bosentan. Half-life of bosentan increased with increasing oral doses; $T_{1/2}$ = 4.7 hours for 100-mg dose and $T_{1/2}$ = 19 hours for the 1000-mg dose.

COMMENTS:

1. The analytical report was incomplete. Details of the standard curve used in assay of plasma samples using{ }were not provided. The sponsor was requested to provide the missing analytical information via a teleconference call on April 24, 2001. Data subsequently submitted by the sponsor in Submission dated June 21, 2001 was incorporated into the review.

2. The sponsor should have measured the concentrations of the major metabolites of bosentan in this study. This would shed some light on the linearity and dose proportionality of metabolite concentrations with increasing bosentan doses.

3. The double peaks in plasma concentrations observed at approximately 10 hours post dose could probably be attributed to biliary excretion of bosentan and subsequent re-absorption from the gut resulting in entero-hepatic recirculation of bosentan.

4. The long half-life of 10 to 20 hours observed at the high dose of 1000 mg probably reflects slow dissolution of bosentan and not the elimination half-life. It is evident from intravenous data that the half-life of bosentan ranges from 2 to 5 hours. Also, when bosentan was administered as an oral solution, the half-life ranged from 4 to 7 hours only.

APPEARS THIS WAY
ON ORIGINAL

STUDY B-14898 – THE EFFECT OF MULTIPLE ORAL DOSE TREATMENT WITH RO 47-0203 ON THE ELIMINATION AND ABSORPTION OF Ro 47-0203 IN HEALTHY MALE VOLUNTEERS.

STUDY INVESTIGATOR AND SITE:

Report No.: B-159037
Volume No.: 6

OBJECTIVES:

1. To investigate the effect of multiple (8 days) oral bosentan dose administration on absorption and elimination of bosentan.
2. To investigate the intra-subject variability after intravenous administration of bosentan.
3. To investigate the effect of food on the tolerability of bosentan.

FORMULATIONS:

Bosentan lyophilisate for i.v. administration (Batch #: GSU 0040)
Bosentan 500 mg tablets (Batch #: GSU 0006)
Matched Placebo for bosentan tablets – (Batch #: GSU 008)

STUDY DESIGN:

This was a single-center, randomized, double-blind, placebo-controlled, multiple dose study in 20 healthy adult male volunteers between the ages of 18 to 36 years (mean: 23 y). On Study Days 1 and 11, all subjects received 250-mg bosentan intravenously as a single dose. Subjects were subsequently randomized to receive either 500 mg oral bosentan (n=12) or placebo (n=8) once daily on Days 3-10. All oral doses were administered in the morning immediately following a standard breakfast.

ASSAY:

All samples were analyzed at

Compound	Matrix	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
							Inter	
Bosentan	Plasma			NP				

Bosentan	Plasma	NP
Bosentan	Plasma	NP
Ro 48-5033	Plasma	NP
Ro 47-8634	Plasma	NP

NP=Not Provided

was used for analysis of plasma bosentan concentrations below ng/ml. method was used for all other samples. Cross-validation was performed to compare the two methods used for analysis. The mean difference between the 2 methods was 3.9% for n=33 samples.

Sample Collection:
Blood samples for measurement of plasma concentrations of bosentan and its metabolites were collected on Day 1 at 0 (predose), 0.25, 0.5, 1, 2, 4, 6, 8, 12, 16, 20, 24, 28, 36 and 48 hours post dose. Blood samples were also collected on Days 3, 4, 5, 10, 11, 12 and 13, however, the study report does not specify the exact times of collection of blood samples on these days.

Twenty-four hour urine samples were collected on Days 1, 3, 5 and 11 for measurement of cortisol, 6-hydroxycortisol and 17-hydroxycorticosteroids. All these analytes were measured using ELISA.

RESULTS:

The pharmacokinetic parameters of bosentan and its metabolites obtained following administration of a multiple 500 mg oral doses of bosentan in healthy males are listed in the following table.

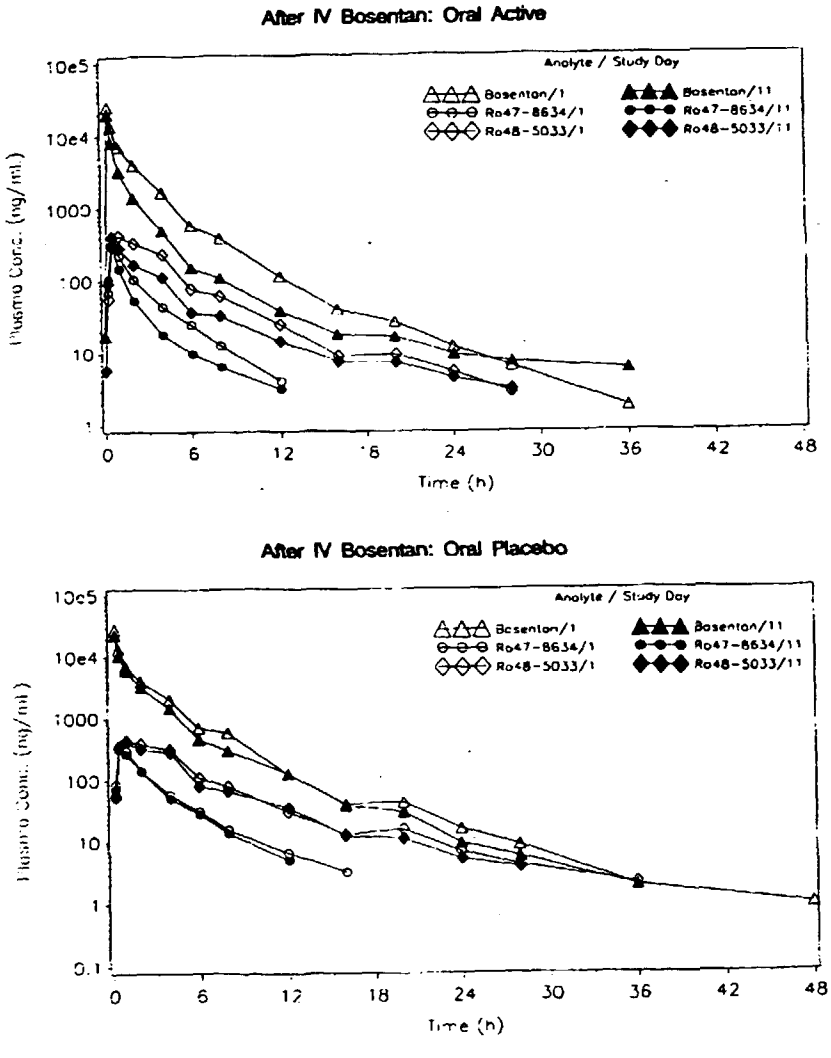
Table 2: Mean (%CV) Pharmacokinetic Parameters of Oral Bosentan and Metabolites

Compound	Day	T_{max} (h)	C_{max} (ng/ml)	$T_{0.5}$ (h)	AUC (ng.h/ml)	CL/F (L/h)	F (%)
Bosentan	3	3.0 (45)	5484 (41)	4.3 (20)	25600 (35)	21.7 (33)	43.2 (20)
	10	2.2 (27)	3637 (41)	5.0 (33)	14430 (34)	39.0 (42)	48.4 (20)
Ro 48-5033	3	4.0 (21)	511.7 (61)	9.3 (57)	2566 (45)		

	10	3.7 (21)	330.8 (62)	16.2 (105)	1576 (47)		
Ro 47-8634	3	2.9 (36)	180.9 (27)	2.9 (31)	773.0 (28)		
	10	2.5 (36)	130.1 (38)	2.7 (45)	491.5 (38)		

Mean Cmax and AUC of bosentan on Day 10 were lower by approximately 43% compared to Day 1. The reduction in Cmax and AUC was paralleled by an approximately 2-fold increase in intravenous clearance of bosentan between Day 1 and Day 11 indicating induction of metabolizing enzymes. The single-dose and steady-state bioavailability of bosentan were similar, approximately 45-48%.

Figure 4. Mean Plasma Concentration Time Profiles of Bosentan and the Metabolites Ro 48-5033 and Ro 47-8634 Following Intravenous Administration of 250 mg Bosentan Before and After a 6 Day Oral Treatment Period



Upon multiple dosing mean Cmax and AUC of the active metabolite Ro 48-5033 and the inactive metabolite Ro 47-8634 decreased by a similar magnitude (about 40%) as bosentan on Day 10 compared to Day 3.

Table 3: Mean (%CV) Pharmacokinetic Parameters of 250-mg Intravenous Bosentan

	Active			*Placebo*		
Day	Vss (L)	CL (L/h)	$T_{0.5}$ *(h)*	Vss (L)	CL (L/h)	$T_{0.5}$ *(h)*
1	17.8 (19)	8.9 (20)	4.7 (29)	17.7 (24)	8.6 (28)	4.8 (34)
11	25.5 (25)	17.7 (21)	8.3 (85)	22.2 (21)	11.1 (39)	4.7 (25)

In addition to increased clearance, volume of distribution at steady-state and half-life of bosentan also increased in subjects receiving active treatment. Mean clearance and half-life increased approximately 2-fold in the active group. While, mean clearance was slightly higher and half-life was unchanged at 5-h in the placebo group. Plasma protein binding remained unchanged at 96% to 97% in both active and placebo groups on Days 1 and 11.

Table 4: Mean (%CV) Pharmacokinetic Parameters of Bosentan Metabolites after 250-mg Intravenous Bosentan

		Active				Placebo			
Compound	Day	T_{max} (h)	C_{max} (ng/ml)	$T_{0.5}$ (h)	AUC (ng.h/ml)	T_{max} (h)	C_{max} (ng/ml)	$T_{0.5}$ (h)	AUC (ng.h/ml)
Ro 48-5033	1	1.4 (68)	438.5 (25)	8.0 (50)	1983 (30)	1.8 (64)	450.6 (29)	7.8 (47)	2455 (47)
	11	0.6 (33)	412.2 (57)	10.2 (7)	1216 (36)	0.9 (22)	429.5 (34)	5.3 (33)	2145 (49)
Ro 47-8634	1	0.6 (33)	280.3 (29)	2.4 (21)	639.6 (31)	0.7 (39)	324.7 (35)	2.9 (27)	837.3 (32)
	11	0.5 (0)	312.8 (27)	2.7 (35)	432.4 (35)	0.7 (39)	351.0 (36)	2.7 (30)	772.0 (34)

Following intravenous administration, a substantial, approx. 40%, decrease in mean AUC of Ro 48-5033 and Ro 47-8634 was observed on Day 11 compared to Day 1. However, there was little change in Cmax and half-life. Cmax of the active metabolite Ro 48-5033 occurred in less than an hour on Day 11 compared to 1.5 h on Day 1 in both the active and placebo groups.

The Cmax and AUC of the active metabolite Ro 48-5033 was about 10% of bosentan after both oral and intravenous dosing.

Intra-subject variability of the 6-hydroxycortisol/cortisol ratio in the placebo group was 25%. On average 6-hydroxycortisol/cortisol ratio increased by a factor of 1.5 in the active group compared to a factor of 0.9 in the placebo group. When 6-hydroxycortisol/17-hydroxycorticosteroid ratio

were compared, the active group exhibited an increase of 1.7 compared to 0.7 in the placebo group. This indicates that bosentan possesses CYP 3A4 enzyme inducing potential upon multiple dosing.

Figure 5a. Plot of the Mean Ratio of 6 -Hydroxycortisol/Cortisol versus Time for the Active and Placebo Group

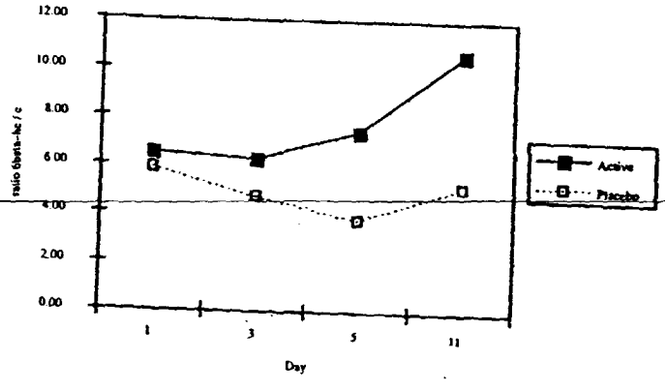
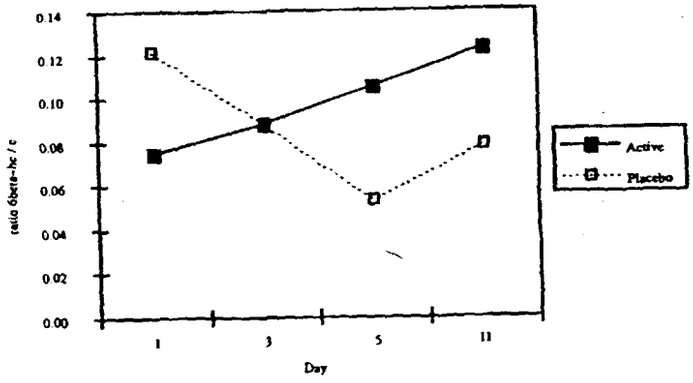


Figure 5b. Plot of the Mean Ratio of 6 -Hydroxycortisol/17-Hydroxycorticosteroids versus Time for the Active and Placebo Group



SAFETY:

There were no reports of deaths, serious adverse events, or withdrawals because of adverse events. Headache was the most frequently reported adverse event.

Decreased hemoglobin values were reported for 20 subjects (4 placebo, 3 bosentan 100 mg, 5 bosentan 200 mg 2 bosentan 500 mg and 6 bosentan 1000 mg) and elevated ALT values were reported for 5 subjects (the largest increase from baseline was 9 fold).

CONCLUSIONS:

Mean Cmax and AUC of bosentan on Day 10 were lower by approximately 43% compared to Day 1. The reduction in Cmax and AUC was paralleled by an approximately 2-fold increase in intravenous clearance of bosentan between Day 1 and Day 11 indicating induction of metabolizing enzymes. The bioavailability of bosentan on Days 3 and 10 were similar, at approximately 45%. Upon multiple dosing mean Cmax and AUC of the active metabolite Ro 48-5033 and the inactive metabolite Ro 47-8634 decreased by a similar magnitude (about 40%) as bosentan on Day 10 compared to Day 3. Mean clearance and half-life increased approximately 2-fold in the active group. Plasma protein binding remained unchanged at 96% to 97% in both active and placebo groups on Days 1 and 11. When 6-hydroxycortisol/17-hydroxycorticosteroid ratio were compared, the active group exhibited an increase of 1.7 compared to 0.7 in the placebo group, indicating CYP 3A4 inducing potential by bosentan upon multiple dosing.

APPEARS THIS WAY
ON ORIGINAL