CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-290/S-001

Clinical Pharmacology and Biopharmaceutics Review

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA: 21-290 SE, Item 5 Amendment **SUBMISSION Dates: December 4/02, September15/03**

BRAND NAME: Tracleer®

GENERIC NAME: Bosentan

SPONSOR: Actelion Pharmaceuticals US, Inc.
Hucleberry Lane
North Andover, MA 01845

DIVISION OFC PHARMACEUTICAL EVALUATION: 1

PRIMARY REVIEWER: Peter H. Hinderling, M.D.

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

In vivo studies in humans indicate that cyclosporine is a potent inhibitor of bosentan with respective 30 and 3.5 fold increases of AUC after single and multiple dose administrations of bosentan to subjects on cyclosporine. Administration of cyclosporine and bosentan is contraindicated. In studies in rats co-administered cyclosporine or tacrolimus increased the exposure to bosentan markedly, by a factor of 17 and 13, respectively. The sponsor claims that the magnitude of the interaction between bosentan and tacrolimus or cyclosporine is too excessive to be explained only by inhibition of the CYP 3A4 mediated metabolism of bosentan by the immunosuppressive agents. This statement is based on the finding in humans that ketoconazole, which is considered to be a potent inhibitor of CYP 3A4, increased the exposure to bosentan at steady state only by a factor of 2. In the mass balance study with oral administration about 95% and 3% of the total radioactivity were recovered in the feces and in urine, respectively. Metabolites constituted 65% (Ro 48-5033: 35 %, Ro 64-1056: 13%, Ro 47-8634: 7 %, minor metabolites: 5% and unknown: 5 %) and bosentan 30% of the administered dose. Thus, the largest fraction of the dose of bosentan is metabolized. The CYP 450 isozymes involved in the metabolism of bosentan have been identified as 3A4 and 2C9. Although

additional mechanisms cannot be excluded the main impact of cyclosporine and possibly tacrolimus on bosentan is likely through inhibition of CYP 3A4 and 2C9.
To justify a contraindication of tacrolimus and bosentan the following standard is applied: In order to fully extrapolate the interaction results between tacrolimus and bosentan in rats to humans using the cyclosporine- bosentan findings, additionally an identical mechanism of inhibition common to both immunosuppressives must be demonstrated.

In a teleconference on August 29 the sponsor was asked to provide mechanistic information from the literature on how tacrolimus and cyclosporine interact with bosentan. The sponsor submitted on September 16 a review article entitled " Contributions of Hepatic and Intestinal Metabolism and P-Glycoprotein to Cyclosporine and Tacrolimus Oral Drug Delivery by M.F. Hebert, Advanced Drug Delivery Reviews (1997); 27:201-214

The review by M.F.Hebert deals with drug-drug interactions involving tacrolimus and cyclosporine, but only from the point of view of how other drugs impact the PK of the two immunosuppressive drugs. The article does not discuss drug interactions where tacrolimus or cyclosporine impact other drugs like bosentan. Thus, the information does not provide mechanistic insights as to how cyclosporine or tacrolimus impact the disposition of tacrolimus.

RECOMMENDATION

In the absence of a mechanistic explanation that is common to both immunosuppressives there is not enough evidence to contraindicate co-administration of bosentan and tacrolimus. However, the possible interaction of tacrolimus and bosentan should be mentioned in the PRECAUTION section of the PI.

LABELING RECOMMENDATION

Co- administration of tacrolimus and bosentan has not been studied in man. Co-administration of cyclosporine A or tacrolimus with bosentan markedly increased the plasma levels of bosentan in animals. Caution should be exercised if tacrolimus and bosentan are used together.

Peter H. Hinderling, MD
Division of Pharmaceutical Evaluation 1

FT Initialed by Patrick J. Marroum, Ph.D.
CC list: HFD 110: NDA 21256;HFD 860: Hinderling, Marroum, Sahajwallah, Mehta;
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/s/

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9/30/03 10:16:31 AM
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9/30/03 10:26:53 AM
BIOPHARMACEUTICS

CLINICAL PHARMACOLOGY and BIOPHARMACEUTICS REVIEW

Division of Pharmaceutical Evaluation I

NDA 21-290 SE
IND 58 317

TRACLEER® (bosentan)
Tablet strengths 62.5 and 125mg
Actelion Pharmaceuticals US, Inc.
56 Huckleberry Lane
North Andover, MA 01845

Submission Dates:
January 17,2002
June 20, 2002
November 11, 2002
December 4, 2002

REVIEWER: Peter H. Hinderling, MD

TYPE OF SUBMISSION: Serial Submissions to IND 58 347

SUBMISSIONS:

Reference is made to the approved NDA 21-290 for Tracleer® (bosentan) film-coated Tablets of 62.5 and 125mg strength. Tracleer® is indicated for the treatment of pulmonary arterial hypertension (PAH) in patients with WHO Class III or IV symptoms, to improve exercise ability and decrease the rate of clinical worsening.

The serial submissions to the IND 58 347 included the final study reports:

- AC-052-356: Breathe 3 "Pharmacokinetics and Tolerability of Tracleer® (Bosentan) in Pediatric Patients with Pulmonary Arterial Hypertension: Single and Multiple Oral Doses"
- AC-052-357: "Multicenter, Open Label, Single Arm, Safety Study of Bosentan in Patients with Pulmonary Arterial Hypertension"
- AC-052-107: Single-and Multiple-Dose Pharmacokinetics of Bosentan in Patients with Impaired Liver Function as Compared to Healthy Volunteers"
- AC-052-108: "A Study to Investigate the Pharmacokinetics of Bosentan in Healthy Subjects when Given Concomitantly Ketoconazole"
- AC-052-109: " A Study to Investigate the Possible Drug-Drug Interaction Between Bosentan and Simvastatin in Healthy Subjects"

SUMMARY OF FINDINGS

BACKGROUND

Bosentan was approved by the Agency in 2001 for the indication treatment of pulmonary arterial hypertension in patients with WHO functional class III and IV symptoms to improve exercise ability and decrease the rate of clinical worsening.

Bosentan is a specific and competitive antagonist at endothelin receptor types ET_A and ET_B. Endothelin-1 is a neurohormone, the effects of which are mediated by binding to ET_A and ET_B receptors in the endothelium and vascular smooth muscle. Endothelin-1 concentrations are elevated in plasma and lung tissue of patients with pulmonary arterial hypertension.

The recommended initial dosing regimen is 62.5mg bid for 4 weeks followed by the maintenance regimen of 125mg bid.

Three (3) of the 5 submitted studies provided new PK information. Study Reports AC-052-136 and AC-052-137 investigated the PK of bosentan in pediatric and adult population with the target disease. Study Report AC-052-107 studied the PK of bosentan in patients with mild liver impairment. The remaining 2 studies AC-052-108 and AC-052-109 represent repetitions of earlier performed drug-drug interaction studies of bosentan with ketoconazole and simvastatin, respectively. Actelion indicated that the reason for performing the 2 repeat studies was that the CRO performing the original studies was investigated by the local authorities in [redacted], because of alleged business, Good Clinical Practice and ethical violations. Actelion conducted a post-trial Trial Master File and source document audit and interviewed the Clinical Investigator and the laboratory personnel responsible for the pharmacokinetic analysis of the original trials and found no evidence of fraud. It is known that in the meantime the CRO has gone out of business.

Study Report AC-052-356: "Pharmacokinetics and Tolerability of Tracleer™ (bosentan) in Pediatric Patients with Pulmonary Arterial Hypertension (PAH)"

The company performed the study in the pediatric population with the target disease without seeking a Written Request from the Agency. A Written Request was also not issued by the Agency.

The goals of the study were to investigate the PK of bosentan when given as single and multiple oral doses on pediatric patients with PAH and the tolerability and safety of bosentan and assess preliminary efficacy data including cardiopulmonary measures, exercise capacity, Borg dyspnoa index, hemodynamics, and WHO functional class. This was an open label, multicenter, non-controlled, parallel group, single- and multiple dose

study with stratification for weight and epoprostenol use. Nineteen (19) male and female pediatric patients with PAH received single doses of 31.25mg, 62.5mg and 125mg of the drug and after an initial treatment phase the subjects received the same doses bid from week 5 to 12. Patients weighing>40 kg received the 125mg dose, patients weighing between 20 and 40 kg received the 62.5mg dose and patients weighing between 10kg and 20kg received the 31.25mg dose. The 31.25mg dose was obtaining by cutting the 62.5mg tablet in half. The tablet strengths for adults were used. No pediatric formulation was provided. The PK parameters of bosentan and its metabolites Ro 47-8634, Ro 48-5033 and Ro 64-1056 were measured in plasma by a specific [ ] method for 24 hours following administration. The pharmacodynamic parameters were measured at baseline and after 12 weeks of treatment. Adverse events were monitored, routine laboratory tests performed and a 12-Lead ECG and vital signs recorded to determine safety.

PK data were available for 18 of the 19 subjects. Repeat administration of bosentan to the pediatric patients resulted in an increase of the oral clearance presumably by induction of CYP 3A4 and 2C9 known to metabolize bosentan. Gender and co-administration of epoprostenol did not appear to impact the PK of bosentan.
The exposure measures for bosentan and metabolites after single and multiple doses of bosentan were different in the 3 weight groups. The AUC and Cmax values after single and multiple dose administration were significantly greater in the heavy and intermediate weight groups than in the light weight group indicating that the applied dose regimen did not provide comparable plasma concentrations of bosentan. After normalization for dose and weight the exposure measures were still different in the 3 groups suggesting development age dependent and/or nonlinear PK of bosentan.

The changes in the exercise parameters varied importantly and none were statistically significant. Five patients improved by one WHO functional class, on patient deteriorated by one WHO functional class and for 12 individuals the WHO functional class remained unchanged. The cardiopulmonary hemodynamic parameters in the children were statistically significantly improved..

STUDY REPORT AC-052-357: " Multicenter, Open Label, Single-Arm, Safety Study of Bosentan in Patients with Pulmonary Arterial Hypertension"

The goals of the study were to obtain safety data on bosentan in patients with PAH and provide bosentan treatment. This was an open, multicenter, single arm, safety study. A pharmacokinetic sub-study was performed in a single center. A total of 115 male and female patients were enrolled in the study with 13 subjects participating in the PK sub-study. The patients received 62.5mg bosentan bid for the first 4 weeks and, dependent on tolerability, the dose was then increased to 125mg bosentan bid. The PK of bosentan and the metabolites Ro 47-8634, Ro 48-5033 and Ro 64-1056 were determined after at least 2 weeks of treatment with the lower and higher dose.

WHO functional class was the evaluated efficacy parameter determined at baseline and after treatment at the 2 dose levels. Adverse events were monitored and laboratory tests with emphasis on liver enzymes and hematology performed repeatedly.
PK parameters of bosentan and its metabolites Ro 47-8634, Ro48-5033 and Ro 64-1056 were measured in plasma for 24 hours following administration using a specific [ ] method.
The data from all 13 enrolled patients were available for analysis at the 62.5mg dose level. At the 125mg dose level data from 11 subjects were at hand. Eight of the subjects were females and 5 males. The PK of bosentan in patients who received 62.5mg and 125mg bosentan bid each for at least 2 weeks were less than dose proportional indicating a dose dependent increase of the oral clearance. The oral clearance in adult patients with PAH was about 50% of that in healthy adult volunteers.

STUDY REPORT AC-052-107:"Single and Multiple Dose Pharmacokinetics of Bosentan in Patients with Impaired Liver Function as Compared to Healthy Subjects"

The objectives of the study were to compare the PK of bosentan and its metabolites after single and multiple dose administration of 125mg to patients with mild liver impairment and healthy volunteers and evaluate the safety and tolerability of bosentan. This was a single center, open label, parallel-group, single and multiple dose study. In the multiple dose phase of the study bosentan was administered bid for 5.5 days. Eight (8) patients and 9 healthy volunteers were enrolled in the study. The PK of bosentan and its metabolites Ro 47-8634, Ro 48-50333 and Ro 64-1056 were followed for 48 hours after single and multiple administrations of the drug. Bosentan and its metabolites were measured by a specific [ ] method. Adverse events were recorded, physical examinations and routine laboratory tests performed, and systolic and diastolic blood pressure in the supine and standing positions as well as a standard a 12-lead ECG at scheduled times before and after drug administration recorded.

The PK data from 8 patients with mild hepatic impairment of Child-Pugh Class A and from 9 healthy volunteers were available. The patients and volunteers were matched for age, gender and body weight. Among the patients 5 were males and 4 females. Among the volunteers 6 were males and 3 females. The measures of exposure in patients with mild hepatic impairment and in matched volunteers treated for 5.5 days with 125mg bosentan bid were comparable. The safety data did not indicate a difference in the tolerability of the treatments between patients with mild liver impairment and matched healthy volunteers. There was a trend for both systolic and diastolic blood pressure to decrease following administration of the drug. This occurred in healthy volunteers and in patients with mild liver impairment. There was no evidence for reflex tachycardia or clinical signs of orthostatic hypotension.

STUDY REPORT AC-052-108: " A Study to Investigate the Pharmacokinetics of Bosentan in healthy Subjects when Given Concomitantly Ketoconazole"

The objectives of the study were to evaluate the influence of concomitant ketoconazole on the PK of bosentan in healthy subjects and assess the single and multiple dose PK after administration of 62.5mg bosentan and to evaluate the tolerability of concomitant ketoconazole and bosentan in healthy subjects. This was a single center, open label, randomized, two period, cross-over study with single and multiple dose administrations of the drug. In Treatment A single doses of 62.5 mg bosentan were administered to the volunteers on Days 1 and 7, and on the intervening Days 2 to 6 the volunteers received bosentan 62.5mg bid. In Treatment B 62.5mg bosentan was administered bid to the volunteers on Days 1 through 5 and a single dose of bosentan was given on Day 6. Ketoconazole 200mg qd was given to the volunteers on Days 1 through 6. Ten (10) healthy male volunteers were enrolled and completed the study. The plasma concentrations of bosentan and Ro 47-8634, Ro 48-5033, and Ro 64-1056 were followed on Days 1 and 7 of Treatment A and on Day 6 of Treatment B for 24hours after drug administration. Bosentan and its metabolites were measured by [ ]
AEs were monitored throughout the study, physical examinations and routine laboratory tests were performed and a 12 Lead ECG recorded at screening and at the end-of-study visit. Vital signs were recorded at screening and at scheduled times prior to and following drug administration.
The co-administration of 200mg ketoconazole qd for 6 days with 62.5mg bosentan bid impacted the exposure measures of bosentan significantly. AUCτ and Cmax were increased by 120.0% and 94.8%, respectively. The observed increases in AUCτ and Cmax in the present study were greater than those in a previous study reported in the NDA with healthy volunteers receiving the same treatment (83% increase in AUCτ, 62% increase in Cmax). The present wording in the label should be kept.

The number of total and drug related AEs was greater in the presence than in the absence of ketoconazole.

STUY REPORT AC-52-109: " A Study to Investigate the Possible Drug-Drug Interaction between Bosentan and Simvastatin in Healthy Subjects"

The objectives of the study were to evaluate the influence of concomitant simvastatin on the PK of bosentan and its metabolites and to evaluate the influence of concomitant bosentan on the PK of simvastatin in healthy subjects and to assess the tolerability of concomitant simvastatin and bosentan in healthy subjects.

This was a single center, open label, multiple dose, randomized, three period cross-over study. In Treatment A the volunteers received bosentan bid for 5.5 days: 125mg bosentan bid on Days 1 through 5 and a single dose on Day 6. In Treatment B the volunteers received simvastatin for 6 days: 40mg simvastatin qd on Days 1 through 6. In Treatment C the volunteers received bosentan 125mg bid for 5.5 days: 125mg on Days 1 through 5 and a single dose on Day 6. Nine (9) healthy, male volunteers were enrolled. The plasma concentrations of bosentan and its metabolites, Ro 47-8634, Ro 48-5033, and Ro 64-156, and of simvastatin and its metabolite, simvastatin-β-hydroxyacid, were followed on Day 6 of the treatments for 12 hours after drug administration. The morning trough

concentrations of all the compounds were also monitored on Days 1 through 5 of the respective treatments. The plasma concentrations of bosentan and metabolites and simvastatin and its metabolite were measured by [ ] methods.
The PK data from all 9 enrolled volunteers were available. Co-administration of 40mg simvastatin qd and bosentan 125mg bid for 6 days resulted in a significant reduction of AUCτ and Cmax of simvastatin (-34.4% and -17.1%, respectively) and its metabolite (-45.6% and –17.8%, respectively. Co-administration of 40mg simvastatin qd for 6 days had no relevant impact on the PK of bosentan. The magnitude of the effects of bosentan on simvastatin and its metabolite in the present study was slightly smaller than in a previous study reported in the NDA (decrease in AUC and Cmax for simvastatin = -49% and -31%, respectively, decrease in AUC and Cmax for simvastatin-β =-60% and -33%, respectively). There was agreement between the studies that simvastatin has no impact on the exposure measures of bosentan.
The number of total and drug related AEs was greatest in Treatment C.

RECOMMENDATION

The Office of Clinical Pharmacology and Biopharmaceutics / Division of Pharmaceutical Evaluation I (OCPB/DPE1) has reviewed the studies for protocols AC-052-356, AC-052-357, AC-052-107, AC-052-108 and AC-052-109 submitted under the IND 58 317.

Study AC-052-356 characterized the PK of bosentan in the pediatric population allowing a comparison of the exposure among the 3 pediatric weight groups and between pediatric and adult patients. The results showed that the dose regimen applied in the pediatric trial using the adult tablets in doses of 31.25mg, 62.5mg and 125mg did not provide comparable measures of exposure in the 3 pediatric groups investigated. In addition, the comparison of the exposure to bosentan between children and adults with the target disease showed that only the intermediate weight group had an exposure to bosentan that was comparable to that in adult patients receiving the same maintenance dose of 62.5mg bid. In the two other weight groups the exposure in the children was significantly smaller than in adult patients receiving the labeled dose regimen of 62.5mg and 125mg bosentan. These results indicate that the PK information does not support the adjusted dose regimens applied in the trial with the 3 pediatric weight groups. **Thus, only if the clinical data support efficacy and safety of bosentan in the children of all three weight groups should the PK information be inserted in the label. However, since the evidence in support of efficacy and safety of bosentan in the pediatric patients was not considered sufficient by the Cardio-renal Division no PK information will be inserted in the label.**
The comparison of the exposure to bosentan ought to be between children and adults with PAH (Study AC-052-357). The comparison of the PK of bosentan in adults with PAH and healthy adults showed that the oral clearance in the patients is 50% smaller.

The sponsor did not provide a pediatric formulation allowing flexibility in dosing. The administration of tablets to preschool children and the need for cutting the 62.5mg tablet

in half results in imprecise dosing. These issues ought to be resolved by the sponsor should bosentan be approved for the treatment of PAH in children.
Study AC-052-357 characterized for the first time the PK of bosentan in the adult population with PAH. The findings ought to be described in the label.

Study AC-052-107 characterized for the first time the PK of bosentan after oral administration in patients with mild liver impairment. The findings should be described in the label.

The findings of **Study AC-052-108** confirmed the results of a previous drug interaction study with ketoconazole. The current version of the label is adequate.

The results of **Study AC-052-109** confirmed the results of a previous drug interaction study with simvastatin. The current version of the label is adequate.

The following revisions to the annotated labeling provided by Actelion are proposed:

1. **Pharmacokinetics**
   General

Applicant's wording: ⊏

]

FDA's wording: " After oral administration, maximum plasma concentrations of bosentan are attained within 3-5 hours and the terminal half-life (t1/2) is about 5 hours **in healthy adult subjects. The exposure to bosentan after intravenous and oral administration** . ⊏

]

2. Metabolism and Elimination
Applicant' wording of third and fourth sentences: ⊏

]

FDA's wording of third and fourth sentences: "Total clearance after single intravenous administration is about **4L/hr in patients with pulmonary arterial hypertension**. Upon multiple **oral** dosing plasma concentrations **in healthy adults** decrease gradually to 50-60% of those seen after single dose administration, probably the effect of auto-induction of the metabolizing liver enzymes."

3. Special populations:

Applicant's wording:"

FDA's wording: " It is not known whether bosentan's pharmacokinetics are influenced by gender, body weight, race or age.

4. *Liver function Impairment*
Applicant's wording:

FDA's wording: in vitro and in vivo evidence showing hepatic metabolism of bosentan suggests that liver impairment could significantly increase exposure of bosentan. In a study comparing 8 patients with mild liver impairment (as indicated by the Child-Pugh method) to 8 controls the single and multiple dose pharmacokinetics was not altered in patients with mild liver impairment.Th e influence of moderate or severe liver impairment on the pharmacokinetics of TRACLEER® has not been evaluated. TRACLEER® should generally be avoided in patients with moderate or severe liver abnormalities and/or aminotranferases elevated >3x ULN (See DOSAGE AND ADMINISTRATION and WARNINGS)).

5. *Pediatric patients*
Applicant's wording:

FDA's wording: delete entire section

6. Warnings

FDA's wording: Move statement on Tacrolimus

[ ] **" into CONTRAINDICATIONS and PRECAUTIONS sections.**

7. Dosage Adjustment in Hepatically Impaired Patients

Applicant's wording: [

]

FDA's wording: Because there is in vitro an in vivo evidence that the main route of excretion of TRACLEER® is biliary, liver impairment could be expected to increase exposure (Cmax, AUC) to TRACLEER®. Mild liver impairment was shown not to impact the pharmacokinetics of TRACLEER®. The influence of moderate or severe liver impairment on the pharmacokinetics of TRACLEER® has not been investigated. There are no specific data to guide dosing in patients with **moderate or severe** hepatic impairment (see **WARNINGS**). TRACLEER® should generally be avoided in patients with **moderate or severe** hepatic impairment.

7. Dosage Adjustment in Children and Adolescents

Applicant's wording: Safety and efficacy in pediatric patients have not been established.

[

]

FDA's version: Keep first sentence and the delete the remainder of the paragraph.

Please convey the Recommendation as appropriate to the sponsor.

Peter H. Hinderling
Division Pharmaceutical Evaluation 1
Office of Clinical Pharmacology and Biopharmaceutics

RD Initialed by Patrick Marroum, Ph.D._____

Briefing held 5/19/03 (Hinderling, Marroum, Mehta, Sahajwalla)

Cc: NDA 21-290, IND 58 317, HFD-860 (Hinderling, Marroum, Mehta, Sahajwalla,Gordon)

ATTACHMENT I

Pharmacokinetic Report Summaries

Protocol AC-052-356

Study Title: Pharmacokinetics and Tolerability of Tracleer™ (bosentan) in Pediatric Patients with Pulmonary Arterial Hypertension (PAH)

Principal Investigator:

Robyn Barst, MD (Principal Investigator), Division of Pediatric Cardiology, New York Presbytarian Hospital, New York, NY

Investigator:

[ ]

Objectives:

1. To investigate the PK of bosentan when given as single and multiple oral doses in pediatric patients with PAH
2. To evaluate tolerability and safety of bosentan and assess preliminary efficacy data including exercise capacity, Borg dyspnoa index, hemodynamics, and WHO functional class.

The PK of bosentan in a pediatric population with the target disease have not been determined up to now.

Subjects:

Nineteen (19) female and male patients with PAH (primary or related to scleroderma or congenital heart defects) and of WHO class II or III on conventional vasodilator/anticoagulant therapy or epoprostenol therapy.
To be eligible the children had to suffer from PAH and be in WHO functional class II or III with or without epoprostenol therapy. PAH could have been primary or associated with scleroderma or congenital heart defects. The subjects had to be in the age between 2 and 17 years with a body weight exceeding 10kg, negative drug screening test, negative pregnancy test (females), stable clinical status if on epoprostenol therapy or vasodilator/anticoagulant therapy. Because bosentan was shown to be teratogenic and fetotoxic in animals, females of child-bearing potential were to have used an acceptable method of contraception that included Barrier type devices in combination with a spermicide, intrauterine devices, injections with Depo-Provera™ (medroxyprogesterone acetate) or levonorgestrel implants.

Exclusion criteria included cardiac index < 2 l/min/m^2, hypotension (<80mmHg systolic blood pressure), left ventricular dysfunction, hemoglobin or hematocrit < 30% of normal, clinically relevant inter-current medical condition possibly interfering with the evaluation of safety, active hepatitis, HIV, history of drug abuse, hypersensitivity to drugs or history of adverse reaction to drugs and symptoms of clinically relevant illness. Because bosentan is a substrate and inducer of CYP3A4 and 2C9 use of glibenclamide, cyclosporine A, tacrolimus (FK 506), inhibitor/inducers of CYP 3A4 and /or CYP 2C9 was a reason for exclusion.

Methodology:

This was an open, multi center, non-controlled, parallel group, single-and multiple dose study with stratification for weight and epoprostenol use. The screening examination (Visit 1) which included the WHO functional assessment of pulmonary hypertension, 12-lead ECG, vital signs, body weight, height, patient history, concomitant medication, Tanner assessment, physical and laboratory examinations, pregnancy test (females of child bearing potential) and patient status on either conventional vasodilator/ anticoagulant or epoprostenol therapy was performed 3-21 days prior to study medication initiation. PK and PD evaluations were done at baseline/initiation (Visit 2) and after 12 weeks of bosentan treatment (Visit 5). On both occasions patients were admitted to the hospital in the evening. The following day a Swan-Ganz catheter was inserted and hemodynamic parameters measured. Blood samples for the evaluation of PK were collected on the third day pre-dose and during a period of 24 hours following dosing. Within 5 days of the PK evaluation period patients ≥ 8 years of age were to have performed the 6 minutes walk test, Borg dyspnea index, and cycle ergometry test. All hemodynamic measurements were made in duplicate during expiration and on room air. At week 12, the hemodynamic measurements were done immediately prior to dosing. At week 4 (Visit 3) the patients returned to the clinic for safety assessments and reevaluation of WHO functional class. Adverse events and change in co-medication were recorded.

Table 1 lists the activity schedule for the 12 week PK phase of the study.

Table 1. Activity Schedule of the PK Phase of the Study

	Pharmacokinetic Study Period				
Treatment Schedule [Study day]	Screening [-21,-3]	Initiation [1]	Week 4 [24-33]	Week 8 [52-61]	Week 12 [80-89]
Visit number	1	2	3	4	5
Informed consent	X				
History	X				
Physical examination	X				
Tanner assessment	X				
ECG 12 each	X	X			X
Laboratory tests	X	X	X	X	X
Exercise tests and Borg dyspnea index		X			X
WHO functional class	X	X	X	X	X
Hemodynamic measurements[1]		X			X
BP, HR, body weight and height	X	X	X		X
Pharmacokinetic evaluation		X			X
Dispense return medication		X	X		X
Adverse events intercurrent illness		X	X	X	X
Concomitant medications	X	X	X	X	X

After the 12 week PK phase of the study the patients could elect to continue in an open-label treatment phase with visits at months 6 and 12 and every 3 months thereafter until end of the study. Telephone follow-ups were scheduled at months 4, 8 and 10. All patients were evaluated at premature withdrawal or at the end of the study.

The treatment with bosentan given in doses of 31.25, 62.5 or 125mg lasted 12 weeks. Table 2 shows the treatment schedule.

Table 2. Dose Regimens of Bosentan in the 3 Body Weight Groups

Body weight	Single dose (Day 1 and at Week-12 visit)	Initial dose (Day 2 through Week-4 visit)	Target dose (starting Week 5)
> 40 kg	125 mg	62.5 mg b.i.d.	125 mg b.i.d.
20 < x ≤ 40 kg	62.5 mg	31.25 mg b.i.d.	62.5 mg b.i.d.
10 ≤ x ≤ 20 kg	31.25 mg	31.25 mg q.d.	31.25 mg b.i.d.

Note: 31.25 mg doses were obtained by cutting 62.5 mg tablets in half.

The doses were adjusted for body weight. On the first and last day of treatment bosentan was given once a day. On Day 2 the patients started with the initial dose given qd (lowest body weight group) or bid (intermediate and largest body weight groups). The dose was up-titrated after 4 weeks. The target doses were given bid. If the target dose was not tolerated the dose could be down-titrated to the initial dose. After 12 weeks the patients could elect to continue treatment.

The 125mg bid target regimen selected for the patients weighing > 40kg was based on the safety and efficacy results of this dose in adult patients of the same weight. The smaller target doses of 62.5 and 31.25mg for the patient groups with lower body weights were found by linear dose normalization assuming that adults receiving 125mg bosentan bid had a body weight of approximately 70kg. As a precautionary measure the plasma samples of the first four patients after receiving the first dose were evaluated immediately.

Study Drug:

Bosentan (Ro 47-0203) 62.5mg tablets, batch number F 0274 A 0001, and 125mg tablets, batch number F 0358 A 001, were supplied by the sponsor. The study medication was manufactured by Patheon Ltd, Canada, and packaged, labeled, and distributed by [redacted] The study medication for each patient was contained

in bottles with labels color coded for each body weight group: yellow for the group with body weight > 40kg, green for the group with body weight 20kg < x ≤ 40kg and white for the group with body weight 10kg ≤ x ≤ 20kg. Each bottle was labeled with the study number, the patient identification number, the batch number, the dose (62.5 or 125mg). The 31.25mg dose was obtained by cutting a 62.5mg tablet in half using a tablet cutter provided with the study medication. The cutting of the 62.5mg tablets was performed by the investigator or study nurse.

Prior and Concomitant Therapy:

All patients received treatment with either epoprostenol or conventional vasodilator/anticoagulant therapy. About half of the patients used epoprostenol and supplemental oxygen. Other medications included loop diuretics (mainly furosemide), digoxin, calcium channel blockers, psycholeptics, analgesics, antihistamines, and antibacterials.

Evaluation:

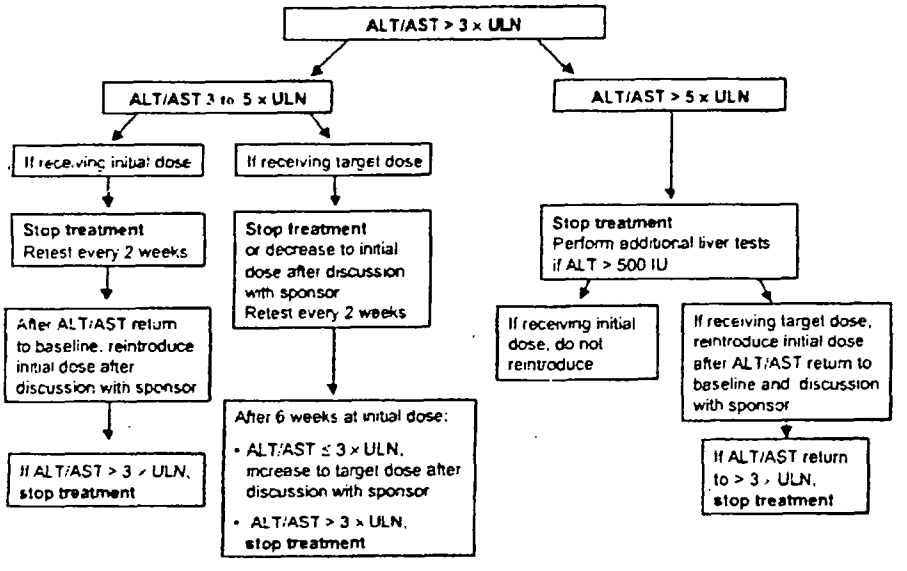
Pharmacokinetics: The PK of bosentan and its metabolites were followed for 24 hours after single and multiple dose administration of the drug.

Efficacy: The pharmacodynamic parameters were assessed at baseline and after 12 weeks of treatment. Exercise capacity was determined by the 6-minute walk test. Maximum dyspnea was assessed using the Borg dyspnea index. This test measures the breathlessness perceived by the patients at the end of the 6-minute walk test. Exercise gas exchange was measured by means of a cycle ergometry test using a calibrated stationary cycle ergometer adjusted to each patient. Exercise time and work rate were recorded and the patients were asked to identify the limiting symptom. During the exercise and recovery periods respiratory gas exchange (VCO_2 and VO_2), end tidal gas tensions and ventilation (VE) were determined on a breath-by-breath basis. Heart rate and rhythm were recorded continuously and systolic blood pressure was measured by cuff. The hemodynamic parameters assessed included cardiac ouput, systolic, diastolic, mean pulmonary and systemic arterial pressures (PAP and SAP), mean pulmonary artery wedge pressure (PCWP), mean right atrial pressure (RAP), pulmonary blood flow (PBF), pulmonary arterial and venous oxygen saturations (PAsat and PVsat), heart rate and systemic arterial and mean venous oxygen saturation (SaO_2 and MVsat). Cardiac index, stroke index, pulmonary vascular resistance (PVR), pulmonary vascular resistance index (PVRI), systemic vascular resistance (SVR), and systemic vascular resistance index (SVRI) were calculated form the experimentally measured parameters.

The pharmacodynamic parameters were expressed as change from baseline.

Safety: Adverse events were monitored continuously. Bosentan is known to have a potential for triggering reversible, dose related abnormalities of the liver function tests (ALT and/or AST). In the event of an elevation in ALT and/or AST the tests were repeated immediately to confirm the results. In cases of abnormal liver function tests without associated symptoms of liver disease, guidelines for the treatment of patients who developed increased liver enzyme values without symptoms of liver disease were followed. They are outlined in Figure 1.

Figure 1. Guidelines for the treatment of patients with increased liver enzymes without symptoms of liver disease.



If ALT and /or AST levels exceeded 500 IU, the Central laboratory was to be requested to assess leucocyte count, differential and IgE. A serum sample for analysis of anti-CYP3A4 and anti-CYP2C9 antibodies was to be collected as well. If ALT and/or AST levels exceeded 1000 IU tests for hepatitis A virus, IgM, hepatitis surface antigen, anti-hepatitis B core antigen, hepatitis C viral RNA, total IgG, and auto-antibodies (antinuclear, anti-smooth muscle cell, and anti-liver/kidney membrane antibodies) were to be performed additionally.

Past experience with bosentan showed that patients with advanced congestive heart failure may experience a decrease in hemoglobin and /or hematocrit. Therefore if hemoglobin was decreased by at least 15% from baseline and was <10g/dL, and/or

hematocrit was decreased by at least 15% from baseline and was < 0.30, a repeat test was performed to confirm the presence of abnormality. In case of an abnormal finding a complete blood count including reticulocytes, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin and red cell distribution width was measured and a direct inspection of blood smears performed. A standard 12-lead ECG with measurements of PR, QRS, QT and QTc, heart rate and rhythm was recorded at screening, treatment initiation, week 12, and premature withdrawal or the end of the study. Supine systolic and diastolic blood pressure and pulse rate were measured at the times of collection of the blood samples for the PK evaluation. A physical examination of the patients was performed at screening and at the final visit.

Blood Sample Collection for PK:

Ideally blood samples were collected at predose and 1, 2.5, 4, 6, 9, 12, 15, and 24 hours post dose through an indwelling catheter. Minimally blood samples were to be taken pre-dose, 2.5, 4, 6, 12 and 15 hours post-dose.

Analytical Methodology:

The plasma samples were analyzed for bosentan, Ro-48-5033, Ro 47-8634, and Ro 64-1065 using a [redacted] method [redacted] Calibration and quality control samples were prepared in plasma and the latter served to analyze the day- to-day performance.

Pharmacokinetic Parameters:

The following parameters were to be determined for bosentan and metabolites:

Cmax	Maximum observed drug concentration
Tmax	Corresponding time
$t1/2(\beta)$	Half life during the terminal log-linear phase of the plasma concentration time curve
AUC(0-t)	Area under the drug concentration time curve up to the time of the last quantifiable concentration
$AUC(0-\infty)$	Area under drug concentration time curve up to infinite time after single dose administration
$AUC(0-\tau)$	Area under the drug concentration time curve up to the

end of the dose interval after multiple dose administration

AUC(0-t) and AUC(0-τ) were obtained by application of the linear trapezoidal rule. AUC(0-∞) was estimated from AUC(0-t) + Ct/β, where Ct is the last quantifiable concentration and β is the terminal elimination rate constant. The value for β was to be estimated from the log-linear least square regression of the plasma concentration time data in the terminal phase

Statistical Methods:

Sample size and power: The total number of subjects to be enrolled in the study or in each of the body weight groups was not based on statistical power considerations. Enrollment of eighteen patients was considered sufficient to meet the objectives of the study.

Analysis of PK Data:

A compartment model independent analysis was performed using Professional WinNonlin Version 3.3. The pharmacokinetic parameters were summarized descriptively. To explore the impact of covariates on the pharmacokinetic parameters analyses of variance (ANOVA) or covariance (ANCOVA) were performed. The dependent variable was either logarithmically transformed or untransformed. With continuous variables ANCOVA was performed. With categorical variables ANOVA was done. Continuous variables included dose, body weight and age. Categorical variables included gender and use of epoprostenol. A stepwise, bottom-up procedure for the inclusion of explanatory variables was used that first examined all variables separately and then took only significant variables into the final model. To explore the impact of body weight on exposure to bosentan and the metabolites the AUC values normalized for body weight were plotted against body weight. Similarly, to explore the impact of concomitant epoprostenol use on the exposure to bosentan and metabolites the AUC and Cmax values of bosentan and metabolites were normalized for dose and body weight.

The pharmacokinetic parameters of bosentan and metabolites obtained in a pediatric population in the present study were compared with those in healthy adults receiving a single administration of bosentan in doses ranging between 31.25 to 250mg or multiple bid administrations of bosentan in doses ranging between 62.5 to 125mg (studies AC-052-106, AC-052-108, AC-052-109, AC-051-110). However, the protocol of the present study stated that the results in the pediatric population were to be compared to those from studies AC-052-101 (healthy adult volunteers) and AC –052-301/301 (adult patients with CHF).

Analysis of PK-PD Data:

To explore a possible PK-PD relationship linear regressions of mean PAP, mean RAP, cardiac index or PVRI on Cmax or AUC were performed.

Analysis of PD Parameters:

Cardiopulmonary hemodynamic parameters and the results of the 6 minute walk test, Borg dyspnea index and cycle ergometry test were summarized descriptively as the change from baseline to week 12 using mean and median 95 % confidence limits. For exploratory purposes p-values were computed for the hemodynamic and exercise parameters using the non-parametric paired signed rank test. The proportion of patients who improved in WHO functional class following treatment were displayed with 95 % confidence limits. Hemodynamic parameters were additionally analyzed descriptively in subgroups defined whether or not they received concomitantly epoprostenol.

Analysis of Safety Data:

Adverse events were coded, tabulated by body system and preferred term, and summarized using frequency counts and proportions. Death, hospitalizations, and premature discontinuations of study treatments were coded by reason or event and were summarized separately as were SAEs. Laboratory test values, quantitative ECG variables, vital signs, body weight, and height were summarized descriptively for all enrolled patients using summary statistics for continuous data. Laboratory values outside the normal range were flagged. Listings of patients with abnormal laboratory values were provided.

RESULTS:

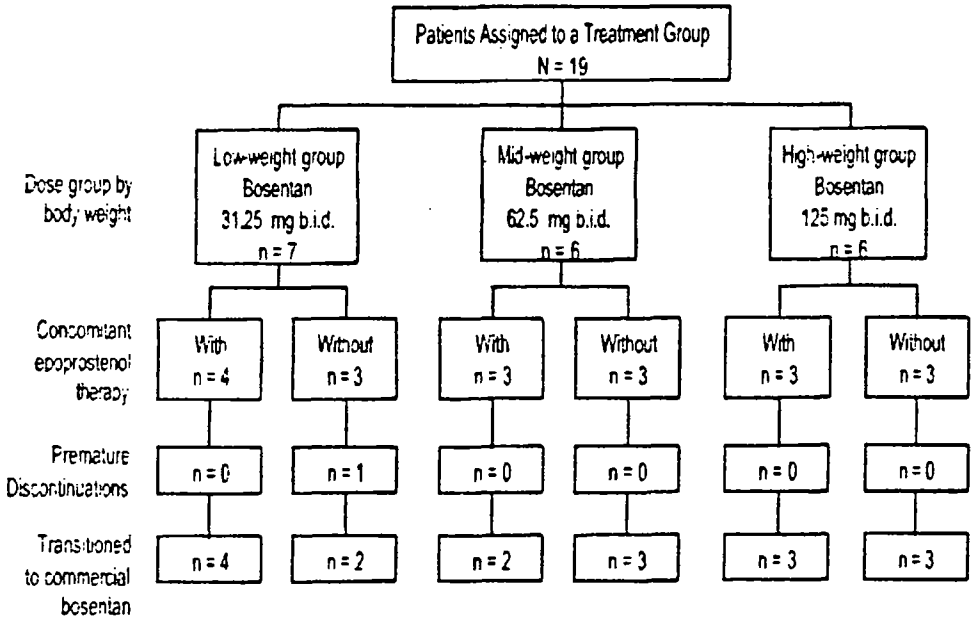
Disposition of Patients:

Nineteen (19) pediatric patients were enrolled in the study. One patient was prematurely discontinued on Day 7 of the treatment because of elevated liver enzymes. An additional patient was discontinued on Day 97 due to an abnormal liver function test. The patient had completed the PK and PD evaluation after 12 weeks of treatment. PK and PD parameters were evaluated in 18 patients and the safety parameters in all 19 subjects enrolled. The respective median body weights of the patients in the three groups were 47, 33 and 16kg which indicated that the individuals in the group weighing > 40kg received a disproportionately larger dose than the others. The majority of patients (78.9%) were Caucasians. The number of female and male patients participating in the study were similar (47.4% males, 52.6% females). The majority of the patients were in WHO functional class II (78.9%) and a minority in Class III (21.1%). All 19 patients suffered from either primary pulmonary hypertension (n=10) or pulmonary arterial hypertension related to congenital systemic to pulmonary communications (n=9). The demographics of the enrolled patients are shown in Table 3 and their disposition in Figure 2.

Table 3. Demographic Characteristics of the Enrolled Pediatric Population

	Patients 10 - 20 kg N=7	Patients >20 - 40 kg N=6	Patients > 40 kg N=6	All N=19
SEX [n (%)]				
n	7	6	6	19
Males	4 57.1%	2 33.3%	3 50.0%	9 47.4%
Females	3 42.9%	4 66.7%	3 50.0%	10 52.6%
AGE (years)				
n	7	6	6	19
Mean	5.7	10.0	14.2	9.7
Standard deviation	1.9	2.4	1.2	4.0
Median	6.0	10.5	14.5	10.0
Min , Max				
AGE [n (%)]				
n	7	6	6	19
< 6 years	5 71.4%	1 16.7%	-	6 31.6%
6 - 12 years	2 28.6%	4 66.7%	1 16.7%	7 36.8%
13 - 17 years	-	1 16.7%	5 83.3%	6 31.6%
WEIGHT (kg)				
n	7	6	6	19
Mean	17.1	31.0	46.5	30.8
Standard deviation	2.5	6.4	5.5	13.3
Median	16.0	33.3	47.3	33.1
Min , Max				
HEIGHT (cm)				
n	7	6	6	19
Mean	108.8	135.9	156.8	132.5
Standard deviation	8.0	11.2	6.1	22.0
Median	108.0	136.4	157.8	133.1
Min , Max				
RACE [n (%)]				
n	7	6	6	19
Caucasian/White	6 85.7%	6 100%	3 50.0%	15 78.9%
Other	1 14.3%	-	3 50.0%	4 21.1%

Figure 2. Disposition of the Patients



Analytical:

The results of the validation report indicated that the performance of the assay fulfilled the requirements for an accurate and precise analytical method. The standard curves for bosentan over the concentration range of . —— ng/mL and for the metabolites (Ro 47-8634, Ro 48-5033, Ro 64-1056) in the range —— ng/mL were linear. The LLOQ was set at — ng/mL for bosentan and — ng/mL for the 3 metabolites. The linearity of the calibration curves was demonstrated by correlation coefficients ≥ 0.996 for bosentan, Ro 47-8634 and Ro 64-1056 and ≥ 0.995 for Ro 48-5033. The inter-assay precision (CV%) of the QC samples was — % for bosentan, — % for Ro 48-5033, — % for Ro 47-8634 and — % for Ro 64-1056. The inter-assay inaccuracy for QC samples was between - — and . —- for bosentan, — and . — for Ro 48-5033, — % and - % for Ro 47-8634 and - — and — % for Ro 64-1056. The assays were performed by

Pharmacokinetics:

The preliminary PK results obtained on the first 4 patients belonging to the intermediate body weight group receiving a single 62.5mg dose of bosentan showed no unexpected differences when compared to healthy adults.

The mean plasma concentrations of bosentan and the metabolites by body weight group are depicted in Figure 3 and the mean parameters are listed in Table 3.

Figure 3. Semilogarithmic Plots of the Arithmetic Mean (SEM) Plasma Concentrations of Bosentan and Metabolites

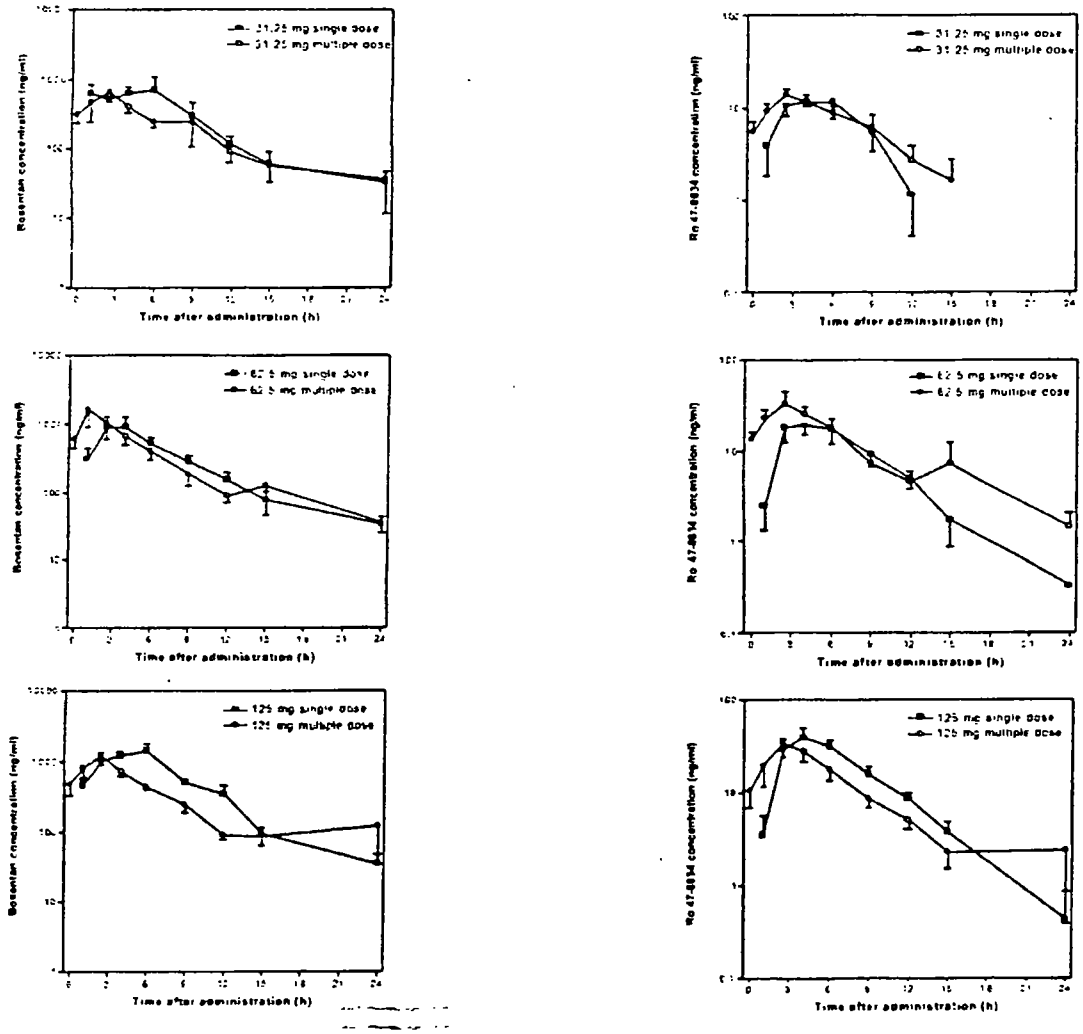
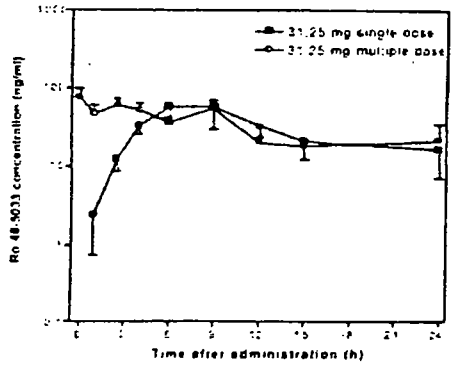
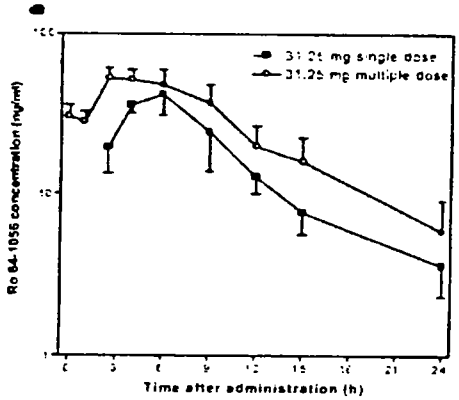
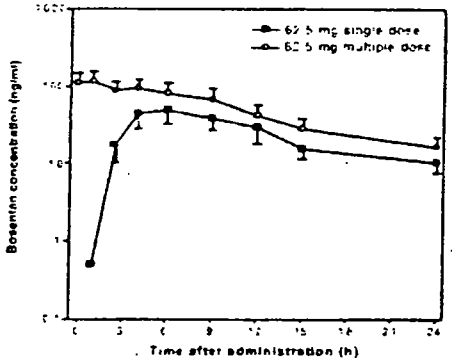
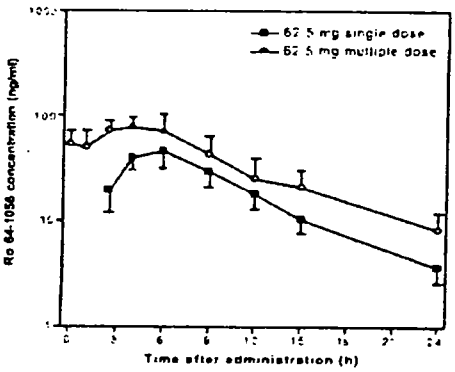


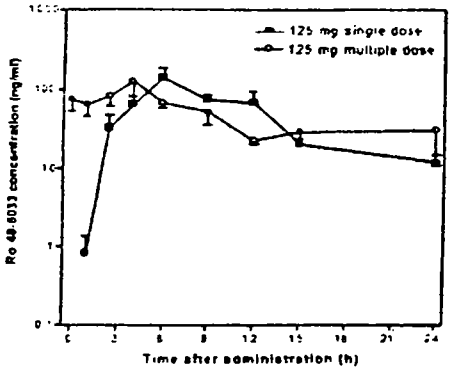
Figure 3. Semilogarithmic Plot of the Arithmetic Mean (SEM) Plasma Concentrations of Bosentan and Metabolites Continued











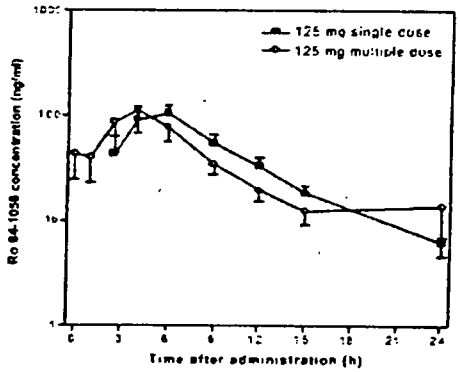


Table 4. Geometric Means (Coefficient of Variation) of Pharmacokinetic Measures and Parameters for Bosentan and its Metabolites in 18 Pediatric Patients after Single (SD) and Multiple Dose (MD) Administration

Bosentan					
Treatment	$AUC_{0-\infty}$ (ng•h/ml)	AUC_{τ} (ng•h/ml)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}(\beta)$ (h)
31.25 mg SD	5453 (56)		959 (69)	1.0 (1.0 - 6.0)	4.7 (40)
31.25 mg MD		3496 (49)	685 (77)	2.5 (0.0 - 9.0)	6.0 (61)
62.5 mg SD	6118 (55)		815 (108)	2.5 (1.0 - 4.0)	5.3 (35)
62.5 mg MD		5428 (79)	1136 (85)	1.0 (0.0 - 2.5)	5.6 (25)
125 mg SD	10777 (32)		1709 (39)	4.0 (2.5 - 6.0)	4.2 (44)
125 mg MD		6124 (27)	1200 (50)	1.8 (1.0 - 6.0)	5.3 (38)
Ro 47-8634					
Treatment	$AUC_{0-\tau}$ (ng•h/ml)	AUC_{τ} (ng•h/ml)	C_{max} (ng/ml)	t_{max} (h)	
31.25 mg SD	71.8 (45)		15.4 (45)	3.3 (2.5 - 6.0)	
31.25 mg MD		91.5 (38)	13.8 (34)	2.5 (1.0 - 9.0)	
62.5 mg SD	136 (72)		18.6 (91)	4.0 (2.5 - 12.0)	
62.5 mg MD		176 (81)	31.8 (72)	2.5 (1.0 - 15.0)	
125 mg SD	253 (52)		43.6 (48)	4.0 (2.5 - 6.0)	
125 mg MD		178 (64)	29.8 (71)	2.5 (2.5 - 4.0)	
Ro 48-5033					
Treatment	$AUC_{0-\tau}$ (ng•h/ml)	AUC_{τ} (ng•h/ml)	C_{max} (ng/ml)	t_{max} (h)	
31.25 mg SD	492 (80)		52.9 (73)	6.0 (4.0 - 12.3)	
31.25 mg MD		511 (41)	87.6 (46)	1.7 (0.0 - 9.2)	
62.5 mg SD	465 (86)		46.3 (110)	6.0 (4.0 - 15.0)	
62.5 mg MD		712 (115)	95.0 (103)	0.0 (0.0 - 3.8)	
125 mg SD	946 (60)		106 (89)	6.0 (4.0 - 6.9)	
125 mg MD		713 (53)	114 (86)	5.0 (0.0 - 9.0)	
Ro 64-1056					
Treatment	$AUC_{0-\tau}$ (ng•h/ml)	AUC_{τ} (ng•h/ml)	C_{max} (ng/ml)	t_{max} (h)	
31.25 mg SD	333 (35)		48.5 (34)	4.0 (2.5 - 6.0)	
31.25 mg MD		450 (37)	61.4 (43)	3.9 (0.0 - 9.0)	
62.5 mg SD	349 (105)		40.4 (116)	6.0 (4.0 - 12.0)	
62.5 mg MD		468 (141)	70.9 (111)	2.5 (0.0 - 6.0)	
125 mg SD	807 (52)		104 (58)	4.0 (4.0 - 6.0)	
125 mg MD		601 (70)	85.8 (98)	4.0 (0.0 - 6.0)	

Table 5 lists the dose and body weight normalized exposure measures in the 3 pediatric groups pediatric patients and the corresponding data in healthy adult volunteers for comparison. The non-normalized data are also presented for the 2 populations.

Table 5. Dose and Body Weight normalized Geometric Mean Measures of Exposure in the 3 Pediatric Groups and Comparison of the Non-Normalized and Normalized Data in the Pediatric Patients and in Healthy Adults

Population	Dose	AUC_{∞} SD (ng•h/ml)	AUC, MD (ng•h/ml)	AUC_{∞} SD* (ng•h/ml)	AUC, MD* (ng•h/ml)
Healthy volunteers	31.25 mg	2049[1]	NA	4616	NA
Pediatric patients	31.25 mg	5453	3496	2879	1846
Healthy volunteers	62.5 mg	4234[2]	2744[2]	5303	3437
Pediatric patients	62.5 mg	6118	5428	3035	2692
Healthy volunteers	125 mg	8791[3]	4586[4]	5486	2862
Pediatric patients	125 mg	10777	6124	4009	2278

Population	Dose	C_{max} SD (ng/ml)	C_{max} MD (ng/ml)	C_{max} SD* (ng/ml)	C_{max} MD* (ng/ml)
Healthy volunteers	31.25 mg	333[1]	NA	750	—
Pediatric patients	31.25 mg	959	685	506	362
Healthy volunteers	62.5 mg	617[2]	516[2]	769	643
Pediatric patients	62.5 mg	815	1136	404	563
Healthy volunteers	125 mg	1612[3]	1006[4]	1006	616
Pediatric patients	125 mg	1709	1200	635	446

* = Corrected for body weight and dose (value divided by dose mean weight).
[1] From Study AC-052-110 (n = 10).
[2] From Study AC-052-108 (n = 10).
[3] From Study AC-052-106 (n = 16).
[4] From Study AC-052-109 (n = 9).
MD = multiple dose, NA = not available, SD = single dose.

The examination of the impact of the dose of bosentan on the PK of the parent drug and the metabolites after single and multiple doses across the 3 pediatric groups is confounded by the difference in body weights and developmental age of the children investigated. The mean doses (range) in mg/kg were 2.7 — mg/kg in the children weighing > 40kg, 2.1 — mg/kg in the children weighing > 20 to 40kg and 1.9 (— ' mg/kg in the children weighing between 10 and 20 kg, indicating that the respective doses in mg/kg of the children of the heavy and intermediate groups were on average 42.1% and 10.5% larger than the mean dose administered to the light weight group. The intersubject variation of the PK parameters of bosentan and the metabolites expressed as coefficient of variation about the geometric mean values ranged between about 30% to 140% and was largest in the intermediate weight group. Among the different covariates

tested including gender, body weight and epoprostenol therapy, only dose after single administration had a statistically significant impact on the PK parameters of bosentan.

After single dose administration the exposure to bosentan and the metabolites were different in the 3 body weight groups. The respective AUC values in the heavy and intermediate weight groups were 97.6% and 12.2% greater than in the light weight group. The Cmax value in the heavy weight group was 78.2% and 109.7%, respectively, greater than the corresponding value in the intermediate and light body weight groups. The dose and body weight normalized AUC value for bosentan in the heavy group exceeded the corresponding values of the intermediate and light body weight groups by 39.2% and 5.4%, respectively. The dose and weight normalized Cmax value of the heavy weight group was 57.2% and 25.5% respectively, greater than the corresponding values of the intermediate and light weight groups.
The results suggested that the dose regimen used in the study did not provide comparable exposures to the children in the 3 weight groups. There was also evidence that the PK of bosentan was either development age dependent and/or nonlinear.

After multiple dose administration the respective mean AUC values in the heavy and intermediate weight groups exceeded the corresponding value in the light weight group by 75.2% and 55.3%, respectively. Similarly, the mean Cmax values of the heavy and intermediate weight groups were 75.2% and 65.8%, respectively, larger than in the light weight group. After normalization for dose and weight the respective AUC values in the heavy and intermediate weight groups were 23.4% and 45.8% greater than in the light weight group. The normalized Cmax values of the heavy and intermediate weight groups were still 23.2% and 55.5% greater than the corresponding value in the light weight group.
The results confirmed that the dose regimen applied in this study did not generate an equivalent exposure to bosentan in the children of the 3 groups studied. The multiple dose data also corroborated that the PK of bosentan are not linear and/or subject to developmental changes.

Consistent with autoinduction of the metablism of bosentan the ratios of AUCτ to AUC0-∞ in the 3 groups tended to be smaller than 1.0 (range: —). The corresponding ratios for the metabolites in the light and intermediate groups tended to exceed 1.0 (range: . —). This was in contrast to the heavy weight group who displayed mean ratios smaller than 1.0 (range: — . The t1/2β values for bosentan after multiple doses tended to be slightly greater than following single dose administration. The terminal half-life of the metabolites could not be determined because the terminal plasma concentrations were below the LLOQ of the assay method. Repeat administration of bosentan resulted in smaller tmax values for bosentan and metabolites compared to single dose administration.

Ro 48-5033, known to exert up to 20% of the activity of bosentan, was the most prominent among the metabolites after single and multiple dose administration. The overall exposure to the metabolites relative to bosentan increased in each of the 3 groups from 15.5% to 18.6% after single dose administration to 24.4% to 30.1% following

repeat dosing. These findings confirmed that repeat administration of the drug increases its oral clearance.

A comparison of the non-normalized data indicated that the exposure to bosentan in the pediatric population was larger than in adult healthy volunteers at identical dose levels suggesting a difference in the oral clearance between the 2 populations. The difference was most pronounced in the pediatric group with the lightest body weight receiving 31.25mg of the drug. In contrast, the data normalized for dose and body weight indicated that when bosentan is administered in single or multiple doses, healthy adult subjects experience a larger exposure than children with the target disease.

Efficacy:

A summary of the PD parameters is depicted in Table 5.

Patient number (wt group)	Diagnosis	WHO class (BL / Wk 12)	Change in mean PAP / cardiac index	6-min walk distance (meters)		Peak VO_2 (ml/min)	
				BL	Week 12	BL	Week 12
With epoprostenol							
1001 (H)	CHD	III / III	↓ / ↑	438	371	682	648
1004 (M)	PPH	III / II	↓ / ↓	397	339	367	311
1005 (L)	CHD	II / II	↓ / ↓	—	—	—	—
1007 (L)	PPH	II / I	↑ / ↑	—	—	—	—
1009 (H)	PPH	II / II	↑ / ↑	456	450	1353	1515
1010 (L)	CHD	II / III	↓ / na	424	429	279	288
2023 (H)	PPH	II / II	↓ / ↑	416	500	926	783
2024 (M)	PPH	II / II	↓ / ↑	523	462	770	751
2027 (L)	PPH	II / II	↓ / ↓	—	—	—	—
2028 (M)	PPH	II / II	↓ / ↑	—	—	—	—
Without epoprostenol							
1002 (M)	CHD	II / II	↓ / ↑	487	505	324	389
1003 (M)	CHD	II / II	↓ / ↓	569	512	908	884
1008 (H)	CHD	III / II	↓ / ↓	485	498	813	797
2021 (M)	PPH	II / II	↑ / ↑*	604	638	993	1299
2022 (H)	PPH	II / I	↓ / ↓	527	643	1602	1887
2025 (L)	CHD	II / II	↓ / ↑	—	—	—	—
2026 (L)	CHD	II / II	na	—	—	—	—
2029 (H)	CHD	III / II	↓ / ↑	573	560	560	660
2030 (L)	PPH	II / II	↓ / ↑	—	—	—	—

↓ Values decreased from baseline to Week 12.

↑ Values increased from baseline to Week 12.

* Improvement seen with bosentan at Week 12; addition of i.v. epoprostenol at 6 months.

— Missing values indicate patient was <8 years; per protocol, no exercise test was performed.

BL = baseline, CHD = congenital heart defect, H = high-weight group, L = low-weight group, M = mid-weight group, na = not available, PPH = primary pulmonary hypertension, WHO = World Health Organization, wt = weight.

Cardiac output could be measured in 17 of the 18 patients completing the study. All the other not cardiac output derived parameters were available from 18 patients. Statistically significant improvements compared to baseline were observed for the cardiopulmonary hemodynamic parameters mean PAP, PVR, PVRI, mean SAP, SVR and SVRI, cardiac output and stroke index. The changes in the exercise parameters were highly variable and none were statistically significant. Five patients belonging to different weight groups improved by one WHO functional class. One subject deteriorated by one functional class and for 12 individuals the WHO functional class did not change during the treatment.

The hemodynamic variables in subjects on bosentan alone or on a combination treatment of bosentan and epoprostenol were similar and there was no statistically significant difference. Patients on bosentan alone showed a trend for a larger decrease in PVRI compared to subjects on both drugs.

PK-PD: Except for a statistically significant correlation between mean PAP and Cmax there existed no other relationships between exposure-and effect measures.

Safety: Safety data were reported for all 19 subjects enrolled in the study. Seventeen (17) of the 19 patients in the study reported at least one AE. Among these were two SAEs. One patient (intermediate body weight group) had ALT elevated to >3•ULN after 5 days of treatment with bosentan. This was considered a SAE by the investigator and the patient was withdrawn on Day 7 of the treatment. Another event in another subject presenting with moderate tachycardia, tremor, hypertension and dizziness led to a prolonged hospitalization and thus the event was classified as SAE. A third patient had bosentan discontinued on Day 197 following recurrent elevation of ALT >3•ULN. The most frequent adverse events were flushing (four patients), headache and abnormal hepatic function (three patients each), dizziness, fluid retention, aggravated PAH, pyrexia and a variety of infections (two patients each). Of these AEs abnormal hepatic function (three patients), flushing, headache, edema, nausea and hypotension (1 patient each) were considered to be possibly or probably drug related. The liver function anomalies in one of the patients with ALT elevations >3•ULN were reversible but only after 8 weeks, whereas in the other patient the exact time to normalization was not known. No decrease in hemoglobin or hematocrit was observed. During the extended treatment period of the trial 3 patients had their concomitant epoprosterol dose gradually decreased while continuing the protocol specified bosentan treatment.
There was no evidence suggesting that the AES were dose or body weight related.

Conclusions:

The exposure measures for bosentan and metabolites in the 3 weight groups of children with PAH WHO functional class II or III who received single and multiple doses of 31.25, 62.5 and 125mg of bosentan were different. The AUC and Cmax values after single and multiple dose administration were significantly greater in the heavy and

intermediate weight groups than in the light weight group indicating that the applied dose regimen did not provide comparable plasma concentrations of bosentan. After normalization for dose and weight the exposure measures were still different in the 3 groups suggesting nonlinear and/or development age dependent PK of bosentan. Repeat administration of bosentan to the pediatric patients resulted in an increase of the oral clearance presumably by autoinduction of CYP 3A4 and 2C9 known to metabolize bosentan. Gender and the co-administration of epoprostenol did not appear to impact the PK of bosentan.
Compared to healthy adult volunteers the exposure measures of bosentan after repeat administration of comparable doses were larger in the pediatric patients suggesting that the oral clearance in the children is smaller than in healthy adults.

Reviewer's Comments:

1.The PK findings of the study ought to be inserted in the label only if based on the clinical endpoints the respectively used dose regimens of 31.25mg , 62.5mg and 125mg bid are determined to be effective and safe in the 3 pediatric groups.
2.The comparison of the pediatric and adult exposure data of bosentan in an attempt to evaluate the doses to be used in the pediatric population is hampered by a lack of information on dose proportionality of the PK of bosentan in the pediatric population. In the trial the children of the 3 weight groups received one dose level only and the doses differed among the groups. In adults patients the oral clearance of bosentan is dose dependent and hence linearity of the PK of bosentan in children cannot be assumed. A comparison of exposure measures in children with PAH and healthy adults, as proposed by the sponsor, is not appropriate. The oral clearance in adult patients is about 50% of that in healthy adults. Table I lists the respective exposure measures observed in adult patients with PAH and adult healthy volunteers

Table I. Comparison between Geometric Mean Exposure Measures in Adult Patients with PAH and Healthy Volunteers

Population	Dose	AUC_τ (ng·h/ml)	C_{max} (ng/ml)
Healthy subjects*	62.5 mg twice daily	2857	544
PAH patients	62.5 mg twice daily	6232	1187
Healthy subjects†	125 mg twice daily	4804	1083
PAH patients	125 mg twice daily	8912	2286

Values are arithmetic means.

* From Study AC-052-108 (n = 10 [21]).

† From Study AC-052-109 (n = 9 [22]).

The data in Table I indicate that the mean oral clearance in adult patients with PAH at the 62.5mg and 125mg dose levels is 54.1% and 46.1% respectively, smaller than in healthy adult volunteers.

Table II lists the respective exposure measures for bosentan in the pediatric and adult populations with PAH.

Table I. Comparison of the Geometric Mean Exposure Measures for Bosentan in Pediatric*and Adult# Populations with PAH

Dose Level mg	AUCτ ng/mL•h		Cmax ng/mL	
	Children	Adults	Children	Adults
31.25	3496	na	685	na
62.5	5428	5739	1136	1062
125	6124	8149	1200	1878

AC 052-356 # AC 052-357 na = not available

The data from Table II indicate that the AUCτ and Cmax values of bosentan in the children of the intermediate weight group receiving 62.5mg bosentan bid were 5% smaller and 7% greater, respectively, than the corresponding values in adults receiving the same regimen. The AUCτ and Cmax values in the children of the heavy weight group were 25% and 36 % smaller than in adults receiving the same 125mg bid regimen. There were no data in adult patients at the 31.25mg bid dose level and the exposure data of the light weight pediatric group were compared to those in adult patients administered 62.5mg or 125mg bid. The comparisons showed that AUCτ and Cmax in the children of the light weight group were 39% and 36%, respectively, smaller than in adult patients receiving the 62.5mg bid regimen and 57% and 64% smaller, respectively than the corresponding values in adult patients receiving 125mg bosentan bid.

In conclusion only the dose regimen of 62.5mg bosentan bid administered to the intermediate weight group provided an exposure to bosentan that was comparable to that in adult patients receiving the same dose regimen. However, even for the intermediate weight group one cannot assume that a treatment with 125mg bosentan bid would result in an exposure comparable to that in adult patients receiving the same regimen. This is because the PK of bosentan in adults are nonlinear (Table II) and thus assuming dose proportionality of the PK of the drug in the children is unwarranted. These findings

indicated that the dose regimens of bosentan applied in this trial resulted in 1. Different exposures among the 3 pediatric weight groups 2. Exposures in at least 2 of the 3 pediatric groups that differed from those in adult patients receiving the labeled dose regimens of 62.5 and 125mg bosentan bid. Unfortunately, the presently available information on the PK of bosentan in the pediatric patients does not allow determination of dose regimens that would result in exposures comparable to those in adult patients receiving 62.5mg or 125mg bosentan bid. The PK information obtained in the pediatric trial does not support the appropriateness of the adjusted dose regimens administered to the children in this trial. The evidence must come from the evaluation of the clinical endpoints.

3. The administration of tablets to preschool children is problematic. The need for cutting the 62.5mg tablet in half leads to imprecise dosing.

AC-052-357 Final Study Report

Study Title: Multicenter, Open Label, Single-Arm, Safety Study of Bosentan in Patients with Pulmonary Arterial Hypertension

Principal Investigator:

This was a multicenter study involving 13 centers in the US. The pharmacokinetic sub-study was performed at one center (RJ Barst, New York Presbyterian Hospital, New York).

Objectives :

Obtain safety data of bosentan in patients with pulmonary arterial hypertension (PAH) and provide bosentan treatment to patients with symptomatic PAH

Subjects:

115 female and male patients were enrolled and 13 patients participated in the PK substudy. To be eligible the patients had to be ≥12 years of age, have a body weight of ≥40 kg and suffer from PAH (primary or secondary to connective tissue or autoimmune disease such as scleroderma or systemic lupus erythematodes or related to HIV), have a mean pulmonary arterial pressure >25mm Hg and pulmonary wedge pressure (PCWP)<15 mm Hg and be in WHO functional class III or IV and prostacyclin naïve. Because bosentan was shown to be fetotoxic and teratogenic in animal studies, females of child bearing potential were to use an acceptable contraceptive method that included

barrier type devices in combination with a spermicide, intrauterine devices, oral contraceptives or hormonal implants.

Exclusion criteria included PAH due to conditions other than those defined in the inclusion criteria, unstable PAH disease, any degree of liver impairment, systolic blood pressure< 85mm Hg, ALT and/or AST > 3•ULN, coadministration of glibenclamide, cyclosporine A, tacrolimus, known drug or alcohol abuse, any illness other than PAH that would reduce life expectancy to less than 6 months.

Methodology:

This was an open, multicenter, single arm, safety study of bosentan. Table 1 shows the schedule of activities.

Table 1. Schedule of Activities

Table 1 Schedule of assessments

Treatment Month (± 5 days)	Initiation	1	2	3	4	5	6	Every 3 months	
Visit	Visit 1	Visit 2	Visit 3 or phone call (a)	Visit 4	Visit 5 or phone call (a)	Visit 6 or phone call (a)	Visit 7	Visits 8+	End of study (b)
Informed consent	X								
PAH history	X								
Relevant concomitant diseases	X								
Concomitant PAH medications	X								
Vital signs (blood pressure, heart rate) and body weight	X								X
Laboratory tests (c)	X(d)	X	X	X	X	X	X	X	X
Serum pregnancy test (d)	X								X
WHO functional class	X	X		X			X	X	X
Pharmacokinetic sampling (e)		X	X						
Dispense/return study medication	X	X		X			X	X	X
Death, other SAEs, and AEs (including MLAs) leading to dose reduction, temporary interruption or permanent discontinuation		X	X	X	X	X	X	X	X

(a) The patient was asked to attend a designated facility (study site clinic or private clinic, based on geographical region of patient's residence) for a blood draw, and samples were to be dispatched to the Central Laboratory for routine laboratory testing. If the patient attended the study clinic, a new bottle of bosentan was dispensed. If the patient attended a private clinic, the investigator was to be immediately informed by fax that the procedure had occurred, whereupon he/she dispatched a new bottle of bosentan to the patient's home by Federal Express.

(b) At permanent withdrawal, when bosentan became commercially available, or when the sponsor stopped the study. However, for an appropriate assessment of safety, a minimum of 3 months of data was to be collected for every enrolled patient, even after bosentan became commercially available.

(c) Laboratory tests included hematology tests (hemoglobin, hematocrit, erythrocytes, leukocytes, differential, and platelets) and liver function tests (AST, ALT, alkaline phosphatase, bilirubin direct and indirect). Reticulocytes and creatinine were measured at baseline (but followed if abnormal) and at the end-of-study visit.

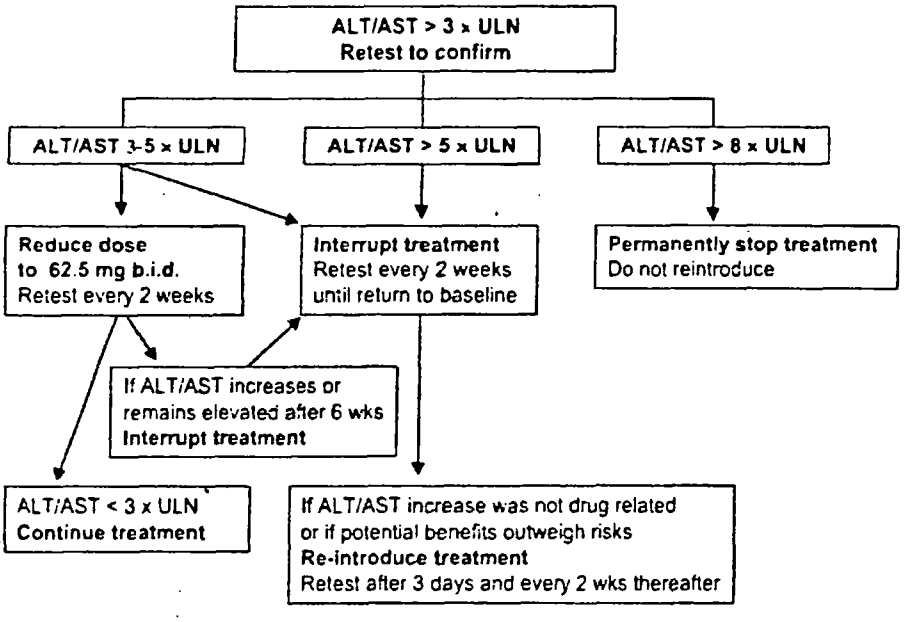
(d) Two blood samples were to be obtained to expedite routine laboratory and pregnancy screens. One sample was to be sent to the local institutional laboratory and results used for patient eligibility screening only. The second sample was to be dispatched to the Central Laboratory, and these results were entered as the baseline laboratory and pregnancy assessments. A serum pregnancy test was also to be performed (as appropriate) at end of the study and in case a pregnancy was suspected, as per the investigator's judgment.

(e) Pharmacokinetic sampling performed in a subset of patients predose and at 1, 2, 3, 4, 6, 9 and 12 hours post dose.

AE = adverse event, MLA = marked laboratory abnormality, PAH = pulmonary arterial hypertension, SAE = serious adverse event, WHO = World Health Organization.

At screening the patients gave written informed consent. Subsequently a medical history was taken, a physical examination performed, the functional WHO class determined and blood samples for laboratory and pregnancy tests (females) taken. The patients had scheduled visits every month for the first 8 months. Later visits occurred every three months until study termination. The treatment consisted of 62.5mg bosentan bid for the first 4 weeks and, provided this dosage level was tolerated well, the dose was increased to 125mg bosentan bid for the remainder of the study. The doses were taken fasted or fed in the morning and in the evening. If the dose of 62.5mg bid was not up-titrated after 4 weeks of treatment the up- titration could be performed at a later visit. If 125mg bid was not tolerated the dose could be reduced to 62.5mg. In this case the dose was to be reduced gradually over a period of 3-7 days. If the patient's clinical status deteriorated the dose could be increased from 125mg to 250mg bid. However this was only permitted if the patient was at least 3 months on therapy, and the monthly conducted liver function tests were < 3 •ULN. At the end-of-study- or permanent withdrawal visit, the vital signs were measured, the WHO functional class determined and routine laboratory tests performed. The study was to end when bosentan became commercially available or when the sponsor stopped the study. Patients were to complete at least 3 months of treatment.

Bosentan has been associated with reversible, dose related increases in liver aminotransferases in previous studies. For ALT and/or AST values ≤ 3 •ULN, the investigator was to continue treatment with either the initial or the target dose, as appropriate. For values > 3•ULN, a new blood sample was to be re-tested immediately by the Central Laboratory to confirm the results. If the abnormal liver function test occurred without associated symptoms of liver disease the investigator was to follow the guidelines of Figure 1.



If elevations in ALT and/or AST >3 •ULN were associated with symptoms of liver disease liver enzymes were to be re-tested immediately to confirm the results. If confirmed bosentan treatment was to be stopped permanently. Liver function tests were to be repeated every 2 weeks until values returned to baseline. Bosentan was not to be reintroduced to patients whose elevated liver enzymes were associated with symptoms of liver disease.
Modest reductions of hemoglobin without evidence of hemolysis, hemorrhage or bone marrow toxicity have been observed in some patients treated with bosentan. If hemoglobin was reduced by at least 15% from baseline and was <10g/dL, and/or hematocrit was decreased by at least 15% from baseline and was < 30 in absolute terms, a repeat test was to be performed to confirm presence of abnormality. If the abnormality was confirmed a complete blood count including reticulocytes, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, red cell distribution width were to be determined and direct inspection of blood smears performed.

Study Drug:

Bosentan (Ro 47-0203) 62.5mg film-coated tablets, Batch number F0277A001, and 125mg film-coated tablets, batch number F0535A001 were supplied by the sponsor. The study medication was manufactured by Patheon Ltd, Canada, packaged, labeled and distributed by [redacted] and dispensed to the US Centers by [redacted]

Prior and Concomitant Therapy:

All patients were to be receiving optimal therapy for PAH, including oral anticoagulants, oral vasodilators, cardiac glycosides, diuretics and/or supplemental oxygen. Concurrent treatment with glibenclamide (glyburide), cyclosporine A, tacrolimus (FK-506) was not permitted.

Evaluation:

Pharmacokinetics: The PK of bosentan and the metabolites Ro 47-8634, Ro 48-5033 and Ro 64-1056 were to be determined at steady-state after at least 2 weeks of treatment with the lower and higher dose.
Efficacy: WHO functional class was assessed in all patients at baseline, and after treatment at the 62.5 and 125mg dose levels.
Safety: Safety was evaluated in all enrolled patients. The parameters assessed included death, other serious adverse events, adverse events leading to dose reductions, or temporary or permanent discontinuation of study medication, and marked laboratory abnormalities of liver enzymes, hemoglobin and hematocrit. Laboratory test including hematology (hemoglobin, hematocrit, erythrocytes, platelets, leucocytes and differential)

and liver function tests (AST, ALT, alkaline phosphatase and bilirubin (direct and indirect)) were performed at each scheduled visit.

Blood Sample Collection for PK:

Patients participating in the PK substudy had blood samples taken over one dosing interval (12 hours) for PK profiling at steady-state after at least 2 weeks of treatment with bosentan 62.5mg bid and again after at least 2 weeks treatment with 125mg bid. Ideally the blood sampling occurred during visits 2 and 3. On these occasions the patients took thei morning dose of 62.5 or 125mg bosentan in the clinic between 7 and 10 am and within 30 minutes after consuming a light breakfast. Four (4) mL blood samples were collected prior to and 1, 2, 3, 4, 6, 9 and 12 hours following drug administration.

Analytical Methodology:

Plasma concentrations of bosentan and the metabolites Ro 47-8634, Ro-5033 and Ro 64-1056 were determined by [ ] method [ ]

Pharmacokinetic Parameters:
Estimates were obtained for the following PK parameters:

Cmax	The observed maximum plasma concentration
tmax	The time to appearance of Cmax
AUCτ	The area under the concentration time curve during the dosing interval

Statistical Methods:

The total number of subjects enrolled in the study or in the PK sub-study was not based on statistical power considerations.

Analysis of PK Data:

AUCτ was obtained on application of the linear trapezoidal rule. Compartment model independent methods using professional WinNonlin Version 3.3. were employed. Overall exposure to the metabolites was obtained by summing up the mean AUCτ values of each of the metabolites and compare the sum to the AUCτ of bosentan. Possible effects of the primary or secondary etiology of PAH on the PK of bosentan were explored. Also the possible impact of gender on the PK of bosentan was explored.

Analysis of Safety Data:

Demographics and baseline characteristic were summarized descriptively. Serious adverse events or adverse events that led to a change in study medication were coded,

tabulated by body system and preferred term, and summarized by simple descriptive statistics. Reasons for death were similarly coded and tabulated. Marked abnormalities of laboratory values were tabulated.
The proportion of patients who improved in WHO functional class were displayed with 95% CI.

RESULTS:

Disposition of Patients

A total of 115 patients were enrolled in the study including the 13 patients who participated in the PK sub-study. Twenty (20) patients were prematurely discontinued from the study. Of these 3 transferred early to commercial bosentan. Four (4) patients died, 10 patients had treatment discontinued because of an adverse event, 1 patient showed a lack of improvement and 2 patients were lost to follow-up. About 70% of the patients had primary PAH and about 20% were in WHO functional class III. The majority of the patients were female and Caucasian. The age ranged between 10 and 80 years. However, there was only 1 patients younger than 18 years old.
A summary of the demographics of the patients is provided in Table 2.

Table 2. Demographics of Enrolled Patients

Protocol: AC-052-357 (Table DEM01: 15JUL02 - Data 15JUL02)

	Bosentan 250mg/d N=115
SEX [n (%)]	
n	115
Males	27 23.5%
Females	88 76.5%
AGE (years)	
n	115
Mean	50.7
Standard deviation	14.9
Median	50.0
Min , Max	
AGE [n (%)]	
n	115
8 - 12 years	1 0.9%
18 - 40 years	26 22.6%
41 - 60 years	56 48.7%
> 60 years	32 27.8%
WEIGHT (kg)	
n	115
Mean	79.6
Standard deviation	22.7
Median	74.8
Min , Max	
HEIGHT (cm)	
n	114
Mean	164.1
Standard deviation	10.7
Median	163.0
Min , Max	
RACE [n (%)]	
n	115
Caucasian/white	90 78.3%
Black	7 6.1%
Asian	6 5.2%
Other	12 10.4%

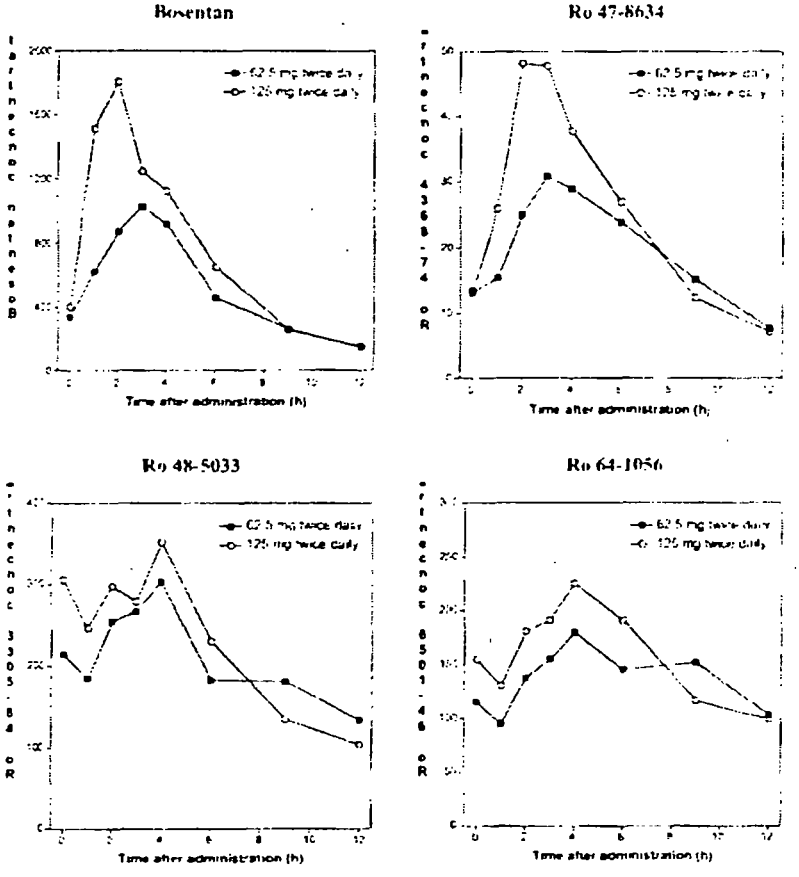
Analytical:

The results of the validation report indicated that the performance of the assay fulfilled the requirements for an accurate and precise analytical method. The standard curves for bosentan in the concentration range from [redacted] ng/mL and for the metabolites (Ro 47-8634, Ro 48-5033, Ro 64-1056) in the concentration range from [redacted] g/mL to [redacted] /mL were linear. The LLOQ was set at [redacted] ng/mL for bosentan and [redacted] ng/mL for the metabolites. The linearity of the analytical method was demonstrated by correlation coefficients ≥ 0.9968 for bosentan, ≥ 0.9944 for Ro 48-5033, ≥ 0.9961 for Ro 47-8634 and ≥ 0.9909 for Ro 64-1056. The interassay precision (CV%) of the QC samples was [redacted] % for bosentan, [redacted] % for Ro 48-5033, [redacted] % for Ro 47-8634 and ≤ [redacted] % for Ro 64-1056. The interassay accuracy was between [redacted] % to [redacted] % for bosentan, between [redacted] % and [redacted] % for Ro 48-5033, between [redacted] % and [redacted] for Ro 47-8634 and between [redacted] % and [redacted] % for Ro 64-1056. The assays were performed at [redacted]

Pharmacokinetics:

Thirteen patients received 62.5mg bosentan bid for 4 to 5 weeks before the first PK assessment. In one patient blood sampling was only started 2.5 hour after dosing and the data for this patient were excluded form the analysis. All 13 patients received the 125mg bid dose. Two patients received the target dose for only 2 and 10 days, respectively, before reduction of the dose. Therefore PK information at the 125mg dose level was available only from 11 patients including 1 patient in whom blood samples were taken up to 6 hours following drug administration. The population tested consisted of 8 females and 5 males in the age between 24 and 68 years. Eight (8) patients were Caucasians, 3 Asian and 1 each Indian and Hispanic. The mean plasma concentrations of bosentan and the metabolites are depicted in Figure 2 and the mean parameters are listed in Table 3.

Figure 2. Arithmetic Mean Plasma Concentration Time Profiles of Bosentan and Metabolites in Patients with PAH after Multiple Dose Administration of 62.5mg and 125mg Bosentan bid.



The exposure (AUCτ) to bosentan was not different between patients with primary pulmonary hypertension or pulmonary hypertension secondary to scleroderma. There was also no overt difference in exposure between female and males.

Table 3 Arithmetic Mean Parameters (95% Confidence Limits)* of Bosentan and Metabolites after Multiple Dose Administration of 62.5mg and 125mg Bosentan bid to Patients with PAH

Bosentan			
Treatment	C_{max} (ng/ml)	t_{max} (h)	AUC_τ (ng·h/ml)
62.5 mg twice daily	1187 (814, 1560)	3.0	6232 (4582, 7881)
125 mg twice daily	2286 (1234, 3337)	2.3	8912 (6296, 11531)
Ro 47-8634			
Treatment	C_{max} (ng/ml)	t_{max} (h)	AUC_τ (ng·h/ml)
62.5 mg twice daily	34.4 (17.4, 51.4)	3.0	238 (79.3, 397)
125 mg twice daily	58.9 (35.1, 82.6)	3.0	295 (176, 415)
Ro 48-5033			
Treatment	C_{max} (ng/ml)	t_{max} (h)	AUC_τ (ng·h/ml)
62.5 mg twice daily	356 (85.2, 627)	4.0	2460 (613, 4307)
125 mg twice daily	429 (59.3, 798)	2.3	2573 (93, 5053)
Ro 64-1056			
Treatment	C_{max} (ng/ml)	t_{max} (h)	AUC_τ (ng·h/ml)
62.5 mg twice daily	196 (61.5, 330)	4.0	1683 (429, 2937)
125 mg twice daily	242 (52.3, 433)	4.0	1833 (17.0, 3649)

*Median (range) for tmax, n=12 for 62.5mg dose level and n=11 for 125mg dose level

The arithmetic mean measures of exposure to bosentan and its metabolites, AUCτ and Cmax, increased less than dose proportional with increasing the dose from 62.5mg bid to 125mg bid. The dose related increases in AUCτ and Cmax tended to be smaller for the metabolites than for bosentan. As a result the overall exposure to the metabolites relative to bosentan at the 125mg dose level decreased from 70.3% to 52.7% at the lower dose level. The Cmax and AUCτ values among the 3 metabolites were largest for the active Ro 48-5033. The AUCτ value of the secondary metabolite Ro 64-1056 relative to bosentan decreased from 27.0% at the lower dose level to 20.7% at the higher dose level. The median tmax values for the parent drug and the metabolites at the lower and higher dose level were similar.

A comparison of the mean parameters of bosentan in patients with PAH and healthy volunteers is presented in Table 4.

Table 4. Comparison of the Arithmetic Mean Exposure Measures for Bosentan in Patients with Pulmonary Arterial Hypertension and Healthy Adult Volunteers Receiving Multiple Doses of Bosentan 62.5mg bid or 125mg bid

Population	Dose	AUC_τ (ng·h/ml)	C_{max} (ng/ml)
Healthy subjects*	62.5 mg twice daily	2857	544
PAH patients	62.5 mg twice daily	6232	1187
Healthy subjects*	125 mg twice daily	4804	1083
PAH patients	125 mg twice daily	8912	2286

Values are arithmetic means.
* From Study AC-052-108 (n = 10 [21]).
† From Study AC-052-109 (n = 9 [22]).
‡ PAH = pulmonary arterial hypertension.

The arithmetic mean exposure measures of bosentan, AUCτ and Cmax, were at both dose levels clearly larger in patients than in healthy adults. At the 62.5mg dose level AUCτ and Cmax of the patients exceeded the corresponding values in healthy adults by 118.1 % and 118.2%, respectively. At the 125mg dose level the respective exposure measures for bosentan in the patients with PAH were 85.5% and 111.1% greater than in healthy adults.

Efficacy:

At baseline 87.6% of the 113 patients were classified as WHO functional class III. After 1 month of treatment at the 62.5mg dose level, 16.8% of the patients showed improvement in WHO functional class. At the end of the study 31.0% of the patients showed an improvement from baseline in WHO functional class. 8.0% of the patients worsened in the WHO functional classification during the course of treatment.

Safety:

The mean treatment duration was 15.8 weeks with 85% of the patients receiving drug for at least 12 weeks. The bosentan dosing was discontinued because of an adverse event in 10 patients, with or without previous dose reduction or temporary interruption in treatment: Worsening of the condition (aggravated PAH) (n=3), elevated liver aminotransferases (n=2), cardiac failure/renal failure (n=1), fluid overload (n=1), hepatitis (n=1), nausea/vomiting (n=1) and renal failure (n=1) were the adverse events. Twelve additional patients had the dose reduced because of an adverse event. Seven patients had a temporary interruption of the treatment that was except for 1 case due to the occurrence of adverse events. Four patients, all from progression of PAH, died during the study. Two additional deaths occurred 16 and 62 days after completion of the study: One was considered to be related to progression of PAH and the other was judged

to be related to a sickle cell crisis by the investigator. None of the 6 deaths was considered to be related to the bosentan treatment.

Among the serious adverse events reported aggravated PAH (n=5) and syncope (n=5) were the most common. Four (4) patients had a marked decrease in hemoglobin concentrations and/or hematocrit. Marked increases in AST and ALT were observed in 10 and 11 patients, respectively. Eight (8) of these patients had elevations >3•ULN: ≤ 5• ULN (3 patient, 5<x<8•ULN (3 patients) x > 8•ULN (2 patients). Bosentan treatment was discontinued in the 2 patients with aminotransferase values >8•ULN and the values returned to baseline after 19 and 45 days, respectively. In 5 of the 6 patients with aminotranferase values 3<x<8•ULN bosentan was down-titrated and in 1 case not uptitrated. By the last study assessment aminotransferase levels were in 4 of the patients near baseline. In 1 patient the aminotransferase levels decreased initially after dose reduction but rebounded later and the treatment with bosentan was stopped. Associated increases in bilirubin and alkaline phosphatase were seen in 3 and 2 of the patients, respectively. Of the patients participating in the PK substudy 3 had alkaline phosphatase and associated bilirubin values exceeding the ULN. In addition several of the patients displayed increase of the aminotransferases above the ULN. Only 1 patient had ALT and AST values >3•ULN.
There was a trend for a decrease in systolic and diastolic blood pressure (-4.3 (15.7) and -3.9(14.3) mmHg, respectively). Pulse rate and body weight was unchanged.

Conclusions:

The PK of bosentan in patients with PAH receiving two dose levels of the drug, 62.5mg and 125mg bid, each for ≥ 2 weeks was less than dose proportional indicating a dose dependent increase of the oral clearance. The oral clearance in adult patients with PAH was importantly smaller than in healthy adult volunteers.

Reviewer's Comments:

The results of the present study with oral administration of bosentan to patients with PAH are consistent with the findings of a previous study with intravenous administration of the drug. The reduction in the oral clearance of the patients relative to healthy volunteers was in the present study –54.1% and 46.1% at the 62.5mg and 125mg dose levels compared to a reduction of the clearance by –57.8% after intravenous administration in a previous study reported in the NDA. The pertinent findings of the study ought to be inserted in the label.

Protocol AC-052-107

Study Title: **Single and Multiple-Dose Pharmacokinetics of Bosentan in Patients with Impaired Liver Function as Compared to Healthy Subjects**

Principal Investigator: **Gabriel Popescu, MD, Apex Research, Landsbergerstrasse 476, 81241 Munich, Germany**

Objectives:

1. To compare the PK of bosentan and its metabolites when the drug is given as single (125mg) and repeated (125mg bid for 5.5 days) oral doses to patients with mildly impaired liver function and healthy volunteers
2. To evaluate safety and tolerability of single (125mg) and repeated (125mg bid for 5.5 days) oral doses of bosentan in healthy subjects and in patients with mild liver impairment

Bosentan is almost exclusively metabolized and the metabolites excreted by the bile. The PK of bosentan and its metabolites may be altered in subjects with impairment of the liver function.

Subjects:

Planned: Eight (8) healthy subjects and 8 patients with mild liver impairment
Analyzed:Nine(9) healthy subjects and 8 patients with mild liver impairment

Inclusion Criteria for eligible healthy volunteers and patients: females (postmenopausal, surgically sterilized, hysterectomized, or using a double-barrier method for contraception) and males in the age between 18 and 65 years of age with a BMI between 19 and 34 kg/m^2 with normal blood pressure (systolic range; 100-140mm Hg, diastolic range: 50-90mmHg) and pulse rate (45-90 bpm) after 5 minutes in supine position, 12 Lead ECG recording without clinically relevant abnormalities (PR interval $\leq$ 200msec, QRS $\leq$ 115msec, QTc $\leq$ 440msec), hematology, biochemistry and urinalysis test results within normal range of the laboratory (except for those related to liver disease for patients) or not considered to be clinically relevant by the investigator, hematocrit>35%,

negative results from drug screen (cocain, cannabinoids, opiates), negative pregnancy test (females only, except postmenopausal or women with documented surgical sterilization or hysterectomy), able to stop alcohol consumption during hospitalization), smoking fewer than 10 cigarette/day, able to stop smoking during hospitalization, no excessive consumption of caffeine and/or methylxanthin containing beverages, able to stop caffeine consumption during hospital stay, normal eating habits, able to communicate well with the investigator in German and to understand and comply with study requirements.

Inclusion criteria for eligible patients with mild liver impairment only: Males or females with liver cirrhosis of alcoholic etiology, degree of liver function impairment in accordance with Child-Pugh classification A, no abnormal physical finding of clinical relevance other than those related to liver disease.

Exclusion Criteria for healthy volunteers and patients: clinically relevant intercurrent medical conditions possibly interfering with meeting objectives of study, history of hepatitis B or C and/or positive hepatitis serology indicating acute or chronic hepatitis B or C (except for vaccinated subjects), positive HIV serology, history of clinically relevant hypersensitivity or severe adverse drug reactions, presence or history of any allergy requiring acute or chronic treatment, bleeding from esophagus varices within 4 weeks prior to study start, participation in another clinical study with an investigational drug during the previous 12-week period or a commercially available drug within 8 weeks prior to study start (except immunosuppressive or immunostimulating agents for which exclusion period is 6 months), ingestion of a drug with well defined potential toxicity to a major organ system, intake of drugs inducing or inhibiting CYP3A4 or CYP2C9 within 4 weeks prior to study start, intake of endothelin antagonists during the 1 year period prior to study start, concomitant treatment with glibenclamide and/or cyclosporine A and/or tacrolimus, loss of 500mL within past 3 months, legal incapacity or limited legal capacity, subjects who cannot be reached in emergency situation, symptoms of clinicallly relevant illness, except liver disease in 4 weeks prior to screening examination.

Exclusion criteria for patients only: History within the past 3 years prior to screening or presence of cardiovascular disease, significant shunt volume as estimated by Duplex sonography.

Exclusion criteria for volunteers only: History or clinical evidence of any disease or alcoholism or drug abuse and/or existence of any other surgical or medical condition which might interfere with the objectives of the study, history or presence of cardiovascular disease.

The Child-Pugh Classification System was used to assess liver function in the patients

Score	Bilirubin (mg/dl)	Albumin (g/dl)	PT (sec prolonged)	Ascites (grade)	Hepatic encephalopathy
1	<2	>3.5	<4	None	None
2	2-3	2.8-3.5	4-6	Mild	1-2
3	>3	<2.8	>6	Severe	3-4

Child-Pugh class: A (mild): 5-6; B (moderate): 7-9; C (severe): ≥ 10

Methodology:

This was a single center, open label, parallel-group, single (Part I)- and multiple (Part II)-dose study. The decision to continue with Part II and the choice of dose was based on upon the single-dose safety and PK results. In Part I the subjects received a single dose of 125mg bosentan on Day 1. In Part II the subjects received 125mg bosentan bid on Days 2-6 and a single dose on Day 7. The dose of 125mg given bid corresponds to the highest labeled dose of bosentan. The study drug was given together with 150mL of water with the subjects in the standing position. During Part II of the study the morning doses were given always at the same time between 7am and 9am. The dosing interval between morning and evening doses was exactly 12 hours. On Day 2 of Part I and on Day 7 of Part II the dose was given to the volunteers in the fasted state. On all other occasions the dose of bosentan was administered irrespective of time of intake of meals.

From screening until Day 4 of Part I and from week 1 prior to the start of the Part II until the end-of-study examination, the subjects were to refrain from strenuous physical exercise and sports activity. The subjects were institutionalized on 2 occasions for a total of 3 days (including 4 nights): A first time from Day 1 of Part I about 12 hours prior to the single dose administration of bosentan until 24 hours thereafter on Day 3 of Part II and a second time on Day 6 of Part II about 12 hours before drug administration on Day 7 until 24 hours thereafter on Day 8 of Part II. On the days of blood sampling for PK purposes (Day 2 of Part I and Day 7 of Part I) the subjects received standardized meals: a warm lunch 4 hours after the 4 hour blood sample, a snack after the 8 hour blood sample, an evening meal 11.5 hours after dosing and breakfast 14 hours after dosing.

Study Drug:

Bosentan was supplied as 125mg tablets, batch number F 0358A001.

Prior and Concomitant Therapy:

Not permitted was: Participation in another clinical study with an investigational drug during the previous 12-week period or a commercially available drug within 8 weeks prior to study start (except immunosuppressive or immunostimulating agents for which exclusion period is 6 months), ingestion of a drug with well defined potential toxicity to a major organ system, intake of drugs inducing or inhibiting CYP3A4 or CYP2C9 within 4 weeks prior to study start, intake of endothelin antagonists during the 1 year period prior

to study start, concomitant treatment with glibenclamide and/or cyclosporine A and/or tacrolimus.

Evaluation:

Pharmacokinetics: The PK of bosentan and its metabolites in plasma were followed for 48 hours after single and multiple dose administration. The PK of bosentan and metabolites in urine were followed for 24 hours after single dose administration and for 12 h after multiple dose administration.

Pharmacodynamics: Total bile salts in serum were followed for 4 hours following single and multiple dose administration of bosentan.

Efficacy: Was not evaluated

Safety: Adverse events were recorded. Physical examinations were performed at screening, 24 hours before and 48 hours after drug intake on both Day 2 of Part I and on Day 7 of Part II. Additional physical examinations were performed on the morning of Day 1 of Part II and at the end-of-study examination. Systolic and diastolic blood pressure measurements were always taken from the same arm after the subjects had rested for 5 minutes in the supine position and then after the subjects remained in a standing position for 1 minute. Measurements were taken during screening, prior to and for 48 hours following drug administration on both Day 2 of Part I and on Day 7 of Part II, on Day 1 of Part II 24 hours before the first repeat dose administration and on Day 6 of Part II 24 hours prior to the last dose administration, and at the end-of study examination.
A standard 12-lead ECG was recorded with the subjects at rest in the supine position for 5 minutes at screening, 24 hours before and 48 hours after drug intake on Day 2 of Part I and Day 7 of Part II, in the morning of Day 1 Part II, and at the end-of-study examination. Three (3) complexes for each standard lead were recorded and the PR, QRS, QTc intervals, the heart rate and the type of rhythm evaluated.
The safety laboratory examination included biochemistry and hematology tests and urinalysis. They were performed at screening, 24 hours before and 48 after drug intake on Day 2 of Part I and Day 7 of Part II, in the morning of Day 1 of Part II and at the end-of-study examination. A drug screen was performed at screening and on Day 1 of Part I and II. Serology was done at screening only.

Portal vein and possible shunt vessels were examined by Duplex ultrasound with the subjects in the supine position for 5 minutes. This examination was performed at screening and only in the subjects with mild liver impairment.

Blood Sample Collection for PK:

On Day 2 of Part I and on Day 7 of Part II blood samples (5mL) were collected prior to and 0.5,1,0, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 15, 18, 24, 36, and 48 hours after drug administration. Additional blood samples were taken just prior to the administration of the morning dose on Days 2 through 6 of Part II.

Urine Sample Collection for PK:

Urine was collected during the 24 hour interval after drug intake on Day 2 of Part I and during the 12 hour interval after drug intake on Day 7 of Part II.

Blood Sample Collection for Pharmacodynamics:

Blood samples for determining total bile salt concentrations in serum were taken on Day 2 of Part I and on Day 7 of Part II immediately prior to drug intake and 0.5, 1.0, 1.5, 2, 2.5, 3, 3.5, and 4 hours after drug administration. Additional blood samples prior to and after drug intake were collected for the determination of plasma protein binding of bosentan.

Analytical Methodology:

Bosentan and its metabolites Ro 47-8634, Ro 48-5033 and Ro 64-1056 in plasma were measured by [redacted]. Calibration and quality control samples were prepared in plasma and the latter served to analyze the day-to-day performance.
The measurements of the total bile salts in serum were performed as part of the clinical chemistry analyses by the analyzing clinical laboratory.

Pharmacokinetic Parameters:

The following parameters estimated:

Cmax	Observed maximum concentration
tmax	Time to appearance of Cmax
t1/2	Terminal elimination half-life
AUC0-t	Area under the plasma concentration time curve from zero to time of last measured concentration above the limit of quantification
AUC0-∞	Area under the plasma concentration time curve from zero to infinity

AUCτ	Area under the plasma concentration time curve during a dose interval (12 hours)
Cl/F	Apparent clearance, where F is the absolute bioavailability
Vd/F	Apparent volume of distribution

AUC0-t was obtained on application of the linear trapezoidal rule. AUC0-∞ was obtained by adding Ct/λz to AUC0-t, where Ct represents the last plasma concentration measured above the LOQ and λz is the first order rate constant associated with the terminal log-linear portion of the plasma concentration time curve. The t1/2 was obtained by dividing ln2 by λz. The CL/F was obtained by dividing AUC0-∞ or AUC0-τ into the dose and Vd/F was obtained by multiplying D/AUC by λz.

Statistical Methods:

Sample size and power: the total number of subjects enrolled in the study was not based on statistical power consideration. However based on previous experience the proposed number of 8 subjects per group was considered adequate to achieve the goals set for the study.

Analysis of PK Data:

Compartment-model independent methods using WinNonlin Professional Version 3.2 were applied to compute the PK parameters of bosentan and the metabolites. Geometric means and 95% confidence intervals were computed for the PK parameters Cmax, AUC0-∞, AUC0-τ, t1/2, Cl/F and Vd/F. Differences in the PK parameters of bosentan and metabolites between healthy subjects and patients with mild liver impairment were explored using the two-sample t-test on the logarithmically transformed values of Cmax, AUC0-∞, AUC0-τ, t1/2, Cl/F and Vd/F. To examine possible differences in tmax between the 2 populations the 2-sample Wilcoxon test was applied using the untransformed tmax values. Differences in the PK parameters between Part I and II within each group were tested by using the one-sample t-test for logarithmically transformed values of Cmax, AUC0-∞, AUC0-τ, t1/2, Cl/F and Vd/F. Possible differences between the tmax values were tested by the one-sample Wilcoxon test using the untransformed values. SAS version 6.12 was employed.

Analysis of Safety Data:

The results of the vital sign- laboratory- and ECG parameters were summarized and expressed as mean, median, SD, minimum, maximum, and number of available observations. The ECG endpoints were expressed as change form the baseline, where baseline was defined from the values that were obtained at screening and on Day 1 of Part I.

RESULTS:

Disposition of Subjects:

Eight (8) patients with mild liver impairment and 9 healthy volunteers were recruited. Except for one of the healthy volunteers who withdrew consent after Part I of the study all completed the trial. Table 2 displays the demographics of the study subjects.

Table 2. Demographics of Enrolled Subjects

	Parameter (mean ±SD)				Gender	
Group	Age (years)	Height (cm)	Weight (kg)	Body Mass Index (kg/m^2)	Male	Female
Patients with mild liver impairment	51.4 ± 8.3	173.9 ± 9.5	77.3 ± 12.0	25.5 ± 2.6	5	3
Healthy subjects	51.0 ± 8.4	171.9 ± 6.3	75.6 ± 11.6	25.6 ± 2.7	6	3

All patients and subjects were Caucasian. All the patients had a Child-Pugh score of 5 or 6 corresponding to Child-Pugh Class A with mild liver impairment. The patients and healthy volunteers were matched for age, weight and gender. At screening 4 of the patients took regularly medication. None of the healthy volunteers was taking regularly medication.

Analytical:

The calibration curves for bosentan were linear in the range of — ng/mL and for the metabolites in the range . — ıg/mL. Linearity of the calibration curves was demonstrated by correlation coefficients ≥ 0.997 for bosentan and Ro 48 5033, and ≥0.995 for Ro 47-8643 and Ro 64-1056.The LLOQ were set at — g/mL for bosentan and — ıg/mL for the metabolites.

The inter-assay precision (CV%) assessed from the QC samples was for bosentan, — % for Ro 48-5033, — % for Ro 47-8634 and . —% for Ro 64-1056. The interassay accuracy for the QC samples ranged between . — . and —' % for bosentan, between —% to — % for Ro 48-5033, between . — % and . — for Ro 47-8634 and between —% and . — % for Ro 64-1056.
The assays were performed by C

Pharmacokinetics:

The arithmetic mean plasma concentrations for bosentan and the metabolites after single and multiple dose administration are displayed semilogarithmically in Figures 3 and 4, respectively.

Figure 3. Semilogarithmic Plot of the Mean Arithmetic Plasma Concentrations of Bosentan and Metabolites after Single Dose Administration

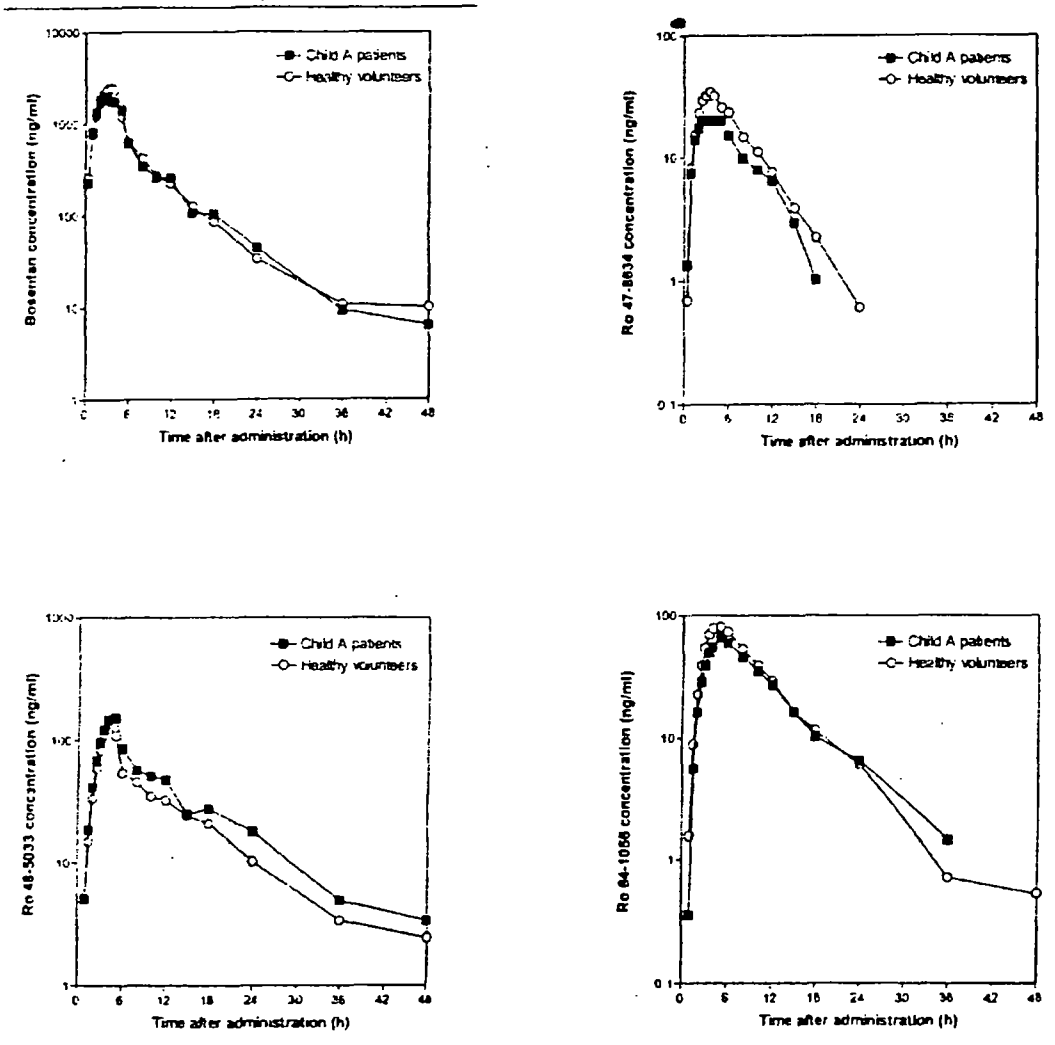
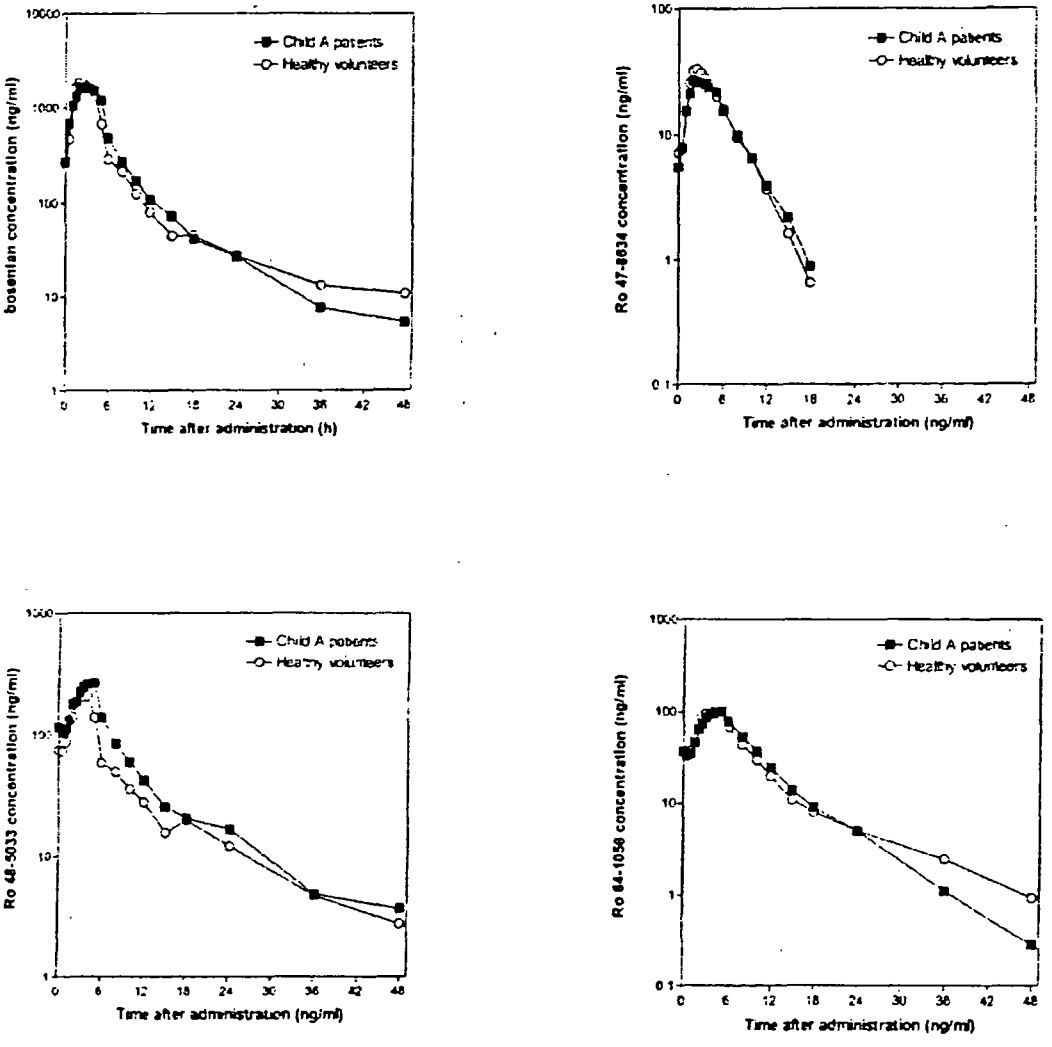


Figure 4. Semilogarithmic Plots of the Arithmetic Mean Plasma Concentrations of Bosentan and Metabolites after Multiple Dose Administration



Tables 3 and 4 list the geometric means of the PK parameters for bosentan and the metabolites in the patients with mild liver impairment and in the healthy volunteers after single and multiple dose administration, respectively.

Table 3. Geometric Means (95% CI) of the PK Parameters for Bosentan and Metabolites after Single Dose Administration of 125mg of the Drug

Bosentan				
Group	C_{max} (ng/ml)	t_{max} (h)	$AUC_{0-\infty}$ (ng·h/ml)	$t_{1/2}$ (h)
Patients	1980	3.3	10781	6.4
	(1345, 2909)	—	(8028, 14479)	(5.6, 7.3)
Healthy subjects	2534	3.2	11957	6.2
	(1968, 3263)	—	(9062, 15776)	(4.9, 7.9)
Ro 47-8634				
Group	C_{max} (ng/ml)	t_{max} (h)	$AUC_{0-\infty}$ (ng·h/ml)	$t_{1/2}$ (h)
Patients	21.3	3.1	180	4.7
	(15.3, 29.8)	—	(141, 230)	(3.8, 5.7)
Healthy subjects	33.5	3.3	233	3.7
	(25.9, 43.4)	—	(164, 332)	(3.2, 4.4)
Ro 48-5033				
Group	C_{max} (ng/ml)	t_{max} (h)	$AUC_{0-\infty}$ (ng·h/ml)	$t_{1/2}$ (h)
Patients	125	4.6	1188	9.7
	(74.1, 211)	—	(814, 1732)	(8.3, 11.3)
Healthy subjects	131	4.1	1009	9.4
	(99.6, 171)	—	(854, 1193)	(7.0, 12.5)
Ro 64-1056				
Group	C_{max} (ng/ml)	t_{max} (h)	$AUC_{0-\infty}$ (ng·h/ml)	$t_{1/2}$ (h)
Patients	63.3	5.0	632	6.7
	(46.7, 85.7)	—	(473, 844)	(5.7, 7.9)
Healthy subjects	83.6	4.5	723	6.5
	(66.3, 105)	—	(520, 1005)	(5.0, 8.5)

Data are expressed as geometric mean (and 95% CI) or, for t_{max}, as median (and range).

Table 4. Geometric Means (95%CI) of the PK Parameters of Bosentan and Metabolites after Administration of 125mg of the Drug bid for 5.5 Days

Bosentan				
Group	C_{max} (ng/ml)	t_{max} (h)	AUC_τ (ng·h/ml)	$t_{1/2}$ (h)
Patients	1715	2.8	7838	7.8
	(1212, 2427)	—	(5460, 11251)	(5.7, 10.7)
Healthy subjects	1831	2.2	7216	10.3*
	(1268, 2643)	—	(4954, 10511)	(8.4, 12.4)

Ro 47-8634				
Group	C_{max} (ng/ml)	t_{max} (h)	AUC_τ (ng·h/ml)	$t_{1/2}$ (h)
Patients	28.0	2.5	160	3.3*
	(20.8, 37.6)	—	(117, 218)	(2.7, 4.0)
Healthy subjects	29.3	2.4	164	3.6
	(19.4, 44.4)	—	(112, 241)	(3.0, 4.3)

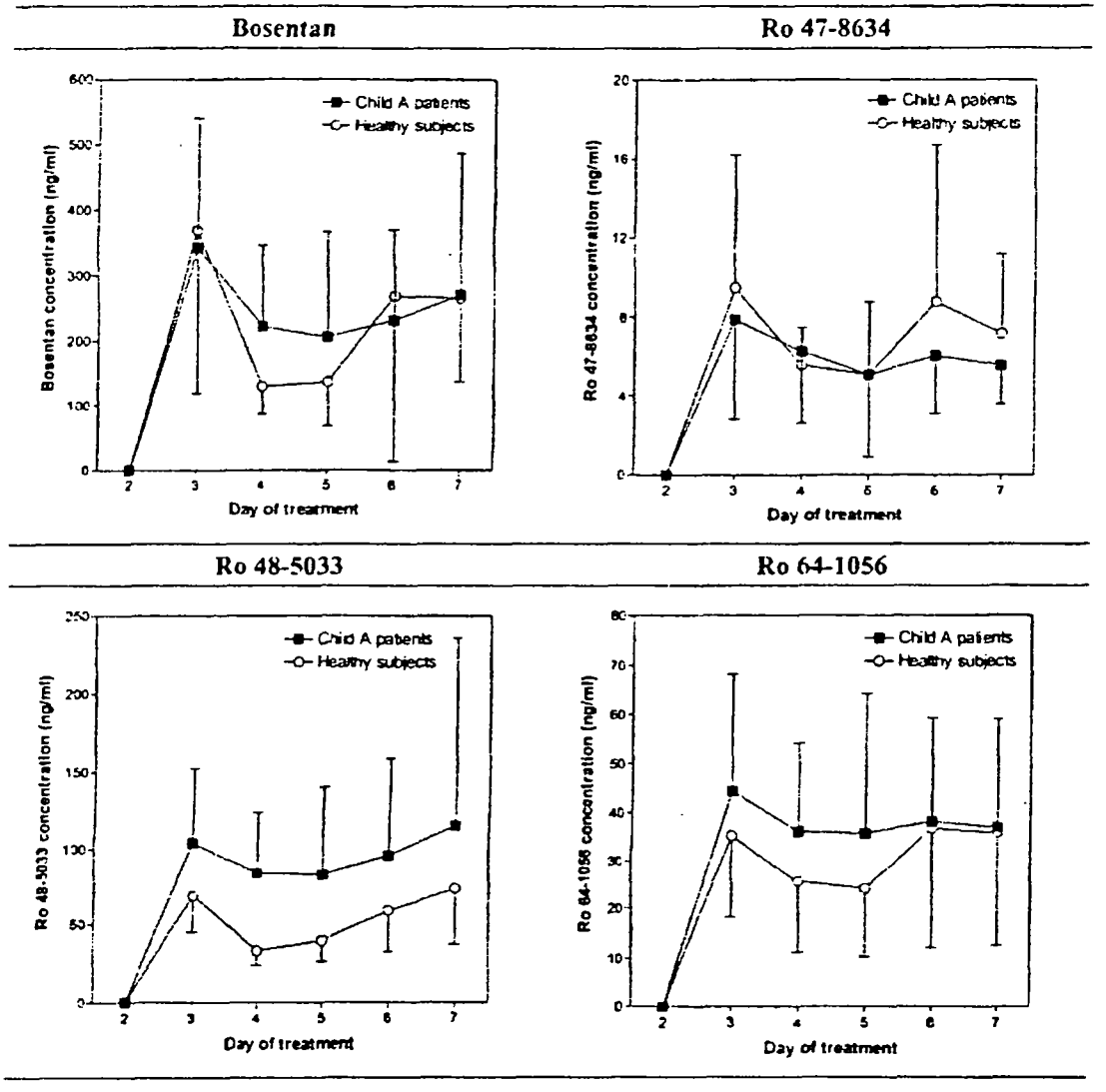
Ro 48-5033				
Group	C_{max} (ng/ml)	t_{max} (h)	AUC_τ (ng·h/ml)	$t_{1/2}$ (h)
Patients	236*	4.1	1339	9.9
	(148, 375)	—	(856, 2096)	(6.6, 14.9)
Healthy subjects	198	3.7	1006	10.2
	(143, 274)	—	(741, 1366)	(8.7, 11.9)

Ro 64-1056				
Group	C_{max} (ng/ml)	t_{max} (h)	AUC_τ (ng·h/ml)	$t_{1/2}$ (h)
Patients	93.6*	3.8*	649	6.2
	(67.4, 130)	—	(468, 900)	(4.8, 8.1)
Healthy subjects	84.7	3.8	565	9.2
	(54.1, 133)	—	(363, 877)	(5.9, 14.4)

Data are expressed as geometric mean (and 95% CI) or, for t_{max}, as median (and range). *P < 0.05 as compared to single dose administration.

The arithmetic mean (SD) morning through plasma concentrations of bosentan and metabolites on different treatment days after multiple doses of the drug for 5.5 days are depicted in Figure 5.

Figure 5. Arithmetic Mean (SD) Morning Trough Plasma Concentrations of Bosentan and Metabolites on Different Treatment Days after Administration of 125 mg of the Drug bid for 5.5 Days



After single dose administration of bosentan the geometric mean AUCo-∞ and Cmax values of the patients with mild liver impairment and the matched healthy volunteers were similar. Similarly, the individual and overall exposures to the metabolites relative to the parent drug were comparable in the two groups. The multiple dose results confirmed

the findings after single dose administration. There were no statistically significant differences in the PK parameters obtained in the two groups. In both groups the exposure to bosentan and the metabolites was decreased following multiple dose administration of the drug for 5.5 days, indicating auto-induction of bosentan's metabolism. The reduction of the exposure in the healthy subjects tended to be larger than in the patients with mild hepatic impairment.
In deviating from the protocol the urine samples were not analyzed for bosentan and metabolites, because the plasma concentrations of bosentan and metabolites were similar in the patients with mild liver impairment and in the healthy volunteers. For the same reason and because the albumin concentration in both groups were similar the determination of the plasma protein binding of bosentan was not performed.

Pharmacodynamics:

The bile salt values showed a considerable degree of intersubject variation. There appeared to be a trend for larger basal bile salt values in the patients with mild hepatic impairment than in the healthy volunteers.

Safety:

A total of 16 AEs were recorded: 12 in healthy volunteers and 4 in the patients with mild hepatic impairment. Six (6) AEs were considered to be remotely or possibly related to drug intake by the investigator. Three of the 8 patients and 5 of the 9 healthy subjects reported at least 1 AE. Headache was the most frequently reported AE. All AEs were of mild to moderate intensity. Whether considering all reported AEs or only drug related AEs there was no evidence that patients with mild impairment tolerated the treatments worse than the matched volunteers. Compared to the predose value taken on Day 1 of Part I there was a trend for both systolic and diastolic blood pressure to decrease following administration of the drug. This occurred in healthy volunteers and in patients with mild liver impairment. There was no evidence for reflex tachycardia or clinical signs of orthostatic hypotension.
The ECG recordings did not show any clinically relevant finding.

Conclusions:

The measures of exposure in patients with mild hepatic impairment and in matched volunteers treated for 5.5days with 125mg bosentan bid were comparable

Reviewer's Comments:

The results of the study ought to be described in the label.

Protocol AC-052-108

Study Title: A Study o Investigate the Pharmacokinetics of Bosentan in Healthy Subjects when Given Concomitantly Ketoconazole

Principal Investigator:

Atef Halabi, MD, Institut fuer klinische Pharmakologie Prof. Dr. Luecker, Zentrum Kiel, Wrangelstrasse 16, 24105 Kiel, Germany

Objectives:

1. To evaluate the influence of concomitant ketoconazole on the PK of bosentan in healthy subjects
2. To evaluate the single and multiple dose PK of 62.5mg bosentan and to evaluate the tolerability of concomitant ketoconazole and bosentan in healthy subjects

Bosentan is metabolized by the CYP 450 iso-enzymes 3A4 and 2C9. Ketoconazole is a strong inhibitor of 3A4.

Subjects:

As planned 10 healthy male subjects participated in the study. To be eligible for the study the volunteers had to be in the age between 18 and 45 years of age, within ± 15% of ideal body weight, non-smokers, have normal blood pressure (systolic blood pressure range:100-140mmHg, diastolic blood pressure range: 50-90mmHg) and pulse rate (45-90 bpm) after 5 minutes in the sitting position, a 12 Lead ECG without clinically relevant abnormalities (PR interval ≤ 200msec, QRS ≤115msec, QTc ≤ 440msec), hematology, biochemistry and urinalysis test results not clinically relevantly deviating from normal range, negative results from drug screen (cannabinoids, cocaine, opiates, benzodiazepines), should have normal eating habits (e.g. should not be vegetarians), consume ≤5 cups of coffee or tea per day or ≤3 bottles (250mL) of cola and able to stop caffeine consumption during institutionalization, be able to communicate well with the Investigator, and give written informed consent.

Exclusion criteria included the following: History or clinical evidence of any disease or alcoholism or drug abuse or existence of any surgical or medical condition that might interfere with absorption, distribution or elimination of the study drugs, history of hepatitis B or C and/or positive results from hepatitis serology indicating acute or chronic hepatitis B or C (except for vaccinated subjects), positive results from HIV serology, history of relevant hypersensitivity or severe adverse reaction to any drug, presence or history of any allergy requiring acute or chronic treatment, participation in another

clinical trial with intake of an investigational drug during the previous 12 weeks or participation in a clinical trial with a marketed drug in the previous 8 weeks (immunostimulating and immunosuppressive drugs during previous 6 months), intake of a drug with a well-defined potential for toxicity to a major organ system during the previous 12 months, intake of drugs inhibiting or inducing the iso-enzymes CYP 3A4 and/or CYP 2C9 within the 4 weeks prior to screening until the end of the study, intake of endothelin antagonists during the previous 12 months, concomitant treatment with any medication, loss of ≥250mL of blood in the previous 3 months, legal incapacity or limited legal capacity, symptoms of a clinically relevant illness in the 4-week period preceding screening.

Methodology:

This was a single center, open label, randomized, two period, cross over study with single and multiple-dose administrations of the drug. In Treatment A single doses of 62.5mg of bosentan were administered to the volunteers on Days 1 and 7 and in the intervening Days 2 to 6 the volunteers received 62.5mg of bosentan bid. In treatment B 62.5mg bosentan was administered bid to the volunteers on Days 1 through 5 and a single dose of the drug was given on Day 6. Ketoconazole was given to the volunteers in Treatment B in a dose of 200mg qd on Days 1 through 6. The chosen design of the study allowed an evaluation of the impact of co-administered ketoconazole on the PK of bosentan in each of the participating volunteers. The lower labeled dose of 62.5mg was chosen for this study to afford the greatest protection to the subjects. A dose of 200mg is the standard dose for ketoconazole in medical practice.

The study medications were given to the volunteers in a sitting position with 150mL of water in the morning between 7 and 9 am and the evening doses were administered exactly 12 hours later. On Days 1 and 7 of Treatment A and on Day 6 of Treatment B the medication was administered to the subjects 30 minutes after they ingested a continental breakfast. On all other study days of Treatments A and B the time of drug administration was independent of food intake. On Days 1 and 7 during Treatment A and on Day 6 of Treatment B the subjects stayed in bed in a semi-supine position from just before receiving the study medications until 4 hours after administration. On these days the volunteers received standardized meals: a continental breakfast (between 0.5 and 1 hour before the morning dose), a lunch (4 hours after the morning dose), a snack (8 hours after the morning dose) and an evening meal (12 hours after the morning dose). From screening to the end-of study-examination the volunteers had to refrain from strenuous exercise and were not allowed to consume any grapefruit juice. The subjects were institutionalized from approximately 12 hours until 24 hours after drug intake on Days 1 and 7 of Treatment A and on Day 6 of Treatment B. The drinking of alcoholic or xanthine containing beverages during the institutionalization was not permitted. The volunteers were encouraged to drink 1500mL of water per day.

Study Drug:

The batch number for the 62.5mg bosentan tablets was F 0357A001. The batch number for the Nizoral® tablets containing 200mg ketoconazole was 00IL354.

Prior and Concomitant Treatment:

Not permitted was: Participation in another clinical trial with intake of an investigational drug during the previous 12 weeks or participation in a clinical trial with a marketed drug in the previous 8 weeks (immunostimulating and immunosuppressive drugs during previous 6 months), intake of a drug with a well-defined potential for toxicity to a major organ system during the previous 12 months, intake of drugs inhibiting or inducing the isoenzymes CYP 3A4 and/or CYP2C9 within the 4 weeks prior to screening until the end of the study, intake of endothelin antagonists during the previous 12 months, concomitant treatment with any medication.

Evaluation:

Pharmacokinetics: The plasma concentration time profile of bosentan and its metabolites were followed on Days 1 and 7 of Treament A and on Day 6 of Treatment B for 24 hours after drug administration. Trough samples were taken on scheduled days during both treatments.

Efficacy: Was not evaluated

Safety: At screening a medical history was taken, a physical examination, routine hematology and biochemistry tests, a urinalysis and virus serology performed, and a 12 lead ECG and vital signs recorded. At the end-of study-evaluation all these activities, except for taking the medical history were repeated.
Adverse events were monitored throughout the duration of the study. Subjects were encouraged to report AEs spontaneously. They were asked "how do you feel" at scheduled times throughout the duration of the study. The vital signs, systolic and diastolic blood pressure and pulse rate were measured indirectly using an automated oscillometric device. The measurements were recorded with the subjects in a semi-supine position after they had rested for 5 minutes at the following times: At screening, immediately prior to and 1, 2, 4, and 12hours after drug administration on Days 1 and 7 of Treatment A and on Day 6 of Treatment B, and at the end-of-study examination. Blood pressure was always measured on the same arm. A 12 lead ECG was performed with the subjects in a supine position at screening and at the-end-of-study evaluation. Prior to the ECG evaluation the volunteers were to have rested for 5 minutes in the supine position. The PR, QRS and QTc intervals, the heart rate (bpm) and the rhythm were evaluated. The QTc intervals were computed on application of Bazett's formula.

Blood Sample Collection for PK:

Blood samples (4mL) for the determination of the plasma concentration time profiles of bosentan and the metabolites were collected on Days 1 and 7 of Treatment A and on Day

6 of Treatment B immediately prior to and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10,12, 14, 16 and 24hours after drug administration. In addition trough samples were taken immediately prior to the morning doses on Day 1 and 2 of Treatment B and on Days 3,4,5 and 6 of Treatment A.

Analytical Methodology:

Bosentan and its metabolites Ro 47-8634, Ro 48-5033 and Ro 64-1056 in plasma were determined by [ ]
Calibration and quality control samples were prepared in plasma and the latter served to analyze the day to day performance.

Pharmacokinetic Parameters:

The following PK parameters were determined:

Cmax	Observed maximum plasma concentration
tmax	Time to appearance of Cmax
β	Elimination rate constant
t1/2β	Corresponding half-life
AUC0-t	Area under plasma concentration time curve from time zero to the last sampling time with a concentration above the LLOQ
AUC0-∞	Area under the plasma concentration time curve from time zero to infinity
AUCτ	Area under the plasma concentration time curve during a dosing interval

Cmax and tmax were read of the plasma concentration time data. The β was obtained from the log-linear least squares regression of the plasma concentration versus time data in the terminal phase. The t1/2β was obtained by dividing β into ln2. AUC0-t and AUC0-τ were estimated by applying the linear trapezoidal rule. Estimates for AUC0-∞ were obtained from AUC0-t + Ct/β, where Ct is the last concentration with a value exceeding LLOQ.

Statistical Methods:

Sample size and power: The total number of volunteers enrolled in this study was not based on statistical power considerations. However, based on previous experience the

proposed number of 10 volunteers was considered adequate for achieving the goals of the study.

Analysis of PK Data:

The effects of ketoconazole on the PK of bosentan were evaluated by comparing log transformed AUCτ-, t1/2β- and Cmax values for bosentan and the metabolites on Day 7 of Treatment A with those of Day 6 of Treatment B using ANOVA with the factors treatment, period, sequence and subject. The signed rank Wilcoxon Test was performed to explore differences in tmax. To test for differences between Days 1 and 7 of Treatment A the one-sample t-test and the Signed Rank Wilcoxon Test were performed.

Analysis of Safety Data:

The safety data analysis used descriptive methods and the data were summarized by mean, median, SD, minimum, maximum, and number of available observations. Individual data of the laboratory tests, vital signs and ECG parameters were listed and flagged if outside the normal range. Concomitant medications were also reported.

RESULTS:

Disposition of Volunteers:

Ten (10) healthy male volunteers were enrolled and completed the study. Their mean (SD) demographic characteristics were: age=29.4(3.6) years, height=182.5(7.7) cm, weight=77.9(8.5) kg.

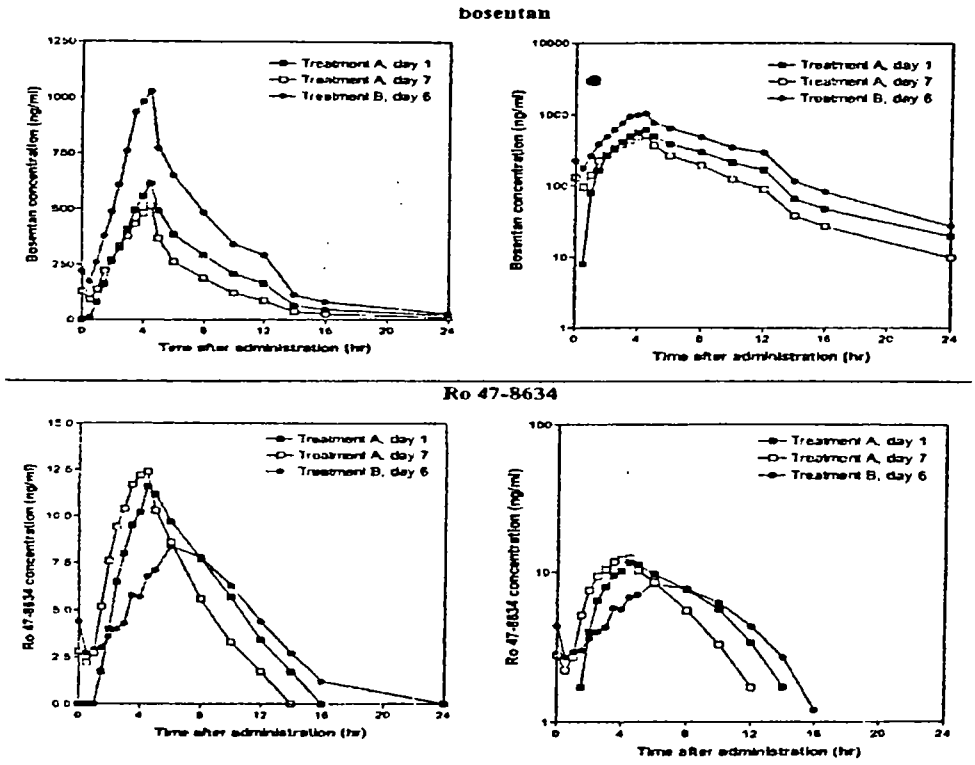
Analytical:

The results of the validation report indicated that the performance of the assay fulfilled the requirements for an accurate and precise analytical method. The standard curves for bosentan over the concentration range of — ng/mL and for the metabolites (Ro 47-8624, Ro 48 5033, Ro 64-1056) in the range of . — ng/mL were linear. Linearity of the calibration curves was demonstrated by correlation coefficients ≥ 0.998 for bosentan, ≥ 0.997 for Ro 48-5033, ≥ 0.996 for Ro 47-8634 and ≥ 0.995 for Ro 64-1056 and residuals — % for bosentan, — % for Ro 48 5033, — % for Ro 47-8634 and . — % for Ro 64-1056 for concentrations above the LLOQ. The LLOQ was set at — ng/mL for bosentan and — g/mL for the metabolites. The interassay precision (CV%) assessed from the QC samples was — % for bosentan, — % for Ro 48-5033, — % for Ro 47-8634 and —% for Ro 64-1056. The percent interassay accuracy of the QC samples ranged between — % and — % for bosentan, between % and — % for Ro 48-5033, between — % and — % for Ro 47-8634 and between — % to — % for Ro 64-1056. The assays were performed by .

Pharmacokinetics:

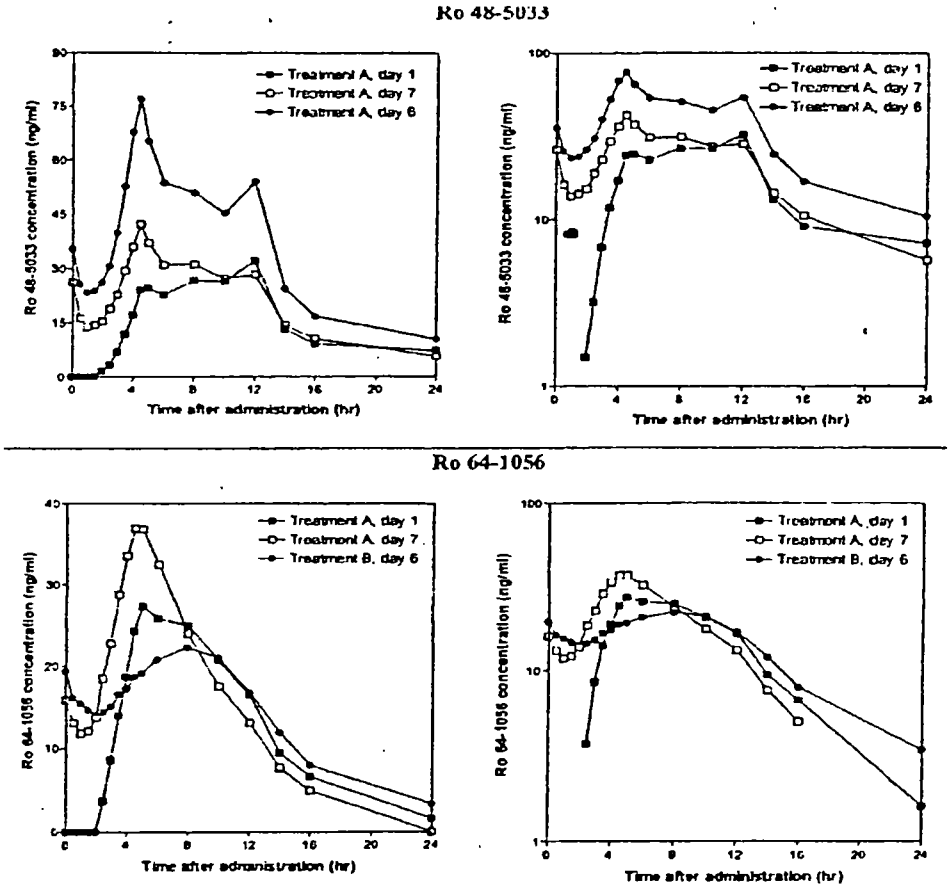
The arithmetic mean plasma concentrations of bosentan and the metabolites in the presence and absence of ketoconazole are depicted in Figure 1.

Figure 1. Linear Plots of the Arithmetic Mean Plasma Concentration Time Profiles of Bosentan and the Metabolites Ro 47-8634, Ro 48-3033 and Ro 64-1056 in the Presence and Absence of Ketoconazole



Treatment A: Bosentan as a single 62.5 mg dose on day 1, bid on days 2 to 6 and a single 62.5 mg dose on day 7.
Treatment B: Bosentan 62.5 mg twice a day on days 1 to 5 and a single 62.5 mg dose on day 6. Concomitant ketoconazole 200 mg once a day on days 1 to 6.

Figure 1. Linear Plots of the Arithmetic Mean Plasma Concentration Time Profiles of Bosentan and the Metabolites Ro 47-8634, Ro 48-5033 and Ro 64-1056 in the Presence and Absence of Ketoconazole Continued



Treatment A: Bosentan as a single 62.5 mg dose on day 1, bid on days 2 to 6 and a single 62.5 mg dose on day 7.

Treatment B. Bosentan 62.5 mg bid on days 1 to 5 and a single 62.5 mg dose on day 6. Concomitant ketoconazole 200 mg once a day on days 1 to 6.

Table 1 lists the values for the PK parameters of bosentan and the metabolites in the presence and absence of ketoconazole.

Table 1. Geometric Means (95% CI) of the PK Parameters* for Bosentan and the metabolites Ro 47-8634, Ro 48-5033 and Ro 64-1056

Bosentan					
Treatment	$AUC_{0-\infty}$ (ng·h/ml)	AUC_{τ} (ng·h/ml)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}(\beta)$ (h)
A, day 1	4234 (3597, 4985)		617 (504, 755)	4.5 —	5.4 (4.5, 6.6)
A, day 7		2744# (2253, 3341)	516 (415, 642)	4.5 —	4.9 (4.0, 5.9)
B, day 6		6093* (5036, 7372)	1005* (776, 1301)	4.5 —	4.6 (4.1, 5.3)
Ro 47-8634					
Treatment	$AUC_{0-\infty}$ (ng·h/ml)	AUC_{τ} (ng·h/ml)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}(\beta)$ (h)
A, day 1	98.4 (79.3, 122)		11.3 (8.6, 14.8)	4.5 —	4.5 (3.6, 5.5)
A, day 7		72.7# (57.4, 92.1)	12.7 (10.1, 16.0)	4.5# —	3.4# (3.0, 3.7)
B, day 6		64.0 (47.2, 86.8)	8.4* (6.3, 11.1)	7.0* —	3.9* (3.3, 4.7)
Ro 48-5033					
Treatment	$AUC_{0-\infty}$ (ng·h/ml)	AUC_{τ} (ng·h/ml)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}(\beta)$ (h)
A, day 1	522 (401, 681)		34.4 (26.7, 44.5)	9.0 —	14.2 (9.6, 21.0)
A, day 7		309# (243, 395)	41.6 (32.9, 52.6)	4.5# —	8.7 (6.6, 11.6)
B, day 6		494* (354, 689)	70.4 (46.3, 107)	4.5 —	9.3 (7.3, 11.9)
Ro 64-1056					
Treatment	$AUC_{0-\infty}$ (ng·h/ml)	AUC_{τ} (ng·h/ml)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}(\beta)$ (h)
A, day 1	283 (244, 328)		28.4 (23.4, 34.5)	6.5 —	4.0 (3.2, 5.1)
A, day 7		259 (210, 319)	37.0 (30.1, 45.4)	4.5# —	3.7 (2.8, 4.8)
B, day 6		215 (177, 262)	25.2 (20.1, 31.4)	8.0 —	5.8 (4.4, 7.6)

Data are expressed as geometric mean (and 95% CI) or, for t_{max}, as median (and range). # $p < 0.05$ as compared to day 1. * $p < 0.05$ as compared to day 7.

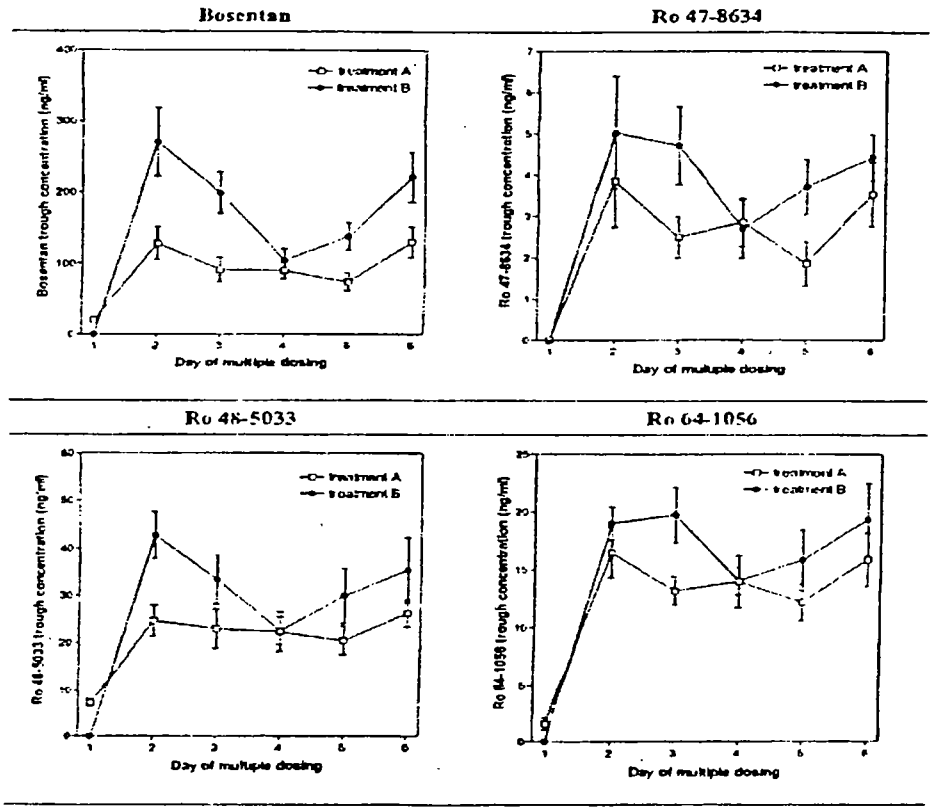
Treatment A: Bosentan as a single 62.5 mg dose on day 1, bid on days 2 to 6 and a single 62.5 mg dose on day 7.

Treatment B: Bosentan 62.5 mg bid on days 1 to 5 and a single 62.5 mg dose on day 6. Concomitant ketoconazole 200 mg once a day on days 1 to 6.

*tmax is expressed as median (range)

Figure 2 shows the arithmetic mean trough plasma concentration time profiles of bosentan and metabolites in he presence and absence of ketoconazole.

Figure 2. Linear Plots of the Arithmetic Mean (SEM) Trough Plasma Concentration Time Profiles of Bosentan and the Metabolites Ro 47-8634, Ro 48-5033 and Ro 64-1056 in the Presence and Absence of Ketoconazole



Treatment A: Bosentan as a single 62.5 mg dose on day 1, bid on days 2 to 6 and a single 62.5 mg dose on day 7.
Treatment B: Bosentan 62.5 mg bid on days 1 to 5 and a single 62.5 mg dose on day 6. Concomitant ketoconazole 200 mg once a day on days 1 to 6.

After multiple doses of the drug the geometric means of the exposure measures AUC and Cmax were 35.2% and 16.4%, respectively, smaller than after single dose administration. The AUC values of the metabolites Ro 47-8634 and Ro 48-5033 were similarly smaller after multiple compared to single dose administration of bosentan. In contrast the multiple dose administration of bosentan did not decrease the Cmax values of the metabolites. The respective AUC values of the metabolites relative to bosentan after

single dose administration ranged between 2.3% and 12.3 %. The pharmacologically active Ro 48-5033 was the most prominent metabolite. These figures did not change importantly after multiple dose administration of bosentan. The values for tmax for the metabolites were smaller after repeat dosing than after single dose administration. These results confirmed the previously observed increase of the oral clearance of bosentan after repeat dosing.

In the presence of ketoconazole the AUCτ and Cmax values of bosentan increased by 122.0% and 94.8%, respectively, indicating that ketoconazole decreased the oral clearance of bosentan importantly and confirming that a significant fraction of the dose of bosentan is metabolized by CYP 3A4. The presence of ketoconazole did not appear to impact the t1/2β values of bosentan and the metabolites. Among the metabolites only Ro 48-5033 showed a significant 59.9 % increase of AUCτ in the presence of ketoconazole. The respective AUCτ values of the secondary metabolite Ro 64-1056 were similar in the presence and absence of ketoconazole, even though the AUCτ of the parent drug was more then doubled in the presence of ketoconazole. The individual and summed up AUCτ values of the metabolites relative to bosentan in the presence of ketoconazole were smaller than in the absence of the inhibitor. The trough concentrations of bosentan and the metabolites on Days 2 to 6 were, with the exception of Day 4, larger in the presence of ketoconazole than in the absence of the inhibitor. A relevant impact of the co-administration of ketoconazole on the exposure measures of bosentan and metabolites was seen on Day 2. An attainment of steady-state for bosentan and the metabolites could not be concluded from the data.

Safety:

Five (5) subjects reported 8 AEs: 2 occurred during Treatment A and 6 during Treatment B. Six (6) of the 8 AEs were judged to be remotely or possibly related to drug administration. Mild to moderate headache (n=4) was the most frequent event reported. One case of mild diarrhoea (n=1) was treated with loperamide. All other AEs resolved spontaneously.

Conclusions:

The co-administration of 200mg ketoconazole qd for 6 days with 62.5mg bosentan bid impacted the exposure measures of bosentan and its metabolites significantly. AUCτ and Cmax were increased by 120.0% and 94.8%, respectively.

Reviewer's Comments:

The observed increases in AUCτ and Cmax of 120.0% and 94.8%, respectively, were greater than those in a previous study reported in the NDA with healthy volunteers receiving the same treatment (83% increase in AUCτ, 62% increase in Cmax). The present wording in the label is adequate.

Protocol AC-052-109

Study Title: A Study to Investigate the Possible Drug-Drug Interaction between Bosentan and Simvastatin in Healthy Subjects

Principal Investigator:

Dieter Saaarschmidt, MD, Pharos GmbH, Hoervelsinger Weg 52-1, 89081Ulm, Germany

Objectives:

1. To evaluate the influence of concomitant simvastatin on the PK of bosentan and its metabolites and to evaluate the influence of concomitant bosentan on the pharmacokinetics of simvastatin in healthy subjects
2. To evaluate the tolerability of concomitant simvastatin and bosentan in healthy subjects

Bosentan induces the activity of the iso-enzymes CYP 3A4 and 2C9 moderately and thus the efficacy of concomitantly administered drugs may be impacted. CYP 3A4 is involved in the metabolism of a wide variety of drugs. Simvastatin was selected as sensitive probe substrate of 3A4 to study the impact of bosentan's induction of CYP 3A4.

Subjects:

It was planned to enroll 9 healthy subjects in the study. Nine (9) subjects were enrolled in the study and completed the study. To be eligible the healthy volunteers had to meet the following inclusion criteria: be male, in the age between 18 and 50 years of age, within ±15% of ideal body weight, have normal blood pressure (systolic blood pressure 100-140mmHg, diastolic blood pressure; 50-90mmHg) and pulse rate (45-90 bpms) after 5 minutes in the sitting position, a 12 Lead ECG without clinically relevant abnormalities (PR ≤200msec, QRS ≤115msec, QTc ≤440msec), hematology, biochemistry and urinalysis test results not clinically relevantly deviating from normal range, negative results from drug screen (cannabinoids, cocaine, opiates, benzodiazepines), should have normal eating habits (eg. should not be vegetarian), consume ≤ 5 cups of coffee or tea per day or ≤ 3 bottles (250mL) of cola and able to stop caffein consumption during institutionalization, be able to communicate well with the Investigator, and give written informed consent.

Exclusion criteria included the following: History or clinical evidence of any disease or alcoholism or drug abuse or existence of any surgical or medical condition that might interfere with the absorption, distribution or elimination of the study drugs, history of hepatitis B or C and/or positive results from hepatitis serology indicating acute or chronic hepatitis B or C (except for vaccinated subjects), positive results from HIV serology, history of relevant hypersensitivity or severe adverse reaction to any drug, presence or history of any allergy requiring acute or chronic treatment, participation in another clinical trial with intake of an investigational drug during the previous 12 weeks or participation in a clinical trial with a marketed product in the previous 8 weeks (immunostimulating or immunosuppressive drugs during previous 6 months), intake of a drug with a well-defined potential for toxicity to a major organ system during the previous 12 months, intake of drugs inhibiting or inducing the isoenzymes CYP 3A4 and/or CYP2C9 within the 4 weeks prior to screening until the end of the study, intake of endothelin antagonists during the previous 12 months, concomitant treatment with any medication, loss of ≥ 250mL blood in the previous 3 months, legal incapacity or limited legal capacity, symptoms of a clinically relevant illness in the 4-week period preceding screening.

Methodology:

This was a single center, open-label, multiple dose, randomized, three period cross over study. In Treatment A the volunteers received bosentan bid for 5.5 days (125mg bosentan bid on Days 1 through 5 and a single dose administration of 125mg bosentan on Day 6. In Treatment B the volunteers received simvastatin for 6 days (40mg simvastatin qd on Days 1 to 6. In Treatment C the volunteers received bosentan 125mg bid for 5.5 days (125mg bosentan on Days 1 through 5 and a single dose administration of 125mg bosentan on Day 6 and concomitantly simvastatin 40mg qd on Days 1-6. The study medication was given to the volunteers in the sitting position together with 150ml water at the same time in the morning between 7 and 9 am.
The time interval between the administration of bosentan in the morning and evenings was exactly 12 hours. On study days 1 through 5 during Treatments A, B and C, study medication was administered irrespective of the time of meal consumption whereas on Day 6 in all treatment periods, study medication was administered to subjects 30 minutes following intake of a continental breakfast. From screening to the end-of study-examination the subjects had to refrain from strenuous physical activity. On the days of blood sampling (Day 6 of each treatment period) the subjects receive standardized meals as follows: A continental breakfast was consumed between 0.5 and 1 hour before drug administration, a lunch was served 4 hours after drug administration, a snack was served 8 hours after drug administration and an evening meal was served 12 hours after drug intake. During each treatment period (A, B, C) the subjects were institutionalized from approximately 12 hours before to 12 hours following intake of medication on Day 6.

The dose of bosentan of 125mg bid corresponds to the highest labeled dose. The dose range of simvastatin administered ranges between 5 and 80mg given bid. The dose of

40mg of simvaststin administered qd proposed for this study corresponds to a clinically effective regimen.

Study Drug:

The batch number for bosentan was F0358A001. The batch number for the Zocor® tablets used in this study was HMS 8250.

Prior and Concomitant Treatment:

Not permitted was: Participation in another clinical trial with intake of an investigational drug during the past previous 12 weeks or participation in a clinical trial with a marketed drug in the previous 8 weeks (immunostimulating and immunosuppressive drugs during the previous 6 months), intake of a drug with a well-defined potential for toxicity to a major organ system during the previous 12 months, intake of drugs inhibiting or inducing the isoenzymes CYP 3A4 and/or 2C9 within the 4 weeks prior to screening until the end of the study, intake of endothelin antagonists during the previous 12 months, concomitant treatment with any medication.

Evaluation:

Pharmacokinetics: The plasma concentration time profiles of bosentan and its metabolites were followed for 12 hours on Day 6 of each treatment period. The plasma concentrations of simvastatin and its metabolite, simvastatin-β-hydroxyacid, were followed on Day 6 of Treatment C for 12 hours following drug administration. In addition morning trough concentrations of all the compounds were monitored on Days 1 through 5 of the respective treatments.

Efficacy: Was not evaluated

Safety: At screening a medical history was taken, a physical examination, routine hematology and biochemistry test, a urinalysis and virus serology performed, and a 12 lead ECG and vital signs recorded. At the end-of study-evaluation all these activities, except for taking the medical history, were repeated. Adverse events were recorded throughout the duration of the study. Subjects were encouraged to report AEs spontaneously. They were asked "how do you feel" at scheduled times throughout the duration of the study. The vital signs, systolic and diastolic blood pressure and pulse rate, were measured indirectly using an automated oscillometric device. Blood pressure was always measured on the same arm. The measurements were recorded with the subjects in a semi-supine position after they had rested for 5 minutes at the following times: At screening, on Day 6 of the treatments immediately prior to and 1, 2, 4 and 12 hours after drug administration and t he end-of-study evaluation. A 12 lead ECG was performed with the subjects in a supine position at screening and at the-end-of -study evaluation. Prior to the ECG evaluation the volunteers were to have rested for 5 minutes in the supine

position. The PR, QRS and QT intervals, the heart rate (bpms) and the rhythm were evaluated. The QTc intervals were computed on application of Bazett's formula.

Blood Sample Collection for PK:

Blood samples for the determination of the plasma concentration profiles of the analytes were collected on Day 6 of Treatments A, B and C immediately prior to drug administration and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 10, and 12 hours after drug administration. Blood samples (4mL) were taken on Day 6 of Treatments A and B to determine the PK of bosentan and metabolites. Blood samples (10mL) were collected on Day 6 of Treatment C to determine the PK of bosentan, its metabolites and simvastatin. In addition morning trough samples were collected on Days 1 though 5.

Analytical Methodology:

Bosentan and its metabolites Ro 48-5033, Ro, Ro 47-8634 and Ro 64-1056 were measured by [redacted]
Calibration and Quality Control (QC) samples were prepared in plasma. Day-to day performance was evaluated by the analysis of the QC samples. The plasma concentrations of simvastatin and β-hydroxyacid simvastatin were determined using a validated [redacted] assay.

Pharmacokinetic Parameters:

The following PK parameters were determined for bosentan, its metabolites and simvastatin and its metabolite:

Cmax	Observed maximum plasma concentration
tmax	Time to appearance of Cmax
AUCτ	Area under the plasma concentration-time curve during a dosing interval
t1/2	Terminal elimination half-life

Cmax and tmax were taken directly from the plasma concentration time data. AUCτ was obtained on application of the linear trapezoidal rule. The values for t1/2 were obtained from log-linear regression analysis of the measured concentrations in the terminal elimination phase.

Statistical Methods:

Sample size and power: The total number of subjects enrolled in the study was not based on statistical power considerations. However, based on previous experience the proposed number of 9 subjects was considered sufficient to achieve the goals of the study.

Analysis of PK Data:

The effects of simvastatin on the PK of bosentan and metabolites were evaluated by comparing log-transformed AUCτ and Cmax values of bosentan and its metabolites on Day 6 of Treatments A and C using ANOVA with the factors treatment, period, sequence and subject. Likewise the log transformed AUCτ and Cmax values of simvastatin and its metabolite on Day 6 of Treatments B and C were compared using ANOVA with the factors treatment, period, sequence and subject to evaluate the effect of bosentan on the PK of simvastatin. The signed Rank Wilcoxon Test was performed to explore differences in tmax between treatments A and C for bosentan and between Treatments B and C for simvastatin.

Analysis of Safety Data:

The safety analysis used descriptive methods and the data were summarized by mean, median, SD, minimum, maximum and number of available observations. The individual data of the laboratory tests, vital signs and ECG parameters were listed and flagged when outside of the normal range. Concomitant medications were also reported.

RESULTS:

Disposition of the Volunteers:

Nine (9) healthy male volunteers were enrolled and completed the study. Their mean (SD) demographic characteristics were: age=34.4 years, height=181.6 (5.2)cm and weight: 76.5 (9.4)kg.

Analytical:

Bosentan and Metabolites

The results of the validation reports indicated that the performance of the assays fulfilled the requirements for accuracy and precision. The standard curves for bosentan in the range of [redacted] ng/mL and for the metabolites (Ro 48-5033, Ro 47-8634 and Ro 64-1056) in the range [redacted] ng/mL were linear. The linearity of the analytical method for bosentan and metabolites was demonstrated by correlation coefficients ≥ 0.998 for bosentan and Ro 48-5033, ≥ 0.994 for Ro 47-8634 and Ro 64-1056.The LLOQ was set at [redacted] ng/mL for bosentan and [redacted] ng/mL for the metabolites. The inter-assay precision

(CV%) assessed from the quality control (QC) samples was . —% for bosentan, % for Ro 48-5033, . — % for Ro 47-8634 and . —% for Ro 64-1056. The percent inter-assay accuracy of the QC samples ranged between . — for bosentan, from — % for Ro 48-5033, from . — 7% for Ro 47-8634 and from ! — to — % for Ro 64-1056.

Simvastatin and Simvastatin-β-hydroxyacid

The standard curves for simvastatin and simvastatin-β-hydroxyacid were linear in the concentration ranges of . — !ng/mL and — ng/mL, respectively. The QC samples for simvastatin had concentrations of — ng/mL that represented the upper, median and lower part of the calibration curve. The corresponding QC samples for simvastatin-β-hydroxyacid had concentrations of — and — /mL. Th linearity of the analytical method for simvastatin and its metabolite was demonstrated by correlation coefficients of 0.9997 for simvastain and 0.9999 for simvastatin-β-hydroxyacid. The within day precision (CV%) for simvastatin and its metabolite were — %, respectively. The within day percent accuracy of the QC samples for simvastatin ranged between — % and for simvastatin-β-hydroxyacid between — %.

Pharmacokinetics:

The arithmetic mean concentrations of bosentan and metabolites in the presence and absence of simvastatin are depicted in Figure 1.

Figure 1. Linear and Semilogarithmic Plots of the Arithmetic Mean Concentrations of Bosentan and Its Metabolites in the Presence and Absence of Simvastatin

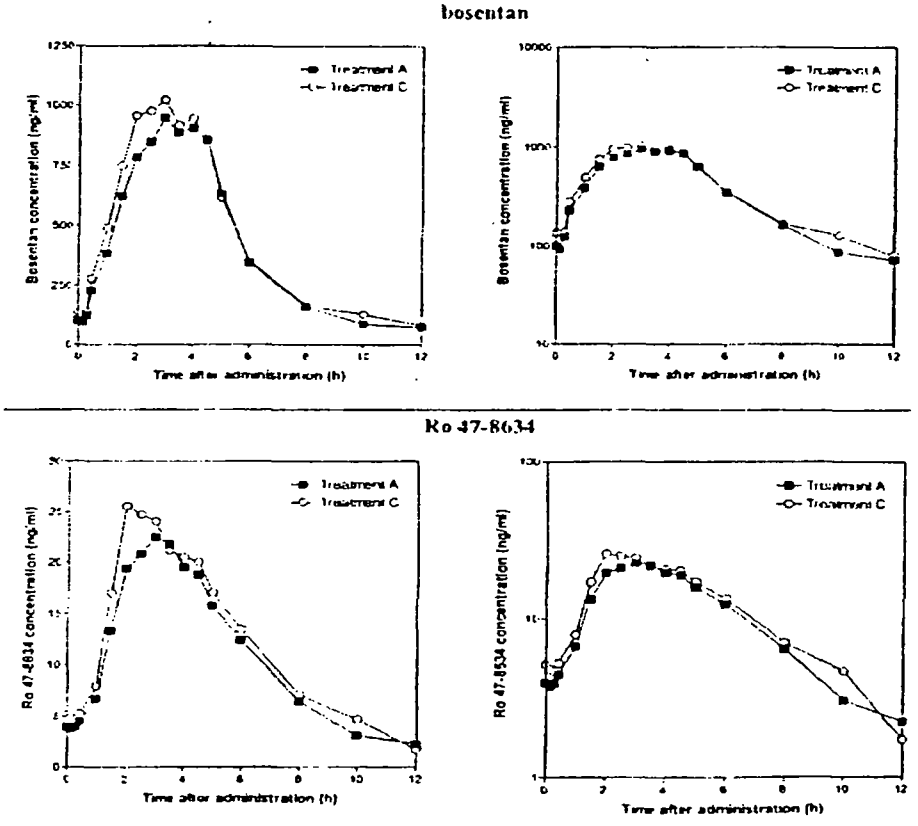
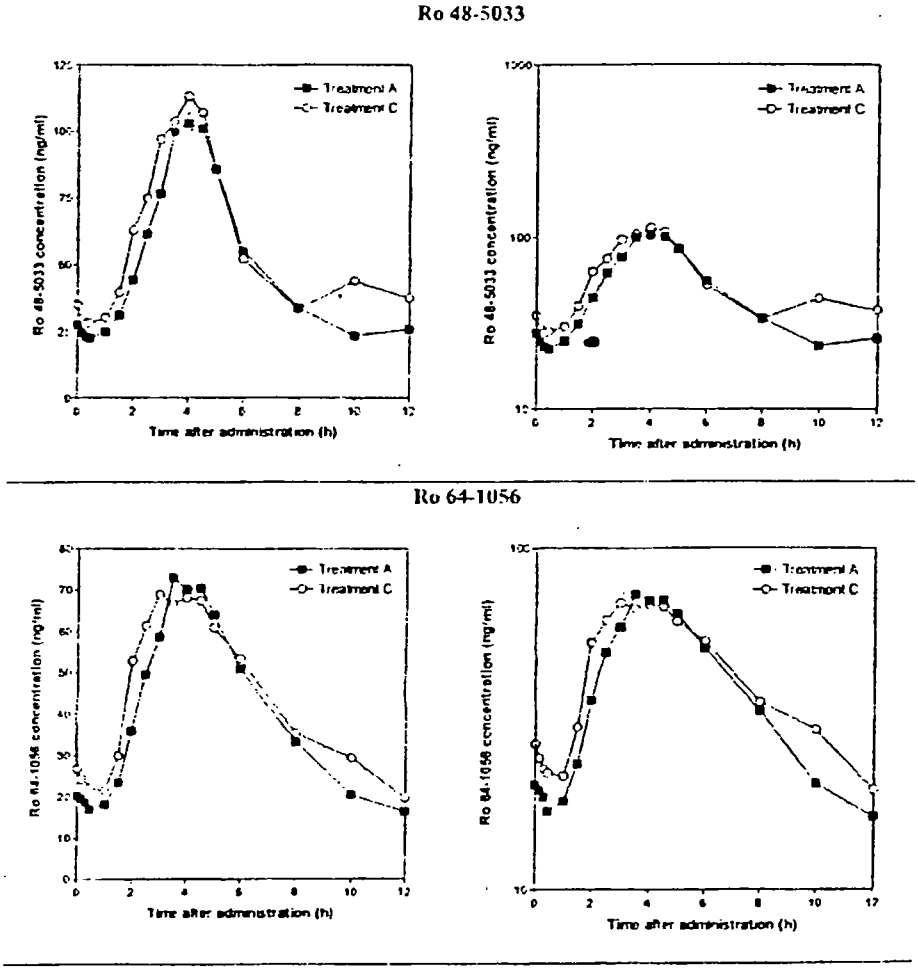


Figure 1. Linear and Semilogarithmic Plots of the Mean Arithmetic Plasma Concentrations of Bosentan and its Metabolites in the Presence and Absence of Simvastatin Continued



Treatment A Bosentan 125 mg twice a day for 5.5 days.
Treatment C Bosentan 125 mg twice a day and simvastatin 40 mg once a day for 5.5 and 6 days, respectively.

Table 1 lists the PK parameters of bosentan and its metabolites.

Table1. Geometric Mean (95% CI) PK Parameters for Bosentan and Metabolites in the Presence and Absence of Simvastatin

Bosentan

Treatment	t_{max} (h)	C_{max} (ng/ml)	AUC_τ (ng·h/ml)
A	3.5	1006	4586
	—	(768, 1318)	(3719, 5656)
C	3.5	1118	4928
	—	(872, 1434)	(3945, 6156)

Ro 47-8634

Treatment	t_{max} (h)	C_{max} (ng/ml)	AUC_τ (ng·h/ml)
A	3.5	25.7	123
	—	(21.9, 30.2)	(105, 144)
C	2.5	29.8	138
	—	(24.5, 36.3)	(117, 163)

Ro 48-5033

Treatment	t_{max} (h)	C_{max} (ng/ml)	AUC_τ (ng·h/ml)
A	4.0	112	551
	—	(85.0, 148)	(451, 674)
C	4.0	114	628
	—	(89.5, 144)	(495, 797)

Ro 64-1056

Treatment	t_{max} (h)	C_{max} (ng/ml)	AUC_τ (ng·h/ml)
A	4.5	81.2	441
	—	(67.5, 97.6)	(359, 542)
C	3.5	81.3	493
	—	(69.3, 95.3)	(409, 595)

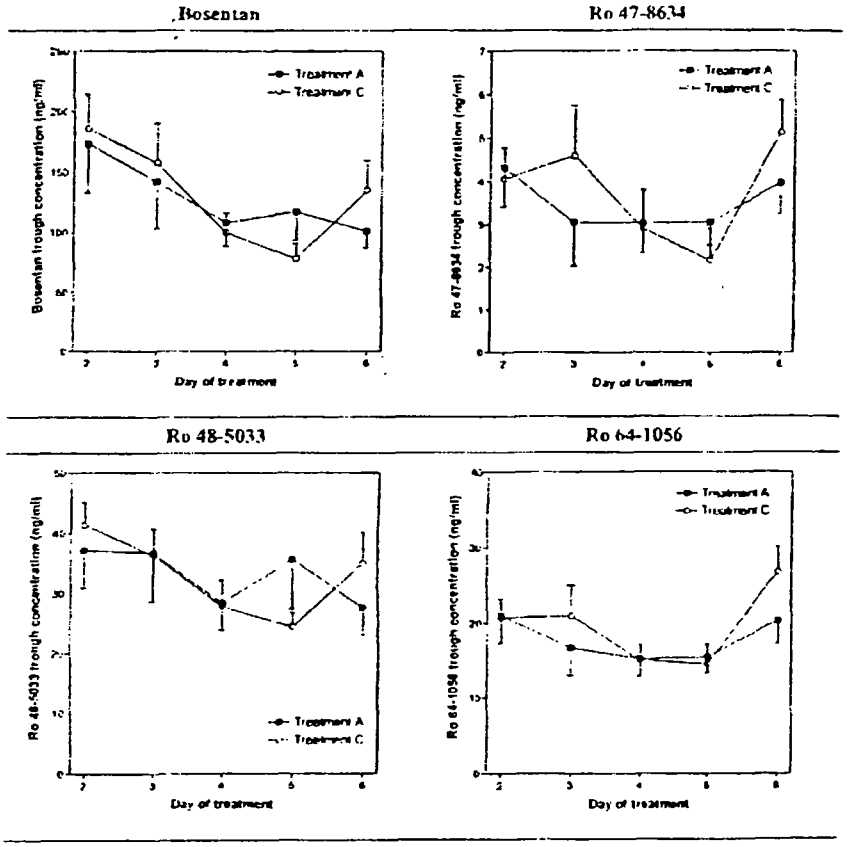
Data are expressed as geometric mean (and 95% CI) or, for t_{max}, as median (and range).

Treatment A: Bosentan 125 mg twice a day for 5.5 days.

Treatment C: Bosentan 125 mg twice a day and simvastatin 40 mg once a day for 5.5 and 6 days, respectively.

Figure 2 shows plots of the trough concentration time profiles of bosentan and metabolites in the presence and absence of simvastatin.

Figure 2. Linear Plots of the Arithmetic Mean (SEM) trough Plasma Concentrations Time Profiles of Bosentan and Metabolites in the Presence and Absence of Simvastatin.

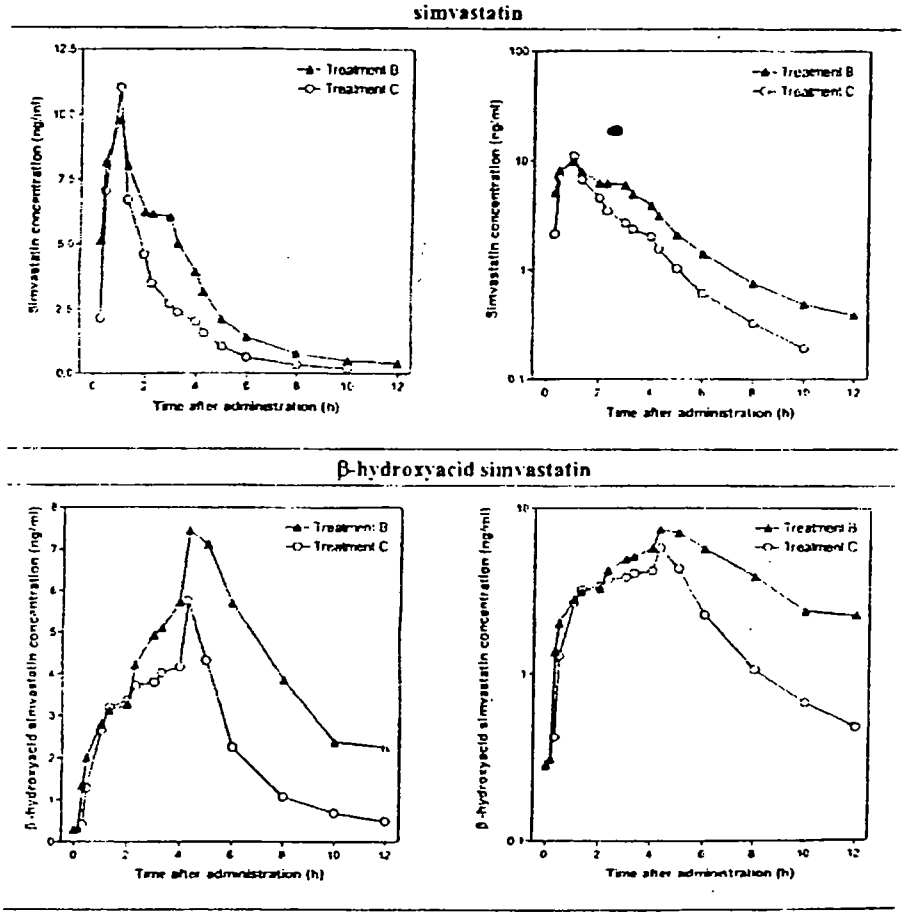


Treatment A: Bosentan 125 mg twice a day for 5.5 days.
Treatment C: Bosentan 125 mg twice a day and simvastatin 40 mg once a day for 5.5 and 6 days, respectively.

The measures of exposure, AUCτ and Cmax, for bosentan and metabolites in the presence and absence of simvastatin were similar with a slight tendency to increase by ≤ 17.9 % in the presence compared to in the absence of simvastatin. The presence of simvastatin also had no impact on the tmax for bosentan and the metabolites. The AUCτ values of the individual and summed up metabolites relative to the parent drug in the presence and absence of simvastatin were comparable with a trend for slightly larger values in the presence compared to in the absence of the statin. The attainment of steady state for bosentan and its metabolites in the presence of simvastatin could not be concluded. These results indicated that co-administration of simvastatin for 6 days had no clinically relevant impact on the PK parameters of bosentan and its metabolites.

Figure 3 depicts the plasma concentration profiles of simvastatin and simvastatin-β-hydroxyacid in the presence and absence of bosentan.

Figure 3. Linear and Semilogarithmic Plots of the Arithmetic Mean Plasma Concentration Profiles of Simvastatin and Simvastatin-β-hydroxyacid in the Presence and Absence of Bosentan



Treatment B: Simvastatin 40 mg once a day for 6 days
Treatment C: Bosentan 125 mg twice a day and simvastatin 40 mg once a day for 5.5 and 6 days, respectively.

Table 3 lists the PK parameters of simvastatin ansd simvastatin-β-hydroxyacid in thepresence and absence of bosentan.

Table3. Geometric Means (95%CI)* of PK Parameters for Simvastatin and Simvastatin-β-Hydroxyacid in the Presence and Absence of Bosentan

	Simvastatin			
Treatment	t_{max1} (h)	C_{max1} (ng/ml)	AUC_τ (ng·h/ml)	$t_{1/2}$ (h)
B	1.5	12.9	30.5	3.2
		(8.1, 20.5)	(23.1, 40.2)	(2.6, 3.8)
C	1.0	10.7	20.0*	2.1
		(7.2, 15.9)	(15.9, 25.1)	(1.6, 2.9)
	β-hydroxyacid simvastatin			
Treatment	t_{max1} (h)	C_{max1} (ng/ml)	AUC_τ (ng·h/ml)	$t_{1/2}$ (h)
B	4.5	7.3	42.0	4.0
		(5.4, 9.7)	(32.1, 57.8)	(3.3, 5.0)
C	4.5	6.0	23.4*	2.9
		(4.2, 8.7)	(16.7, 32.6)	(2.4, 3.6)

Data are expressed as geometric mean (and 95% CI) or, for t_{max}, as median (and range) * $p < 0.05$ as compared to treatment B.

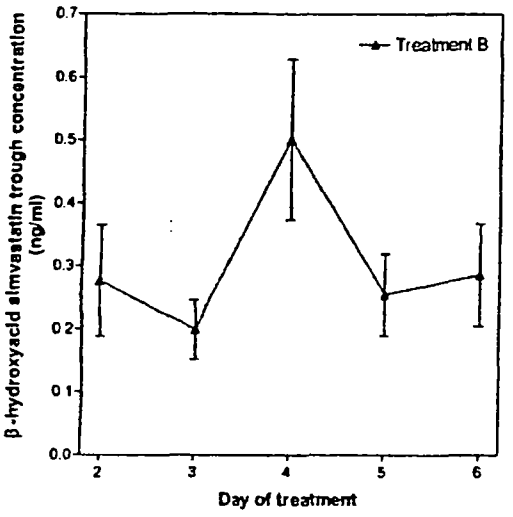
Treatment B: Simvastatin 40 mg once a day for 6 days.

Treatment C: Bosentan 125 mg twice a day and simvastatin 40 mg once a day for 5.5 and 6 days, respectively.

*Median (range) for tmax

Figure 4 shows the arithmetic mean trough plasma concentration profiles of simvastatin-β-hydroxyacid.

Figure 4. Linear Plot of the Arithmetic Mean (SEM) Trough Plasma Concentration Profiles of Simvastatin-β-Hydroxyacid



Treatment B: Simvastatin 40 mg once a day for 6 days.

The geometric means of AUCτ for simvastatin and its metabolite decreased significantly by 34.4% and 45.6%, respectively, in the presence of bosentan. The respective Cmax values showed the same tendency (-17.1% and -17.8%). The co-administration of bosentan also tended to decrease the t1/2 of simvastatin and its metabolite. In the absence of bosentan the ratio of the AUCτ values for the metabolite to simvastatin was 1.41. This value decreased to 1.17 in the presence of bosentan. These results indicated that bosentan, a known inhibitor of the iso-enzymes CYP 3A4 and 2C9 inhibited the metabolism of simvastatin and simvastatin-β-hydroxyacid. The attainment of steady state of simvastatin-β-hydroxyacid could not be concluded from the morning trough concentrations. A large majority of the morning trough concentrations for simvastatin were below the LLOQ and the time course of the plasma concentrations of the parent drug could not be determined.

Safety:

All subjects reported at least one AE during the course of the study. Of the 38 reported AEs, 12 occurred during Treatment A, 5 during Treatment B and 21 during Treatment C. Mild to oderate headache was the most frequently reported event (11 cases). Thirty (30) AEs were judged by the Investigator to be possibly related to the medications. The number of total and drug related AEs was greatest in Treatment C. One case of moderate headache was treated with ibuprofen, all other AEs resolved spontaneously.

Conclusions

Co-administration of 40mg simvastatin qd and bosentan 125mg bid for 6 days results in a significant reduction of AUCτ and Cmax of simvastatin (-34.4% and -17.1%) and its metabolite (-45.6% and -17.8%). Co-administration of simvastatin has no relevant impact on the PK of bosentan.

Reviewer's Comments

The magnitude of the effects of bosentan on simvastatin and its metabolite in the present study was smaller than the decrease in AUC and Cmax for simvastatin (-83% and -62%, respectively) and simvastatin-β-hydroxyacid (-60% and -33%, respectively) in a previous study reported in the NDA. There was agreement between the studies that simvastatin has no impact on the exposure measures of bosentan. The present wording of the label is adequate.

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

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/s/

Peter Hinderling
5/20/03 04:48:27 PM
PHARMACOLOGIST

Patrick Marroum
5/20/03 05:34:45 PM
BIOPHARMACEUTICS

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

General Information About the Submission

	Information		Information
NDA Number	21 290	Brand Name	Tracleer
OCPB Division (I, II, III)	Div 1	Generic Name	Bosentan
Medical Division	CardioRenal	Drug Class	Endothelin Receptor Anatgonist
OCPB Reviewer	Peter Hinderling	Indication(s)	Pulmonary Arterial Hypertension
OCPB Team Leader	Patrick Marroum	Dosage Form	62.5 mg and 125mg Tablets
		Dosing Regimen	31.25mg , 62.5mg and 125mg QD
Date of Submission	December 4/02	Route of Administration	oral
Estimated Due Date of OCPB Review	May 30/03	Sponsor	Actelion
PDUFA Due Date	June 4/03	Priority Classification	S
Division Due Date	May 30/03		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies				
HPK Summary				
Labeling				
Reference Bioanalytical and Analytical Methods	X			
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:				
multiple dose:				
Patients-				
single dose:				
multiple dose:	X			
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:	X			
geriatrics:				

renal impairment:				
hepatic impairment:				
PD:				
Phase 2:				
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		2		

Filability and QBR comments		
	"X" if yes	Comments
Application filable ?	X	Reasons if the application is not filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?
Comments sent to firm ?		Comments have been sent to firm (or attachment included). FDA letter date if applicable.
QBR questions (key issues to be considered)		
Other comments or information not included above		
Primary reviewer Signature and Date	Peter Hinderling , 1/21/03	
Secondary reviewer Signature and Date	Patrick Marroum, 1/22/03	

CC: NDA 21-290, HFD-860(Electronic Entry or Lee), HFD-110(CSO), HFD-860(TL, DD, DDD)

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/s/

Peter Hinderling
2/5/03 05:31:15 PM
PHARMACOLOGIST

Patrick Marroum
2/6/03 08:36:09 AM
BIOPHARMACEUTICS