

Office of Clinical Pharmacology and Biopharmaceutics
New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	21-290	Brand Name	TRACLEER
OCBP Division (I, II, III)	I	Generic Name	Bosentan
Medical Division	Cardio-Renal Drug Products	Drug Class	Endothelin Receptor Antagonist
OCBP Reviewer	Gabriel J. Robbie	Indication(s)	Pulmonary Arterial Hypertension
OCBP Team Leader	Patrick J. Marroum	Dosage Form	Tablets
		Dosing Regimen	62.5 mg b.i.d. for 4 weeks 125 mg b.i.d. thereafter
Date of Submission	11/17/00	Route of Administration	Oral
Sponsor	Actelion	Priority Classification	1-S

Clin. Pharm. and Biopharm. Information

	“X” if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
I. Clinical Pharmacology				
Mass balance:	X	1	1	
Isozyme characterization:	X	2	2	
Blood/plasma ratio:	X	1	1	
Plasma protein binding:	X	4	4	
Pharmacokinetics (e.g., Phase I) -				
<u>Healthy Volunteers-</u>				
acute dose:	X	2	2	
chronic dose:	x	2	2	
Patients-				
acute dose:	X	1	1	
chronic dose:				
Dose proportionality -				
fasting / non-fasting acute dose:				
fasting / non-fasting chronic dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:	X	7	7	
In-vivo effects of primary drug:	X	7	7	
In-vitro:	X	1	1	
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:	X	1	1	
hepatic impairment:				
PD:				
Phase 2:				
Phase 3:	X	2	2	
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:	x	1	1	

Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability:	X	2	2	
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:	X	2	2	
Bioequivalence studies -				
traditional design; acute / multi dose:	X	1	1	
replicate design; acute / multi dose:				
Food-drug interaction studies:	X	2	2	
Dissolution:	X	1	1	
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies				
<i>Filability and QBR comments</i>				
	"X" if yes	<i>Comments</i>		
Application filable ?	x	Reasons if the application <u>is not</u> filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?		
Comments sent to firm ?	N/A	Comments have been sent to firm (or attachment included). FDA letter date if applicable.		
QBR questions (key issues to be considered)	<ol style="list-style-type: none"> 1. Pharmacokinetics of bosentan in PPH patients? 2. Can we extrapolate observed magnitude of change in bosentan concentrations observed in the special population study and drug interaction studies to PPH patients? 3. Appropriateness of sponsor proposed dissolution medium, containing 1% surfactant, and dissolution specification? 			
Other comments or information not included above				
Primary reviewer Signature and Date PM reviewer Signature and Date	Gabriel J. Robbie			
Secondary reviewer Signature and Date	Patrick J. Marroum			

CC: NDA 21-290, HFD-850(Lee), HFD-110(GORDONM,CSO), HFD-860(Marroum, Mehta), Biopharm (CDER)

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA:	21-290	SUBMISSION DATES
IND:	49,073	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 0.5 % sodium lauryl sulfate in water with a dissolution specification of Q not less than 80% 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.

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

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

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

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, 1 each in severe renal impairment, chronic heart failure, coronary artery disease and essential hypertension patients, and, 2 studies each in migraine and subarachnoid hemorrhage patients. The studies in chronic heart failure, coronary artery disease, essential hypertension, migraine, subarachnoid hemorrhage patients were not reviewed.

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 IC₅₀ 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 C_{max} 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 (V_{ss}) 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 Day 1.

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 V_{ss} (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 70% in 30 minutes. The concentration of sodium lauryl sulfate is too high. A more appropriate medium with similar performance would be 0.5 % sodium lauryl sulfate in water with a specification of Q not less than 80 % 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, HPLC/UV and LC/MS/MS. 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 LC/MS/MS was 0.5 ng/ml.

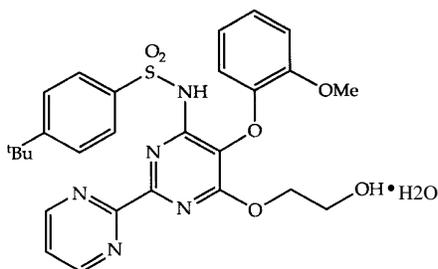
QUESTION BASED REVIEW

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: $C_{27}H_{29}N_5O_6 \cdot H_2O$

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	6.959	13.918
Pre-gelatinized Starch	3.125	6.25
Sodium starch glycolate	3.750	7.50
Povidone K90	0.825	1.65
Glyceryl behenate	2.475	4.95
Magnesium stearate	0.825	1.65
Film Coat		
Hydroxypropylmethyl cellulose	1.560	2.340
Triacetin	0.200	0.300
Talc	0.720	1.080
Titanium Dioxide CI 77891	0.991	1.486
Iron Oxide Yellow CI 77492	0.007	0.011
Iron Oxide Red CI 77491	0.002	0.003
Ethylcellulose Aqueous Dispersion	0.520	0.780

Bosentan drug substance is manufactured by Roche Ireland Ltd., Ireland. Bosentan for commercial distribution will be manufactured, packaged, and labeled by Patheon Inc. Toronto Region Operations, 2100 Syntex Court, Mississauga, Ontario, L5N 7K9, 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, HPLC/UV and LC/MS/MS. Both these methods were sensitive, specific, precise and accurate. The limit of quantification of bosentan in plasma using LC/MS/MS was 0.5 ng/ml. The limit of quantification for all 3 metabolites, Ro 48-5033, Ro 47-8634 and Ro 64-1056, in plasma was 5 ng/ml using LC/MS/MS. Cross validation of the two methods using 33 samples was performed in Study B-14898 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 C_{max} 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 C_{max} 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

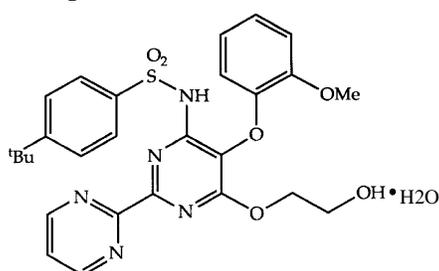
Bosentan is extensively metabolized in the liver and subsequently eliminated in the bile. In an ADME study, following a single oral dose mean recovery of radioactivity in feces was 94% of which 30% was unchanged bosentan. Only 5% of an intravenous dose was excreted unchanged in the urine. The major enzymes 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.

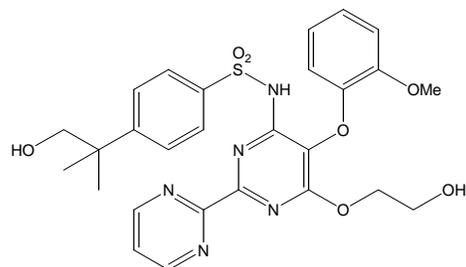
n.m.=not measured

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%.

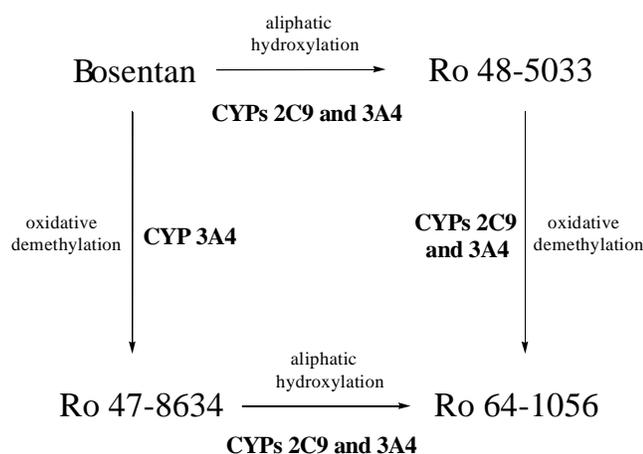
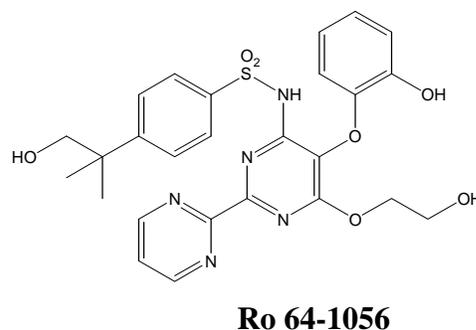
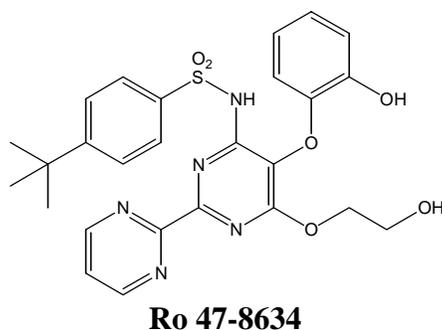
The binding of Ro 47-8634 to ET_A receptor was >300-fold less potent and its binding to ET_B receptor was approximately 30-fold less potent than bosentan. Ro 64-1056 was approximately 30-fold less potent than bosentan.



Bosentan



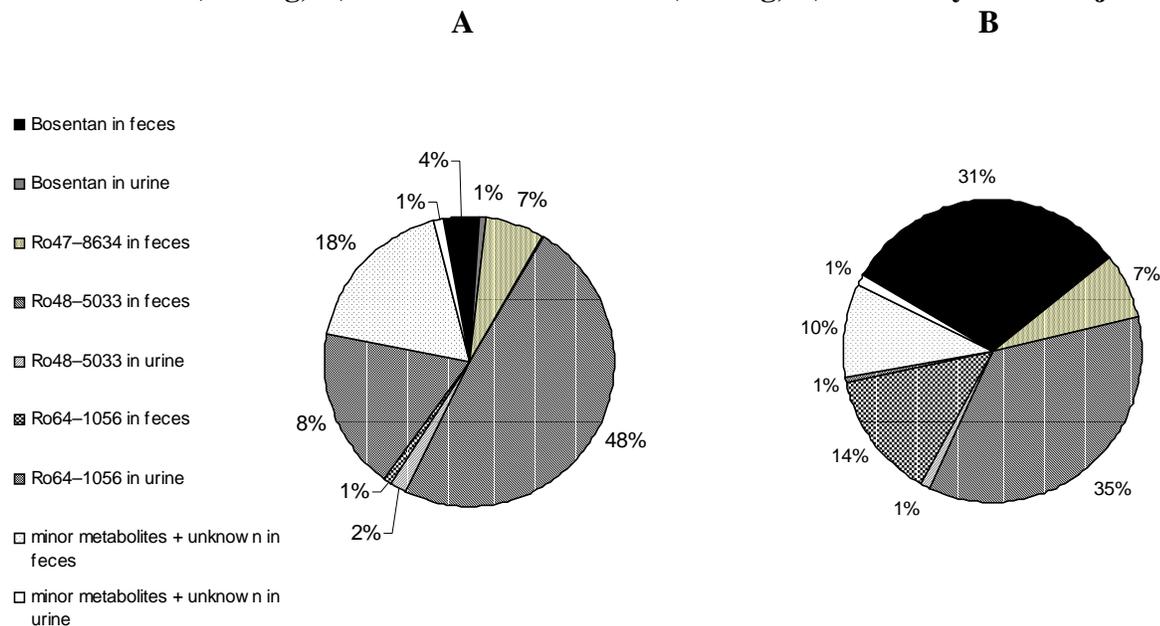
Ro 48-5033



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 94.5% to 99.2%). 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



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 93.1% to 102.1%). 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.

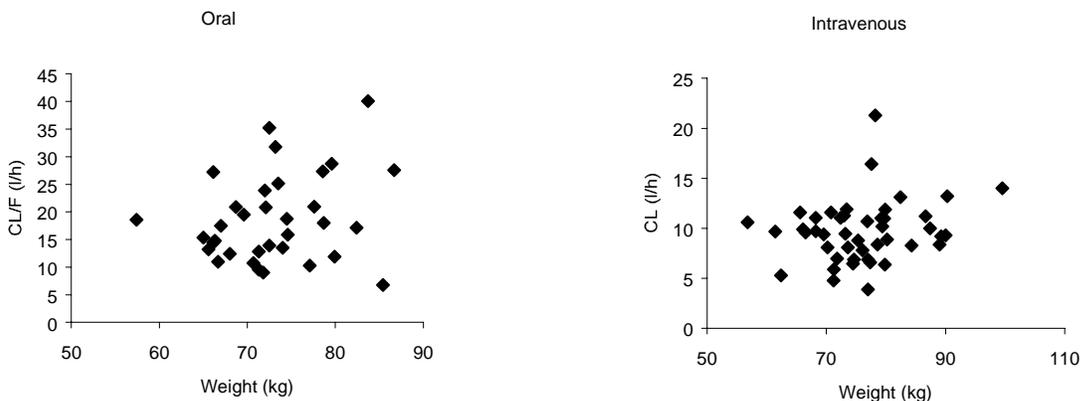
- **WHAT ARE THE VARIABILITIES OF PK PARAMETERS IN VOLUNTEERS AND PATIENTS?**

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

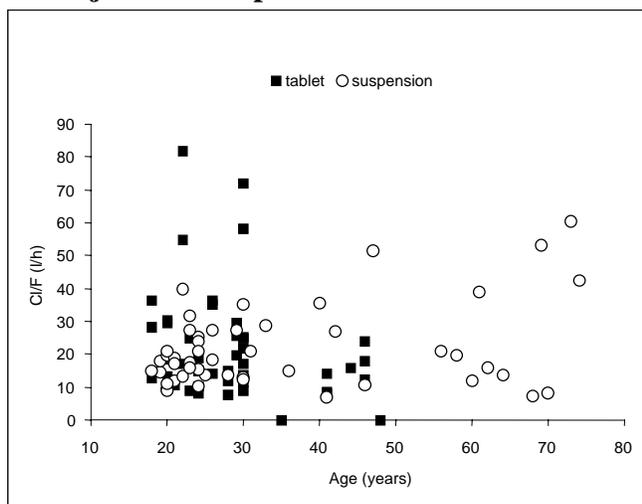
Ethnic group	N	CL/F (l/h)
Black	8	15.4 (9.1, 26.0)
Caucasian	58	15.8 (14.0, 15.9)

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 CHF and SAH 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 CHF and SAH 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 contraindicated.

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 162% and 100%, respectively, compared to single dose trough concentration in the absence of CsA. The magnitude of increase in steady-state bosentan C_{max} 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 C_{max} 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 C_{max} , AUC and trough concentration values by 26%, 49% and 62%, 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 (B-159037) 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 concomitantly. 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 C_{max} and AUC of bosentan by 62% and 83%, respectively. The increase in bosentan C_{max} 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 C_{max} 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 C_{max} 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 SAH 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 SAH 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 37⁰C with a specification of Q not less than 70% in 30 minutes. The concentration of surfactant used is relatively high. Similar dissolution performance is obtained with 0.5 % sodium lauryl sulfate in water. Therefore, the biopharmaceutics reviewer proposes the following dissolution method, medium and specification; dissolution not less than 80% (Q) dissolved in 30 min in 0.5 % sodium lauryl sulfate in water at 50 rpm using USP Apparatus II (paddle).

LABELING RECOMMENDATIONS

CLINICAL PHARMACOLOGY

Clinical Pharmacokinetics

1. Change bioavailability value from 70% to 45%. The amended sentence should read,

“The absolute bioavailability of bosentan is approximately 45% and is unaffected by food.”

2. Change value of volume of distribution at steady-state from 121 L to 21 L. Change value of clearance from 15.6 L/h to 3.8 L/h and state that these parameters were obtained in PPH patients. The amended sentence should read,

“In primary pulmonary hypertension patients, the apparent volume of distribution (V_{ss}) is 21 L and the apparent clearance is 3.8 L/h.”

CLINICAL PHARMACOLOGY

Special Populations

3. Amend the following sentence,

“The pharmacokinetics of bosentan are not influenced by gender, weight, race or age.”

To,

“The influence of gender, weight, race or age on the pharmacokinetics of bosentan is not known.”

4. Reword the following sentence,

“Because there is in vitro and in vivo evidence of an extensive hepatic contribution to the metabolism and elimination of bosentan, liver impairment would be expected to affect its pharmacokinetic and metabolism.”

To,

“In vitro and in vivo evidence suggests extensive hepatic metabolism of bosentan, therefore, liver impairment is expected to significantly affect the pharmacokinetics of bosentan”

PRECAUTIONS

Drug Interactions

5. Move the sentence describing enzyme induction before enzyme inhibition,

The amended PRECAUTIONS/Drug Interactions section should read as follows,

“Bosentan is a mild to moderate inducer of CYP 3A4 and CYP2C9. Consequently, plasma”

“Bosentan had no relevant inhibitory effect of any CYP450 isoenzymes tested.....”

6. Bosentan is expected to alter plasma concentrations of other statins that are predominantly metabolized by CYP 3A4, such as, simvastatin, lovastatin, cerivastatin and atorvastatin.

Therefore, the following amendments are recommended.

a) Change title from “Simvastatin” to “Simvastatin and other statins”

b) Add the following sentence,

“Bosentan is also expected to reduce plasma concentrations of other statins that are predominantly metabolized by CYP 3A4, such as, simvastatin, lovastatin, cerivastatin and atorvastatin.”

The amended simvastatin drug interaction section should read,

“Simvastatin and other statins: Co-administration of bosentan decreased the plasma concentrations of simvastatin (a CYP3A4 substrate), and its active β -hydroxy acid metabolite, by approximately 50%. The plasma concentrations of bosentan were not affected. Bosentan is also expected to reduce plasma concentrations of other statins that are predominantly metabolized by CYP 3A4, such as, lovastatin, cerivastatin and atorvastatin. The possibility of reduced statin efficacy should be considered. An increase in statin dose should be considered when bosentan is started.”

7. Bosentan is expected to alter plasma concentrations of other oral hypoglycemic agents that are predominantly metabolized by CYP 2C9.

Therefore, the following addition is recommended.

“Bosentan is also expected to reduce plasma concentrations of other oral hypoglycemic agents that are predominantly metabolized by CYP 2C9.”

The amended Glyburide (glibenclamide) drug interaction section should read,

“Glyburide (glibenclamide): Co-administration of bosentan decreased the plasma concentrations of glyburide by approximately 30%. The plasma concentrations of bosentan were also decreased by approximately 30%. Bosentan is also expected to reduce plasma concentrations of other oral hypoglycemic agents that are predominantly metabolized by CYP

2C9. *The possibility of reduced hypoglycemic response at therapeutic doses should be considered. 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.*”

8. Although not measured, ketoconazole is expected to increase bosentan concentrations several-fold immediately after co-administration, similar to the 30-fold increase in bosentan concentrations observed with the first dose of cyclosporine.

The Clinical Pharmacology Medical reviewer and the biopharmaceutics reviewer propose contra-indicating coadministration of bosentan and ketoconazole.

9. The potential for drug interaction with oral contraceptives should be highlighted with a separate section as follows,

“Oral Contraceptives: Specific interaction studies have not been performed to evaluate the effect of co-administration of bosentan on oral contraceptives. Since many of these drugs are metabolized by CYP 3A4, there is a possibility of failure of contraception when TRACLEER™ is co-administered. Women may need to use an additional contraceptive method when taking bosentan which may make hormonal contraceptives less effective.”

Use in Elderly Patients

10. Due to lack of data, the information in elderly patients should be changed to the following,

“Clinical studies of TRACLEER™ were not adequate to determine whether subjects aged 65 and over respond differently than younger subjects, greater sensitivity to TRACLEER™ cannot be ruled out. Clinical circumstances, some of which may be more common in the elderly, such as hepatic or renal impairment, can have a clinically significant effect on TRACLEER™ pharmacokinetics (see “CLINICAL PHARMACOLOGY, Clinical Pharmacokinetics”). Close clinical monitoring is prudent for elderly patients. The lowest effective dose should be used to prevent the occurrence of side effects (see “DOSAGE AND ADMINISTRATION”).

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: ¹⁴C-bosentan (0.086 to 348 µg/ml) was separated from plasma, α₁-acid glycoprotein, albumin by a Spectra/Por 2 (12,000-14,000 MW) membrane in a dialysis cell. Equilibrium dialysis was conducted for 3 hours at 37⁰C and the concentration of parent drug was measured by concentration of radioactivity. Fraction unbound was calculated after dialysis using the following equation.

$$fu = \frac{C_{buffer}}{C_{plasma}}$$

Red Blood Cell Binding: Whole blood was spiked with ¹⁴C-bosentan to give concentrations over a range of 0.2 to 208 µg/ml and incubated at 37⁰C. 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

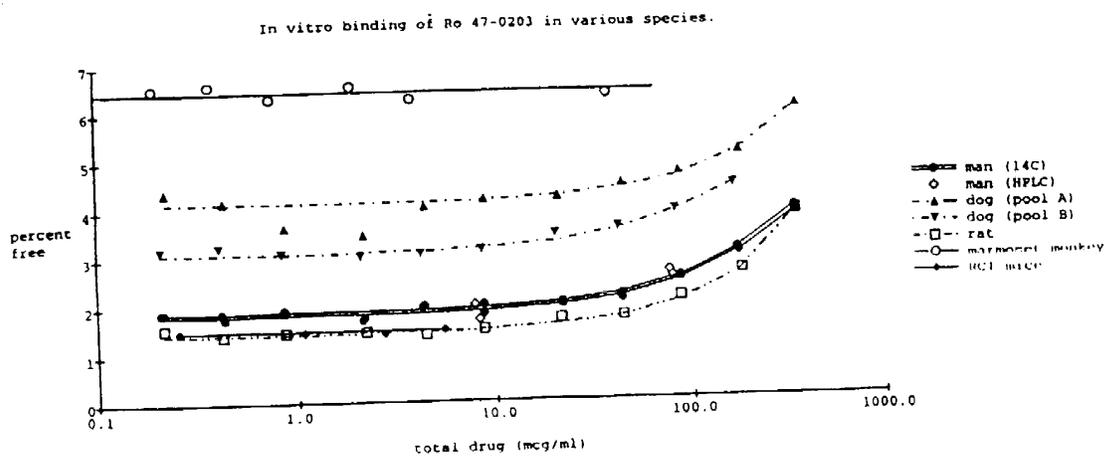
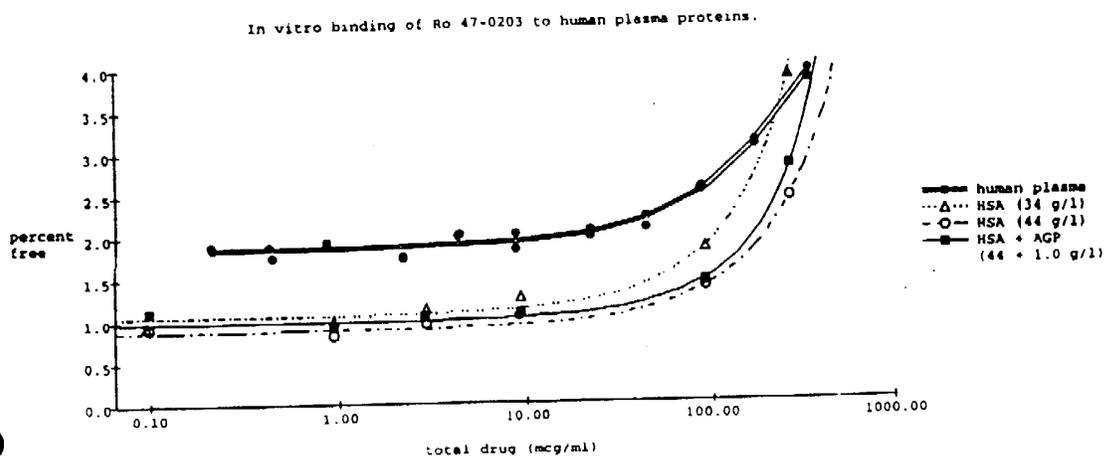


Figure 2



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.

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 37⁰C in a buffer containing 0.05 M potassium phosphate (pH 7.4), 0.1 mM EDTA, 3 mM MgCl₂, microsomal protein, and substrate. An NADPH generating system was used which contained 10 mM glucose-6-phosphate (G-6-P), 1 U/ml G-6-P dehydrogenase, and 0.5 mM NADP⁺. The following table lists the details and results of the inhibition study.

CYP P450	Substrate	Bosentan Conc. Range (µM)	Amt. m'som (pmoles)	Reac.ti me (min)	Assay	IC₅₀ Bosentan (µM)
1A2	Tacrine 1- & 7-hydroxylation	0, 15, 30, 50, 100	208	17.5	HPLC-UV	No Effect
2D6	Dextromethorphan O-demethylation	0, 10, 20, 50	136	30	HPLC-fluorescence	No Effect
2C19	Hydroxylation of S-mephenytoin	0, 30, 70, 125	1000	30	HPLC-radioactivity	203
2C9	4-hydroxylation of diclofenac	0, 5, 10, 25, 50	33	5	HPLC-UV	22
3A4/5	Hydroxylation of Midazolam	0, 5, 10, 25, 50	66	10	HPLC-UV	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 IC₅₀ 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 IC₅₀ values are larger than the highest concentration employed in the experiment.
3. The inhibitory activities of bosentan metabolites were not studied.

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:

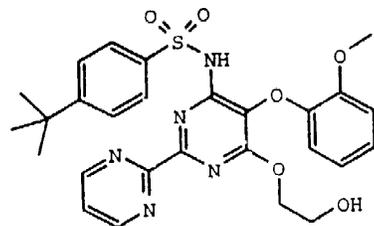
Bosentan (3.6 μM to 108 μM) was incubated with the human liver microsomes (0.5 mg/ml or 1 mg/ml) with a P450 content of 0.354 nmoles/mg protein. Specific inhibitors and competitors were used to inhibit metabolism of bosentan such as: 10 μM methoxypsoralen (CYP 2A6 inhibitor), 7 μM quinidine (CYP 2D6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

RESULTS:

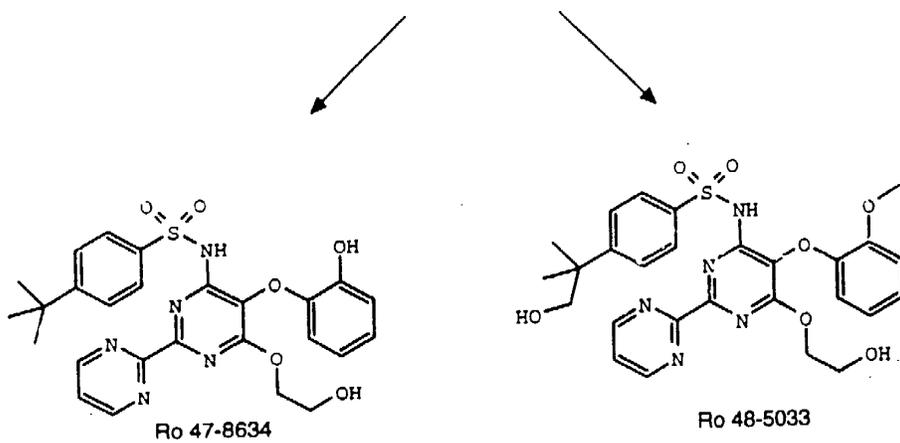
The in vitro human liver microsomal studies indicated that,

- 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.

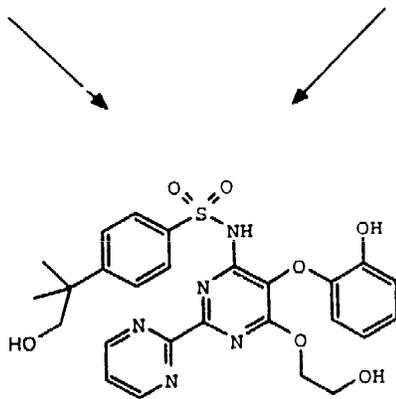


Ro 47-0203/009



Ro 47-8634

Ro 48-5033



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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: J.H.G. Jonkman, Ph.D., F.C.P. R.Ph.

Pharma Bio-Research Int. B.V.
P.O. BOX 200
NL-9470 AE Zuidlaren
The Netherlands

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 [¹⁴C]-Ro 47-0203 (Batch #: GSU 0080)

Bosentan oral suspension – 50 mg/ml, 3.7 MBq [¹⁴C]-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 [¹⁴C]-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 [¹⁴C]-labeled bosentan (3.7 MBq) within 10 min of a standard breakfast.

ASSAY:

All samples were analyzed at F. Hoffmann-La Roche Ltd.
Department of PRPK, Bioanalytical Section
Basel, Switzerland.

Compound	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)	
	<i>Matrix</i>							
Bosentan	Plasma	HPLC/	200 -	NP	2.0	5	13.4	+5.5

		MS		20000		75	3.4	+1.8
						200	0.9	+0.4
						500	1.9	-1.3
						1000	2.1	-2.6
						2000	2.7	+3.6
						5000	1.1	-2.2
						10000	1.9	+3.4
						17500	0.3	-1.4
						20000	1.6	+2.2
Ro 48-5033	Plasma	HPLC/ MS	NP	NP	2.0	5	13.3	+7.4
						75	10.4	+7.9
						200	0.3	-0.1
						500	0.2	+0.4
						1000	0.8	+0.5
						2000	1.3	-1.5
						5000	1.9	-2.7
						10000	1.9	+4.4
						17500	0.9	-3.5
						20000	2.7	+2.6
Ro 47-0634	Plasma	HPLC/ MS	NP	NP	2.0	5	12.5	+5.6
						75	11.7	+1.2
						200	2.9	+1.0
						500	11.5	-2.4
						1000	7.7	+3.4
						2000	0.02	-6.3
						5000	1.2	-5.0
						10000	1.9	+0.6
						17500	1.4	0.4
						20000	1.7	+8.3

NP=Not Provided

¹⁴C-radioactivity was measured using a liquid scintillation analyzer. HPLC/MS was used for analysis of plasma bosentan concentrations below 200 ng/ml and HPLC/UV 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

Substance	Radioactivity Excreted (% of Dose)			
	Intravenous		Oral	
	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

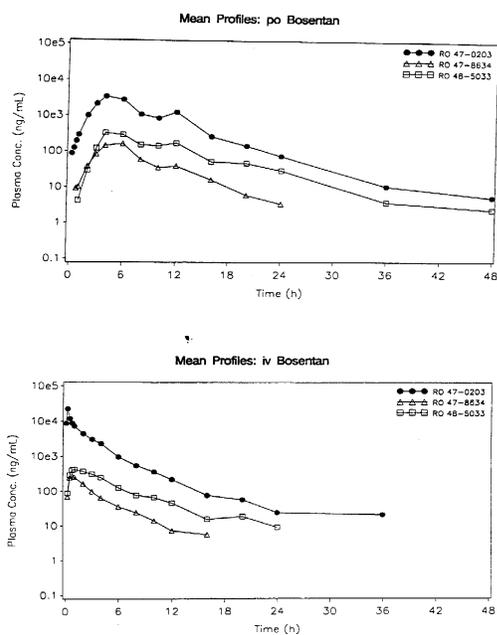
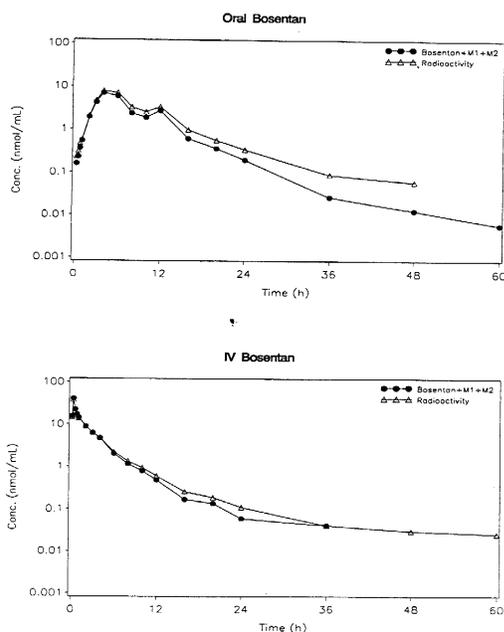


Figure 3. Mean Plasma Concentration Time Profiles of Radioactivity (nmol-equivalents) and the Sum of Bosentan and its two Major Metabolites Ro 48-5033 and Ro 47-8634



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)	V _{ss} (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 C_{max} and AUC ratio of metabolite Ro 48-5033 to bosentan were 11% and 13%, respectively. The metabolite to parent ratio of both C_{max} 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: J.H.G. Jonkman, Ph.D., F.C.P. R.Ph.

Pharma Bio-Research Int. B.V.
NL-9471 GP ZUIDLAREN
The Netherlands

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 F. Hoffmann-La Roche Ltd.
Department of PRPK, Bioanalytical Section
Basel, Switzerland.

Compound	Matrix	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%		Accuracy (% Bias)
							Intra	Inter	
Bosentan	Plasma	HPLC/ UV	10 - 10000	NP	20	50	NP	9.5	-2.2
						500	NP	5.0	-2.2
						5000	NP	6.3	-1.2
Bosentan	Plasma	HPLC/ LC/MS	0.5 - 500	NP	0.5	2.38	NP	6.31	-0.57
						79.1	NP	4.20	-0.48
Bosentan	Urine	HPLC/ UV	50 - 25000	NP	50	50	NP	1.9	-1.0
						506	NP	3.2	-4.5
						1021	NP	2.2	-5.7

NP=Not Provided

HPLC/UV 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 HPLC/UV. For all other subjects the analytical results of the LC/MS 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

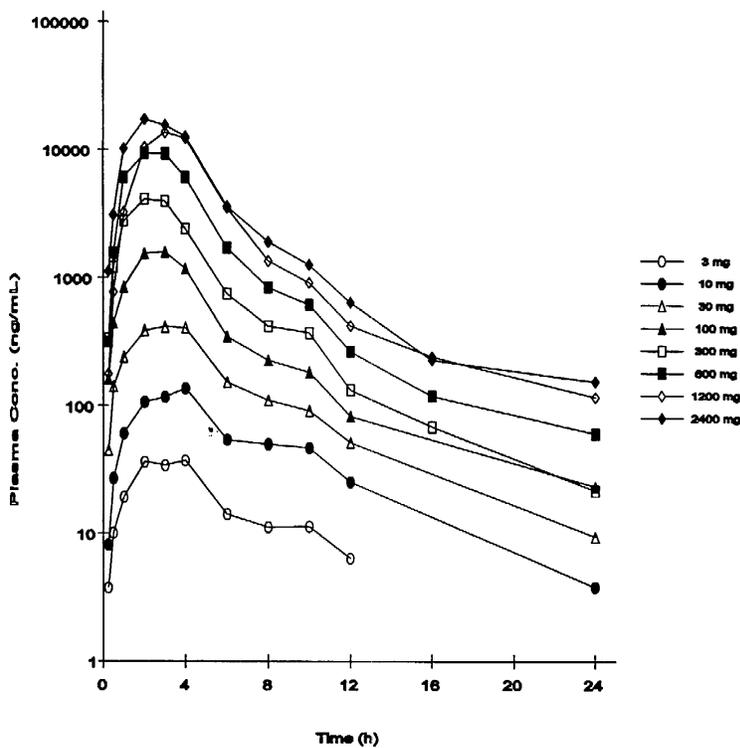
	C _{max} (ng/ml)	T _{max} (h)	T _{0.5} (h)	AUC (ng.h/ml)	CL/F (L/h)
3 mg	38.7 (51)	2.8 (47)	3.7 (22)	267.8 (30)	12.2 (32)
10 mg	139.1 (53)	2.7 (45)	4.0 (10)	1009 (35)	11.0 (35)

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)

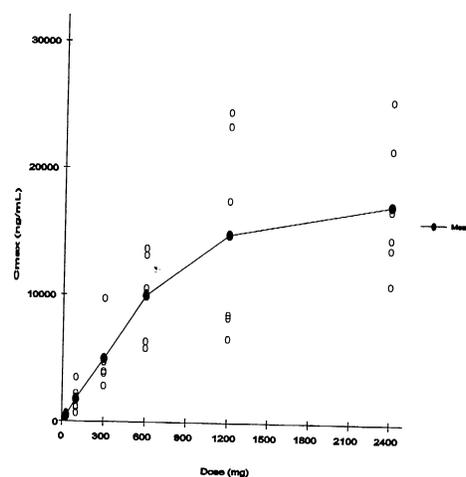
Upon oral dosing, bosentan concentrations increased with a T_{max} between 2 and 3 hours. Secondary peaks were observed in all subjects at the higher dose levels around 10 hours post dose. After attaining C_{max} plasma concentrations of bosentan declined with a $T_{1/2}$, which increased with dose, 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

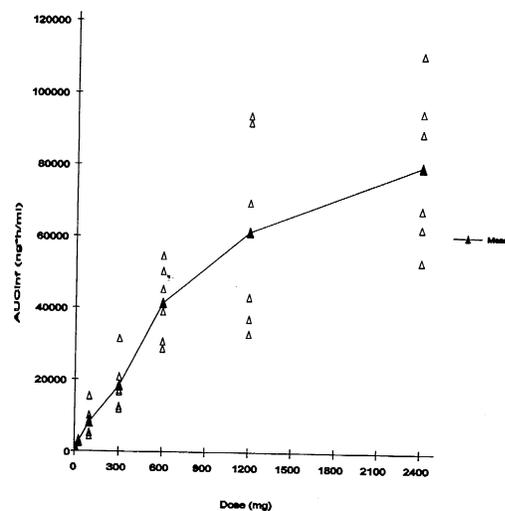
Mean Plasma Concentration Time Profiles of Ro 47-0203 Following Single P.O. Doses of 3 to 2400 mg of Ro 47-0203/016



Relationship between C_{max} and Dose: Single P.O. Doses of 3 to 2400 mg Ro 47-0203/016



Relationship between $AUC_{0-\infty}$ and Dose: Single P.O. Doses of 3 to 2400 mg Ro 47-0203/016.



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 Laser 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 C_{max} at higher doses could be due to limited absorption because of low solubility of bosentan. The mean dose-normalized AUC and C_{max} 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: J.H.G. Jonkman, Ph.D., F.C.P. R.Ph.

Pharma Bio-Research Int. B.V.
NL-9471 GP ZUIDLAREN
The Netherlands

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 F. Hoffmann-La Roche Ltd.
Department of PRPK, Bioanalytical Section
Basel, Switzerland.

Compound	Matrix	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%		Accuracy (% Bias)
							Intra	Inter	
Bosentan	Plasma	HPLC/ UV	NP	NP	50	50	NP	12.6	+7.5
						500	NP	7.9	+3.0
						5000	NP	7.9	+2.1
Bosentan	Plasma	HPLC/ LC/MS	NP	NP	0.5	2.31	NP	10.2	+9.6
						79.09	NP	6.9	-0.2
Bosentan	Urine	HPLC/ UV	NP	NP	50	NP	NP	NP	NP
						NP	NP	NP	NP

NP=Not Provided

LC/MS was used for the lower dose groups of 10 and 50 mg and for all samples below 200 ng/ml. HPLC/UV 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:

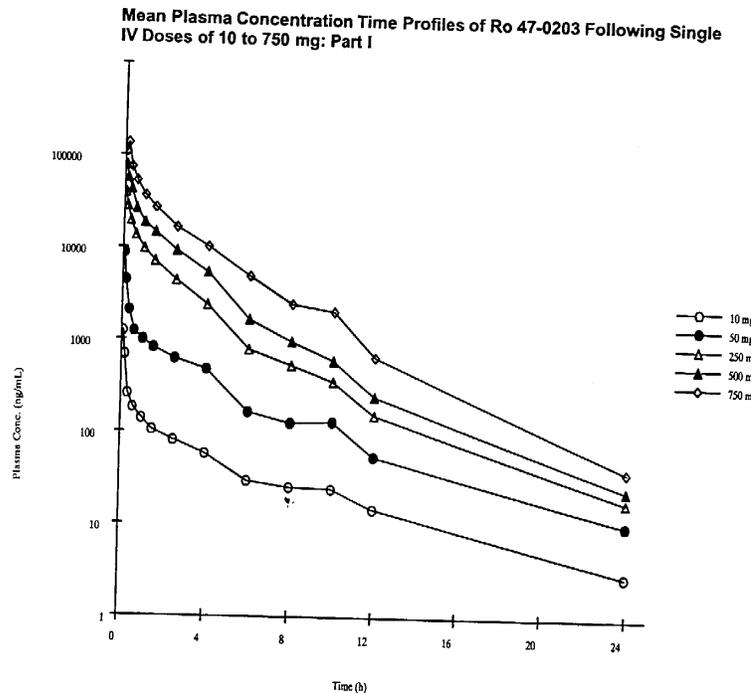
Part I:

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)	0.78 (54)
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)	
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.

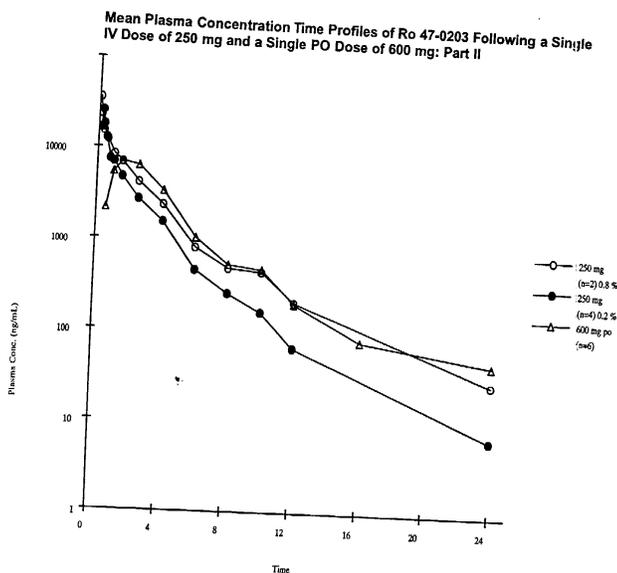
Part II:

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.

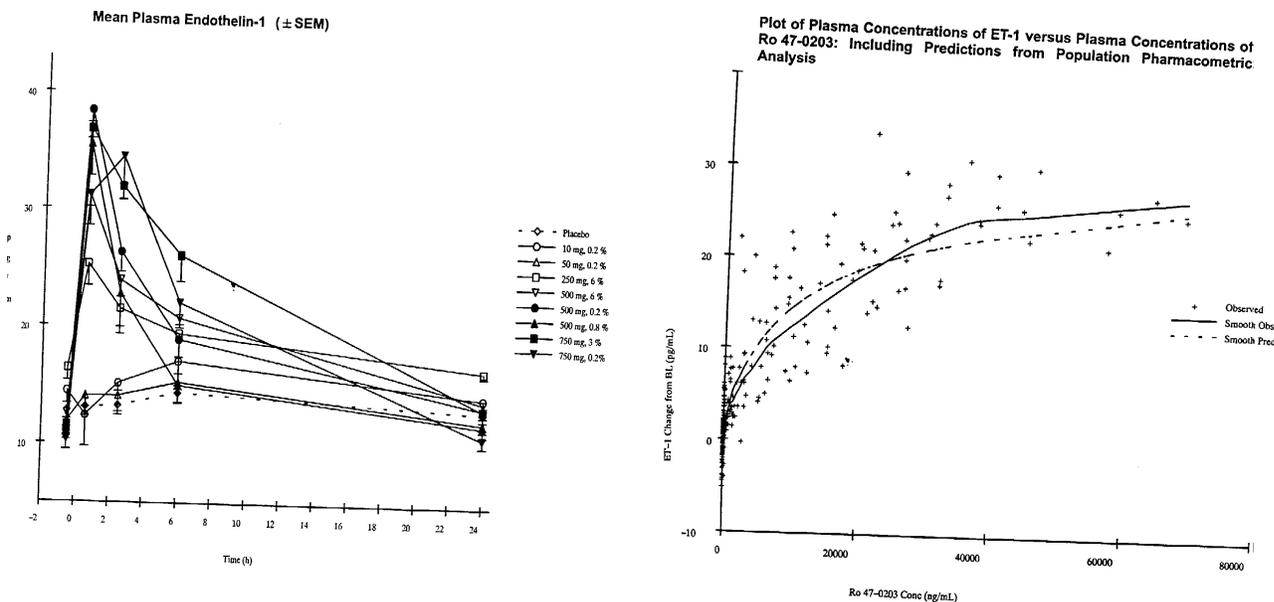


PHARMACODYNAMICS:

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 $\mu\text{g}/\text{ml}$ 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 $\mu\text{g/ml}$ 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 HPLC/UV or HPLC/MS 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: J.H.G. Jonkman, Ph.D., F.C.P. R.Ph.

Pharma Bio-Research Int. B.V.
NL-9471 GP ZUIDLAREN
The Netherlands

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 F. Hoffmann-La Roche Ltd.
Department of PRPK, Bioanalytical Section
Basel, Switzerland.

Compound	Matrix	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
Bosentan	Plasma	HPLC/ UV	10 - 10000	NP	10	50	11.6	+4.7
						500	8.3	+3.9
						5000	6.5	+1.8
Bosentan	Plasma	HPLC/ LC/MS	0.5 - 200	NP	0.5	2.56	7.1	+2.4
						82.65	7.7	+3.3
Bosentan	Urine	HPLC/ UV	50 - 12500	NP	50	125	10.4	-1.6
						461	8.6	+3.3
						2558	10.6	+6.8
						4880	7.7	+9.2

NP=Not Provided

HPLC/MS was used for analysis of plasma bosentan concentration at the lower dose groups of 100 and 200 mg. HPLC/UV 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)

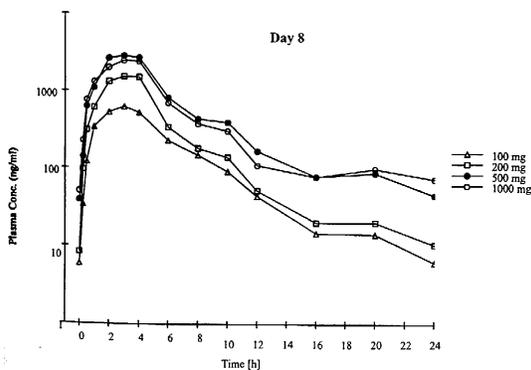
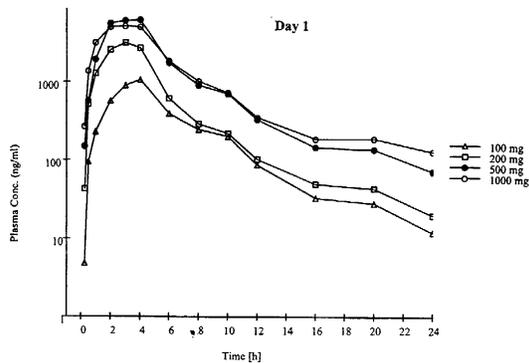
1000 mg	1	3.0 (30)	5441 (58)	13.4 (77)	32880 (54)	39.7 (58)	0.17 (45)
	8	3.2 (24)	2859 (38)	19.6 (32)	13310 (26)	79.7 (26)	0.15 (35)

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

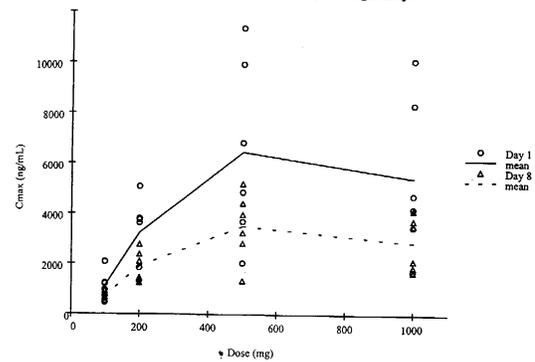
The T_{max} of bosentan occurred between 2 and 3 hours. Secondary peaks were observed in most subjects approximately 10 hours post dose. The T_{1/2} of about 5-8 hours increased to 14-20 hours at the high dose of 1000 mg. Fraction of the administered dose excreted unchanged in the urine, about 0.10%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

C_{max} and AUC of bosentan increased less than proportionally with dose especially after 500 mg. The C_{max} 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 C_{max} 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.

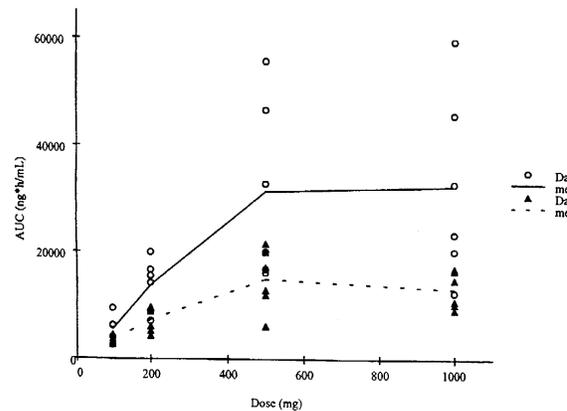
Mean Plasma Concentration Time Profiles of Ro 47-0203 Following Multiple P.O. Doses of 100 to 1000 mg of Ro 47-0203/025 and /026 Once Daily for Eight Days



Relationship between C_{max} and Dose: Multiple P.O. Doses of 100 to 1000 mg Ro 47-0203/025 and /026 Once Daily for Eight Days



Relationship between AUC_{0-∞} (Day 1) and Dose and between AUC_{0-24h} (Day 8) and Dose: Multiple P.O. Doses of 100 to 1000 mg Ro 47-0203/025 and /026 Once Daily for Eight Days



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 C_{max} and AUC following administration of 1200 mg suspension was 14830 ng/ml and 61420 ng.h/ml, respectively, which was significantly higher than the C_{max} 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:

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

C_{max} and AUC of bosentan increased less than proportionally with dose especially after 500 mg. The C_{max} 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 C_{max} 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 HPLC/MS 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.

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: J.H.G. Jonkman, Ph.D., F.C.P. R.Ph.

Pharma Bio-Research Int. B.V.
NL-9471 GP ZUIDLAREN
The Netherlands

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 F. Hoffmann-La Roche Ltd.
Department of PRPK, Bioanalytical Section
Basel, Switzerland.

Compound	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
Bosentan	Plasma	HPLC/ UV	NP	50	100	2.8	+1.0
					150	2.1	-0.1

						500	3.2	+4.9
						2000	3.4	+3.4
						4000	2.4	+1.8
						5025	3.9	+1.8
						9823	3.5	+2.3
Bosentan	Plasma	HPLC/ UV	5 - 250	NP	5	14.67	2.1	+1.8
						19.20	10.8	+4.6
						51.56	2.0	-2.5
						100	5.4	-1.2
						160.4	2.7	-4.2
Bosentan	Plasma	HPLC LC/MS	0.5 - 200	NP	0.5	2.47	4.54	+0.3
						78.24	5.42	-3.8
Ro 48-5033	Plasma	HPLC LC/MS	2 - 1000	NP	2.0	2.5	11.1	-9.5
						5.0	16.9	-2.9
						75	16.7	-0.14
Ro 47-8634	Plasma	HPLC LC/MS	2 - 1000	NP	2.0	2.5	11.3	-11.8
						5.0	11.7	-1.01
						75	13.5	+2.8

NP=Not Provided

HPLC/MS/MS was used for analysis of plasma bosentan concentrations below 5 ng/ml. HPLC/UV 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.

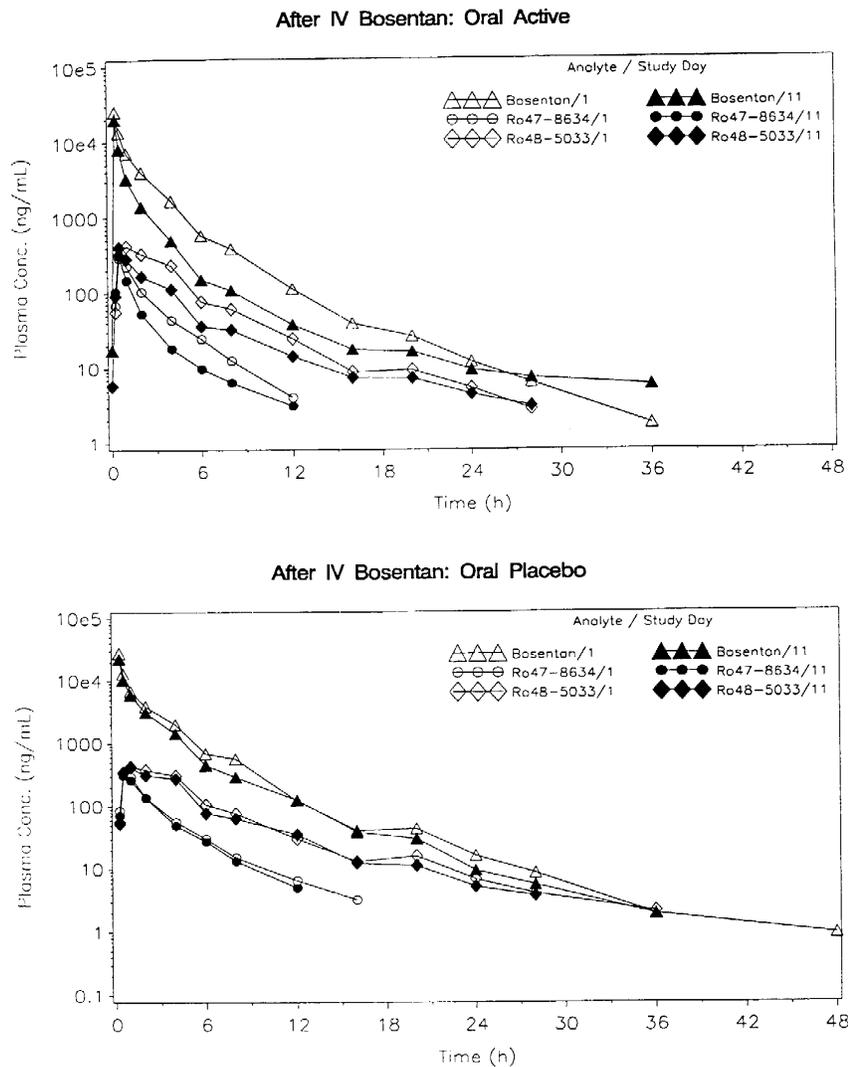
Table 2: Mean (%CV) Pharmacokinetic Parameters of Oral Bosentan and Metabolites

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 8 Day Oral Treatment Period



Upon multiple dosing mean C_{max} 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

Day	Active			Placebo		
	V _{ss} (L)	CL (L/h)	T _{0.5} (h)	V _{ss} (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

Compound	Day	Active				Placebo			
		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 C_{max} and half-life. C_{max} 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 C_{max} 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

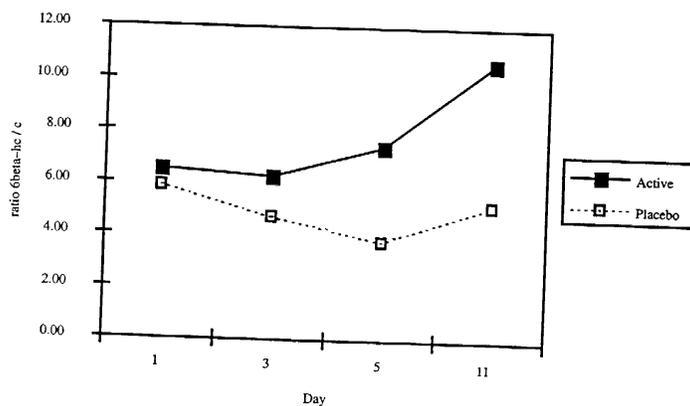
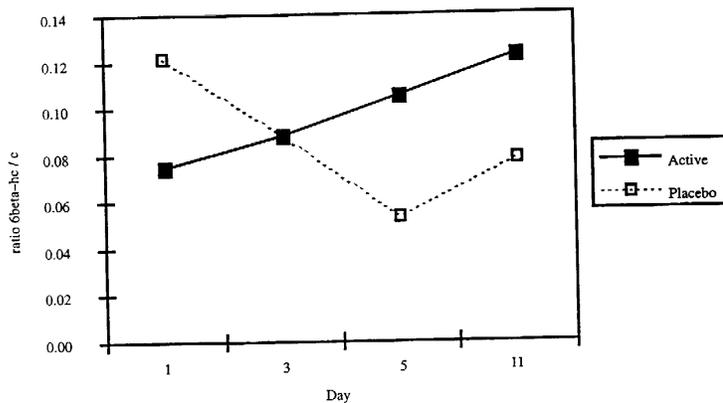


Figure 5b. Plot of the Mean Ratio of 6-Hydroxycortisol/17-Hydroxycorticosteroids versus Time for the Active and Placebo Group



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.

STUDY B-159036 – A STUDY OF THE BIOAVAILABILITY OF RO 47-0203 FROM THE 100 MG TABLETS RO47-0203/026 AND FROM THE 500 MG TABLETS RO 47-0203 (FASTING OR AFTER FOOD) RELATED TO THAT FROM THE AQUEOUS SOLUTION RO 47-0203/016.

STUDY INVESTIGATOR AND SITE: N. Freundlich

Newark Beth Israel Medical Center
201 Lyons Avenue
Newark, NJ 07112

Report No.: B-159036

Volume No.: 7

OBJECTIVES:

1. To assess the relative bioavailability of bosentan 100 mg and 500 mg tablets versus oral suspension following single oral 500 mg doses.
2. To assess the effect of food intake on oral bioavailability of bosentan solid dosage form.

FORMULATIONS:

Bosentan tablets – 100 mg (Batch #: G Lu 007)

Bosentan tablets – 500 mg (Batch #: G Lu 006)

Bosentan oral suspension – 500 mg in 10 ml water (Batch #: GFR 0030)

STUDY DESIGN:

This was a single-center, randomized, open-label, single dose, four-period, cross-over study in 12 healthy adult male volunteers between the ages of 18 to 50 years. Subjects were randomized to one of 4 treatment sequences, DCBA, BDAC, CADB or ABCD, where, A = 500 mg oral suspension, fasted; B = 5 x 100 mg tablets, fasted; C = 1 x 500 mg tablet, fasted and; D = 1 x 500 mg tablet, fed. The washout period between periods was 5-10 days.

ASSAY:

All samples were analyzed at F. Hoffmann-La Roche Ltd.

Department of PRPK, Bioanalytical Section

Basel, Switzerland.

Compound	Matrix	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%		Accuracy (% Bias)
							Intra	Inter	
Bosentan	Plasma	HPLC/ UV	50 - 20000	NP	50	50	NP	5.5	-0.6
						107	NP	3.3	-3.1
						500	NP	7.8	+5.4
						550	NP	2.2	-2.7

						2175	NP	2.1	-2.1
						4325	NP	1.2	-1.2
						5000	NP	10.7	+2.4
Bosentan	Plasma	HPLC/ LC/MS	0.5 -200	NP	0.5	2.47	NP	7.6	+0.08
						78.24	NP	5.7	-0.22

NP=Not Provided

HPLC/MS was used for analysis of plasma bosentan concentrations below 200 ng/ml and HPLC/UV 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

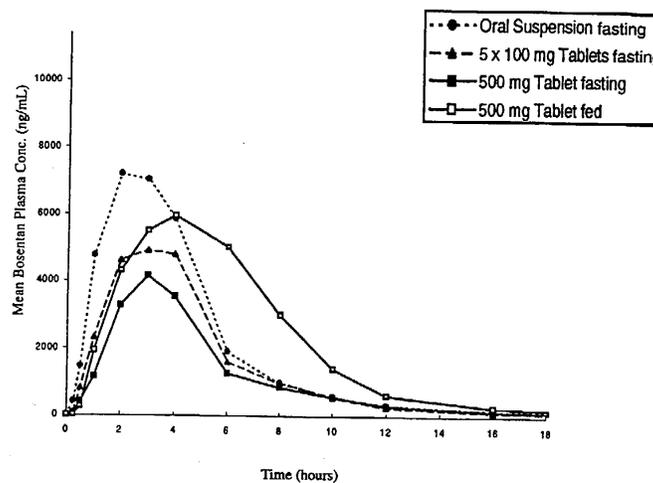
The pharmacokinetic parameters of bosentan obtained from the 4 treatments are listed in the following table.

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

Treatment	C _{max} (ng/ml)	T _{max} (h)	T _{0.5} (h)	AUC (ng.h/ml)	CL/F (L/h)	Rel.Bio (95% CI)
500 mg Oral Suspension, fasted (A)	8165 (48)	2.4 (41)	6.7 (45)	37075 (48)	17.7 (61)	-
5 x 100 mg tablets, fasted (B)	5917 (67)	3.1 (29)	9.6 (45)	27743 (57)	25.5 (65)	72 (59, 87)
500 mg tablet, Fasted (C)	4491 (62)	3.0 (25)	9.6 (38)	21995 (52)	31.8 (71)	58 (48, 70)*
500 mg tablet, Fed (D)	8079 (38)	3.8 (48)	6.1 (31)	43199 (35)	13.2 (40)	215 (177, 260)**

*C/A AUC ratio; **D/C AUC ratio

2. Mean Plasma Bosentan Concentration-Time Profiles for All Treatments
Mean Concentrations over 18 Hours



C_{max} and AUC of bosentan from 5 x 100 tablets were not bioequivalent to 500 mg oral suspension of bosentan. Both C_{max} and AUC were lower by about 25% when administered as 5 x 100 tablets. When bosentan was administered as a single 500-mg tablet, C_{max} and AUC decreased by 40% compared to oral suspension.

A significant food effect was observed on 500 mg tablets of bosentan. Mean C_{max} and AUC of bosentan when administered with food increased by 100%. The T_{max} was increased by 1 hour. The C_{max} obtained with the 500-mg tablet with food was comparable to the C_{max} obtained with oral suspension in the fasted state, but the T_{max} occurred about 1.5 hours later with food. The AUC was higher with food indicating prolonged absorption probably due to decreased gastric transit time and/or improved solubility (bosentan solubility is pH dependent).

SAFETY

There were no deaths. Subject # 0011 (500 mg oral suspension) developed asymptomatic biphasic T waves and T wave inversion starting about 2 hours after dosing (at C_{max}). ECG at baseline was normal. He was hospitalized and underwent a cardiac evaluation. Cardiac enzymes remained within normal limits. The only confirmed abnormality found on exam was mitral valve prolapse with regurgitation. The subject was discharged without further treatment. The C_{max} and AUC of bosentan for this subject were somewhat higher than mean values.

Overall, reported adverse events were no more common in the fed state (despite increased mean C_{max} and AUC values) than in the fasting state.

CONCLUSIONS:

A significant food effect was observed when 500 mg tablet was administered in the fed and fasted states. Food increased the C_{max} and AUC of bosentan by 100%, probably due to increased gastric transit time and/or improved solubility.

The bioavailability of bosentan from tablets was significantly lower than oral suspension. The C_{max} and AUC from 5 x 100 tablets and 1 x 500 mg tablet were lower by about 25% and 40% compared to the 500 mg oral suspension.

COMMENTS:

1. The label recommends administration of bosentan with or without food. This is because at the proposed dose of 125 mg, food slightly increased C_{max} and AUC (Study AC 052-106). The contrasting results between the two studies is probably due to the significant effect of food on dissolution especially at higher dose of bosentan, as seen in the present study.
2. The sponsor should have measured the concentrations of the active metabolite of bosentan in this study.

STUDY AC-052-106 – A SINGLE-DOSE STUDY TO INVESTIGATE THE RELATIVE BIOAVAILABILITY OF AN 125 MG TABLET OF BOSENTAN IN COMPARISON WITH AN ORAL SUSPENSION OF 125 MG BOSENTAN AND TO INVESTIGATE THE EFFECT OF FOOD ON THE BIOAVAILABILITY OF THE 125 MG TABLET AND THE 62.5 MG TABLET, GIVEN AS 2 x 62.5 MG, IN HEALTHY MALE SUBJECTS

STUDY INVESTIGATOR AND SITE: W. Tetzloff, MD

Phoenix International Iphar
Arnikastrasse 4
85635 Hohenkirchen-Siegersbrunn, Germany

Report No.: AC 052-106

Volume No.: 10

OBJECTIVES:

1. To evaluate the relative bioavailability of bosentan 125 tablet versus an oral suspension of 125 mg bosentan, under fasted conditions.
2. To evaluate the effect of food on the bioavailability of 125 mg bosentan tablet.
3. To evaluate the bioavailability of 62.5 mg bosentan tablet, given as 2 tablets, relative to that of 125 mg tablet under fed conditions.

FORMULATIONS:

Bosentan tablets – 125 mg oral suspension (Batch #: 419/2000)

Bosentan tablets – 62.5 mg tablets (Batch #: PT 2242 T 53)

Bosentan tablets – 125 mg tablets (Batch #: PT 2241 T 53)

STUDY DESIGN:

This was a single-center, randomized, open-label, single dose, four-period, cross-over study in 16 healthy adult male volunteers between the ages of 18 to 50 years (mean age =35 y). Subjects were randomized to one of 4 treatment sequences, DCBA, BDAC, CADB or ABCD, where, A = 125 mg oral suspension, fasted; B = 125 mg tablet, fasted; C = 125 mg tablet, fed (FDA high fat meal) and; D = 2 x 62.5 mg tablet, fed (FDA high fat meal). The washout period between periods was 7 days.

ASSAY:

All samples were analyzed at CEPHAC
90 Avenue des Hauts de la Chaume
France

Compound	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
Matri							

X								
Bosentan	Plasma	HPLC/ MS/MS	1 - 1000	NP	1	2	15.44	+2.60
						100	4.49	+8.10
						800	6.00	+1.69
						5000	7.44	-3.16
Ro 48-5033	Plasma	HPLC/ MS/MS	5 - 200	NP	5	7.5	14.6	+3.2
						50	9.53	+6.4
						150	6.90	+5.33
						1500	5.52	+3.33
Ro 47-8634	Plasma	HPLC/ MS/MS	5 - 200	NP	5	7.5	9.44	+2.67
						50	7.88	+1.80
						150	6.73	+0.00
						1500	9.38	-2.67
Ro 64-1056	Plasma	HPLC/ MS/MS	5 - 200	NP	5	7.5	13.28	-0.53
						50	7.22	-1.60
						150	7.95	-2.67
						1500	6.15	-5.33

NP=Not Provided

HPLC/MS/MS was used for analysis of plasma bosentan and metabolite concentrations.

Sample Collection:

Blood samples (8-ml) for measurement of plasma concentrations of bosentan and its metabolites were collected on Day 1 of all 4 periods at 0 (predose), 0.33, 0.67, 1, 1.33, 1.67, 2, 2.5, 3, 3.5, 4, 6, 8, 10, 12, 15, 18, 24, 36 and 48 hours post dose.

RESULTS

The pharmacokinetic parameters of bosentan and metabolites obtained from the 4 treatments are listed in the following table.

Table 2: Mean (90% CI) Pharmacokinetic Parameters of Bosentan and Metabolites

Treatment	C _{max} (ng/ml)	T _{max} (h)	T _{0.5} (h)	AUC (ng.h/ml)
BOSENTAN				
125 mg Oral Suspension, Fasted (A)	1293 (1044, 1806)	3.0 (1.7, 8.0)	5.80 (4.85, 7.64)	7832 (5941, 11890)
125 mg tablets, Fasted (B)	1317 (1062, 1855)	3.5 (1.7, 8.0)	5.38 (4.73, 6.44)	7983 (6499, 11200)
125 mg tablet, Fed (C)	1612 (1294, 2343)	4.0 (2.5, 8.0)	5.19 (4.36, 6.80)	8791 (6946, 12670)
2 x 62.5 mg tablet, Fed (D)	1573 (1321, 2024)	3.8 (2.5, 8.0)	6.01 (5.06, 7.68)	8926 (7251, 12240)
Ro 48-5033				
125 mg Oral Suspension, Fasted (A)	67.2 (56.2, 94.6)	4.0 (4.0, 12.0)	4.65 (3.94, 6.13)	625 (532, 843)
125 mg tablets, Fasted (B)	65.1 (54.9, 85.1)	6.0 (3.5, 12.0)	4.97 (4.11, 6.69)	650 (549, 869)

125 mg tablet, Fed (C)	70.0 (54.7, 100)	6.0 (3.5, 12.0)	5.32 (4.38, 7.29)	657 (547, 894)
2 x 62.5 mg tablet, Fed (D)	79.0 (64.7, 104)	6.0 (4.0, 12.0)	4.95 (4.13, 6.61)	649 (550, 851)
Ro 64-1056				
125 mg Oral Suspension, Fasted (A)	47.4 (40.5, 60.5)	4.0 (2.5, 8.0)	3.50 (3.02, 4.41)	358 (313, 442)
125 mg tablets, Fasted (B)	48.4 (42.3, 59.7)	6.0 (3.0, 8.0)	3.52 (3.04, 4.34)	346 (303, 426)
125 mg tablet, Fed (C)	48.4 (41.0, 62.4)	6.0 (3.5, 8.0)	3.70 (3.14, 4.69)	370 (318, 472)
2 x 62.5 mg tablet, Fed (D)	44.1 (38.8, 52.7)	6.0 (3.5, 8.0)	3.05 (2.69, 3.64)	335 (292, 414)

The C_{max} and AUC of bosentan obtained following administration of 125 mg suspension or tablet in the fasted state were similar, about 1300 ng/ml and 7900 ng.h/ml, respectively. When the 125 mg tablet was administered with food, the mean C_{max} of bosentan increased by 300 ng/ml to 1600 ng/ml and mean AUC increased by 800 ng.h/ml to 8791 ng.h/ml. The C_{max} and AUC of bosentan obtained following administration of 2 x 62.5 mg tablets of bosentan were similar to those obtained following 1 x 125 mg tablet. The half-life of bosentan in all 4 treatments were similar, between 5 and 6 hours.

The C_{max} and AUC of Ro 48-5033 (active metabolite) obtained following administration of 125 mg suspension or tablet in the fasted state were similar, 65 ng/ml and 650 ng.h/ml, respectively. When the 125 mg tablet was administered with food both mean C_{max} and AUC of Ro 48-5033 remained unchanged. The half-life of Ro 48-5033 in all 4 treatments was similar, between 4 and 5 hours.

The mean C_{max} and AUC of Ro 64-1056 obtained from all 4 treatments were similar, 48 ng/ml and 350 ng.h/ml, respectively.

The pharmacokinetic parameters of inactive metabolite Ro 47-8634 were not presented in the study report.

Table 3: Point Estimates and 90% Confidence Intervals for Relative Bioavailability and Food Effect of Bosentan

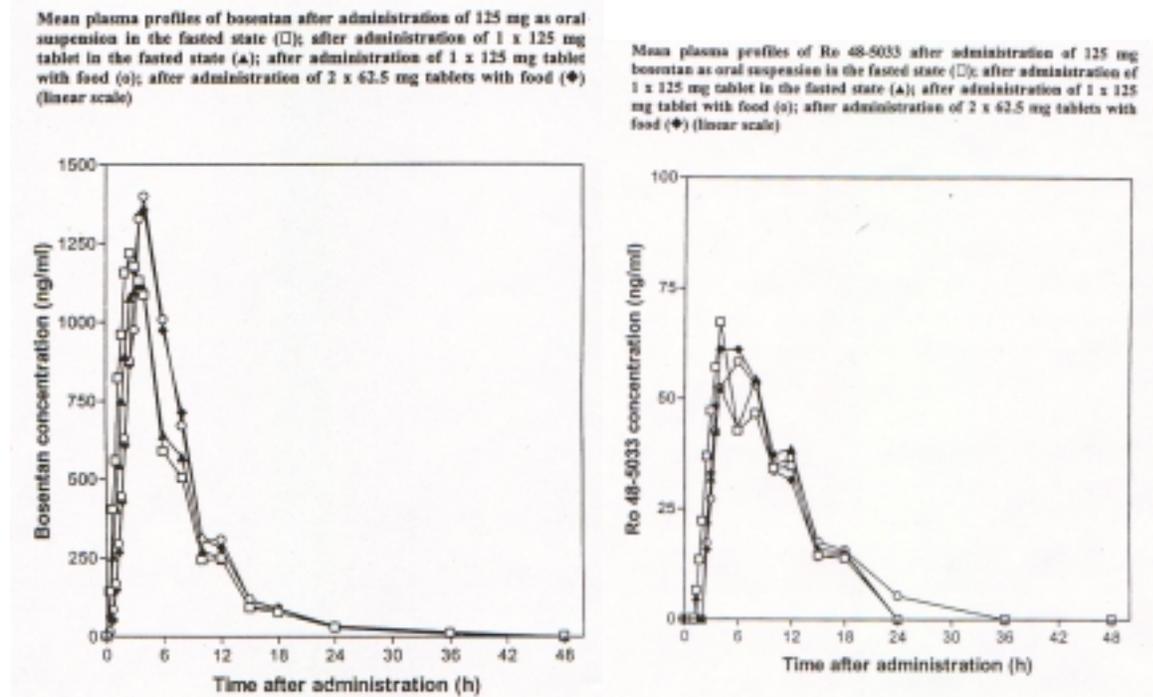
Treatment	C _{max}		AUC	
	Rel.Bio (95% CI)	Food Effect (95% CI)	Rel.Bio (95% CI)	Food Effect (95% CI)
125 mg Oral Suspension, Fasted (A)	-	-	-	-
125 mg tablets, Fasted (B)	1.02 (0.82, 1.26)	-	1.02 (0.90, 1.16)	-
125 mg tablet, Fed (C)	-	1.22 (0.99, 1.52)	-	1.10 (0.97, 1.25)
2 x 62.5 mg tablet, Fed (D)	0.98 (0.79, 1.21)	1.19 (0.96, 1.48)*	1.02 (0.89, 1.15)	1.12 (0.98, 1.27)*

*D/B

The 125 mg tablets of bosentan were bioequivalent to the suspension with regard to both C_{max} and AUC. C_{max} and AUC from the 125-mg oral suspension were lower by 37% and 15%,

respectively, (after dose normalization) compared to the 500-mg oral suspension used in Study # B-159036. Two 62.5-mg tablets of bosentan were bioequivalent to the 125 mg tablet with regard to AUC but the lower limit of the 90% confidence interval for C_{max} was slightly below the 0.8 lower limit for bioequivalence.

The C_{max} and AUC of bosentan in the fed state was not bioequivalent to the fasted state. Mean C_{max} was 22% higher and mean AUC was 10% higher in the fed state compared to the fasted. However, this increase in C_{max} and AUC is not expected to affect safety or efficacy of bosentan.



SAFETY:

There were no reported deaths or serious adverse events. There were 4 reports of headache, 1 report of nausea, and 1 report of influenza-like illness. There were 6 reports of abnormally low hemoglobin and 2 reports of abnormally high ALT values.

CONCLUSIONS:

The C_{max} and AUC of bosentan obtained following administration of 125 mg tablet was bioequivalent to the tablet in the fasted state. Mean C_{max} and AUC were approximately 1300 ng/ml and 7900 ng.h/ml, respectively. Food increased mean C_{max} and AUC by 22% and 10%, respectively. In the fed state, 2 x 62.5 mg tablets of bosentan was bioequivalent to 1 x 125 mg tablet. The C_{max} and AUC of Ro 48-5033 (active metabolite) and Ro 64-1056 were not affected by food.

COMMENTS:

1. In a previous study (B-159036), the early tablet formulation exhibited 100% increase in C_{max} and AUC in the presence of food. This could be due to both formulation and also poor dissolution at the high dose of 500 mg. In the present study, at the lower dose of 125-mg, food did not affect C_{max} and AUC significantly. The biopharmaceutics reviewer concurs with the sponsor that bosentan can be administered with or without food.
2. C_{max} and AUC from the 125-mg oral suspension were lower by 37% and 15%, respectively, (after dose normalization) compared to the 500-mg oral suspension used in Study # B-159036. The sponsor's view that the bioavailability of the new tablet formulation is greater is not supported by the data. In order to list the bioavailability of bosentan as 70% instead of the observed 45%, the sponsor will have to perform an absolute bioavailability study.
3. The pharmacokinetic parameters of inactive metabolite Ro 47-8634 were not presented in the study report.

STUDY B-162292 – AN EXPLORATORY TRIAL OF THE ENDOTHELIN ANTAGONIST BOSENTAN IN PATIENTS WITH PRIMARY PULMONARY HYPERTENSION. PART I: OPEN-LABEL, SINGLE ASCENDING IV DOSES. PART II: DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTIPLE ORAL DOSES.

STUDY INVESTIGATOR AND SITE: D. Williamson

A. Keogh
St. Vincent's Hospital
Sydney, Australia

Report No.: B-162292

Volume No.: 2.22

BACKGROUND:

This study was originally planned to study the effects of intravenous (Part I, 1 day) and oral (Part II, 8 weeks) to be conducted in 30 patients with primary pulmonary hypertension. However, the sponsor prematurely stopped the study when 2 patients randomized to placebo died early in Part II. At the time the study was stopped, 7 patients had completed Part I and had been randomized to either bosentan 1000 mg b.i.d. or placebo in Part II. Only 1 placebo and 1 bosentan patient had completed the trial.

OBJECTIVE:

To assess the safety, efficacy, pharmacokinetics and pharmacodynamics of single ascending intravenous doses and repeated oral bosentan administration.

FORMULATIONS:

Bosentan Lysophilizate for intravenous administration (GSU 0041)

Bosentan tablets – 500 mg (Batch #: GLU0028)

Matching Placebo tablets (Batch #: GLU0008)

STUDY DESIGN:

This was a single-center exploratory trial conducted in 2 parts in primary pulmonary hypertension patients between 18 and 70 years of age with mean pulmonary arterial pressure (MPAP) > 25 mm Hg on Day 1. Part I was an open-label design with each patient receiving 3 single intravenous ascending doses of bosentan, 50 mg, 150 mg and 300 mg at 0 h, 2 h and 4 h, respectively. After completion of Part I of the study, subjects entered Part II of the study which was a double-blind, randomized, placebo-controlled design where patients were randomly allocated to receive oral doses of either placebo or bosentan 1000 mg b.i.d. for 8 weeks. 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 suspension and 2 were randomized to matching placebo. All doses were administered in the morning following an overnight fast.

ASSAY:

All samples were analyzed at F. Hoffmann-La Roche Ltd.
Department of PRPK, Bioanalytical Section
Basel, Switzerland.

Compound	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
	Matri x						
Bosentan	Plasma	HPLC/ UV	NP	50	100	6.1	-1.7
					500	10.0	-1.0
					1000	5.0	+0.2
					5000	4.5	-0.9
					10000	5.7	-0.4

NP=Not Provided

Sample Collection:

Blood samples for measurement of plasma concentrations of bosentan were collected at: 5, 15, 30, 45, 60, 115, 130, 135, 150, 165, 180, 235, 255, 270, 285, 300 and 360 minutes post 50 mg intravenous dose.

RESULTS

The pharmacokinetic parameters of intravenous bosentan obtained following single doses of 50, 150 and 300 mg to 7 patients with PPH are listed in the following table.

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

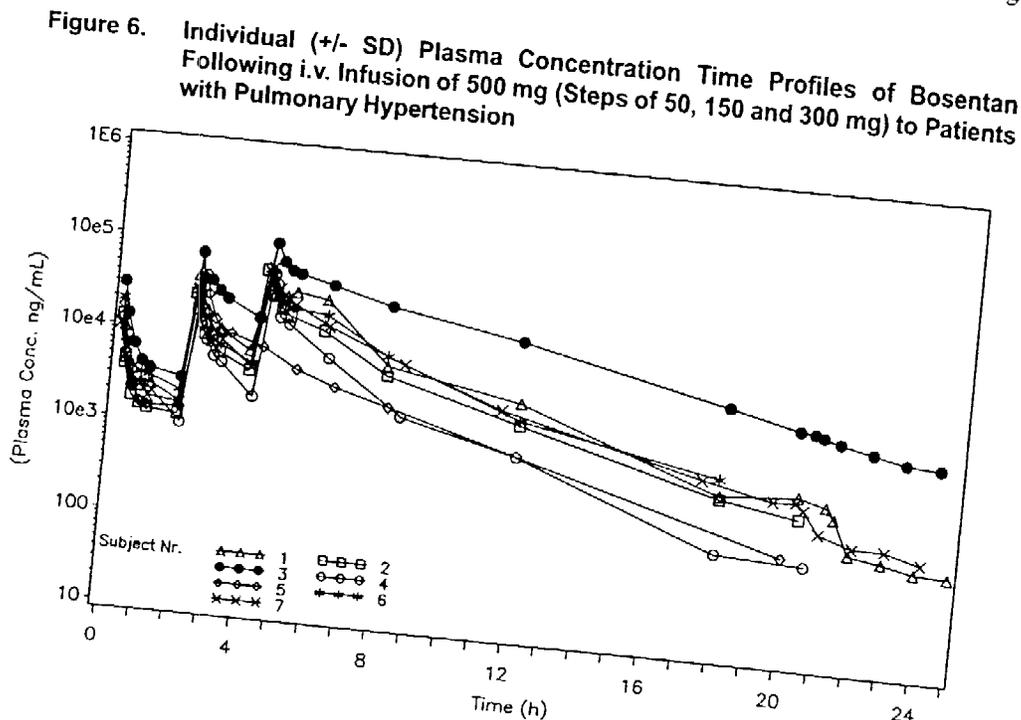
Total Dose	C _{max,1} (ng/ml)	C _{max,2} (ng/ml)	C _{max,3} (ng/ml)	T _{0.5} (h)	AUC (ng.h/ml)	CL (L/h)	V _{ss} (L)
500 mg	15310 (44)	35510 (47)	58250 (38)	3.8 (26)	171710 (74)	3.8 (48)	21.0 (42)

C_{max,1}, C_{max,2} and C_{max,3} represents the maximum concentrations observed following administration of 50 mg, 150 mg and 300 mg doses. Although, substantial residual concentrations remained in the body prior to administration of the next dose, the increase in C_{max} with increasing doses of intravenous bosentan was less than proportional. Based on this

observation, it is evident that solubility limitation might not be the only factor accounting for the less than proportional increase in C_{max} and AUC seen with increasing oral doses. Saturation of plasma protein binding occurred in vitro at concentrations above 20 $\mu\text{g/ml}$. Therefore, it is possible that the less than proportional increase in concentrations are probably due to saturation of plasma protein binding at the high concentrations seen after intravenous administration.

Compared to healthy volunteers, the clearance of bosentan in patients with PPH was at least 50% lower. In Studies B-162287 and B-159041 upon administration of 250 mg intravenous bosentan to healthy volunteers, observed clearance values were 10.3 L/h and 9.3 L/h, respectively, while in Study B-162287 a clearance of 6.6 L/h was estimated following a 500-mg dose. The %CV ranged between 27% and 45% in the 3 studies, which was comparable to observed %CV in the present study

The half-life and steady-state volume of distribution of bosentan in PPH patients was similar to those observed in healthy volunteers.



There were 2 deaths in this study, Patient #3 and #7. Bosentan clearance in Patient # 3 was only 1/3 of the mean clearance, consequently, following intravenous administration maximum concentrations in Patient #3 were 2-fold higher than other patients. Clearance and maximum concentrations of bosentan in Patient #7 were close to average values. Both patients died within 2 days of receiving intravenous bosentan.

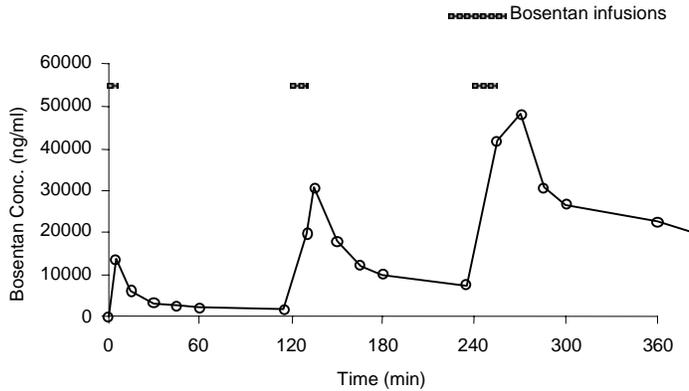
Only 1 patient (Patient #2) received 1000 mg oral bosentan twice daily for 8 weeks.

PHARMACODYNAMICS:

The mean changes from baseline in cardiac index, stroke index, PAP and pulmonary arterial pressures versus time for the 3 infusions (arrows) are shown below. The reliability of the results from this small, uncontrolled, open label trial is questionable.

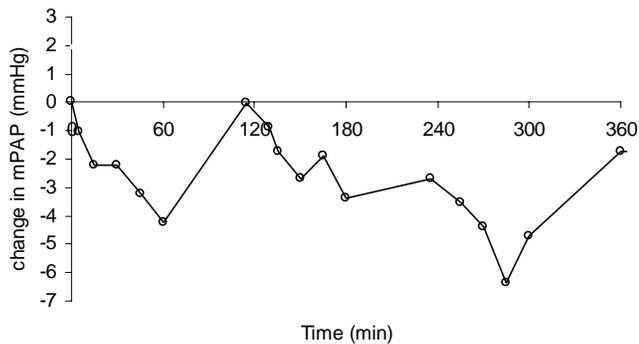
During infusion of bosentan at the highest concentration (300 mg/15 min), mean (\pm SEM) reduction in mean PAP was 6.5 ± 4.5 mmHg and mean reduction in TPVR was $23.7 \pm 5.9\%$. These reductions lag slightly behind plasma bosentan concentrations.

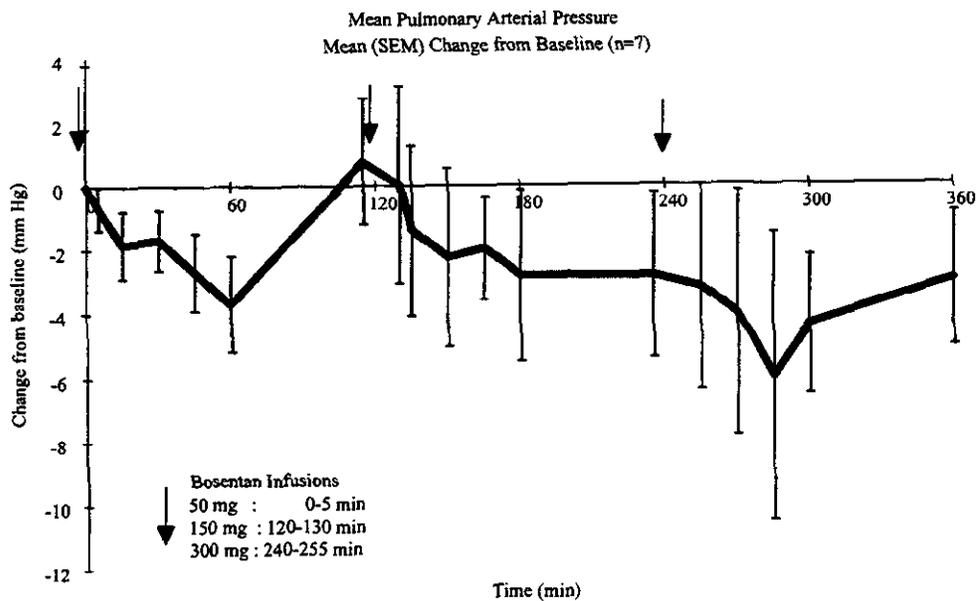
Mean bosentan plasma concentrations versus time over 6 h



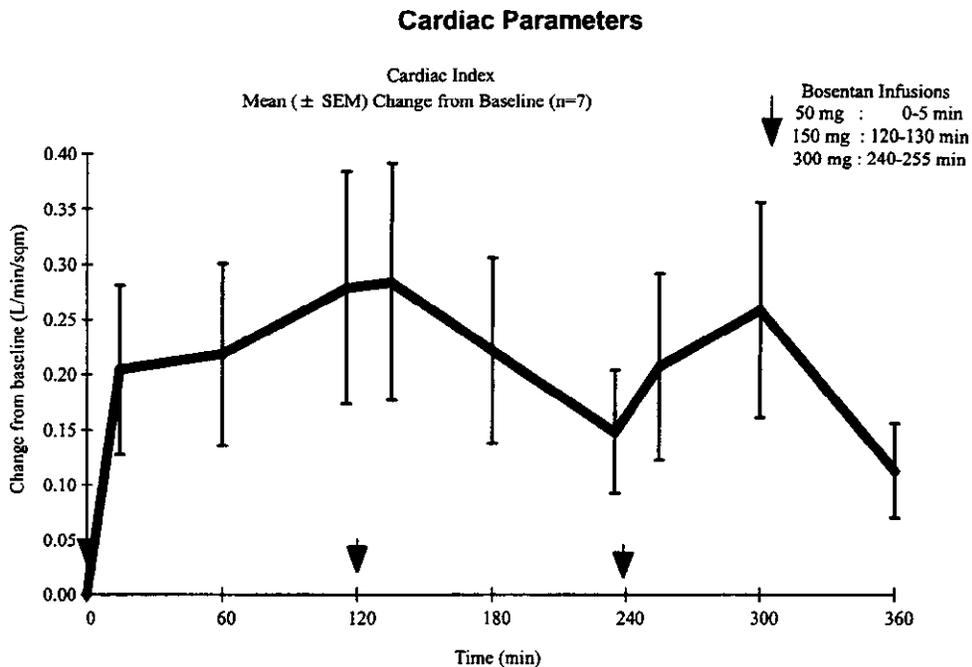
Mean PAP:

Mean pulmonary artery pressure, absolute change from baseline versus time over 6 h

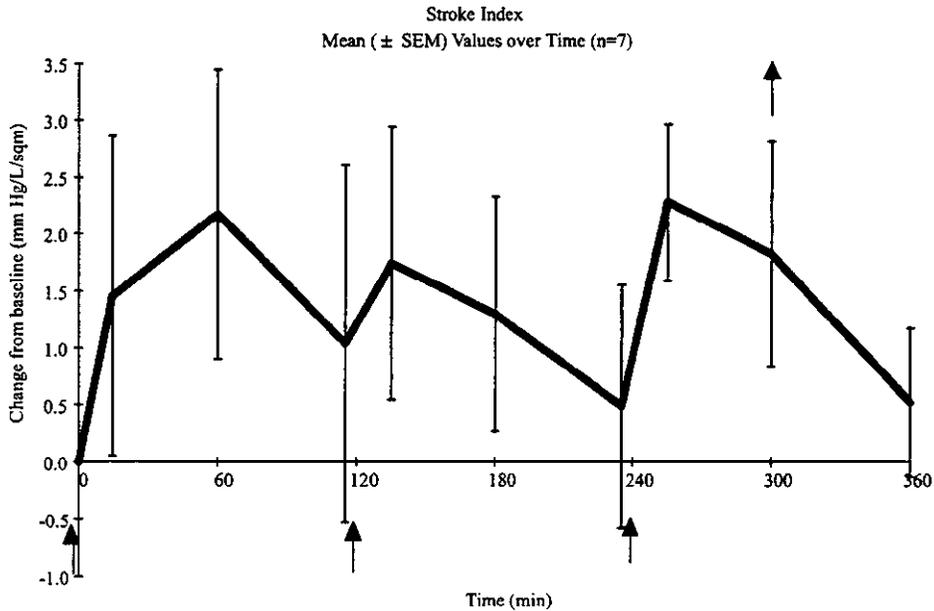




Mean cardiac index:



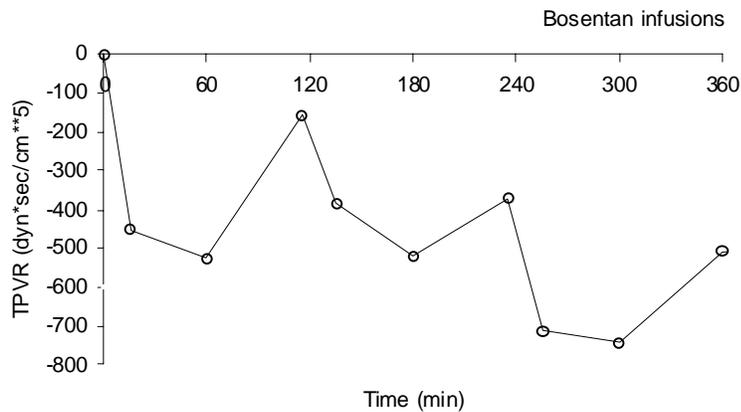
Mean stroke index:



In this study, bosentan tended to increase mean cardiac and stroke index and decrease mean PAP. There is no dose response information.

Pulmonary Vascular Resistance:

Relationship between plasma concentrations and mean pulmonary vascular resistance in patients with pulmonary arterial hypertension receiving bosentan (50 mg, 150 mg, and 300 mg i.v.)



In patients with pulmonary arterial hypertension treated with 3-step infusions of bosentan (50 mg, 150 mg, followed by 300 mg), the decrease of blood pressure reached values close to 25 mmHg for systolic BP and 15 mmHg for diastolic BP.

SAFETY:

Deaths

Of the 7 patients who received iv bosentan, 2 died approximately 1 day after receiving 2 of the 3 planned infusions. The first patient (#3) developed hypotension, which did not respond to dopamine, adrenaline and iv fluids. She became dyspneic, oliguric, and decreased platelet count. She died a short time later. No autopsy was performed. The clearance of bosentan in this patient was very low (15% the clearance of healthy volunteers) and, compared to the other study patients, her Cmax was about 2 fold higher. This patient had evidence of some liver disease at baseline.

The second patient (#7) underwent the infusion uneventfully but felt cold and clammy immediately after the Swan-Ganz catheter was removed the next day. She was normotensive but complained of throat tightness, breathlessness and nausea. She started to improve within 45 minutes and was transferred to the ICU for observation. There was another recurrence of symptoms, which were treated with nitrates and sublingual nifedipine. Hypotension ensued, followed by oliguria. She continued to deteriorate and died later that day. Post mortem revealed pulmonary edema and bilateral pulmonary effusions.

Prothrombin time

Of the 4 patients receiving concomitant oral bosentan and warfarin, 3 had prolonged prothrombin times (see bosentan-warfarin interaction study).

Withdrawals

Three of the 4 patients randomized to oral bosentan dropped out because of adverse events. These patients are discussed below There were 4 patients reporting a total of 8 serious events.

#5	28-year-old woman who received the first two infusions of bosentan; the third was not administered because her systemic BP fell to 91/57 at 3 h after the start of the first infusion. On study day 2, she received reduced dose oral bosentan because of the decrease in BP. She was discharged from hospital, but received further observation. At 17:00 she was readmitted to the hospital because of exertional dyspnea, pleuritic chest pain, fever and hypotension (75-80/palp mmHg). The next dose was held and she was re-hospitalized. The hypotension, chest pain and dyspnea resolved. Broad-spectrum antibiotic therapy was started. The patient was discharged from hospital with reduced dose of study drug (1 tablet bid). This was increased to 2 tablets twice daily. Four weeks later she presented with cough, sore throat, fever, malaise, myalgia, fatigue, and decreased exercise tolerance without worsening dyspnea. A few days later she was still afebrile and acyanotic on examination with onset of nausea, vomiting, mild hematemesis. The patient discontinued bosentan of own accord and developed rigors. Her usual dyspnea worsened and she became unable to get out of bed. She took Augmentin. One week later she was hospitalized because of progressive deterioration. On admission she was unwell, slightly deteriorated and had severe cyanosis. She was afebrile and reported erythema of throat and had elevated JVP and expiratory wheezes. Peripheral oliguria was noted while in hospital. Laboratory tests were: hemoglobin = 105 g/L; WBC = 7.1 x10 ⁹ /mm ³ ; INR 3.6;
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	APTT 66; urea = 46 mg/dL; creatinine = 0.31 mg/dL; urinalysis: protein = ++, blood = +. Although she was treated for presumed respiratory tract infection, bosentan dose was reduced to 500-mg bid. twice daily She received various interventions and her oxygen saturation, coagulopathy and renal function (creatinine 0.23 mg/dL) continued to improve. She had severe nausea and vomiting . Although enterobacter was isolated from mid-stream urine, other cultures were negative. One week after hospital admission she generally improved but remained hypoxic. Iron deficiency was noted, coagulopathy resolved and warfarin was re-started. Nausea and vomiting persisted together with weight loss and wasting. Renal impairment improved (creatinine = 0.12 mg/dL). Bosentan was discontinued and she continued to improve.
#6	49 year old female developed hypotension during the third infusion of bosentan. Blood pressure responded to iv metaraminol. Oral bosentan was started at a reduced dose and she recovered
#4	50-year-old white female received the three bosentan infusions. After receiving 4 weeks of oral bosentan (1000 mg bid), the patient reported nausea and vomiting. Her condition deteriorated rapidly with increasing vomiting, poor oral intake and vomiting of study drug. She became febrile and developed diarrhea. Two days later she was admitted to hospital. High leukocyte count (19.0×10^9) and increased INR (on warfarin) were noted. She was treated with i.v. fluids and oral ciprofloxacin. Bosentan was discontinued and in the following two days she deteriorated further with confusion, fever, rigors, diarrhea, vomiting, worsening of dyspnea and cyanosis were recorded. She was transferred to another hospital where she was found to be hypoxic and severely acidotic. Bosentan was restarted and slight improvement was reported. Traces of clostridium difficile toxin were found in the stool and interpreted as a positive result. Treatment with oral antibiotic was started. High volume diarrhea persisted, thought to be consistent with a pseudomembranous colitis. Events eventually resolved.
#2	68-year-old female who experienced generalized weakness, severe exertional dyspnea, subcostal discomfort on exercise and palpitations 10 days after completing treatment with bosentan. She was admitted to hospital for review. ECG monitoring revealed frequent supraventricular complexes (up to 19 beats/min). She improved clinically but remained dyspneic and was discharged from hospital. Elevated LFTs were reported.

CONCLUSIONS:

Mean clearance of bosentan was at least 50% lower (3.8 L/h) in patients with primary pulmonary hypertension compared to healthy volunteers. Less than proportional increases in C_{max} and AUC of bosentan were observed following intravenous administration of 50 mg, 150 mg and 300 mg of bosentan. The protein binding characteristics of bosentan in PPH patients is not known. It is possible that the less than proportional increases in C_{max} and AUC could be due to saturation of plasma protein binding of bosentan. The half-life and steady-state volume of distribution of bosentan in PPH patients were similar to those observed in healthy volunteers.

The pharmacokinetics of bosentan following oral administration is not known in PPH patients.

The mean changes from baseline in cardiac index, stroke index, PAP and pulmonary arterial pressures versus time for the 3 infusions showed a tendency to decrease with increasing dose. The reductions in

PAP and TPVR lag slightly behind plasma bosentan concentrations indicating equilibration delay. The reliability of the results from this small, uncontrolled, open label trial is questionable.

COMMENTS:

1. A major deficiency in this study and the entire NDA is the lack of information regarding the pharmacokinetics of oral bosentan in PPH patients. Important information such as single dose and steady-state concentrations following 62.5 mg and 125 mg doses, half-life, extent of enzyme induction, protein binding etc. are not known.
2. Since, the different doses were not administered in parallel, it is difficult to say whether the increasing reduction in PAP and TPVR are a result of increasing doses or a time effect with maximum pharmacodynamic effect significantly lagging concentrations.
3. The sponsor should have measured the concentrations of the major metabolites of bosentan in this study.

STUDY AC 052-101 – A STUDY TO ASSESS THE PHARMACOKINETICS OF BOSENTAN (Ro 47-0203) IN SUBJECTS WITH SEVERE RENAL DYSFUNCTION COMPARED TO SUBJECTS WITH NORMAL RENAL FUNCTION

STUDY INVESTIGATORS AND SITES: R. Gellert, MD
VanTx Research
Poland Sp. Zo. O.
Banacha Ia
02 097 Warszawa, Poland

Report No.: VTX 99/O/007

Volume No.: 2.21

OBJECTIVES:

1. To evaluate the single dose pharmacokinetics of bosentan in subjects with severe renal impairment compared to normal renal function.

FORMULATIONS:

Bosentan – 250-mg tablets (batch #: PT 2227 T 68)

STUDY DESIGN:

This was an open-label, parallel, single-dose study in a total of 16 male subjects; 8 with severe renal impairment and 8 with normal renal function. All subjects received a single 125-mg dose of bosentan with meals in the morning on Day 1. The mean age of subjects in the renal impairment and healthy groups were 48 years and 23 years, respectively. Mean creatinine clearance (CLcr) in renal impairment subjects and normal subjects was 23 ml/min and 116 ml/min, respectively.

ASSAY:

Compound	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)	
Bosentan	Plasma	LC/MS/MS	1 - 4096	≥ 0.990	1.0	2.97	8.0	-1.4
						90.8	6.2	+3.9
						912	4.7	+1.8
						3512	0.0	-4.1
Ro 48-5033	Plasma	LC/MS/MS	2 - 512	≥ 0.990	2.0	6.04	7.0	-2.6
						84.1	2.8	-1.5
						418	4.2	-1.0
						451	3.2	+1.8
Ro 47-8634	Plasma	LC/MS/MS	2 - 512	≥ 0.990	2.0	6.11	8.9	+0.5
						82.1	4.2	-0.3
						401	5.6	+0.5
						443	3.9	+1.1

Ro 64-1056	Plasma	LC/MS/MS	2 - 512	≥ 0.990	2.0	5.88	7.5	+1.0
						81.6	5.5	+6.0
						410	5.5	-2.9
						453	6.4	+0.6

Sample Collection:

Blood samples (4.5-ml) were collected for analysis of bosentan and its metabolites on Day 1 pre-dose and at 0.33, 0.67, 1, 2, 3, 4, 6, 8, 10, 12, 15, 18, 24, 30 and 36 hours post-dose.

RESULTS

The pharmacokinetic parameters of bosentan and its metabolites obtained following a single oral dose of 125-mg in normal and renal impairment subjects are listed in the following table.

Table 1: Geometric Mean (95% CI) Pharmacokinetic Parameters of Bosentan and its Metabolites

Compound	Subjects	C _{max} (ng/ml)	T _{max} (h)	T _{1/2} (h)	AUC _{0-∞} (ng.h/ml)
Bosentan	Normal	1763 (1182 – 2771)	4.0 (3.0 – 4.0)	6.01 (4.99 – 7.26)	7182 (5095 – 10374)
	Renal Impairment	1112 (737 - 1684)	4.0 (4.0 – 6.0)	5.12 (4.43 – 5.95)	6427 (3391 – 11241)
Ro 48-5033	Normal	87.6 (65.2 – 123)	4.0 (4.0 – 10.0)	4.42 (4.99 – 7.26)	527 (428 – 654)
	Renal Impairment	70.7 (53.3 – 95.7)	5.0 (4.0 – 10.0)	7.51 (5.29 – 10.6)	834 (585 – 1193)
Ro 47-8634	Normal	21.7 (14.5 – 32.9)	4.0 (3.0 – 6.0)	2.33 (1.87 – 2.92)	111 (77.6 – 161)
	Renal Impairment	29.9 (18.6 – 48.7)	5.0 (3.0 – 6.0)	4.17 (2.23 – 7.40)	224 (104 – 441)
Ro 64-1056	Normal	64.9 (52.7 – 81.0)	4.0 (4.0 – 6.0)	3.93 (2.88 – 5.44)	421 (336 – 535)
	Renal Impairment	91.7 (74.3 – 116)	6.0 (4.0 – 8.0)	7.71 (5.40 – 11.2)	881 (567 – 1357)

Table 2: Point estimate and 95% CI for PK parameters of bosentan and metabolites

Compound	Parameter	Renal Impairment vs. Healthy Subjects	
		Point Estimate	95% Conf. Interval
Bosentan	C _{max} (ng/ml)	0.63	0.38, 1.06
	AUC _∞ (ng.h/ml)	0.89	0.55, 1.47
Ro 48-5033	C _{max} (ng/ml)	0.80	NP
	AUC _τ (ng.h/ml)	1.58	NP
Ro 47-8634	C _{max} (ng/ml)	1.38	NP
	AUC _τ (ng.h/ml)	2.01	NP
Ro 64-1056	C _{max} (ng/ml)	1.41	NP

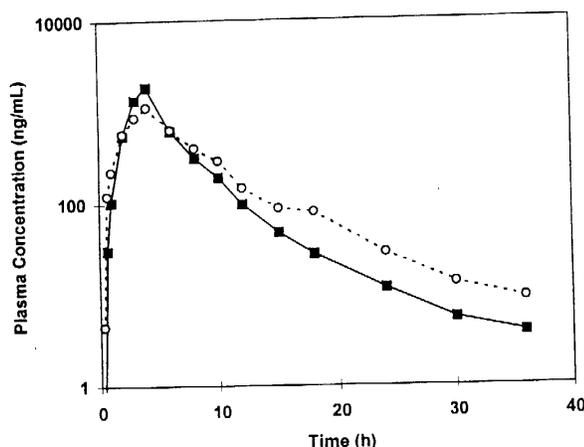
AUC τ (ng.h/ml)	2.09	NP
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NP=Not Provided by Sponsor

Bosentan:

Mean C_{max} was 37% lower and mean AUC was 11% lower in severe renal impairment patients compared to healthy subjects. Median T_{max} and $T_{1/2}$, however, were similar in both groups. The reason for the lower concentrations, especially peak concentrations of bosentan in severe renal impairment patients is not known.

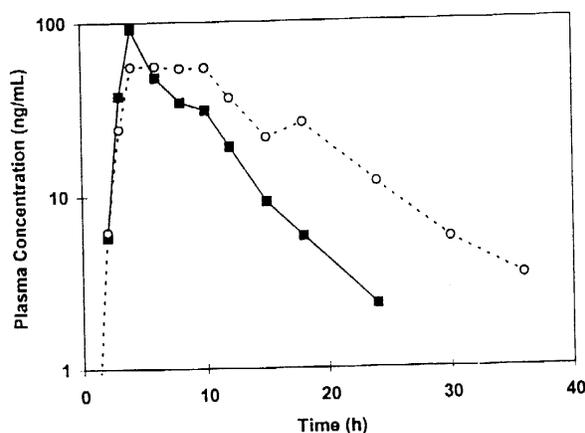
Mean plasma concentration versus time curves of bosentan (Ro 47-0203) after single oral administration of 125 mg bosentan in healthy subjects and in patients with impaired renal function (semi-logarithmic scale)



Ro 48-5033:

Mean C_{max} was 20% lower, while, mean AUC was 58% higher in patients with severe renal impairment compared to healthy subjects. Median T_{max} occurred 1 h later and median $T_{1/2}$ was approximately 3 h longer in severe renal impairment patients compared to healthy subjects. Ro 48-5033 concentrations in

Mean plasma concentration versus time curves of Ro 48-5033 after single oral administration of 125 mg bosentan in healthy subjects and in patients with impaired renal function (semi-logarithmic scale)



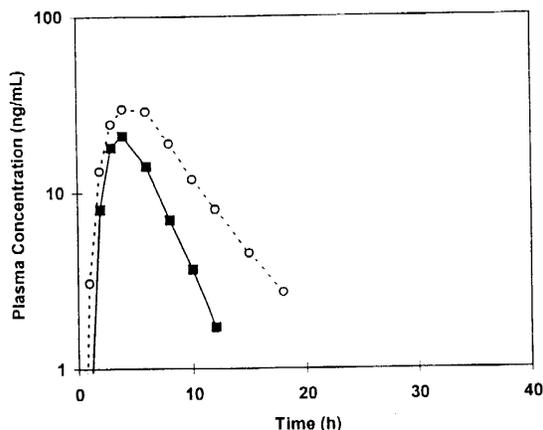
severe renal impairment patients and healthy subjects was <10% of parent drug concentrations. The increase in Ro 48-5033, active metabolite, concentrations in severe renal impairment patients was lower

than that seen with the other metabolites of bosentan (Ro 47-8634 and Ro 64-1056). This indicates that the renal route might not be the only pathway of elimination for Ro 48-5033.

Ro 47-8634:

Mean C_{max} and AUC were higher by 38% and 100%, respectively, in renal impairment patients compared to healthy subjects. Median T_{max} occurred 1 h later and median $T_{1/2}$ was approximately 2 h longer in severe renal impairment patients compared to healthy subjects.

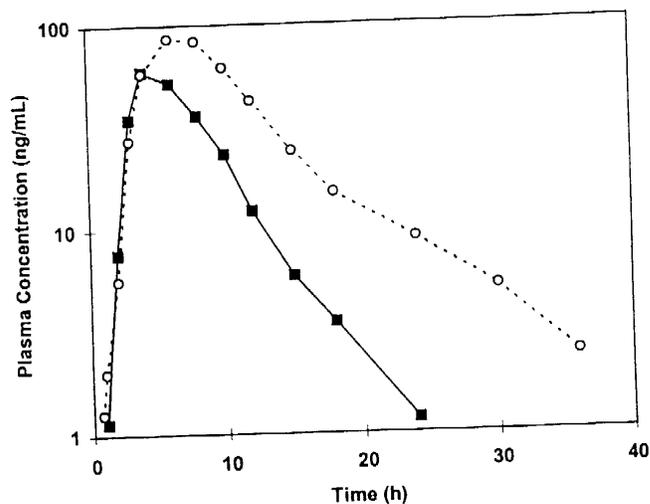
1 Mean plasma concentration versus time curves of Ro 47-8634 after single oral administration of 125 mg bosentan in healthy subjects and in patients with impaired renal function (semi-logarithmic scale)



Ro 64-1056:

Mean C_{max} and AUC of Ro 64-1056 in renal impairment patients were higher by 41% and 109%,

6 Mean plasma concentration versus time curves of Ro 64-1056 after single oral administration of 125 mg bosentan in healthy subjects and in patients with impaired renal function (semi-logarithmic scale)



respectively, compared to healthy subjects. The median T_{max} occurred 1 h later and median $T_{1/2}$ was approximately 4 h longer in severe renal impairment patients compared to healthy subjects. The increase in Ro 47-8634 and Ro 64-1056 concentrations and slow elimination in renal impairment patients indicates

that the renal route is a significant pathway of elimination for Ro 64-1056.

SAFETY

There were no reported deaths or serious adverse events. The only subject reporting an adverse event was a healthy subject with headache and weakness. Blood pressure was lowered to a greater extent in the renal impairment subjects. It is not possible to draw conclusions about blood pressure effects since the study was open label. There were no reports of hypotension.

CONCLUSIONS:

Mean C_{max} and AUC of bosentan were 37% and 11% lower in severe renal impairment patients compared to healthy subjects. Median T_{max} and $T_{1/2}$, however, were similar in both groups. The reason for the lower concentrations, especially peak concentrations, of bosentan in severe renal impairment patients is not known. The concentrations of the active metabolite Ro 48-5033 increased in severe renal impairment patients but were <10% of parent drug concentrations. Concentrations of the other metabolites, Ro 47-8634 and Ro 64-1056, increased by 100% in severe renal impairment patients compared to normal subjects indicating that the renal route is a major route of elimination of the metabolites of bosentan.

In the absence of a concentration-effect relationship, the effect of reduced bosentan concentrations on efficacy in renal impairment patients cannot be assessed. Dosage adjustment is not recommended by the sponsor.

COMMENTS:

1. The sponsor has not studied the effect of mild and moderate renal impairment on the pharmacokinetics of bosentan. Bosentan is primarily metabolized and less than 3% is excreted unchanged in the urine. Therefore, the present study in severe renal impairment patients only is acceptable. The current study helps in understanding the maximum extent of increase in metabolite concentrations.
2. The sponsor did not provide the lower and upper limits of the 95% confidence intervals for the ratios of C_{max} and AUC in renal impairment patients to normal subjects for Ro 48-5033, Ro 47-8634 and Ro 64-1056.
3. The extent of decrease in C_{max} and AUC of bosentan in severe renal impairment subjects should be included in the label.
4. Concentrations of the active metabolite, Ro 48-5033, increased by 58% in severe renal impairment patients compared to normal subjects. This increase is not expected to increase pharmacodynamic activity significantly. The concentrations of the inactive metabolites, Ro 47-8634 and Ro 64-1056, were significantly higher in severe renal impairment subjects; the toxicity of these metabolites, however, is not known.

STUDY B-162293– A RENAL HEMODYNAMIC AND PHARMACOKINETIC STUDY OF BOSENTAN (Ro 47-0203) AND CYCLOSPORINE A (SANDIMMUN NEORAL) IN HEALTHY VOLUNTEERS

STUDY INVESTIGATORS AND SITES: Prof. G. Thiel, MD
Dr. I. Binet, MD
Dept. of Internal Medicine, Nephrology
Kantonsspital Basel
Switzerland

Report No.: B-162293

Volume No.: 2.15

OBJECTIVES:

1. To assess the effect on renal hemodynamics and safety of bosentan alone and in combination with cyclosporin A (CsA).
2. To assess the pharmacokinetics of bosentan and CsA during combined multiple oral dose treatment.

FORMULATIONS:

Bosentan – oral suspension containing 100 mg/ml bosentan (Batch # GFR0075)

Placebo – oral suspension matched to bosentan (Batch # GFR0076)

Cyclosporin A – capsules of 25, 50 and 100 mg, Sandimmun Neoral[®], Sandoz Ltd.

STUDY DESIGN:

This was a double-blind, randomized, placebo-controlled, cross-over study in 8 healthy male between 20 and 28 years of age. 7 subjects completed the study. The subjects were healthy male volunteers between the ages of 18 and 65 years (mean: 51 years). On Day 1 of Period 1, all subjects were randomized to receive either Treatment A – 500 mg BID bosentan + 300 mg BID cyclosporin A for 8 days, or Treatment B – 300 mg BID cyclosporin A + placebo for 8 days. Cyclosporin treatment was initiated concomitant with the second dose of bosentan or placebo on Day 1 of both treatment periods. Only the morning dose was administered on Day 8. In Period 2, subjects received the alternate treatment. The washout period between treatment periods was 12 days.

The initial intended dose of bosentan was 1000 mg BID; however, the dose was reduced to 500 mg BID because of severe headache and gastric disturbance in the first 2 subjects. Based on the trough levels of CsA, the dosage was to be adjusted in both treatment periods after 2 and 4 days of CsA treatments according to the following schedule to reach a target trough plasma concentration of 200 –250 ng/ml CsA at steady state.

CsA dosage adjustment starting with the evening dose on day 3:	If morning CsA trough level < 100 ng/mL:	Increase of the Sandimmun Neoral dose by 100 mg/day (i.e., +50 mg in the morning and in the evening)
	If morning CsA trough level between 100-149 ng/mL:	Increase of the Sandimmun Neoral dose by 50 mg/day (i.e., +25 mg in the morning and in the evening)
	If morning CsA trough level between 150-250 ng/mL:	No change
	If morning CsA trough level > 250 ng/mL:	Decrease of the Sandimmun Neoral dose by 50 mg/day (i.e., -25 mg in the morning and in the evening)
	If morning CsA trough level > 300 ng/mL:	Decrease of the Sandimmun Neoral dose by 100 mg/day (i.e., -50 mg in the morning and in the evening)
CsA dosage adjustment starting with the evening dose on day 5:	If morning CsA trough level < 150 ng/mL:	Increase of the Sandimmun Neoral dose by 100 mg/day (i.e., +50 mg in the morning and in the evening)
	If morning CsA trough level between 150-199 ng/mL:	Increase of the Sandimmun Neoral dose by 50 mg/day (i.e., +25 mg in the morning and in the evening)
	If morning CsA trough level between 200-250 ng/mL:	No change
	If morning CsA trough level > 250 ng/mL:	Decrease of the Sandimmun Neoral dose by 50 mg/day (i.e., -25 mg in the morning and in the evening)
	If morning CsA trough level > 300 ng/mL:	Decrease of the Sandimmun Neoral dose by 100 mg/day (i.e., -50 mg in the morning and in the evening)

ASSAY:

Compound	Matrix	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
Bosentan	Plasma	HPLC/ UV	50 - 1000	NP	50	50	9.5	+0.2
						250	2.5	+0.5
						500	4.0	+1.2
						5000	6.3	+1.4
						10000	4.6	-2.8
Cyclosporin A	Plasma	FPIA*	0-1500	NP	100	150	NP	NP
						400		
						800		

- Fluorescent Polarization Immunoassay

Sample Collection:

On Days 8 of both treatment periods, blood samples for measurement of plasma concentrations of bosentan and CsA were collected at 0 (predose), 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hours post-dose. Blood samples were also collected on Day 1 of both treatment periods for measurement of bosentan concentrations at the same times as indicated above. Blood samples were collected for trough measurement of bosentan on Days 3 and 5, and for trough measurement of cyclosporin on Days 3, 5 and 8.

RESULTS

Effect of Bosentan on Cyclosporin A:

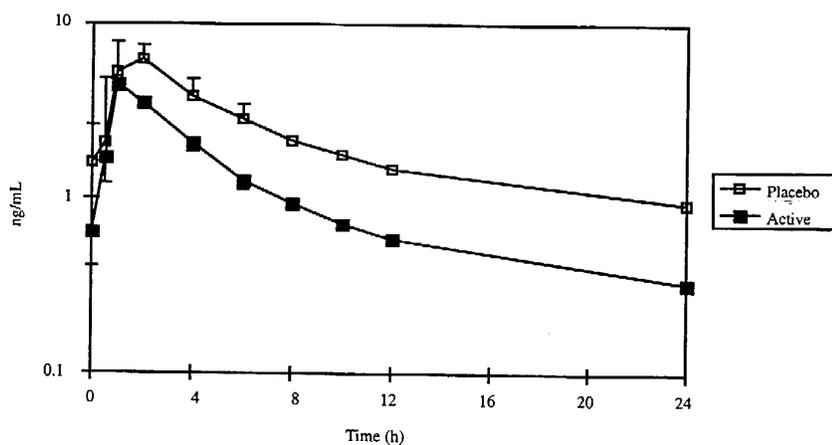
The pharmacokinetic parameters of cyclosporin A in the presence of placebo and bosentan are listed in the following table.

Table 1: Mean (SD) Steady-state Pharmacokinetic Parameters of Cyclosporin A

Parameter	CSA + Placebo	CSA + Bosentan
T _{max} (h)#	1.7 (30)	1.4 (38)
C _{max} (ng/ml)	1362 (24)	1512 (13)
AUC ₀₋₁₂ (ng.h/ml)	7819 (31)	6520 (20)
AUC/Dose (ng.h/ml)	38.1 (34)	20.5 (25)
CL _o (L/h)	29 (33)	51 (24)
T _{1/2} (h)	15.8 (26)	14.0 (24)
C _{trough} /Dose at S.S (ng/ml)	1.51 (45)	0.61 (32)

Concomitant administration of bosentan reduced the C_{max} and AUC_τ of CsA at steady-state. Mean C_{max} of CsA in the presence of bosentan decreased by 26%. In the presence of bosentan steady-state trough concentration of CsA decreased by 62% and steady-state AUC decreased by 49%. The decrease in CsA C_{max}, C_{trough} and AUC in the presence of bosentan can be attributable to the enzyme inducing property of bosentan.

Figure 10. Dose Corrected Mean (+/- SD) Blood Concentration Time Profiles of Cyclosporine A Following Multiple Oral Doses Given BID



Effect of Cyclosporin A on Bosentan:

The pharmacokinetic parameters of bosentan in the absence and presence of cyclosporin are listed in the following table.

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

Parameter	Day 1 No CsA	Day 8 With CsA
C _{max} (ng/ml)	4743 (49)	7916 (54)
T _{max} (h)	2.9 (41)	4.3 (50)
AUC ₀₋₁₂ (ng.h/ml)	24780 (54)	48900 (49)
CL _o (L/h)	29.3 (57)	12.4 (43)
T _{1/2} (h)	3.3 (31)	3.4 (34)

On Day 1, volunteers received 500-mg bosentan alone which resulted in mean trough concentration at the end of the dosing interval (12 h) of 495 ng/ml. After the first dosing interval, both 500-mg bosentan and cyclosporin were administered and only trough concentrations were measured at the end of the dosing interval. The mean trough concentration of bosentan in the presence of cyclosporin increased to 10425 ng/ml, a 21-fold increase. Maximum increases in bosentan concentrations up to 30-fold were observed.

Upon coadministration of multiple doses of bosentan and CsA, the higher trough levels decreased from a 20 to 30-fold increase to a 2-fold higher steady-state level by Day 5. The observed decrease in bosentan trough levels with chronic dosing could be attributable to the enzyme inducing effect of bosentan. At steady-state, the mean trough concentration was 1300 ng/ml which is about 162% higher than the mean trough concentration of 495 ng/ml obtained on Day 1 with bosentan alone. The observed mean increase in C_{max} and AUC on Day 8 compared to Day 1 was approximately 100%. But this increase was characterized by high variability; range=18% to 180% for C_{max} and, range=22% to 140% for AUC.

The cause of the interaction is unknown. Since bosentan is metabolized by CYP 3A4, it is hypothesized that the increased levels of bosentan could be attributable to competitive inhibition of CYP 3A4 metabolism of bosentan by CsA. It is also hypothesized that the decreased elimination of bosentan could be due to decreased biliary excretion of bosentan. Bosentan is a substrate of P-glycoprotein, an active transport system, which could be inhibited by CsA. In the rat, CsA has been shown to inhibit biliary secretion in a dose-dependent manner; however, the effect of CsA on biliary secretion in man is not known.

In a multiple dose study of bosentan in healthy volunteers (STUDY B-159037), Day 8 C_{max} and AUC were both approximately 50% of Day 1 values for all doses (100, 200, 500 and 1000 mg). Based on this information, the magnitude of increase in steady-state bosentan C_{max} and AUC is expected to be higher than 100%, which value was obtained by incorrectly comparing steady-state bosentan C_{max} and AUC in the presence of CsA to single dose bosentan C_{max} and AUC in the absence of CsA.

Figure 7. Mean (+/- SD) Plasma Concentration Time Profiles of Bosentan Following Multiple Oral Doses of 500 mg BID

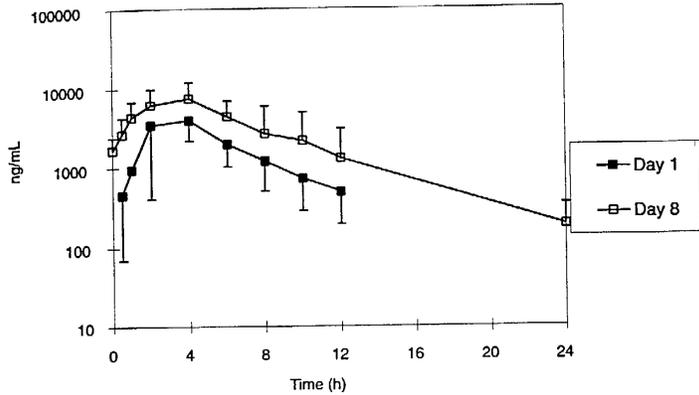
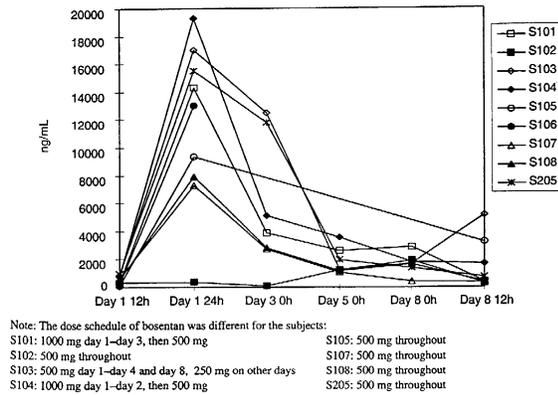


Figure 8. Individual Trough Plasma Concentration Time Profiles of Bosentan Following Multiple Oral Doses of 250-1000 mg BID (corrected for 500 mg doses)



CONCLUSIONS:

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 162% and 100%, respectively, compared to single dose trough concentration in the absence of CsA. The magnitude of increase in steady-state bosentan C_{max} and AUC is actually higher than the reported increase of 100%, obtained by incorrectly comparing steady-state bosentan C_{max} and AUC in the presence of CsA to single dose bosentan C_{max} 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 C_{max} , AUC and trough concentration values by 26%, 49% and 62%, respectively, probably by inducing metabolizing enzymes.

The concomitant use of bosentan and cyclosporin should be contraindicated.

COMMENTS:

1. In view of the high variability in bosentan concentrations, the sponsor should have enrolled more subjects into the study. This study is under powered and therefore, the true magnitude of effect of CsA on bosentan concentrations cannot be gained from 8 subjects.

2. This study is conducted using 500-mg of bosentan which 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.
3. In a multiple dose study of bosentan in healthy volunteers (STUDY B-159037), Day 8 C_{max} and AUC were approximately 50% of Day 1 values for all doses (100, 200, 500 and 1000 mg). Based on this information, it can be assumed that although the C_{max} and AUC of bosentan increased by 100% in the presence of CsA, the true extent of interaction is probably higher than 100%.

The sponsor should have designed the study with a multiple-dose bosentan only arm. This would serve as a reference and would aid in understanding the true extent of interaction at steady-state upon concomitant administration of bosentan and cyclosporin A.

4. The analytical report for cyclosporin A was not in English. The sponsor was requested to submit the analytical report in English on April 24, 2001. The sponsor submitted a translation of the original analytical report in submission dated June 21, 2001 and the submitted information was subsequently incorporated into the review.

STUDY B-159044 – THE EFFECT OF BOSENTAN ON THE PHARMACOKINETICS OF DIGOXIN IN HEALTHY MALE SUBJECTS

STUDY INVESTIGATORS AND SITES: R. Schulz, MD

Roche Clinical Pharmacology Unit
F-67064 Strasbourg
Cedex, France

Report No.: B-159044

Volume No.: 2.16

OBJECTIVES:

1. To investigate the effect of multiple oral dose of bosentan (500 mg BID, 7 days) on the steady-state pharmacokinetics of digoxin.

FORMULATIONS:

Bosentan – 500 mg tablets (batch #: GLU 0064/09)

Lanoxin[®] - 0.125 mg and 0.25 mg digoxin tablets (Wellcome)

STUDY DESIGN:

This was an open-label, randomized, two-period, crossover study in 18 healthy male subjects between the ages of 20 and 40 years (mean: 27 years) with a mean body weight of 71 kg. On Day 1 of Period A subjects were randomized to receive either **Treatment A:** Digoxin 0.375 mg BID orally on Day 1 followed by 0.375 mg orally once-daily on Days 2 to 13, or **Treatment B:** digoxin 0.375 mg BID on Day 1 followed by digoxin 0.375 mg once-daily on Days 2 to 13 + bosentan 500 mg BID from Days 8 to 14. Subjects received the alternate treatment in Period B, the washout period ranged from 10 days to 4 weeks. Digoxin doses were taken within 10 minutes after consumption of a meal.

ASSAY:

Compound	Matri x	Method	Range (ng/ml)	Linear ity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
Bosentan	Plasma	HPLC/ UV	5.0 - 5000	NP	5.0	10	6.0	-2.4
						50	2.0	-0.7
						100	3.6	-2.4
						1000	2.7	-2.4
						2000	3.1	+2.2
Digoxin	Serum	Radioimmunoassay	0.15 - 5.0	NP	0.15	0.15	9.6	-3.3
						0.30	4.4	-1.4
						0.74	6.2	+2.2
						1.60	3.0	+3.2
						2.58	2.4	+0.5
						5.01	3.7	-2.8
Digoxin	Urine	Radioimmunoassay	NP	NP	6.0	NP	NP	NP

NP = not provided by sponsor

Sample Collection:

Blood samples (5-ml) for analysis of digoxin concentrations were collected on Days 1, 8 and 14 before dosing and at 1, 2, 4, 6, 8, 10, 12 and 24 hours post-dose.

Blood samples (7-ml) for analysis of bosentan concentrations were collected on Day 8 pre-dose and at 4 h and 12 hours post dose and on Day 14 pre-dose and at 1, 2, 4, 6, 8, 10 and 12 hours post-dose.

Urine was collected 0 - 24 hours post-dose for analysis of digoxin concentrations on Days 1, 8 and 14.

RESULTS

Effect of Bosentan on Digoxin:

The pharmacokinetic parameters of digoxin in the absence and presence of bosentan are listed in the following table.

Table 1: Mean (SD) Pharmacokinetic Parameters of Digoxin

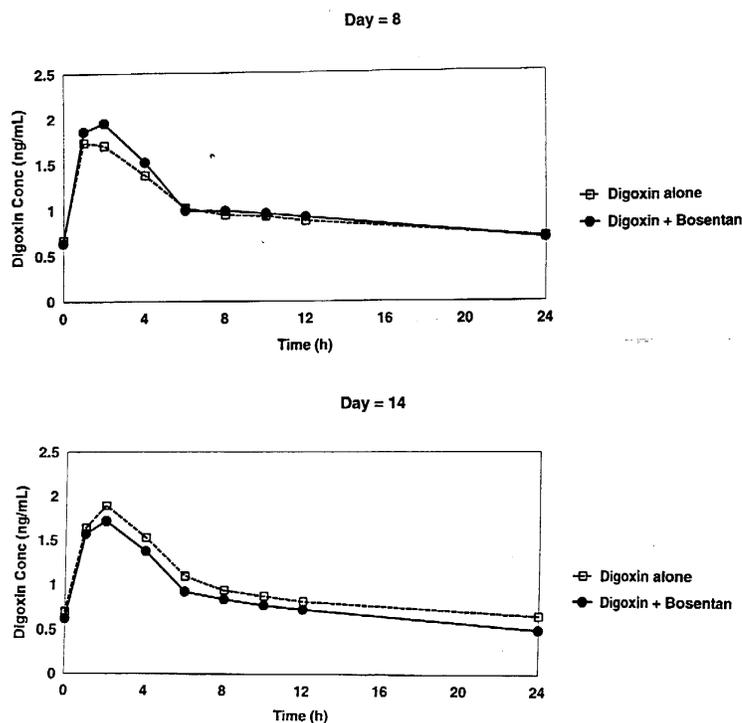
Parameter	Treatment Day	Digoxin + Bosentan (TEST)	Digoxin Alone (REF)	Point Estimate	90% Confidence Interval
		Arithmetic means	Ratio of test/reference means		
C _{max} (ng/ml)	Day 8	2.29 (0.62)	2.01 (0.56)	114	99 - 131
	Day14	2.00 (0.61)	2.12 (0.31)	91	80 - 104
AUC ₀₋₂₄ (ng.h/ml)	Day 8	24.5 (5.7)	23.3 (6.0)	106	97 - 115
	Day14	20.2 (4.66)	23.1 (5.94)	88	79 - 98
C _{min} (ng/ml)	Day 8	0.68 (0.26)	0.69 (0.27)		
	Day14	0.50 (0.21)	0.65 (0.26)		
T _{max} (h)	Day 8	1.7 (1.0)	1.6 (1.0)		
	Day14	2.0 (1.1)	1.7 (0.8)		
T _{1/2} (h)	Day 8	51.3 (50.6)	43.7 (30.6)		
	Day14	25.8 (14.6)	42.3 (39.3)		
CL/F (L/h)	Day 8	16.1 (3.8)	17.2 (4.6)		
	Day14	19.5 (4.5)	17.3 (4.4)		
CL _R /F (L/h)	Day 8	5.8 (2.7)	5.6 (1.3)		
	Day14	5.8 (2.4)	5.1 (1.4)		

In the absence of bosentan, steady-state digoxin concentrations were achieved in 7 days. Steady-state C_{max} of digoxin was between 2.0 and 2.1 ng/ml with a T_{max} of 1.6 to 1.7 h post-dose.

Concomitant administration of bosentan and digoxin lowered Day 14 C_{max} and AUC_τ of digoxin by 9% and 12%, respectively. The 90% confidence intervals for C_{max} of digoxin following multiple dosing of 500-mg bosentan BID for 7 days was contained within the bioequivalence limits of 0.80 and 1.25. However, the lower limit of the 90% confidence interval for AUC₀₋₂₄

was slightly below 0.80. The Day 14 C_{min} of digoxin decreased by 30% upon coadministration with 500-mg BID bosentan.

Figure 1. Mean Serum Concentration Time Plots of Digoxin at Steady-State During Treatment with 0.375 mg Once Daily without or with Concomitant Treatment with Bosentan 500 mg BID for 7 Days



Effect of Digoxin on Bosentan:

The pharmacokinetic parameters of bosentan in the presence of digoxin are listed in the following table.

Table 2: Mean (SD) Pharmacokinetic Parameters of Bosentan Obtained following 500-mg BID for 7 Days

Parameter	Treatment Day	Present Study 500-mg BID	Another 500-mg QD Study (B-159037)
C_{max} (ng/ml)	Day 14	3260 (1040)	3491
AUC_{0-12} (ng.h/ml)	Day 14	12600 (3630)	15030
$T_{1/2}$ (h)	Day 14	4.5 (4.5)	7.1
C_{trough} (ng/ml)	Day 8	991 (1150)	-
C_{trough} (ng/ml)	Day 14	177 (171)	-

Tmax (h)#

Day 14

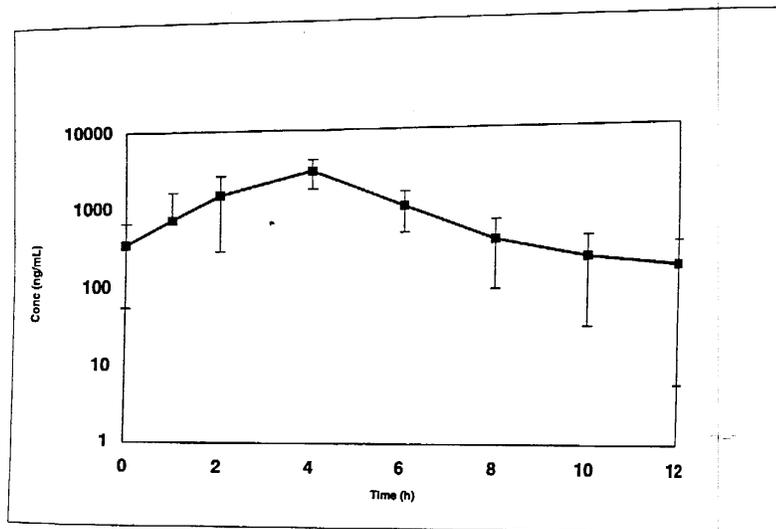
3.7 (0.8)

3.0

Day 14 C_{max} and AUC of bosentan were similar to values obtained in another study (B-159037) in healthy volunteers, where bosentan 500-mg was administered once-daily for 8 days. This indicates that multiple dose digoxin did not alter the pharmacokinetics of bosentan. Although, bosentan was dosed once-daily in Study B-159037 compared to the present study where bosentan was administered twice-daily, the C_{max} and AUC values should be comparable because of the short $T_{1/2}$ of bosentan (4 to 7 hours).

The trough concentrations of bosentan decreased from 991 ng/ml on Day 8 to 177 ng/ml on Day 14 probably due to induction of its metabolizing enzymes.

Figure 2. Mean (. SD) Plasma Concentration Time Plot of Bosentan Following Treatment with 500 mg BID for 1 week with Concomitant Digoxin Treatment of 0.375 mg Once Daily



SAFETY

There were 19 subjects entered into the study. There were no deaths and no serious adverse events. One subject withdrew because of mild 2nd degree AV block after receiving digoxin alone and one subject withdrew because of bronchitis. The reporting of adverse events was similar for the 2 groups.

CONCLUSIONS:

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 (B-159037) in healthy individuals indicated no effect of concomitant administration of digoxin on bosentan pharmacokinetics.

The concomitant use of digoxin and bosentan is not expected to pose a safety concern.

COMMENT:

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.

STUDY B-159043 – THE EFFECT OF BOSENTAN ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF WARFARIN IN HEALTHY MALE SUBJECTS

STUDY INVESTIGATORS AND SITES: R. Schulz, MD

Roche Clinical Pharmacology Unit
F-67064 Strasbourg
Cedex, France

Report No.: B-159043

Volume No.: 2.17

OBJECTIVES:

1. To investigate the effect of multiple oral dose treatment with bosentan on the pharmacokinetics and pharmacodynamics of single dose warfarin in healthy male subjects

FORMULATIONS:

Bosentan – 500 mg tablets (batch #: GLU 0037)

Placebo –tablets matching bosentan tablets (batch #: GLU 0030)

Warfarin sodium - Coumadine[®], 2 and 10 mg tablets

STUDY DESIGN:

This was a double-blind, randomized, placebo-controlled, two-period, cross-over study in 12 healthy male subjects between the ages of 19 and 29 years (mean: 24 years) with a mean body weight of 70 kg. On Day 1 of Period I all subjects are to be randomized to receive either, **Treatment A:** 500 mg BID bosentan for 10 days and single 26-mg dose of warfarin with morning dose of bosentan on 6th day of bosentan treatment, or **Treatment B:** Placebo matching bosentan for 10 days and single 26-mg dose of warfarin together with morning dose of placebo on 6th day of placebo treatment. Subjects received the alternate treatment in Period II following a 2-3 week washout interval.

ASSAY:

Compound		Method	Range (ng/ml)	Linear ity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
	Matri x							
Bosentan	Plasma	HPLC/ UV	5.0 - 2000	NP	5.0	10 50 100 1000 2000	NP 7.1 9.6 2.1 1.7	+5.7 +7.1 +2.0 +2.0 +4.0
R-Warfarin	Plasma	HPLC/Fluorescence	50 - 2750	NP	50	196 588 1230 2210	2.7 1.9 2.2 2.5	+1.4 +3.9 +2.6 +1.6
S-Warfarin	Plasma	HPLC/Fluorescence	50 -	NP	50	196	3.8	+0.8

2750	588	2.3	+4.1
	1230	1.7	+3.1
	2210	3.1	+3.1

Sample Collection:

Blood samples were collected for analysis of R- and S-warfarin concentrations prior to dosing on Day 6 and at 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 96 and 120 h post-dose.

Blood samples were collected for analysis of bosentan concentrations prior to dosing on Day 6 and at 12 h and 120 h.

RESULTS

The pharmacokinetic parameters of R- and S-warfarin in the presence of bosentan and placebo are listed in the following table.

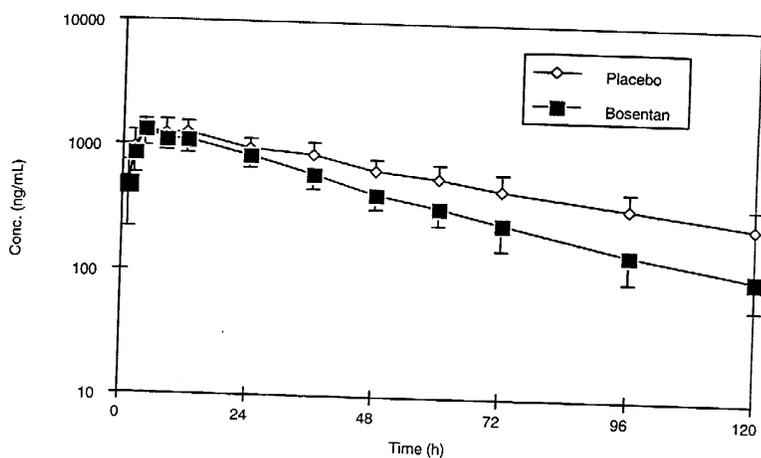
Table 1: Mean (SD) Pharmacokinetic Parameters of Warfarin with Bosentan and Placebo

Parameter	Warfarin + Bosentan (TEST)	Warfarin + Placebo (REF)	Point Estimate	95% Confidence Interval
R-WARFARIN				
Cmax (ng/ml)	1370 (326)	1370 (327)	100	93 - 108
Tmax (h)	4.3 (1.9)	6.8 (4.0)		
T1/2 (h)	32.1 (5.6)	50.9 (12)		
AUC ₀₋₁₂₀ (ng.h/ml)	54800 (12400)	77200 (19900)	72	68 - 76
AUC _{0-∞} (ng.h/ml)	59500 (14500)	97200 (31600)	62	57 - 68
CL/F (L/h)	0.46 (0.10)	0.29 (0.09)		
S-WARFARIN				
Cmax (ng/ml)	1380 (278)	1340 (268)	103	96 - 110
Tmax (ng/ml)	4.0 (1.5)	4.3 (1.9)		
T1/2 (h)	25.1 (6.3)	37.7 (10)		
AUC ₀₋₁₂₀ (ng.h/ml)	42700 (10300)	55900 (15000)	77	72 - 82
AUC _{0-∞} (ng.h/ml)	44600 (11600)	63100 (19600)	71	66 - 77
CL/F (L/h)	0.63 (0.18)	0.45 (0.14)		

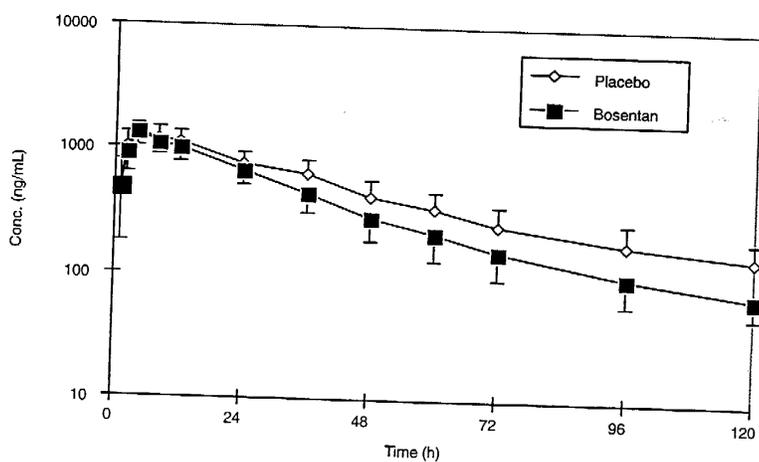
Concomitant administration of multiple doses of 500-mg BID bosentan increased the elimination of R- and S-warfarin following a single dose of 26-mg warfarin. The CL/F of R-warfarin and S-warfarin increased by 59% and 40%, respectively, and the half-life of R-warfarin and S-warfarin decreased by 37% and 33%, respectively, in the presence of bosentan compared to placebo. The decreased half-life is consistent with faster elimination of warfarin. Concomitant administration of bosentan did not affect the Cmax of R-warfarin and S-warfarin. It is hypothesized that the increased elimination of warfarin is probably due to induction of both CYP2C9 and CYP3A4.

Figure 3. Mean Plasma Concentration Time Plots of R- and S-Warfarin Following a Single Oral Dose of 26 mg Warfarin During Placebo or Bosentan Treatment with 500 mg BID

R-Warfarin



S-Warfarin



PHARMACODYNAMICS:

The pharmacodynamic measures of prothrombin time and Factor VII for warfarin in the presence of bosentan and placebo are listed in the following table.

Table 1: Mean (SD) Pharmacodynamic Parameters of Warfarin Alone and with Bosentan

Parameter	Warfarin + Bosentan (TEST)	Warfarin + Placebo (REF)	Point Estimate	95% Confidence Interval
Baseline PT (INR)	1.03 (0.10)	1.03 (0.07)		
Tmax (h)	33.9 (8.6)	36.9 (8.0)		
PTmax,cor (INR)	0.58 (0.29)	0.75 (0.35)	77	68 - 87
AUC _{PT,cor} (INR.h)	27.2 (17.4)	42.9 (28)	62	54 - 70

During placebo treatment, the single dose of 26-mg warfarin increased mean prothrombin time by 1.7-fold. The maximal prothrombin time ($PT_{\max,cor}$) was achieved 37 hours postdose. The baseline corrected AUCs for prothrombin time ($AUC_{PT,cor}$) was 42.9 INR.h and factor VII activity ($AUC_{VII,cor}$) was 6315%.h.

Concomitant administration of bosentan reduced the anticoagulation action of warfarin by decreasing the plasma concentration of R- and S-warfarin. Primary pharmacodynamic measures such as $PT_{\max,cor}$ decreased by 23% and $AUC_{PT,cor}$ decreased by 38%. Consistent with the unaltered C_{\max} of R- and S-warfarin, the time to maximum effect and time course of warfarin effect on prothrombin time and factor VII activity were not altered by bosentan.

Figure 1. Mean (SD) Prothrombin Time versus Time Plot Following a Single Oral Dose of 26 mg Warfarin during Placebo or Bosentan Treatment with 500 mg BID

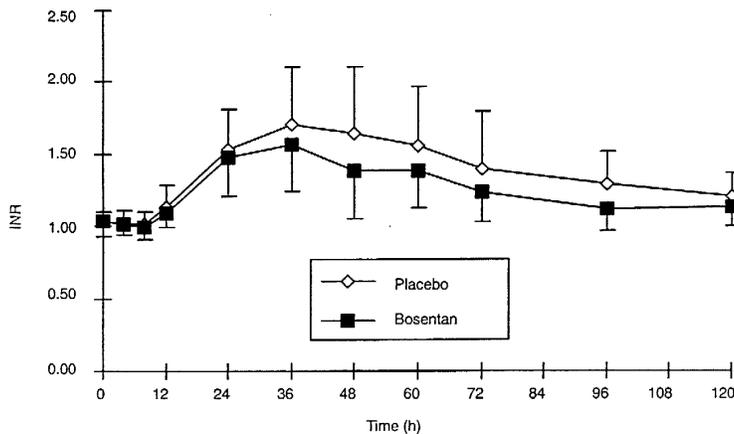
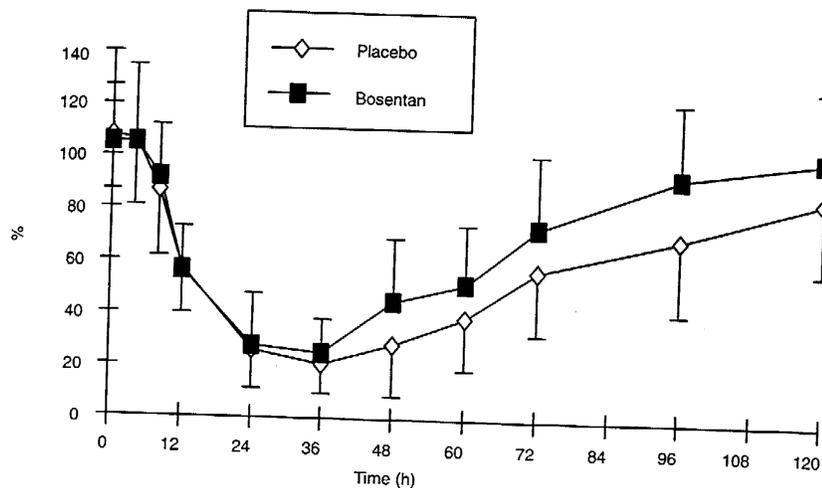


Figure 2. Mean (SD) Factor VII Activity versus Time Plot Following a Single Oral Dose of 26 mg Warfarin during Placebo or Bosentan Treatment with 500 mg BID



EFFECT ON THE PHARMACOKINETICS OF BOSENTAN:

Concomitant administration of a single dose of 26-mg warfarin with bosentan decreased bosentan trough concentrations significantly. Mean trough plasma concentration of bosentan 12-h after administration of the single dose of warfarin decreased by 63%, (388 ng/ml to 143 ng/ml). The reason for this interaction is not known at present. Mean trough concentration of bosentan recovered to its pre-warfarin steady-state trough concentration 120 hours after the single dose administration of warfarin (404 ng/ml).

SAFETY

There were 12 subjects. No subject died, reported a serious adverse event, or dropped out of the study because of an adverse event. Of the subjects receiving warfarin plus bosentan, 92% reported headaches compared to 26% of subjects receiving warfarin alone. There is considerable concern regarding the reduction of the anticoagulation effect of warfarin when used in conjunction with bosentan.

CONCLUSIONS:

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 concomitantly.

COMMENTS:

1. 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.

STUDY AC 052-101 – A STUDY ON THE POSSIBLE INTERACTION BETWEEN THE ENDOTHELIN RECEPTOR ANTAGONIST BOSENTAN, THE ANTIFUNGAL AGENT KETOCONAZOLE AND THE ANGIOTENSIN II RECEPTOR ANTAGONIST LOSARTAN IN HEALTHY VOLUNTEERS

STUDY INVESTIGATORS AND SITES: R. Jovic, MD
C. H. Kleinbloesem, Ph.D.
VanTx Research AG
St. Jakobsstrasse 41-43
4132 Muttenz
Switzerland

Report No.: AC 052-101

Volume No.: 2.18

OBJECTIVE:

To determine the pharmacokinetics of bosentan under steady-state conditions when given alone and in combination with ketoconazole or losartan.

FORMULATIONS:

Bosentan – 250-mg tablets (batch #: PT2227T68)

Ketoconazole – 200-mg tablets of Nizoral[®] by Janssen-Cilag (batch #: 98I03/610, 95K27/840 and 97A23/966)

Losartan – 50-mg tablets of Cozaar[®] by Merck Sharp & Dohme (batch #: HG 80360 and HG67240)

STUDY DESIGN:

This was a open-label, randomized, three-period crossover, multiple-dose study in 13 healthy subjects of either gender (8 M/5 F) between the ages of 21 and 37 years. On Day 1 of Period 1 all subjects were randomized to receive 1 of 3 treatments, **Treatment A:** 125 mg BID bosentan on Days 1-4 and a single dose on Day 5, or **Treatment B:** 125 mg BID bosentan on Days 1-4 and a single dose on Day 5 + 200 mg QD ketoconazole on Days 1-5, or **Treatment C:** 125 mg BID bosentan on Days 1-4 and a single dose on Day 5 + 100 mg QD losartan on Days 1-5. Subjects received alternate treatments in Periods 2 and 3. There was a 7-day washout interval between treatment Periods. Bosentan, ketoconazole and losartan were administered with food.

ASSAY:

Compound		Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
	<i>Matri</i>							
	<i>x</i>							
Bosentan	Plasma	LC/MS/MS	0.05 - 25.0	>0.996	5.0	15.0 300 1200	5.50 3.17 4.34	-8.35 -0.93 -2.58

Ro 48-5033	Plasma	LC/MS/MS	NP	NP	2.37	7.10 71.0 284	4.56 5.75 5.30	-5.63 -2.21 -0.19
Ro 47-8634	Plasma	LC/MS/MS	NP	NP	1.23	3.69 36.9 148	4.98 3.05 3.77	-2.37 -0.76 +2.16
Ro 64-1056	Plasma	LC/MS/MS	NP	NP	2.44	7.33 73.3 293	8.05 6.65 7.35	-5.26 -3.54 -2.28

Sample Collection:

On Day 5 of each of the 3 treatment Periods blood samples were collected, for analysis of bosentan and its metabolites, pre-dose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10 and 12 hours after the morning dose of bosentan.

RESULTS

The pharmacokinetic parameters and 90% confidence intervals of bosentan when administered alone and in the presence of 200 mg QD ketoconazole and 100-mg QD losartan are listed in the following tables.

Table 1: Geometric Mean (SD) Pharmacokinetic Parameters of Bosentan on Day 5

Parameter	125-mg BID Bosentan	125-mg BID Bosentan +200-mg QD Ketoconazole	125-mg BID Bosentan +100-mg QD Losartan
C _{max} (ng/ml)	996 (448)	1610 (838)	857 (350)
T _{max} * (h)	3.7 (1.1)	4.0 (1.5)	3.0**(1.5)
AUC ₀₋₁₂ (ng.h/ml)	4304 (1629)	7896 (3788)	3672 (1554)
MRT (h)	233 (76)	208 (49)	244 (50)

*arithmetic mean; **median

Table 2: Point estimate and 90% Confidence Intervals for Bosentan and its Metabolites

Compound	Parameter	(Ketoconazole + Bosentan) vs. (Bosentan Alone)		(Losartan + Bosentan) vs. (Bosentan Alone)	
		Point Estimate	90% Conf. Interval	Point Estimate	90% Conf. Interval
Bosentan	C _{max} (ng/ml)	1.62	1.34, 1.94	0.86	0.72, 1.04
	AUC _τ (ng.h/ml)	1.83	1.52, 2.21	0.85	0.71, 1.03
Ro 47-8634	C _{max} (ng/ml)	0.66	0.51, 0.87	1.02	0.78, 1.34
	AUC _τ (ng.h/ml)	0.88	0.69, 1.11	0.83	0.66, 1.06
Ro 48-5033	C _{max} (ng/ml)	1.39	1.04, 1.85	0.94	0.71, 1.26
	AUC _τ (ng.h/ml)	1.31	0.95, 1.81	0.85	0.62, 1.17
Ro 64-1056	C _{max} (ng/ml)	0.70	0.49, 0.99	0.76	0.53, 1.08
	AUC _τ (ng.h/ml)	0.84	0.61, 1.15	0.70	0.51, 0.96

EFFECT OF KETOCONAZOLE ON BOSENTAN AND ITS METABOLITES:

Concomitant administration of 200-mg QD ketoconazole significantly increased steady-state C_{max} and AUC of bosentan by 62% and 83%, respectively. The T_{max} of bosentan, however, remained unchanged in the presence of ketoconazole.

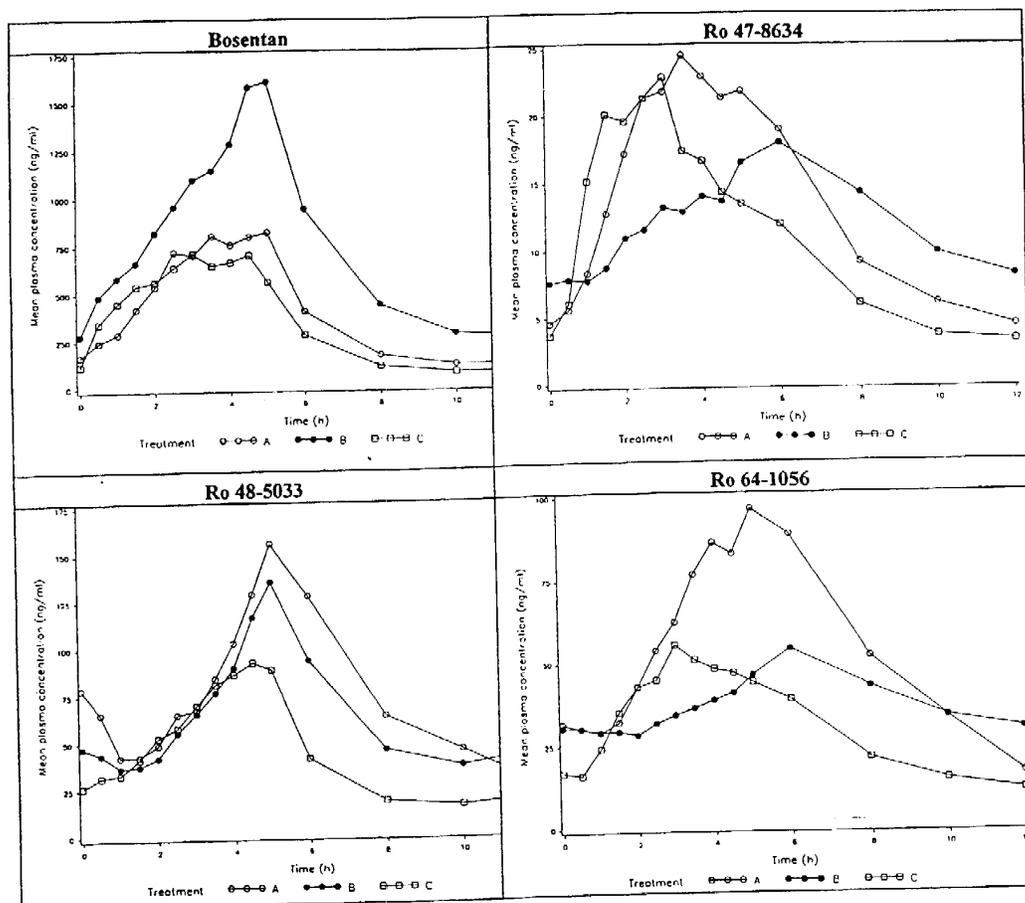
The increase in bosentan C_{max} and AUC in the presence of ketoconazole can be attributed to inhibition of bosentan metabolism via CYP3A4. This was evident from the decreased concentration of metabolites of bosentan, except Ro 47-5033, in the presence of ketoconazole. The C_{max} and AUC of the active metabolite, Ro 47-8634, were lower by 33% and 12%, respectively, in the presence of ketoconazole. A similar reduction in C_{max} and AUC of metabolite Ro 64-1056 by 30% and 16%, respectively, was observed in the presence of ketoconazole. Contrary to the other metabolites of bosentan, Ro 48-5033 concentrations increased in the presence of ketoconazole; the C_{max} and AUC of Ro 48-5033 were 39% and 31% higher.

The sponsor did not measure bosentan concentrations after coadministration of the 1st dose of ketoconazole. In the study assessing the effect of concomitant administration of bosentan and cyclosporin, bosentan trough concentrations increased by 30-fold after coadministration of the 1st dose of cyclosporin. A similar magnitude of increase in bosentan concentrations is expected after coadministration of the 1st dose of ketoconazole.

EFFECT OF LOSARTAN ON BOSENTAN AND ITS METABOLITES:

Concomitant administration of 100-mg QD losartan lowered both C_{max} and AUC of bosentan by 15%. Losartan decreased the C_{max} and AUC of metabolite Ro 64-1056 by 24% and 30%, respectively. The AUC of the other metabolites, Ro 47-8634 and Ro 48-5033 decreased by approximately 15%, while the C_{max} was unaffected.

Figure 1: Mean plasma concentrations (ng/ml) on day 5 of bosentan, Ro 47-8634, Ro 48-5033 and Ro 64-1056: linear scale



SAFETY

There were 12 study subjects. No adverse events were reported. One subject (#5) dropped out during the bosentan only phase; the sponsor gave no explanation. Five subjects had decreases from baseline in both hemoglobin and hematocrit laboratory values.

CONCLUSIONS:

Concomitant administration of 200-mg QD ketoconazole significantly increased the steady-state C_{max} and AUC of bosentan by 62% and 83%, respectively. The increase in bosentan C_{max} 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 C_{max} and AUC of the active metabolite, Ro 47-8634, were lower by 33% and 12%, respectively, in the presence of ketoconazole.

Concomitant administration of bosentan and CYP 3A4 inhibitors should be

contraindicated.

Concomitant administration of 100-mg QD losartan lowered both C_{max} and AUC of bosentan by 15%. Losartan decreased the C_{max} and AUC of metabolite Ro 64-1056 by 24% and 30%, respectively. The AUC of the other metabolites, Ro 47-8634 and Ro 48-5033 decreased by approximately 15%, while the C_{max} was unaffected.

COMMENTS:

1. In view of the metabolism inducing effect of bosentan, the sponsor should have evaluated the effect of bosentan on the concentrations of ketoconazole and losartan and their metabolites. This is especially important in the case of losartan since losartan is metabolized by CYP3A4 and CYP2C9 to an active carboxylic acid metabolite which is reported to be 10 to 40 times as potent as the parent compound.
2. The sponsor should have collected blood concentrations of bosentan on Day 1 in the presence of ketoconazole and losartan. This is because, in a previous multiple dose study (STUDY B-159037), bosentan concentrations decreased progressively upon multiple dosing, by about 50% compared to initial concentrations, due to self-induction of metabolizing enzymes. Therefore, a greater magnitude of interaction is anticipated after the first co-administered dose compared to steady-state (Day 5).
3. The Agency recommends the sponsor to incorporate into the label information regarding the significant increase in bosentan C_{max} and AUC in the presence of ketoconazole.
4. Bosentan is expected to affect ketoconazole concentrations, however, since ketoconazole concentrations were not measured in the present study the magnitude of such an interaction not known.

STUDY AC-052-102 – A STUDY ON THE POSSIBLE INTERACTION BETWEEN THE ENDOTHELIN RECEPTOR ANTAGONIST BOSENTAN AND THE HMG-CoA REDUCTASE INHIBITOR SIMVASTATIN IN HEALTHY VOLUNTEERS

STUDY INVESTIGATORS AND SITES: R. Jovic, MD

C. H. Kleinbloesem, Ph.D.
VanTx Research AG
St. Jakobsstrasse 41-43
4132 Muttenz
Switzerland

Report No.: VTX 98/I/257

Volume No.: 2.19

OBJECTIVES:

1. To assess the steady-state pharmacokinetics of bosentan and simvastatin in healthy adult volunteers when administered alone and when administered concomitantly.

FORMULATIONS:

Bosentan – 250 mg scored tablets (batch #: PT2227T68)

Simvastatin – Zocor[®] 40 mg (Merck Sharp and Dohme, Batch #: HH09800)

STUDY DESIGN:

This was an open-label, randomized, two-period cross-over study in 12 healthy subjects of either gender (4 M/8 F) between the ages of 18 and 29 years (mean: 21 years). On Day 1 of Period I, volunteers were randomized to receive either **Treatment A:** 125-mg BID bosentan from Days 1-9 + 40 mg QD simvastatin from Days 6-10, or, **Treatment B:** 40-mg QD simvastatin on Days 1-10 + 125-mg BID bosentan on Days 6-9 and single dose on Day 10. Following a 7-day washout interval all subjects received alternate treatment on Day 1 of Period II. Both bosentan and simvastatin were administered with standardized meals.

ASSAY:

Compound		Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
	<i>Matri</i>							
	<i>x</i>							
Bosentan	Plasma	LC/MS/MS	5 - 1600	≥ 0.996	5.0	15.0 300 1200	5.92 4.15 4.86	-8.75 -1.79 -4.41
Ro 48-5033	Plasma	LC/MS/MS	2.5 - 400	≥ 0.996	2.5	7.5 75 300	6.31 7.21 6.77	-7.78 -2.71 -4.24
Ro 47-8634	Plasma	LC/MS/MS	1.25 -	≥ 0.996	1.25	3.75	5.90	-1.65

			200			37.5 150	3.68 3.78	+2.15 +2.89
Ro 64-1056	Plasma	LC/MS/MS	2.5 - 400	≥ 0.996	2.5	7.5 75 300	7.23 8.17 7.74	-4.70 -0.62 -1.91
Simvastatin	Plasma	LC/MS/MS	1.0 - 40	≥ 0.995	1.0	3 15 30	10.8 10.4 13.0	-2.15 -5.83 +0.88
β-hydroxy simvastatin	Plasma	LC/MS/MS	0.5 - 40	≥ 0.995	0.5	1.5 15 30	11.2 9.53 10.3	-0.07 -6.83 +1.26

Sample Collection:

Blood samples (5-ml) were collected for analysis of simvastatin, β-hydroxy acid simvastatin, bosentan and metabolite concentrations on Days 5 and 10 of both treatment periods prior to dosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10 and 12 hours post-dose. Trough samples were also collected in both treatment periods prior to the morning dose on Days 1, 2, 3, 4, 7, 8 and 9. An additional trough blood sample was collected on the morning of Day 6 and additional samples were also collected at 16 and 24 hours post dose on Days 5 and 10 from subjects receiving Treatment B only.

RESULTS

EFFECT OF SIMVASTATIN ON BOSENTAN:

Steady-state pharmacokinetic parameters and 90% confidence intervals of the PK parameters of bosentan and its metabolites obtained following administration of 125-mg BID bosentan alone, in the presence of 40-mg QD simvastatin are listed in the following table.

Table 1: Geometric Mean (SD) Pharmacokinetic Parameters of Bosentan and metabolites

Compound	TREATMENT	C _{max} (ng/ml)	T _{max} (h)*	AUC _{0-T} (ng.h/ml)	MRT (h)*
Bosentan	Bosentan alone	841 (340)	3.2 (1.4)	3644 (1226)	4.30 (0.7)
	Bosentan + Simvastatin	829 (395)	2.4 (1.6)	3408 (1194)	3.67 (0.6)
Ro 47-8634	Bosentan alone	31.5 (5.5)	3.2 (1.2)	153 (45)	4.58 (0.5)
	Bosentan + Simvastatin	33.8 (14)	2.3 (0.8)	143 (49)	3.95 (0.6)
Ro 48-5033	Bosentan alone	94.3 (45)	4.4 (1.7)	507 (193)	5.47 (0.6)
	Bosentan + Simvastatin	105 (47)	3.9 (1.2)	530 (224)	5.14 (0.6)
Ro 64-1056	Bosentan alone	92.6 (23)	3.9 (0.9)	514 (147)	5.30 (0.6)
	Bosentan + Simvastatin	111 (48)	3.3 (0.8)	556 (205)	4.72 (0.6)

*arithmetic mean

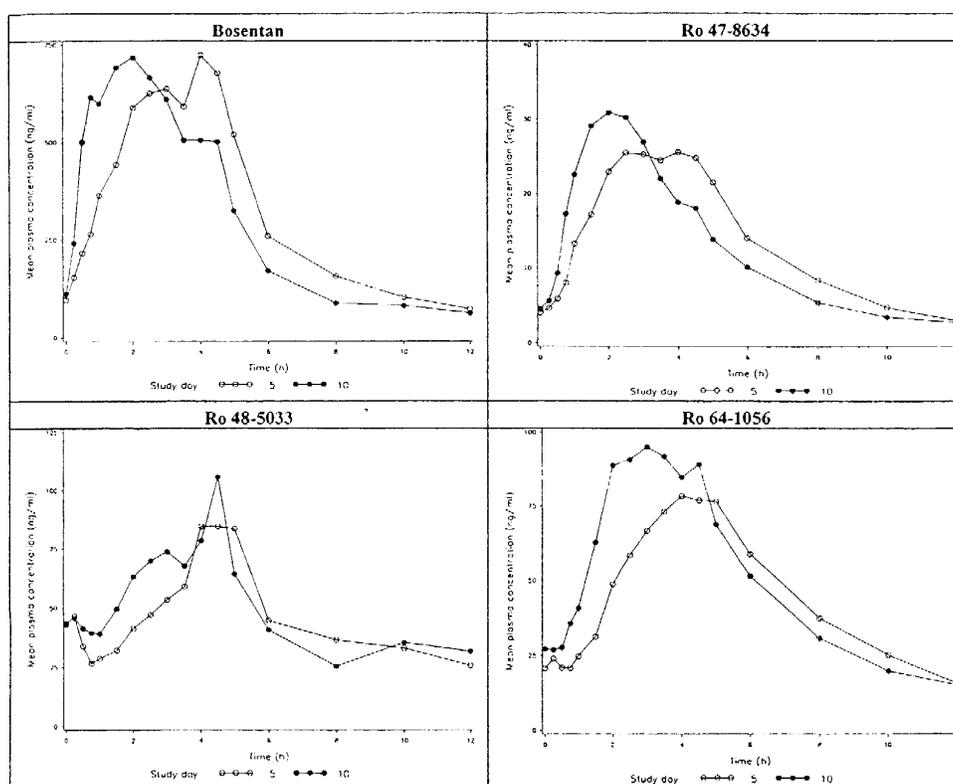
Table 2: Point estimate and 90% CI for PK parameters of bosentan and metabolites

Compound	Parameter	(Simvastatin+ Bosentan) vs. (Bosentan Alone)	
		Point Estimate	90% Conf. Interval
Bosentan	C _{max} (ng/ml)	0.99	0.82, 1.19
	AUC _t (ng.h/ml)	0.94	0.83, 1.06

Ro 47-8634	C _{max} (ng/ml) AUC _τ (ng.h/ml)	1.07 0.93	0.91, 1.26 0.87, 1.01
Ro 48-5033	C _{max} (ng/ml) AUC _τ (ng.h/ml)	1.12 1.05	0.89, 1.40 0.90, 1.22
Ro 64-1056	C _{max} (ng/ml) AUC _τ (ng.h/ml)	1.20 1.08	1.05, 1.38 0.98, 1.19

Concomitant administration of simvastatin did not affect the pharmacokinetic parameters of bosentan or its metabolites, except for T_{max}, which occurred earlier in the presence of simvastatin.

Figure 3: Mean plasma concentrations (ng/ml) on days 5 and 10 of bosentan, Ro 47-8634, Ro 48-5033 and Ro 64-1056: linear scale



EFFECT OF BOSENTAN ON SIMVASTATIN & β -HYDROXY SIMVASTATIN:

Steady-state pharmacokinetic parameters and 90% confidence intervals of the PK parameters of simvastatin and its metabolite, β -hydroxy simvastatin, obtained following administration of 40-mg QD simvastatin alone, in the presence of 125-mg BID bosentan are listed in the following table.

Table 3: Geometric Mean (SD) Pharmacokinetic Parameters of Simvastatin and metabolite

Compound	TREATMENT	C _{max} (ng/ml)	T _{max} (h)*	AUC _{0-T} (ng.h/ml)	MRT (h)*	Metabolite:Parent Ratio
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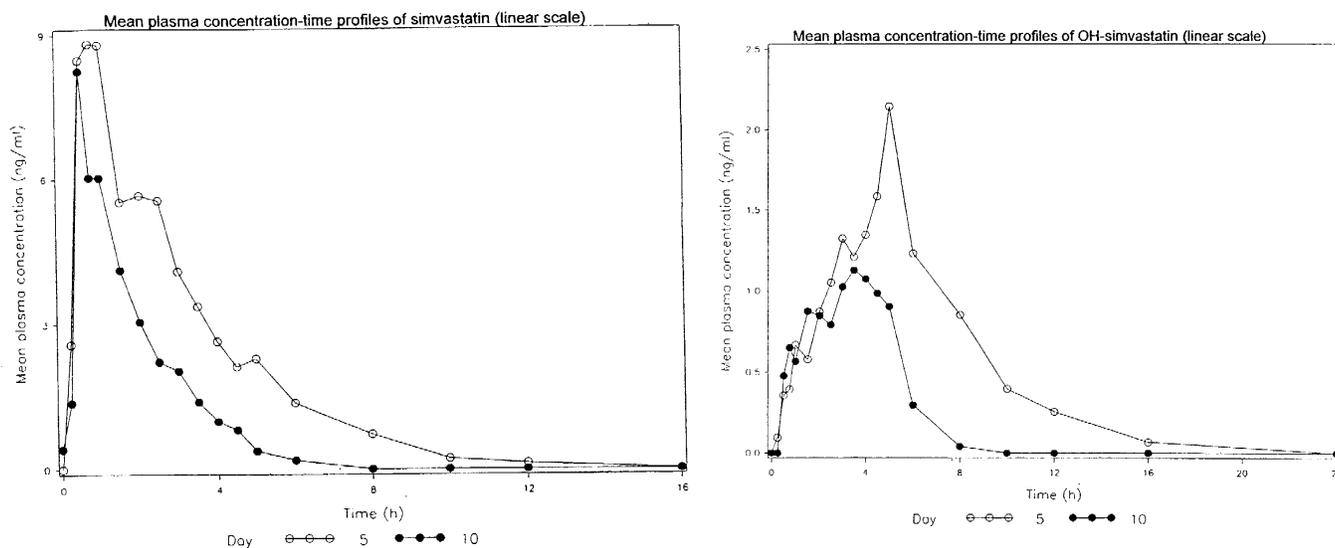
Simvastatin	Simvastatin Alone	12.6 (9.1)	1.17 (0.9)	25.6 (13.6)	2.78 (1.2)	
	Simvastatin + Bosentan	8.63 (8.4)	0.81 (0.4)	12.9 (5.7)	1.90 (0.6)	
β -hydroxy simvastatin	Simvastatin Alone	2.23 (1.6)	4.02 (1.7)	8.0 (11)	4.94 (1.8)	0.32
	Simvastatin + Bosentan	1.49 (1.1)	2.52 (1.9)	3.16 (6.3)	3.10 (1.0)	0.24

*arithmetic mean

Table 4: Point estimate & 90% CI for PK parameters of Simvastatin and Metabolite

Compound	Parameter	(Simvastatin+ Bosentan) vs. (Bosentan Alone)	
		Point Estimate	90% Conf. Interval
Simvastatin	C _{max} (ng/ml)	0.69	0.43, 1.10
	AUC _τ (ng.h/ml)	0.51	0.40, 0.63
β -hydroxy simvastatin	C _{max} (ng/ml)	0.67	0.46, 0.96
	AUC _τ (ng.h/ml)	0.40	0.19, 0.85

Coadministration of bosentan and simvastatin significantly decreased steady-state C_{max} and AUC of simvastatin on Day 10 by 31% and 49%, respectively. Steady-state C_{max} and AUC of the active metabolite, β -hydroxy simvastatin, decreased to a greater extent by 33% and 60%, respectively. The T_{max} of both simvastatin and β -hydroxy simvastatin occurred earlier in the presence of bosentan from 1.2 vs 0.8 h and from 4.0 vs. 2.5 h, 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. This indicates that the reduction in β -hydroxy simvastatin AUC in the presence of bosentan is probably not entirely due to reduced metabolite formation but might also be due to increased metabolism of β -hydroxy simvastatin.

SAFETY:

There were 12 subjects: 4 male and 8 female. There were no serious events and no drop outs. Reported adverse events included flu (1) and headache (2). There were a few minor changes in LFTs. One subject had a decrease in hemoglobin/hematocrit from 12.6/36.9 at baseline to 10.6/31.5 at study end.

CONCLUSIONS:

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.

COMMENTS:

1. The Agency recommends the sponsor to incorporate into the label information regarding the significant lowering of C_{max} and AUC of both simvastatin and its active metabolite, β -hydroxy simvastatin, in the presence of bosentan.

STUDY AC-052-103 – A STUDY ON THE POSSIBLE INTERACTION BETWEEN THE ENDOTHELIN RECEPTOR ANTAGONIST BOSENTAN AND THE ANTI-DIABETIC GLIBENCLAMIDE IN HEALTHY VOLUNTEERS

STUDY INVESTIGATORS AND SITES: R. Jovic, MD
C. H. Kleinbloesem, Ph.D.
VanTx Research AG
St. Jakobsstrasse 41-43
4132 Muttenz
Switzerland

Report No.: AC-052-103

Volume No.: 2.20

OBJECTIVES:

1. To assess the steady-state pharmacokinetics of glibenclamide, bosentan and its metabolites in healthy adult volunteers when either drug is administered alone or concomitantly.

FORMULATIONS:

Bosentan – 250 mg scored tablets (batch #: PT2227T68)

Glibenclamide – Semi-Daonil[®] 2.5 mg (Hoechst Marion Roussel, Batch #: 40 N646)

STUDY DESIGN:

This was an open-label, randomized, multiple dose, two-period cross-over study in 12 healthy non-smoking subjects of either gender (9 M/3 F) between the ages of 18 and 35 years (mean: 21 years). On Day 1 of Period I, volunteers were randomized to receive either **Treatment A:** 125-mg BID bosentan from Days 1-9 and a single dose on Day 10 + 2.5 mg BID glibenclamide from Days 6-9 and a single dose on Day 10, or, **Treatment B:** 2.5 mg BID glibenclamide on Days 1-9 and a single dose on Day 10 + 125-mg BID bosentan on Days 6-9 and single dose on Day 10. Following a 19-day washout interval all subjects received alternate treatment on Day 1 of Period II. Both bosentan and glibenclamide were administered with standardized meals.

ASSAY:

Compound		Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
	<i>Matri</i>							
	<i>x</i>							
Bosentan	Plasma	LC/MS/MS	5 - 1600	≥ 0.996	5.0	15.0 300 1200	3.75 2.46 3.13	-6.99 -0.79 -2.35
Ro 48-5033	Plasma	LC/MS/MS	2.5 - 400	≥ 0.996	2.5	7.5 75 300	4.14 6.16 4.65	-7.96 -2.34 -3.68
Ro 47-8634	Plasma	LC/MS/MS	1.25 -	≥ 0.996	1.25	3.75	4.56	-1.23

			200			37.5	2.03	+0.81
						150	3.03	+2.16
Ro 64-1056	Plasma	LC/MS/MS	2.5 - 400	≥ 0.996	2.5	7.5	7.28	-3.35
						75	6.70	-3.20
						300	6.59	-4.27
Glibenclamide	Plasma	LC/MS/MS	5.0 - 125	≥ 0.997	0.5	15	6.53	-5.02
						50	4.52	-0.22
						100	6.70	+1.22

Sample Collection:

Blood samples (5-ml) were collected for analysis of glibenclamide, bosentan and its metabolite concentrations on Days 5 and 10 of both treatment periods prior to dosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10 and 12 hours post-dose. Trough samples were also collected in both treatment periods prior to the morning dose on all of the treatment days.

RESULTS

EFFECT OF GLIBENCLAMIDE ON BOSENTAN:

Steady-state pharmacokinetic parameters and 90% confidence intervals of the PK parameters of bosentan and its metabolites obtained following administration of 125-mg BID bosentan alone, in the presence of 2.5-mg BID glibenclamide are listed in the following table.

Table 1: Geometric Mean (SD) Pharmacokinetic Parameters of Bosentan and metabolites

Compound	TREATMENT	C _{max} (ng/ml)	T _{max} (h)*	AUC _{0-T} (ng.h/ml)	MRT (h)*
Bosentan	Bosentan alone	754 (298)	2.8 (1.3)	3495 (1191)	4.32 (0.77)
	Bosentan + Glibenclamide	571 (258)	3.0 (0.8)	2475 (1084)	4.18 (0.57)
Ro 47-8634	Bosentan alone	26.9 (15.1)	3.1 (1.3)	132 (64.3)	4.61 (0.70)
	Bosentan + Glibenclamide	21.6 (13.1)	3.3 (0.8)	98.0 (63.8)	4.45 (0.57)
Ro 48-5033	Bosentan alone	70.8 (29.4)	4.7 (2.7)	428 (177)	5.57 (0.75)
	Bosentan + Glibenclamide	53.0 (21.7)	4.1 (1.1)	320 (131)	5.63 (0.46)
Ro 64-1056	Bosentan alone	75.0 (27.5)	4.2 (1.6)	382 (125)	5.20 (0.63)
	Bosentan + Glibenclamide	61.0 (27)	3.8 (0.7)	299 (136)	5.12 (0.44)

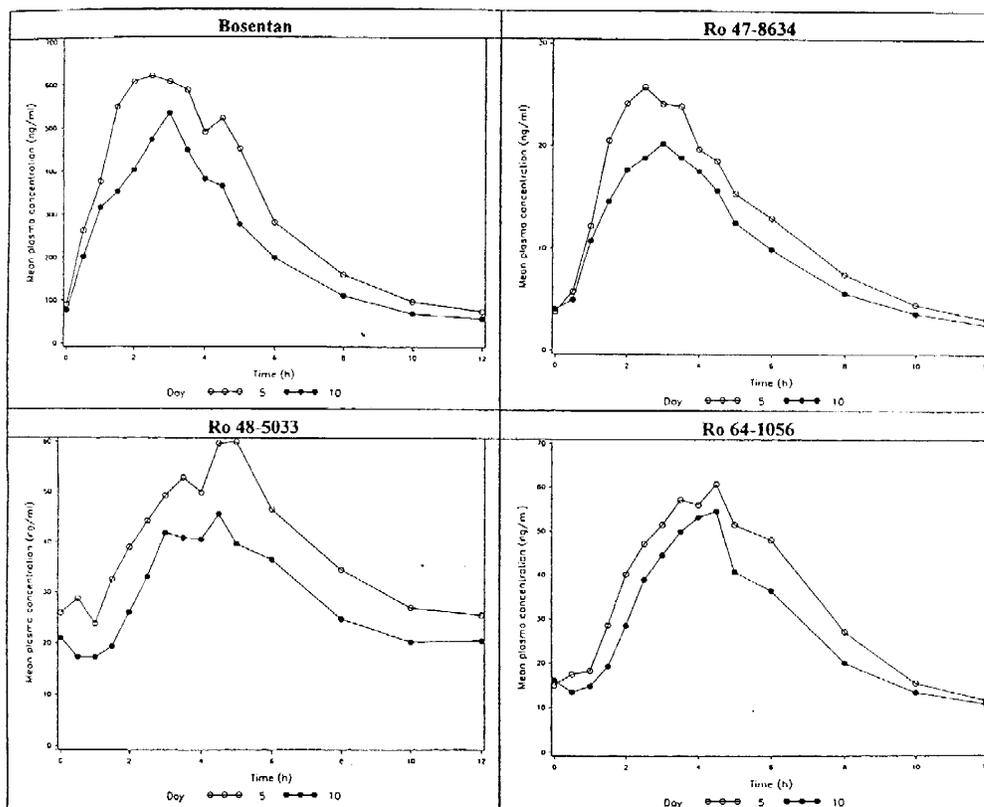
*arithmetic mean

Table 2: Point estimate and 90% CI for PK parameters of bosentan and metabolites

Compound	Parameter	(Glibenclamide+ Bosentan) vs. (Bosentan Alone)	
		Point Estimate	90% Conf. Interval
Bosentan	C _{max} (ng/ml)	0.76	0.63, 0.91
	AUC _τ (ng.h/ml)	0.71	0.60, 0.83
Ro 47-8634	C _{max} (ng/ml)	0.80	0.69, 0.93
	AUC _τ (ng h/ml)	0.74	0.64, 0.87
Ro 48-5033	C _{max} (ng/ml)	0.75	0.60, 0.94
	AUC _τ (ng h/ml)	0.75	0.63, 0.89

Ro 64-1056	C _{max} (ng/ml)	0.82	0.69, 0.97
	AUC _τ (ng h/ml)	0.78	0.67, 0.92

Figure 3: Mean plasma concentrations (ng/ml) on days 5 and 10 of bosentan, Ro 47-8634, Ro 48-5033 and Ro 64-1056: linear scale



Concomitant administration of glibenclamide significantly decreased concentrations of bosentan and its metabolites, the T_{max}, however, was unaffected. Glibenclamide decreased the C_{max} and AUC of bosentan by 24% and 29%, respectively. The decrease in bosentan concentrations were probably not due to increased metabolism, since, the concentrations of the metabolites of bosentan were also lower in the presence of glibenclamide. Glibenclamide decreased the C_{max} of Ro 47-8634, Ro 48-5033, Ro 64-1056 by 20%, 25% and 18%, respectively, and decreased AUC by 26%, 25% and 22%, respectively. Because of the decreased metabolite and parent concentrations, it is hypothesized that glibenclamide decreases the concentrations of bosentan and its metabolites by induction of p-glycoprotein transport.

EFFECT OF BOSENTAN ON GLIBENCLAMIDE

Steady-state pharmacokinetic parameters and 90% confidence intervals of the PK parameters of glibenclamide obtained following administration of 2.5-mg BID glibenclamide alone, in the presence of 125-mg BID bosentan are listed in the following table.

Table 3: Geometric Mean (SD) Pharmacokinetic Parameters of Glibenclamide

Compound	TREATMENT	C _{max} (ng/ml)	T _{max} (h)*	AUC _{0-T} (ng.h/ml)	MRT (h))*
Glibenclamide	Glibenclamide Alone	68.3 (22.9)	3.3 (1.5)	369 (122)	4.3 (0.88)
	Glibenclamide + Bosentan	53.3 (22.3)	3.0 (1.0)	223 (64)	3.8 (0.72)

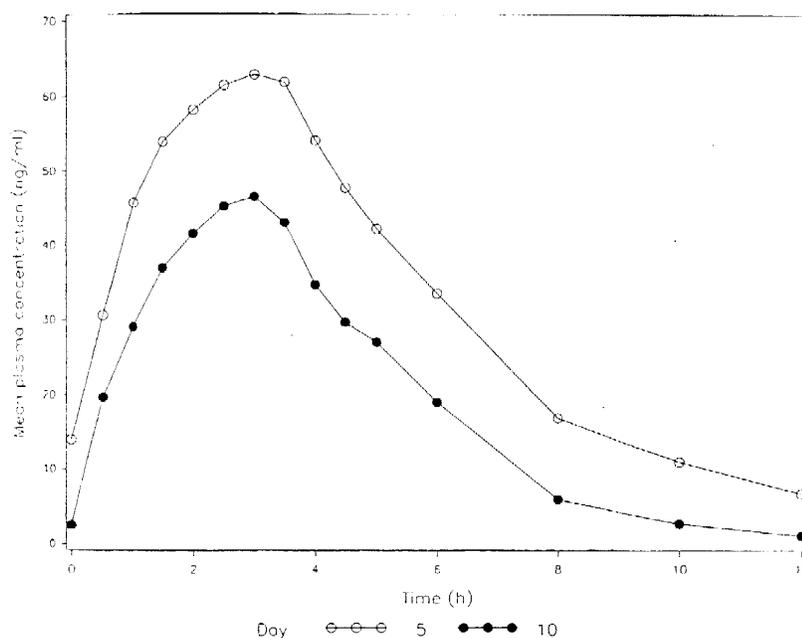
*arithmetic mean

Table 4: Point estimate & 90% CI for PK parameters of Glibenclamide

Compound	Parameter	(Glibenclamide+ Bosentan) vs. (Bosentan Alone)	
		Point Estimate	90% Conf. Interval
Glibenclamide	C _{max} (ng/ml)	0.78	0.66, 0.92
	AUC _τ (ng.h/ml)	0.60	0.56, 0.65

Coadministration of bosentan and glibenclamide significantly decreased steady-state C_{max} and AUC of glibenclamide on Day 10 by 22% and 40%, respectively. The reduction in glibenclamide AUC in the presence of bosentan could probably be attributable to induction of liver enzymes (2C9) or induction of p-glycoprotein transport systems.

Figure 1: Mean plasma concentrations (ng/ml) on days 5 and 10 of glibenclamide: linear scale



SAFETY:

There were 12 subjects. There were no reported deaths, serious adverse, or study withdrawals because of an adverse event. Four subjects reported adverse events: headache, headache and fatigue, abdominal pain and increased LFT, increased LFTs. Hematology values were obtained only at screening.

CONCLUSIONS:

Coadministration of bosentan and glibenclamide significantly decreased steady-state C_{max} and AUC of both bosentan and glibenclamide. Steady-state C_{max} 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.

Concomitant use of bosentan and glibenclamide could result in decreased effectiveness of glibenclamide at therapeutic doses. Alternative hypoglycemic agents should be considered, since, an increase in glyburide dose to offset decrease in hypoglycemic response could increase the risk of elevated liver enzymes.

COMMENTS:

1. The Agency recommends the sponsor to incorporate into the label information regarding the significant lowering of C_{max} and AUC of both glibenclamide and bosentan when administered concomitantly.
2. Bosentan induced decrease in glibenclamide concentrations could result in reduced hypoglycemic response at therapeutic doses. This effect may be observed with other sulfonylurea hypoglycemic agents which are also metabolized by CYP2C9.

STUDY B-162290 – A PILOT STUDY ON THE SAFETY AND TOLERABILITY OF Ro 47-0203 IN PATIENTS RECEIVING NIMODIPINE AFTER SURGICAL CLIPPING FOR ANEURYSMAL SUBARACHNOID HEMORRHAGE

STUDY INVESTIGATORS AND SITES: Multi-center Study

Report No.: B-162290

Volume No.: 2.49

OBJECTIVES:

1. To monitor the plasma concentrations of bosentan and nimodipine to investigate possible pharmacokinetic interactions.

FORMULATIONS:

Bosentan – Lyophilisate for intravenous injection (Batch #: GSU 0040)

Nimodipine – Source not provided

STUDY DESIGN:

This was an open-label, multi-center study in 6 aneurysmal subarachnoid hemorrhage (SAH) patients. There were 2 males and 4 females whose mean age was 56 years. All patients received an intravenous infusion of nimodipine from the time of admission which was titrated to a maximum of 33 µg/kg/h. Patients underwent surgical clippings of the aneurysm. On Study Day 1, ≤10 days after the SAH event, patients received a single intravenous dose of bosentan 500 mg, infused over 30 minutes.

ASSAY:

Plasma concentrations of bosentan were measured at Roche, Basel, Switzerland. Plasma concentrations of nimodipine were measured at ANAWA Laboratories AG, Wangen, Switzerland.

Compound		Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
	<i>Matri</i>							
	<i>x</i>							
Bosentan	Plasma	HPLC/UV	50 - 12500	NP	50	50 100 500 1900 3790	11.0 13.3 3.9 4.8 6.7	+0.0 +2.2 +1.5 +4.0 -1.3
Bosentan	Plasma	LC/MS/MS	NP	NP	0.5	2.467 78.24	5.50 5.61	+2.23 +1.49
Nimodipine	Plasma	GC/ECD	1.25 - 200	≥ 0.996	1.0	4.19 10.5	3.0 2.5	+4.8 +4.7

10.3 3.5 +3.3
20.6 5.6 +2.8

NP=not provided

Sample Collection:

Blood samples (5-ml) were collected for analysis of bosentan concentrations predose and at 15, 30, 40 min and 1, 2, 3, 4, 6, 8, 10, 12 and 24 hours after start of the infusion.

Blood samples (5-ml) were also collected for measurement of nimodipine concentrations at 30 min and 4, 12 and 24 hours after start of bosentan infusion.

RESULTS

EFFECT OF NIMODIPINE ON BOSENTAN:

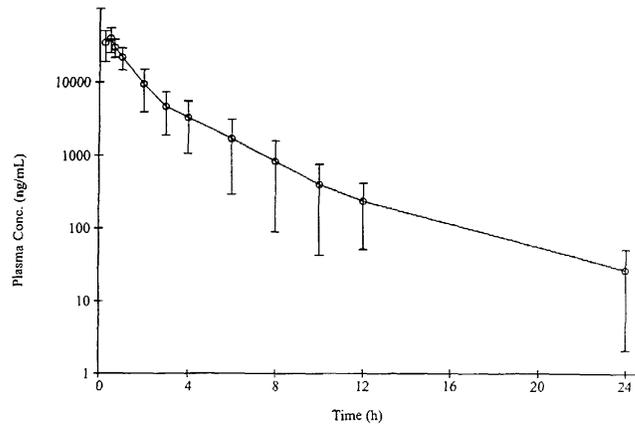
The pharmacokinetic parameters of bosentan obtained following administration of 500-mg bosentan infusion in the presence of nimodipine are listed in the following table.

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

Total Dose	T _{0.5} (h)	AUC (ng.h/ml)	CL (L/h)	V _{ss} (L)
500 mg	3.4 (16)	61429 (47)	9.5 (39)	17.5 (21)

The pharmacokinetic parameters of bosentan in the presence of steady-state nimodipine in SAH patients were similar to those seen in healthy volunteers. However, the pharmacokinetics of bosentan in SAH patients in the absence of nimodipine is not known.

Figure 8. Mean (± SD) Plasma Concentration Time Profiles of Bosentan Following a Single I.V. Dose of 500 mg on Top of Nimodipine to Patients with SAH



Individual patient data on bosentan plasma concentrations and their actual sampling times are shown in Appendix 6. Individual and mean bosentan plasma concentrations are shown in Appendix 7.

EFFECT OF BOSENTAN ON NIMODIPINE

Except for 1 patient (#5), plasma concentrations of nimodipine were in the range of 15.7 and 42.6 ng/ml. Patient # 5 showed a 10-fold (before bosentan) and a 30-fold (24-h after bosentan) higher plasma concentrations. Nimodipine concentrations in Patient # 5 was 296 ng/ml (pre-bosentan) which increased to 938 ng/ml 24 hours after start of bosentan infusion. In all other patients nimodipine levels were not different before and after bosentan treatment.

CONCLUSIONS:

The pharmacokinetic parameters of bosentan in SAH 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 SAH patients.

COMMENTS:

1. This was an open-label study with no placebo-arm in 6 patients only. The pharmacokinetics of bosentan in the absence of nimodipine in SAH patients is not known.
2. Upon multiple dosing of bosentan, nimodipine concentrations could decrease because of enzyme induction.

**COMPARISON OF CLINICAL AND COMMERCIAL (TO BE MARKETED)
FORMULATIONS**

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

DISSOLUTION METHOD DEVELOPMENT:

Bosentan is sparingly soluble in water and its solubility is pH dependent. Bosentan exhibits enhanced solubility at pH values greater than 8.5. During the development of bosentan the sponsor had used a dissolution medium of phosphate buffer pH 8.5 for dissolution testing. Since, the FDA Guidance to Industry “Dissolution Testing of Immediate Release Solid Oral Dosage Forms” states that the pH of the dissolution medium should be between 1 and 8, the sponsor has developed a dissolution medium with surfactant sodium lauryl sulfate to aid dissolution.

SELECTION OF DISSOLUTION MEDIUM:

The following table lists mean % bosentan dissolved from 125-mg tablets in different media.

Time (min)	% Release in Different Dissolution Media				
	Phosphate Buffer (pH 7.5)	Phosphate Buffer (pH 7.7)	Phosphate Buffer (pH 7.9)	1% Sodium Lauryl Sulfate	1% Sodium Lauryl Sulfate (pH 7.5)
5	24.4	27.9	47.2	37.7	43.1
10	41.4	51.5	68.3	68.7	73.0
20	57.6	72.6	85.9	94.0	96.2
30	68.8	84.1	94.0	100.4	102.0
45	78.3	93.1	98.7	103.0	103.9
60	85.7	97.8	101.2	103.4	104.7

Addition of 1% sodium lauryl sulfate greatly enhanced dissolution of bosentan in water (\approx pH 6.6) or water adjusted to pH 7.5.

SELECTION OF SURFACTANT CONCENTRATION:

The effect of different concentrations of sodium lauryl sulfate on mean % dissolved from 125-mg tablets of bosentan is presented in the following table.

Time (min)	% Release in Different Dissolution Media		
	0.5% Sodium Lauryl Sulfate	1% Sodium Lauryl Sulfate	1.5% Sodium Lauryl Sulfate
5	36.1	36.8	37.0
10	63.2	71.0	70.1
20	86.4	91.6	94.8
30	96.1	99.1	100.9
45	101.5	102.1	103.0
60	102.9	103.0	103.6

Based on the above data, the sponsor selected 1% sodium lauryl sulfate for dissolution. In the opinion of the biopharmaceutics reviewer, the lowest concentration of 0.5% sodium lauryl sulfate aided dissolution to a similar degree as the higher concentrations of surfactant.

SELECTION OF APPARATUS AND SPEED:

The effect of apparatus type (paddle vs. basket) and rotational speed on mean % bosentan dissolved from 125-mg tablets in 1% sodium lauryl sulfate is presented in the following table.

Time (min)	DISSOLUTION APPARATUS AND SPEED			
	Paddle at 50 rpm	Paddle at 75 rpm	Basket at 50 rpm	Basket at 75 rpm
5	36.8	38.2	16.4	28.8
10	71.0	73.0	39.0	55.0
20	91.6	95.7	55.3	72.9
30	99.1	100.5	60.8	81.0
45	102.1	103.4	63.9	88.6
60	103.0	102.0	64.4	91.9

The paddle apparatus yielded greater and complete dissolution compared to basket apparatus. Also, similar dissolution was observed at paddle speeds of 50 rpm and 100 rpm. Therefore, the sponsor selected USP Apparatus 2 (paddle) at a speed of 50 rpm for dissolution testing.

SPONSOR PROPOSED DISSOLUTION METHOD:

Based on the above data, the sponsor proposed dissolution method and specifications is listed below:

Dosage Form:	Tablet
Strengths:	62.5 and 125 mg
Apparatus Type:	USP Apparatus 2 (paddle)
Media:	Water with 1% Sodium Lauryl Sulfate at 37 ⁰ C
Volume:	900 ml
Speed of Rotation:	50 rpm
Brief Description of Dissolution Analytical Method:	HPLC with UV detection at 272 nm
Sampling Times	5, 10, 15, 30, 45, 60 min
Proposed Dissolution Specification	Q=70% not less than 30 min

Dissolution greater than 90% is obtained in 20 minutes in 1% sodium lauryl sulfate dissolution medium. Therefore, the sponsor proposed dissolution specification of Q not less than 70% dissolution in 30 min is considered less stringent.

The biopharmaceutics reviewer proposes the following dissolution method, medium and specification; dissolution not less than 80% (Q) dissolved in 30 min in 0.5 % sodium lauryl sulfate in water.

RECOMMENDATION:

The Office of Clinical Pharmacology and Biopharmaceutics finds the sponsor proposed dissolution medium not acceptable for the following reasons.

1. The proposed concentration of sodium lauryl sulfate is considered to be high. Similar dissolution performance is obtained with 0.5% sodium lauryl sulfate in water.
2. The biopharmaceutics reviewer proposes the following dissolution method, medium and specification; dissolution not less than 80% (Q) dissolved in 30 min in 0.5% sodium lauryl sulfate in water using USP Apparatus II (paddle) at a speed of 50 rpm.