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:

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 .

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

				NP NP NP		
Bosentan	Plasma		NP	NP NP		

NP=Not Provided

[ ] was used for analysis of plasma bosentan concentrations below ( ) ng/ml and 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

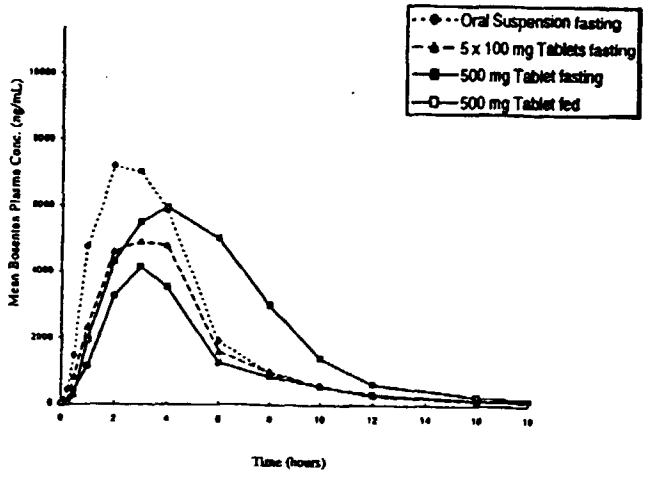
The pharmacokinetic parameters of bosentan obtained from the 4 treatments are listed in the following table.

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



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

A significant food effect was observed on 500 mg tablets of bosentan. Mean Cmax and AUC of bosentan when administered with food increased by 100%. The Tmax was increased by 1 hour. The Cmax obtained with the 500-mg tablet with food was comparable to the Cmax obtained with oral suspension in the fasted state, but the Tmax 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 Cmax). 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 Cmax 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 Cmax 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 Cmax 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 Cmax 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 Cmax and AUC (
   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 – 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:

Report No.:
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

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

	x		
Bosentan	Plasma	1	NP
Ro 48-5033	Plasma		NP
Ro 47-8634	Plasma		NP
Ro 64-1056	Plasma		NP

NP=Not Provided

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 Cmax 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 Cmax 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 Cmax 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 Cmax 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 Cmax 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 Cmax 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

	Cmax		AUC	
Treatment	**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

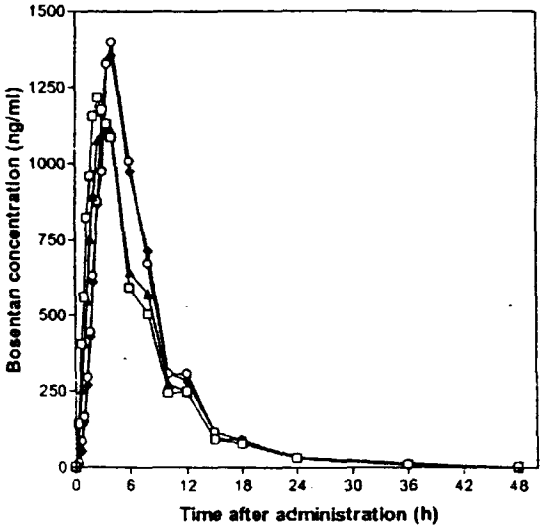
The 125 mg tablets of bosentan were bioequivalent to the suspension with regard to both Cmax and AUC. Cmax and AUC from the 125-mg oral suspension were lower by 37% and 15%,

respectively, (after dose normalization) compared to the 500-mg oral suspension used in Study #

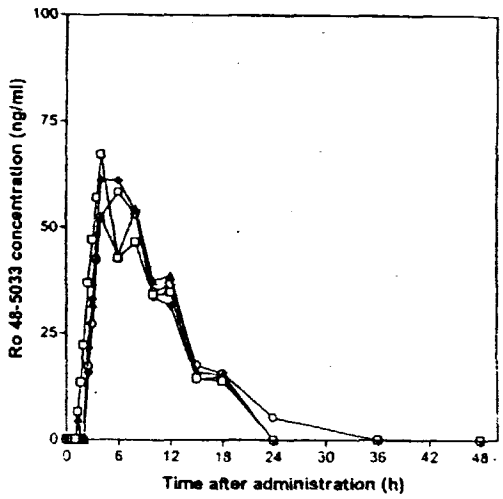
Two 62.5-mg tablets of bosentan were bioequivalent to the 125 mg tablet with regard to AUC but the lower limit of the 90% confidence interval for Cmax was slightly below the 0.8 lower limit for bioequivalence.

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

Mean plasma profiles of bosentan after administration of 125 mg as oral suspension in the fasted state (□); after administration of 1 x 125 mg tablet in the fasted state (▲); after administration of 1 x 125 mg tablet with food (○); after administration of 2 x 62.5 mg tablets with food (◆) (linear scale)



Mean plasma profiles of Ro 48-5033 after administration of 125 mg bosentan as oral suspension in the fasted state (□); after administration of 1 x 125 mg tablet in the fasted state (▲); after administration of 1 x 125 mg tablet with food (○); after administration of 2 x 62.5 mg tablets with food (◆) (linear scale)



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 Cmax and AUC of bosentan obtained following administration of 125 mg tablet was bioequivalent to the tablet in the fasted state. Mean Cmax and AUC were approximately 1300 ng/ml and 7900 ng.h/ml, respectively. Food increased mean Cmax 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 Cmax and AUC of Ro 48-5033 (active metabolite) and Ro 64-1056 were not affected by food.

COMMENTS:

1. In a previous study the early tablet formulation exhibited 100% increase in Cmax 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 Cmax and AUC significantly. The biopharmaceutics reviewer concurs with the sponsor that bosentan can be administered with or without food.
2. Cmax 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 . 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 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:

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

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

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)	Vss (L)
500 mg	15310 (44)	35510 (47)	58250 (38)	3.8 (26)	171710 (74)	3.8 (48)	21.0 (42)

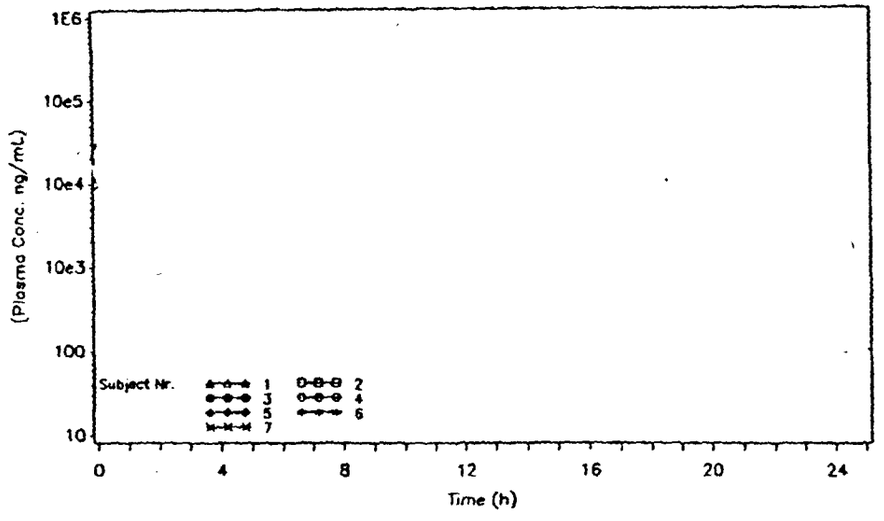
$C_{max,1}$, $C_{max,2}$ and $C_{max,3}$ represents the maximum concentrations observed following administration of 50 mg, 150 mg and 300 mg doses. Although, substantial residual concentrations remained in the body prior to administration of the next dose, the increase in C_{max} with increasing doses 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 µg/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 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 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.

Figure 6. Individual (+/- SD) Plasma Concentration Time Profiles of Bosentan Following i.v. Infusion of 500 mg (Steps of 50, 150 and 300 mg) to Patients with Pulmonary Hypertension



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.

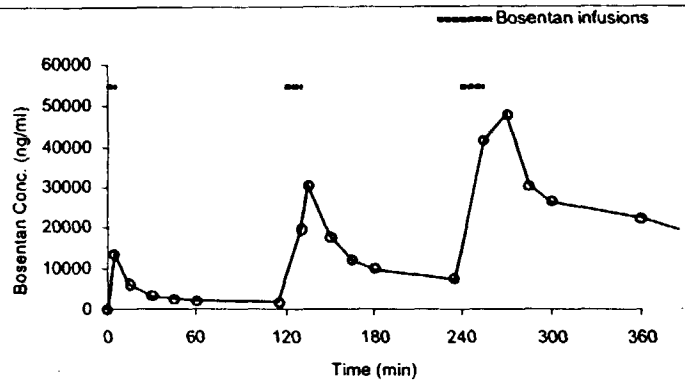
Only 1 patient (Patient #2) received 1000 mg oral bosentan twice daily for 8 weeks.

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.

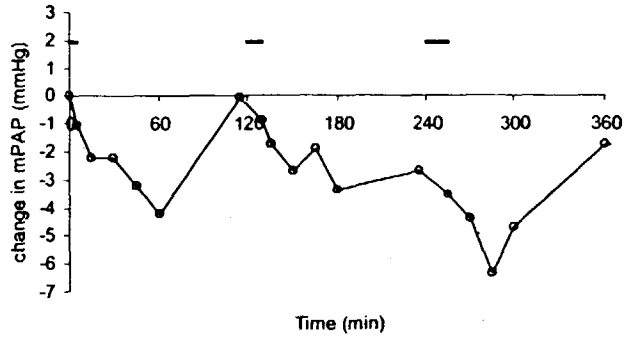
During infusion of bosentan at the highest concentration (300 mg/15 min), mean (± SEM) reduction in mean PAP was 6.5 ± 4.5 mmHg and mean reduction in TPVR was 23.7 ± 5.9%. These reductions lag slightly behind plasma bosentan concentrations.

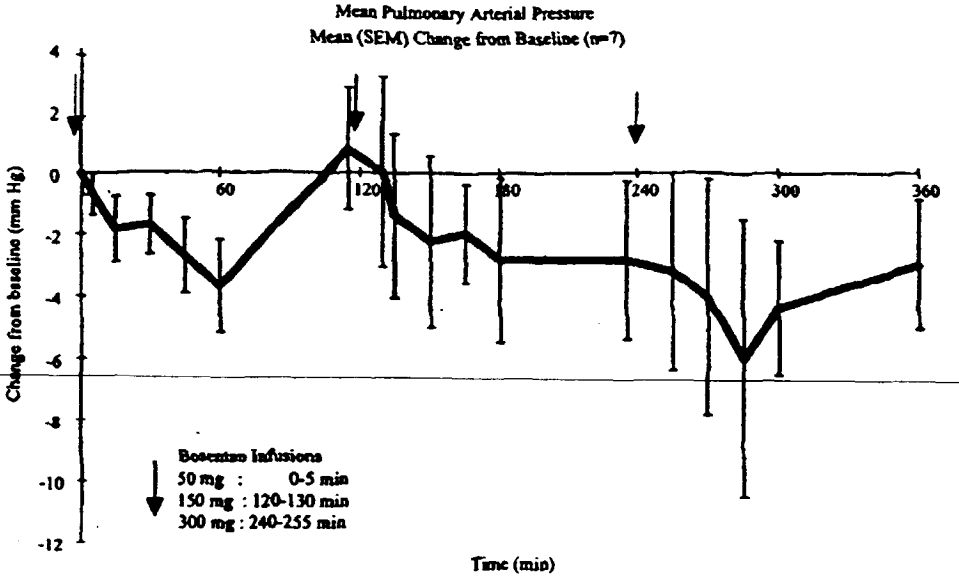
Mean bosentan plasma concentrations versus time over 6 h



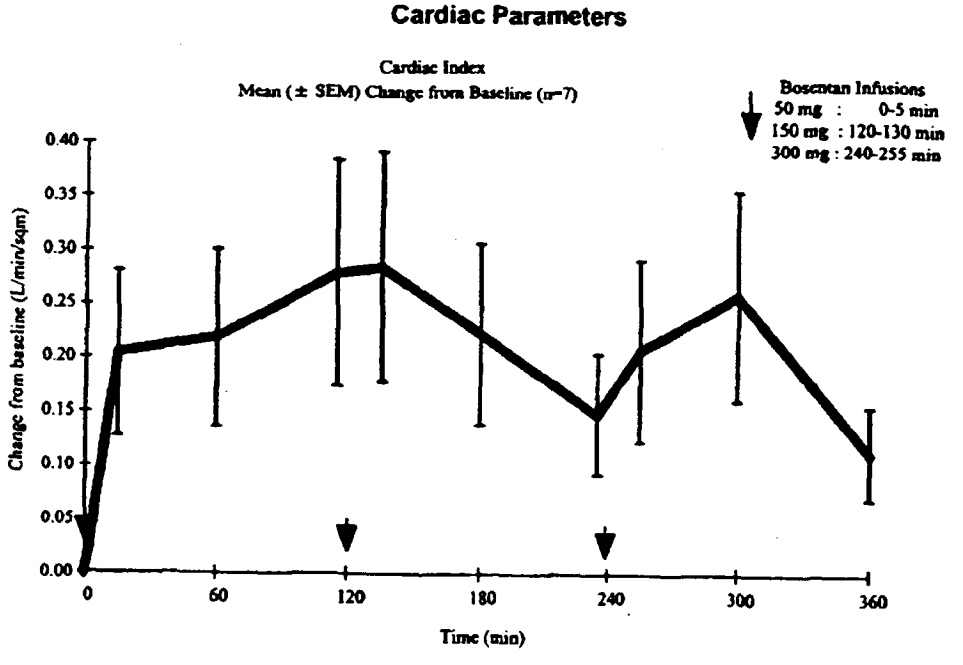
Mean PAP:

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

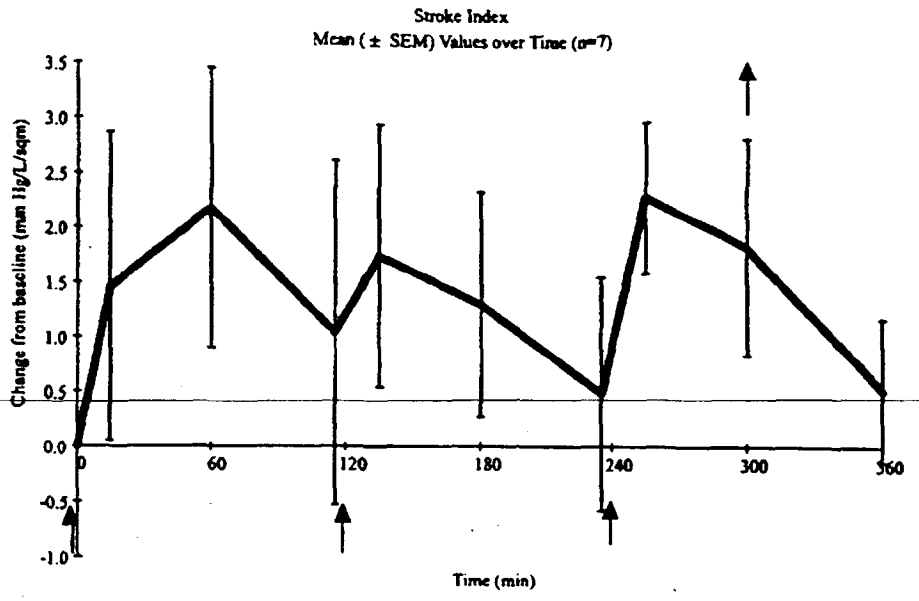




Mean cardiac index:



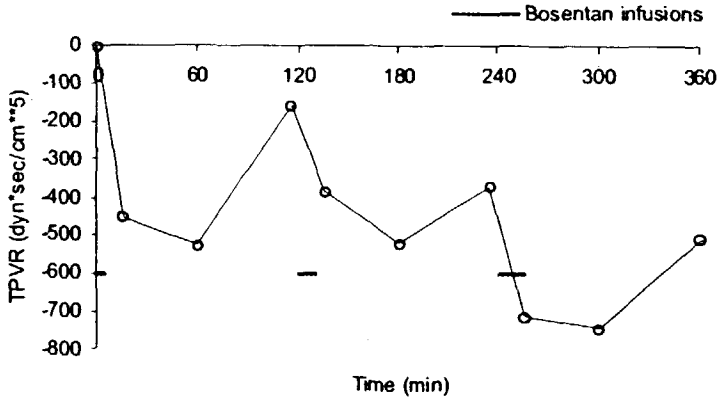
Mean stroke index:



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;

	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 Cmax 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 Cmax 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 SUBJECS WITH SEVERE RENAL DYSFUNCTION COMPARED TO SUBJECTS WITH NORMAL RENAL FUNCTION

STUDY INVESTIGATORS AND SITES:

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	Matrix	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
Bosentan	Plasma							
Ro 48-5033	Plasma							
Ro 47-8634	Plasma							

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)	$AUC_{0-\infty}$ (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

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

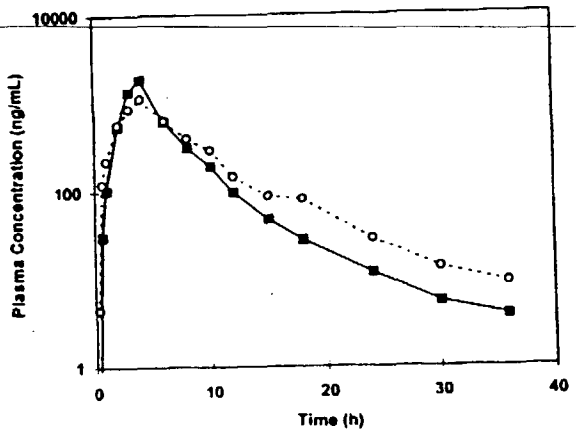
	AUCτ (ng.h/ml)	2.09	NP

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 Tmax 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.

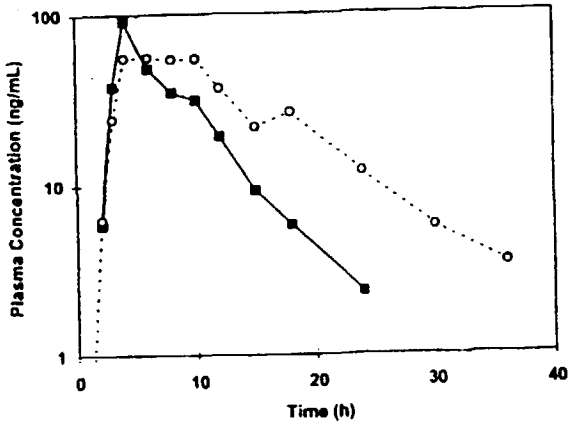
Mean plasma concentration versus time curves of bosentan (Ro 47-0203) after single oral administration of 125 mg bosentan in healthy subjects and in patients with impaired renal function (semi-logarithmic scale)



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 Tmax 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)

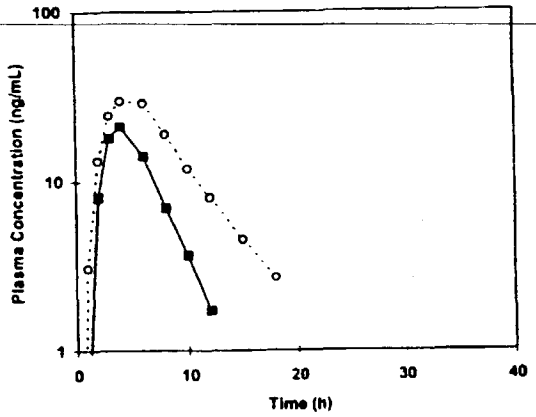


severe renal impairment patients and healthy subjects was <10% of parent drug concentrations. The increase in Ro 48-5033, active metabolite, concentrations in severe renal impairment patients was lower

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.

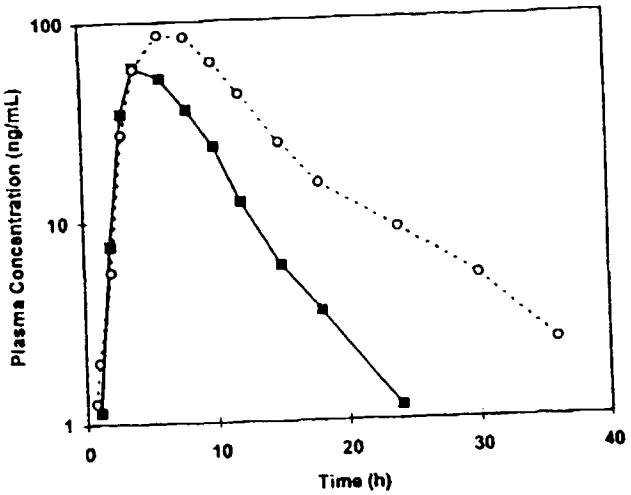
Ro 47-8634:
Mean C_{max} and AUC were higher by 38% and 100%, respectively, in renal impairment patients compared to healthy subjects. Median Tmax occurred 1 h later and median $T_{1/2}$ was approximately 2 h longer in severe renal impairment patients compared to healthy subjects.

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 Tmax 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 drawn 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 Tmax 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:

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 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			NP				
Cyclosporin A	Plasma			NP				

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:

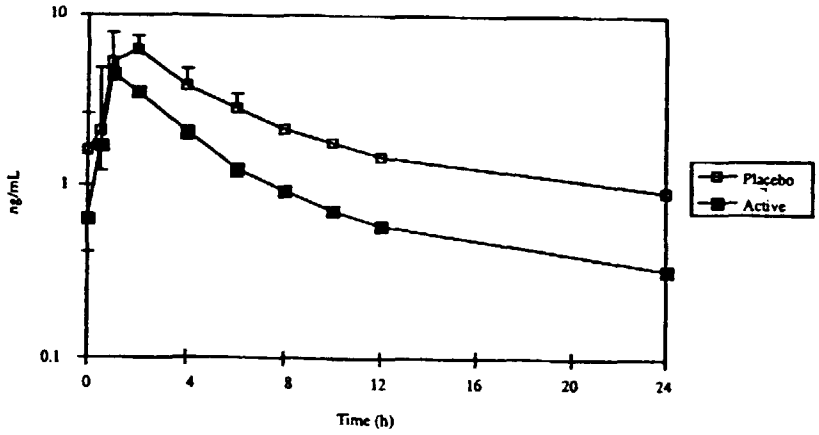
The pharmacokinetic parameters of cyclosporin A in the presence of placebo and bosentan are listed in the following table.

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

Parameter	CSA + Placebo	CSA + Bosentan
Tmax (h)#	1.7 (30)	1.4 (38)
Cmax (ng/ml)	1362 (24)	1512 (13)
$AUC_{0\text{-}12}$ (ng.h/ml)	7819 (31)	6520 (20)
AUC/Dose (ng.h/ml)	38.1 (34)	20.5 (25)
CLo (L/h)	29 (33)	51 (24)
$T_{1/2}$ (h)	15.8 (26)	14.0 (24)
Ctrough/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 Cmax, Ctrough 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
Cmax (ng/ml)	4743 (49)	7916 (54)
Tmax (h)	2.9 (41)	4.3 (50)
AUC_{0-12} (ng.h/ml)	24780 (54)	48900 (49)
CLo (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= % to % for C_{max} and, range= % to % 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 , 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 Cmax 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 Cmax and AUC in the absence of CsA.

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

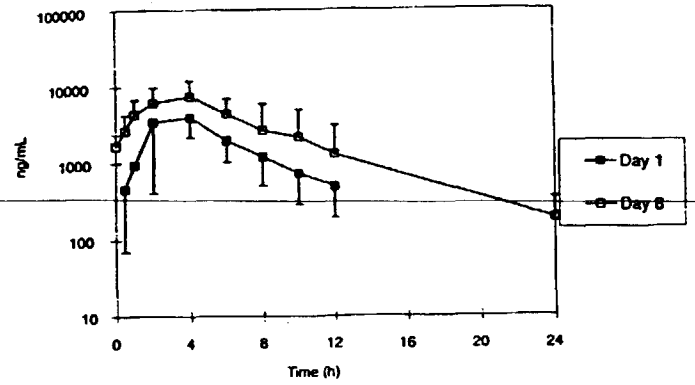
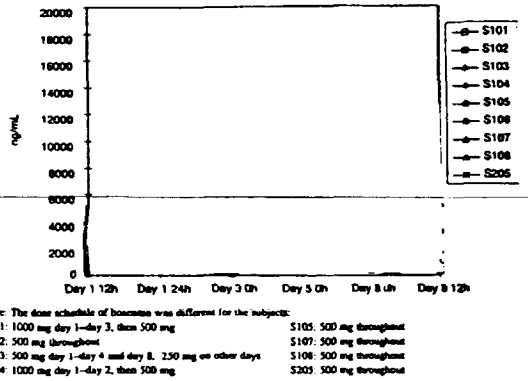


Figure 8. Individual Trough Plasma Concentration Time Profiles of Bosentan Following Multiple Oral Doses of 250-1000 mg BID (corrected for 500 mg doses)



Note: The dose schedule of bosentan was different for the subjects:
S101: 1000 mg day 1–day 3, then 500 mg
S102: 500 mg throughout
S103: 500 mg day 1–day 4 and day 8, 250 mg on other days
S104: 1000 mg day 1–day 2, then 500 mg
S105: 500 mg throughout
S107: 500 mg throughout
S108: 500 mg throughout
S205: 500 mg throughout

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, Cmax 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 Cmax 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 Cmax and AUC in the absence of CsA. This is because bosentan concentrations decline upon multiple dosing to 50% of their single dose concentrations because of enzyme auto-induction.

Bosentan decreased cyclosporin steady-state Cmax, AUC and trough concentration values by 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 ( ), Day 8 Cmax 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 Cmax 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.

APPEARS THIS WAY
ON ORIGINAL

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

STUDY INVESTIGATORS AND SITES:

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

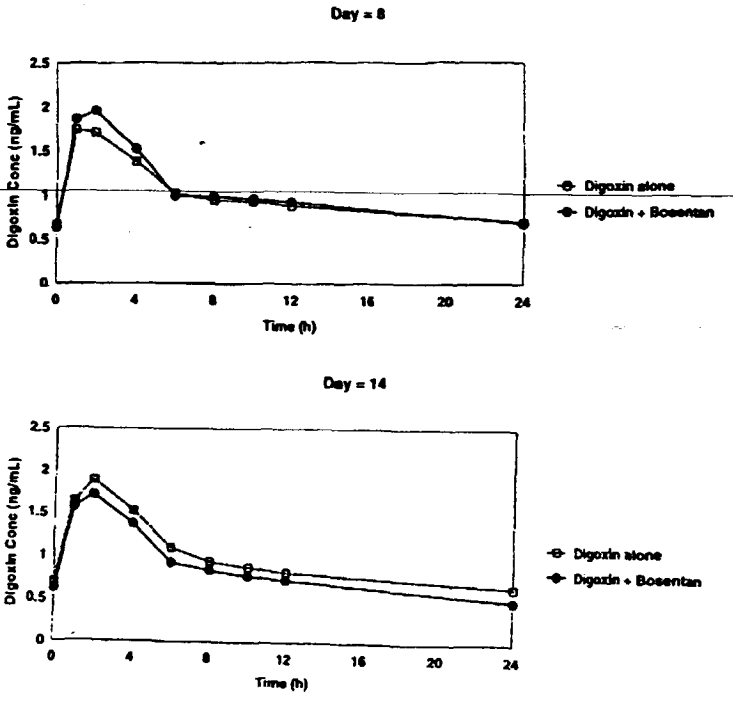
Parameter	Treatment Day	Digoxin + Bosentan (TEST)	Digoxin Alone (REF)	Point Estimate	90% Confidence Interval
		Arithmetic means		Ratio of test/reference means	
Cmax (ng/ml)	Day 8	2.29 (0.62)	2.01 (0.56)	114	99 - 131
	Day14	2.00 (0.61)	2.12 (0.31)	**91**	**80 - 104**
AUC_{0-24} (ng.h/ml)	Day 8	24.5 (5.7)	23.3 (6.0)	106	97 - 115
	Day14	20.2 (4.66)	23.1 (5.94)	**88**	**79 - 98**
Cmin (ng/ml)	Day 8	0.68 (0.26)	0.69 (0.27)		
	Day14	0.50 (0.21)	0.65 (0.26)		
Tmax (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

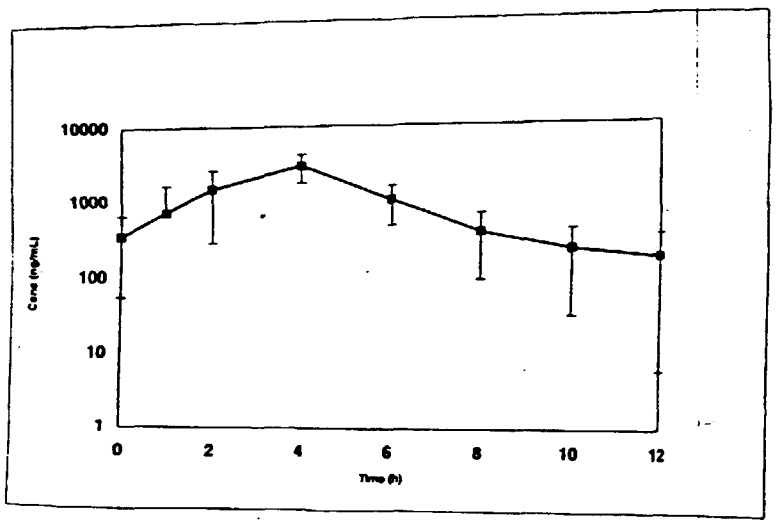
Parameter	Treatment Day	Present Study 500-mg BID	Another 500-mg QD Study (B-159037)
Cmax (ng/ml)	Day 14	3260 (1040)	3491
AUC_{0-12} (ng.h/ml)	Day 14	12600 (3630)	15030
$T_{1/2}$ (h)	Day 14	4.5 (4.5)	7.1
Ctrough (ng/ml)	Day 8	991 (1150)	-
Ctrough (ng/ml)	Day 14	177 (171)	-

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 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 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 ng/ml on Day 8 to 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 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:

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	Matrix	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
Bosentan	Plasma			NP				
R-Warfarin	Plasma			NP				
S-Warfarin	Plasma			NP				

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.

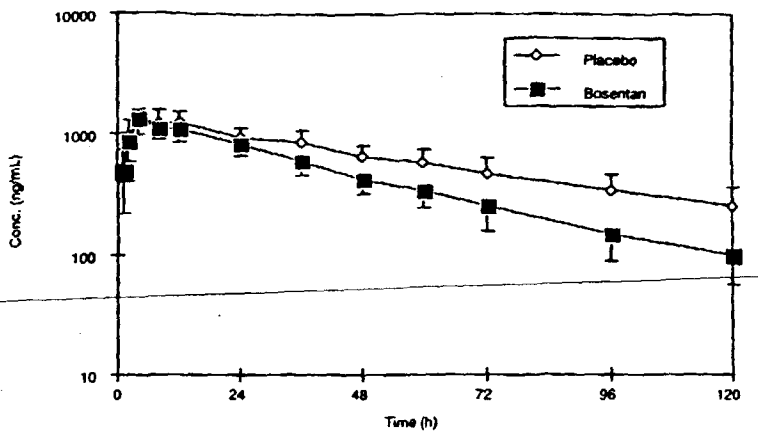
Table 1: Mean (SD) Pharmacokinetic Parameters of Warfarin with Bosentan and Placebo

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
$AUC_{0-\infty}$ (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
$AUC_{0-\infty}$ (ng.h/ml)	44600 (11600)	63100 (19600)	71	66 - 77
CL/F (L/h)	0.63 (0.18)	0.45 (0.14)		

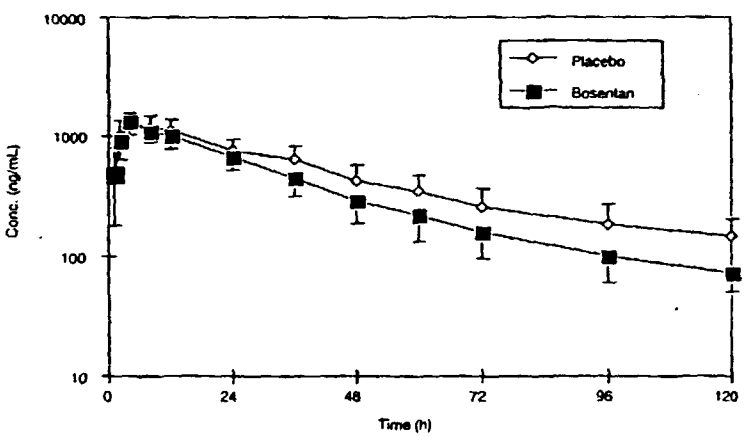
Concomitant administration of multiple doses of 500-mg BID bosentan increased the elimination of R- and S-warfarin following a single dose of 26-mg warfarin. The CL/F of R-warfarin and S-warfarin increased by 59% and 40%, respectively, and the half-life of R-warfarin and S-warfarin decreased by 37% and 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

$AUC_{VIII,cor}$ (%.h)	4549 (1490)	6315 (1648)	70	59 - 83

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 Cmax 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

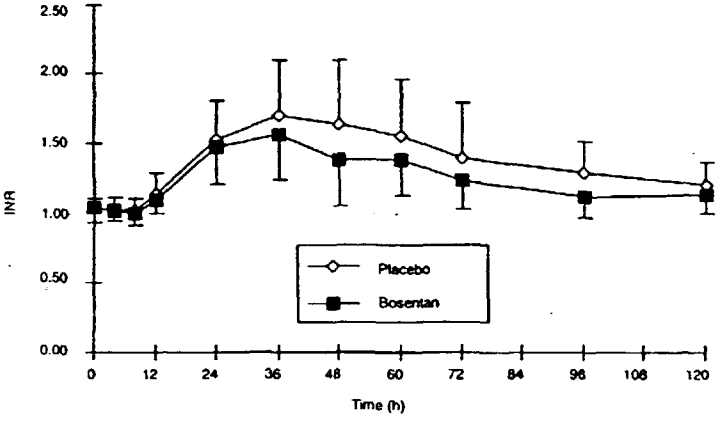
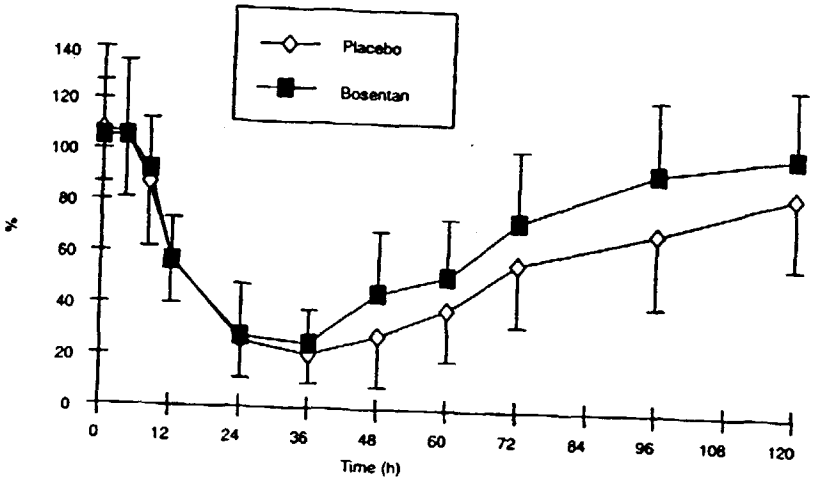


Figure 2. Mean (SD) Factor VII Activity versus Time Plot Following a Single Oral Dose of 26 mg Warfarin during Placebo or Bosentan Treatment with 500 mg BID



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:

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

Ro 48-5033	Plasma	NP	NP
Ro 47-8634	Plasma	NP	NP
Ro 64-1056	Plasma	NP	NP

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
Cmax (ng/ml)	996 (448)	1610 (838)	857 (350)
Tmax* (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

		(Ketoconazole + Bosentan) vs. (Bosentan Alone)		(Losartan + Bosentan) vs. (Bosentan Alone)	
Compound	Parameter	Point Estimate	90% Conf. Interval	Point Estimate	90% Conf. Interval
Bosentan	Cmax (ng/ml)	1.62	1.34, 1.94	0.86	0.72, 1.04
	AUCτ (ng.h/ml)	1.83	1.52, 2.21	0.85	0.71, 1.03
Ro 47-8634	Cmax (ng/ml)	0.66	0.51, 0.87	1.02	0.78, 1.34
	AUCτ (ng.h/ml)	0.88	0.69, 1.11	0.83	0.66, 1.06
Ro 48-5033	Cmax (ng/ml)	1.39	1.04, 1.85	0.94	0.71, 1.26
	AUCτ (ng.h/ml)	1.31	0.95, 1.81	0.85	0.62, 1.17
Ro 64-1056	Cmax (ng/ml)	0.70	0.49, 0.99	0.76	0.53, 1.08
	AUCτ (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 Cmax and AUC of bosentan by 62% and 83%, respectively. The T_{max} of bosentan, however, remained unchanged in the presence of ketoconazole.

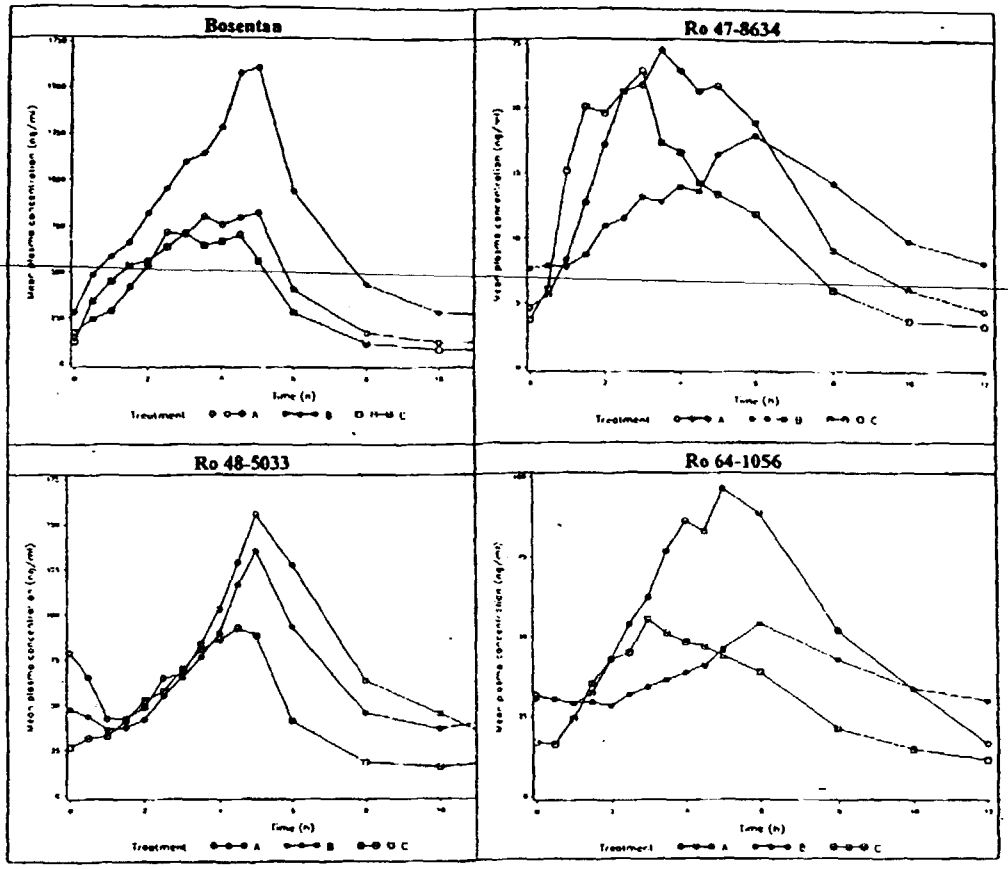
The increase in bosentan Cmax and AUC in the presence of ketoconazole can be attributed to inhibition of bosentan metabolism via CYP3A4. This was evident from the decreased concentration of metabolites of bosentan, except Ro 47-5033, in the presence of ketoconazole. The Cmax and AUC of the active metabolite, Ro 47-8634, were lower by 33% and 12%, respectively, in the presence of ketoconazole. A similar reduction in Cmax 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 Cmax and AUC of Ro 48-5033 were 39% and 31% higher.

The sponsor did not measure bosentan concentrations after coadministration of the Ist 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 Ist dose of cyclosporin. A similar magnitude of increase in bosentan concentrations is expected after coadministration of the Ist dose of ketoconazole.

EFFECT OF LOSARTAN ON BOSENTAN AND ITS METABOLITES:

Concomitant administration of 100-mg QD losartan lowered both Cmax and AUC of bosentan by 15%. Losartan decreased the Cmax 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 Cmax 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 Cmax and AUC of bosentan by 62% and 83%, respectively. The increase in bosentan Cmax and AUC in the presence of ketoconazole can be attributed to inhibition of bosentan metabolism via CYP3A4. This was evident from the decreased concentration of metabolites of bosentan, except Ro 47-5033, in the presence of ketoconazole. The Cmax and AUC of the active metabolite, Ro 47-8634, were lower by 33% and 12%, respectively, in the presence of ketoconazole.

Concomitant administration of bosentan and CYP 3A4 inhibitors should be

contraindicated.

Concomitant administration of 100-mg QD losartan lowered both Cmax and AUC of bosentan by 15%. Losartan decreased the Cmax 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 Cmax 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 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:

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 Days1-9 + 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	*Matrix*	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
Bosentan	Plasma							
Ro 48-5033	Plasma							
Ro 47-8634	Plasma							

Ro 64-1056	Plasma
Simvastatin	Plasma
β-hydroxy simvastatin	Plasma

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	Cmax (ng/ml)	Tmax (h)*	AUC_{τ} (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

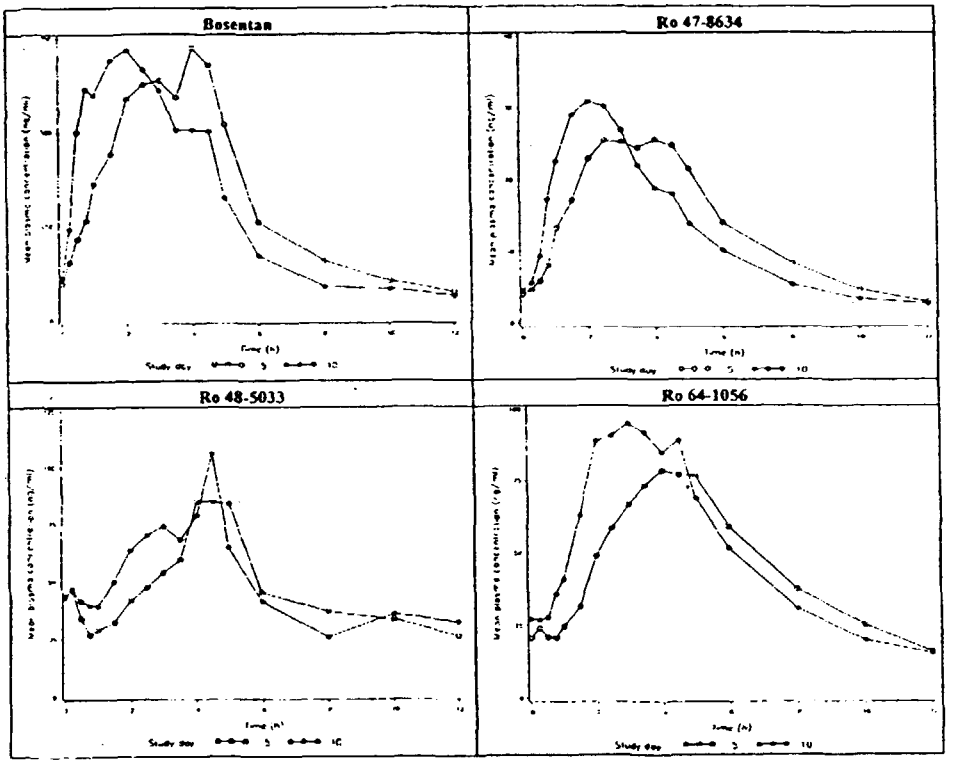
Table 2: Point estimate and 90% CI for PK parameters of bosentan and metabolites

		(Simvastatin+ Bosentan) vs. (Bosentan Alone)	
Compound	Parameter	Point Estimate	90% Conf. Interval
Bosentan	Cmax (ng/ml)	0.99	0.82, 1.19
	AUC_{τ} (ng.h/ml)	0.94	0.83, 1.06

Ro 47-8634	Cmax (ng/ml)	1.07	0.91, 1.26
	AUCτ (ng.h/ml)	0.93	0.87, 1.01
Ro 48-5033	Cmax (ng/ml)	1.12	0.89, 1.40
	AUCτ (ng.h/ml)	1.05	0.90, 1.22
Ro 64-1056	Cmax (ng/ml)	1.20	1.05, 1.38
	AUCτ (ng.h/ml)	1.08	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	Cmax (ng/ml)	Tmax (h)*	AUC_{0-T} (ng.h/ml)	MRT (h)*	Metabolite:Parent Ratio

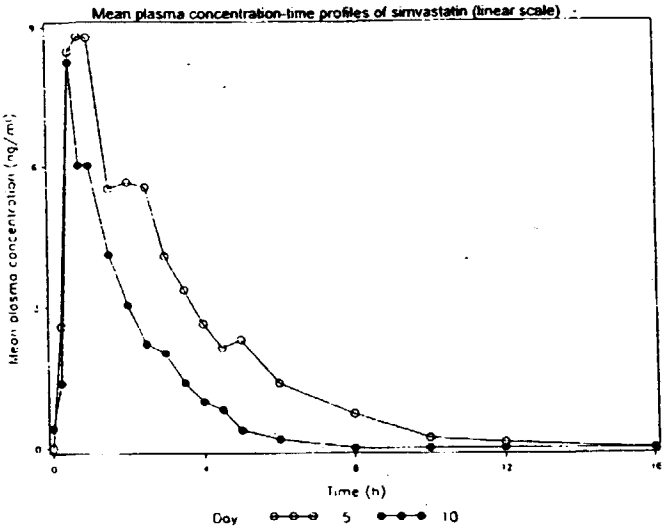
Simvastatin	Simvastatin Alone	12.6 (9.1)	1.17 (0.9)	25.6 (13.6)	2.78 (1.2)	
	Simvastatin + Bosentan	8.63 (8.4)	0.81 (0.4)	12.9 (5.7)	1.90 (0.6)	
β-hydroxy simvastatin	Simvastatin Alone	2.23 (1.6)	4.02 (1.7)	8.0 (11)	4.94 (1.8)	0.32
	Simvastatin + Bosentan	1.49 (1.1)	2.52 (1.9)	3.16 (6.3)	3.10 (1.0)	0.24

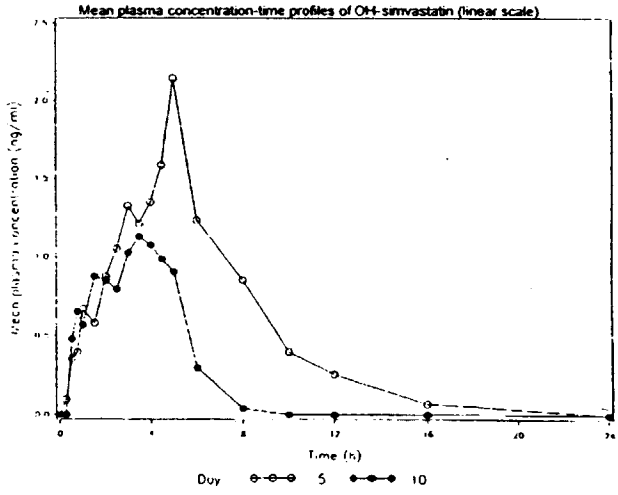
*arithmetic mean

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

		(Simvastatin+ Bosentan) vs. (Bosentan Alone)	
Compound	**Parameter**	**Point Estimate**	**90% Conf. Interval**
Simvastatin	Cmax (ng/ml)	0.69	0.43, 1.10
	AUCτ (ng.h/ml)	0.51	0.40, 0.63
β-hydroxy simvastatin	Cmax (ng/ml)	0.67	0.46, 0.96
	AUCτ (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:

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 Days1-9 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	*Matrix*	Method	Range (ng/ml)	Linearity	LOQ (ng/ml)	QC (ng/ml)	CV%	Accuracy (% Bias)
Bosentan	Plasma							
Ro 48-5033	Plasma							
Ro 47-8634	Plasma							

Ro 64-1056 Plasma

Glibenclamide Plasma

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	Cmax (ng/ml)	Tmax (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)

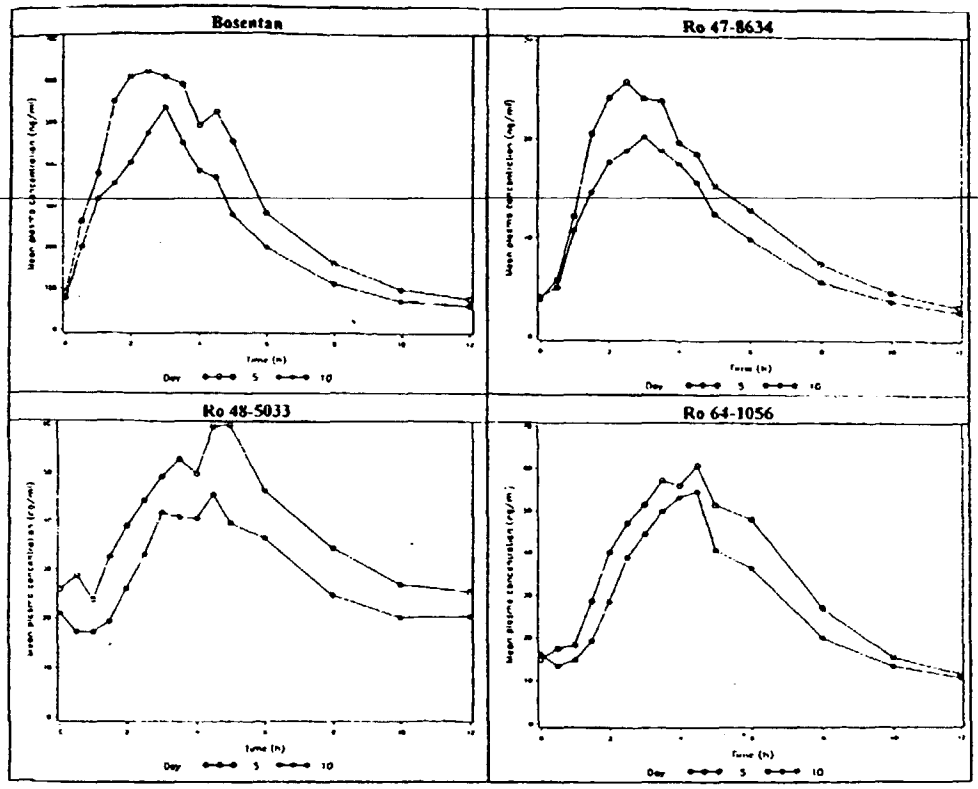
*arithmetic mean

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

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

Ro 64-1056	Cmax (ng/ml)	0.82	0.69, 0.97
	AUCτ (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 Tmax, however, was unaffected. Glibenclamide decreased the Cmax 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	Cmax (ng/ml)	Tmax (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)

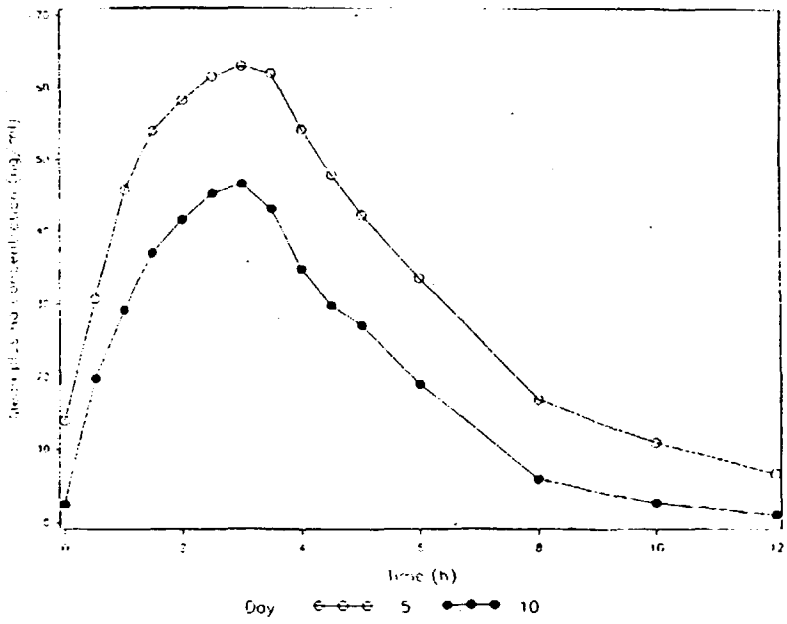
*arithmetic mean

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

		(Glibenclamide+ Bosentan) vs. (Bosentan Alone)	
Compound	Parameter	Point Estimate	90% Conf. Interval
Glibenclamide	Cmax (ng/ml)	0.78	0.66, 0.92
	AUCτ (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 Cmax and AUC of bosentan decreased by 24% and 29%, respectively, while that of glibenclamide decreased by 22% and 40%, respectively. This interaction is probably due to induction of liver enzymes/p-glycoprotein transport and/or increase in bile flow.

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

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 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 event, patients received a single intravenous dose of bosentan 500 mg, infused over 30 minutes.

ASSAY:

Plasma concentrations of bosentan were measured at . Plasma concentrations of nimodipine were measured at

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

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:

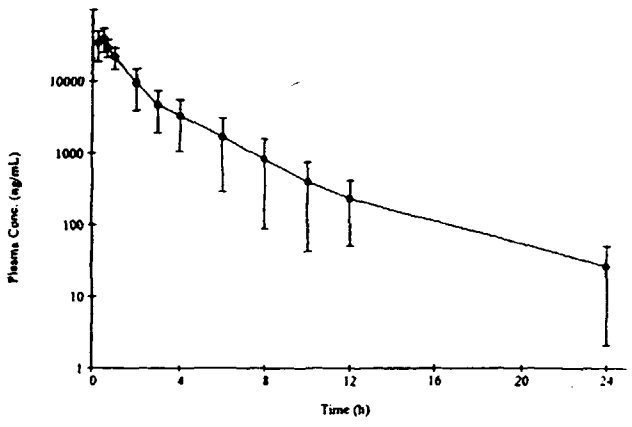
The pharmacokinetic parameters of bosentan obtained following administration of 500-mg bosentan infusion in the presence of nimodipine are listed in the following table.

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

Total Dose	$T_{0.5}$ (h)	AUC (ng.h/ml)	CL (L/h)	Vss (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 patients were similar to those seen in healthy volunteers. However, the pharmacokinetics of bosentan in 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 .



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 and 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 patients obtained following a single 500-mg intravenous dose of bosentan in the presence of steady-state nimodipine was similar to those obtained in healthy volunteers in other studies. Except for one patient, single intravenous dose of bosentan did not alter steady-state nimodipine concentrations in patients.

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 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 (≈ 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 37^0C
Volume:	900 ml
Speed of Rotation:	50 rpm
Brief Description of Dissolution Analytical Method:	
Sampling Times	5, 10, 15, 30, 45, 60 min
Proposed Dissolution Specification	Q=)% not less than 30 min

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

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

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( )sodium lauryl sulfate in water.
2. The biopharmaceutics reviewer proposes the following dissolution method, medium and specification; dissolution not less than( )% (Q) dissolved in 30 min in( )sodium lauryl sulfate in water using USP Apparatus II (paddle) at a speed of 50 rpm.

ADDENDUM TO STUDY AC 052-101 TITLED "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"

Report No.: AC 052-101
Volume No.: 2.18

OBJECTIVE:

To determine the pharmacokinetics of bosentan when administered alone and following concomitant administration of 200 mg QD ketoconazole on Days 2, 3 and 4.

FORMULATIONS:

Bosentan – 250-mg tablets (batch #: PT2227T68)
Ketoconazole – 200-mg tablets of Nizoral® by Janssen-Cilag (batch #: 98I03/610, 95K27/840 and 97A23/966)

STUDY DESIGN:

This was an 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.

Sample Collection:
Only trough samples were collected for analysis of bosentan concentration on Dosing Days 2, 3 and 4. 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

Trough concentrations of bosentan on Days 2, 3, 4 and 5 when administered alone and in the presence of ketoconazole are presented in the following table.

Table 1: Mean (SD) and Maximum Trough Concentrations of Bosentan When Administered Alone and in the presence of Ketoconazole

	Mean (SD)		Maximum Trough Conc.	
Trough Conc. On Day	125-mg BID Bosentan Alone	125-mg BID Bosentan +200-mg QD Ketoconazole	125-mg BID Bosentan Alone	125-mg BID Bosentan +200-mg QD Ketoconazole
2	223 (187)	261 (242)		
3	119 (76.5)	172 (196)		
4	199 (262)	263 (241)		
5	168 (180)	278 (257)		

EFFECT OF KETOCONAZOLE ON BOSENTAN:

Mean trough concentrations of bosentan increased 17%, 44%, 32% and 65% on Days 2, 3, 4, and 5, respectively, in the presence of 200 mg QD ketoconazole. Based on the 17% to 65% increases in bosentan trough concentrations in the presence of ketoconazole it can be implied that ketoconazole affects bosentan concentrations which is not expected to be clinically significant.

The caveat to extrapolating interaction magnitude based on bosentan trough concentrations alone is that, at the time of trough sampling (24 hours following ketoconazole administration) ketoconazole concentrations will be significantly reduced compared to peak concentrations which occur between 1 - 2 hours post-dose, assuming an initial half-life of 2 hours (first 10 hours) and terminal half-life of 8 hours. The reduced concentrations of ketoconazole at 24 hours post-dose might produce a mild interaction, compared to peak concentrations.

Based on the following points it can be inferred that ketoconazole increases bosentan concentrations, however, the increase is not expected to be clinically significant.

1. Interaction with ketoconazole is not as profound as cyclosporine + bosentan, where a 30-fold increase in bosentan trough concentrations was observed following the first coadministered dose.

2. Day 5 increase in bosentan C_{max} and AUC of 62% and 83%, respectively, in the presence of ketoconazole is comparable to the increase observed with the trough concentrations on Days 2, 3, 4 and 5.

CONCLUSIONS:

Mean trough concentrations of bosentan increased 17%, 44%, 32% and 65% on Days 2, 3, 4, and 5, respectively, in the presence of 200 mg QD ketoconazole. Based on the trough concentrations it can be implied that ketoconazole increases bosentan concentrations, but the increase in bosentan concentration is not expected to be clinically significant.

COMMENTS:

1. The sponsor should have collected a complete plasma profile of bosentan concentrations following the first coadministered dose of ketoconazole on Day 1. \

2. The dose of ketoconazole used in the present study, 200 mg QD, is less than the maximal dose used clinically, 400 mg QD. Therefore, a larger increase in bosentan concentrations is expected at the clinical dose of 400 mg QD ketoconazole.

RECOMMENDATIONS:

The Office of Clinical Pharmacology and Biopharmaceutics finds the addendum acceptable. No further action is warranted at this time.

/S/ 9/7/01

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

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