1. A more frequent sample collection would have allowed a better definition of the time course of the endothelin-1 plasma concentrations and hence of the endothelin receptor antagonistic effect of ambrisentan.
2. Female subjects (no childbearing potential) should have been included.
3. A pre-dose urine sample should have been collected

Study Report: EE-002 "A Randomized, Double-Blind, Placebo Controlled, Multiple Ascending Dose Trial to Investigate the Tolerability, Safety, Pharmacokinetics- and Dynamics of the Oral BSF 20875 in Healthy Male Subjects"

Study Site and Investigator: [redacted]

Objectives

Primary

To investigate the safety and tolerability of multiple ascending oral doses of BSF 208075 in healthy male subjects

Secondary

To assess the multiple dose pharmacokinetics of BSF 209075 in plasma and urine
To evaluate the pharmacodynamic effects of BSF 208075 on cardiovascular parameters
To screen the potential effects of BSF 208075 on endogenous endothelin-1 plasma concentrations
To detect a potential enzyme induction by BSF 208075

Formulations

5 mg tablets (Batch No. L0003020,P272/2) and 10 mg tablets (Batch No. L0003024,P272/22) and matching 5 and 10 mg placebo tablets (Batch No. L0003022,P269/2) were provided by the sponsor.

Design

A randomized, double-blind, placebo controlled, parallel group design was used. Healthy male subjects in the age between 18-45 years were to be enrolled. The planned ascending doses were 5 mg qd, 10 mg qd and 20 mg qd. They were to be administered to the subjects in the morning

after an overnight fast together with 240 mL water. The doses were to be administered for 10 days. Study Days 1 and 10 were planned as in-house stays. A follow-up was to be conducted 72 h after the last dosing. The enrollment of 30 subjects was planned with 10 subjects per dose group (8 on active drug and 2 on placebo).

Table 1 shows the scheduled study activities:

Table 1 Study flow chart

	Pre dose	Dosing group I - III													Follow-up
Day	-14 to -7	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Informed consent	X														
Anamnesis	X														
Physical examination	X														X
Ambulatory[c]	X			X	X	X	X	X	X	X	X		X	X	X
In-house stay[b]		X		X							X		X		
Urine drug test/alcohol breath test	X	X									X				
Serology	X				X										
Laboratory (blood and urine)[f]	X		X[a]		X		X[a]		X			X			X
ECG	X		X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]			
Vital signs - Respiratory frequency and core body temperature	X		X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]			
Impedance cardiography	X		X				X					X[g]			
Blood pressure, pulse	X		X	X	X	X	X	X	X	X	X	X			
Dosing[b]			X	X	X	X	X	X	X	X	X	X			
Pharmacokinetics (blood)[h]			X	X	X	X	X	X	X	X	X	X	X	X	X
Urine collection[i]		X	X	X								X	X	X	X
Pharmacodynamics[g] (blood)			X	X		X		X		X		X	X	X	
Monitoring of unwanted events			X	X	X	X	X	X	X	X	X	X	X	X	X

a) Urine analysis only on Days 1, 5 and within 72 hours after the last dosing.
b) From Day 0 approx. 8.00 until Day 2, 4 hours after dosing at approx. 11.30. From Day 9 approx. 8.00 until Day 11, 24 hours after the last dosing, approx. 8.00.
c) Ambulatory for screening and follow-up examinations. On Days 3-8 two times, in the morning for dosing until 4 hours after dosing approx. 11.30, and in the evening 12 hours after dosing. On Day 2 only in the evening 12 hours after dosing, on Day 11 only in the evening 36 hours after the last dosing.
d) Day 1 and 10: pre-dose, 1, 2, 4, 8, 12, 24 hours post-dose; Days 2-9 1, 2, 4, 12 hours post-dose.
e) Days 1 and 10: pre-dose, 2, 4, 6, 12, 24 hours post-dose; Days 2-9 only 2 hours post-dose.
f) Day 10: 2 and 24 hours after the last dose.
g) Day 1: pre-dose, 2 and 10 hours post-dose; Day 10: 2, 10, 24 hours post-dose; Day 2, 4, 6, 8: 2 hours post-dose.
h) Days 1 and 10: pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16 hours post-dose and Day 10: 24, 36, 48 hours post-dose; Day 2, 4, 6, 8: hours pre-dose; Day 3, 5, 7, 9: 2 hours post-dose.
i) Day 0: 1-24 hours pre-dose; Day 10: 0-24, 24-48, 48-72 hours after the last dose.

Vital signs, blood pressure, pulse rate, ECG, stroke volume, cardiac output, ejection time and pre-ejection period (by impedance cardiography) and the coagulation parameters (thromboplastin time, partial thromboplastin time, thrombin time, concentrations of antithrombin III, fibrinogen) hematology, urinalysis and chemistry were determined at scheduled times during the study. The results on the liver enzymes SGPT, SGOT and γ-GT and erythrocyte count were analyzed by a repeated measurement ANOVA model with main effect terms for "dose" (between subjects) and "day" (Days 1, 3, 5, 7, 10, and 13 within subjects) and a term for the dose-by-day interaction, the latter being relevant for the detection of dose related changes over time.

Pharmacokinetic Profiling

Blood samples were collected on Day 1: Pre-dose and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16 h after administration
Day 10: Pre-dose and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24, 36, 48, 72 h after the last dose administration
Days 2, 4, 6, 8: pre-dose
Days 3, 5, 7, 9: 2 h post-dose
Total urine volumes were collected on Days 0-1: -24-0 h, and on Days 10-13: 0-24, 24-48, and 48-72 h after the last dose on Day 10

Bioassay

The BSF 208075 concentrations in plasma and urine were determined by a HPLC-MS/MS assay with LLOQ in plasma and urine [redacted], respectively. The assays were performed [redacted]

Pharmacokinetic Data Analysis

The parameters AUC0-24, AUC, Cave (AUC0-24/24h), Cmax, Cmin (lowest concentration observed), Cpre (trough concentration observed in morning pre-dose), CL/F, tmax, λz, t1/2, PTF [peak-trough fluctuation (%) PTF= 100 • (Cmax,ss- Cmin,ss)/Cave,ss], Ae0-24, and CLr (=Ae0-24/AUC0-24) were determined using compartment model independent methods.

To evaluate dose proportionality of the PK of BSF 208075 the geometric means of log transformed Cmax and AUC were plotted against log transformed dose and linear least square regressions performed. The slope of the regression line was tested for a significant difference from 1.

Pharmacodynamics

Blood samples for the determination of endothelin-1 plasma concentrations were collected on
Day 1: Pre-dose, 2 and 10 h after BSF 208075 administration
Day 10: 2, 10, 24 and 48 h after BSF 208075 administration
Days 2, 4, 6, 8: 2 h after BSF 208075 administration

The method used to measure the plasma concentrations of endothelin-1 is not indicated.

Enzyme Induction Potential

To determine whether BSF 208075 induces CYP3A 6 β-hydroxycortisol total urine volumes were collected on:
Days 0-1: -24-0 h prior to administration of BSF 208075
Days 1 and 10: 0-24 h after administration of BSF 208075

The method used to measure 6β-hydroxycortisol is not indicated.

Results

Thirty subjects were enrolled and 28 completed the study. The subjects were males and ranged in age between 22 and 41 y and in weight between 64.4 k and 84.6 kg. Two subjects dropped out of the study due to adverse events after receiving the 10 mg qd dose. One subject developed fever and diarrhea on Day 7 and the other subject reported moderate headache on Day 3. Only the 5 mg and 10 mg dose levels were tested. Because of safety concerns the 15 mg qd dose regimen was not administered. Instead the third dose group received an intermediate dose of 7.5 mg qd.

Tolerability/Safety

The adverse events recorded (number, severity, seriousness) are listed in Table 8:

Table 8 Adverse event summary

	Placebo	5.0 mg/day	7.5 mg/day	10.0 mg/day
Number of subjects reporting	N = 6	N = 8	N = 8	N = 8
any adverse event	5	7	8	8
at least probably related AEs	3	1	8	8
severe adverse events	none	none	none	none
serious adverse events (SAEs)	none	none	none	none
AEs leading to withdrawal	none	none	none	2

Data source: Appendix 2.7, Table 2.7.5

Headache was the most frequently reported adverse event and occurred more often (100%) in subjects receiving the 7.5mg qd and 10 mg qd treatments.

There were no overt drug induced changes in hematology and coagulations parameters noted.

Mean SGPT and SGOT in the different dose groups and individual SGPT and SGOT in subjects with elevated values are listed in Tables 10 and 11:

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Table 10 Mean ALAT (GPT) and ASAT (GOT) concentrations over time

		Screening	Pre-dose Day 1	Day 7	Day 10	Day 13
ALAT (GPT) (U/l)	Placebo	30.58	27.90	29.92	28.12	36.92
	5 mg/day	35.21	31.98	41.96	19.46	60.15
	7.5 mg/day	27.74	25.19	28.00	32.01	40.81
	10 mg/day	32.35	29.78	33.90	44.50	56.87
ASAT (GOT) (U/l)	Placebo	20.92	19.48	20.45	19.18	24.97
	5 mg/day	25.04	22.40	28.51	28.75	37.70
	7.5 mg/day	22.38	20.75	25.88	23.25	28.59
	10 mg/day	25.45	22.80	24.04	27.68	30.28

Data source: Appendix 2.8, Table 1.2.1.1-4 and Table 1.2.2.1-4

Table 11 Individual ASAT (GOT) and ALAT (GPT) concentrations over time

	Dose group	Subject	Screen	Day 1 pre	Day 3	Day 5	Day 7	Day 10	Day 13	follow up
ALAT[b] (GPT) [U/l]	5 mg/day	5	38.8	40.8	50.6	64.6	101.5↑	138.7↑	142.1↑	99.9↑
	7.5 mg/day	23	37.9	40.0	55.2	59.6	66.0↑	75.1↑	113.4↑	NA
	10 mg/day	12	46.5	42.1	39.1	40.7	48.3	76.2↑	90.5↑	NA
	10 mg/day	13	45.8	42.5	42.7	53.9	56.7	74.7↑	84.0↑	NA
ASAT[c] (GOT) [U/l]	5 mg/day	5	20.4	21.4	26.5	34.0	53.3	52.3	55.0	31.2
	7.5 mg/day	23	26.6	24.3	33.0	32.6	36.0	34.9	50.6	NA
	10 mg/day	12	40.9	29.3	25.1	25.9	30.0	45.1	38.6	NA
	10 mg/day	13	23.6	24.7	20.9	29.3	29.7	32.0	35.8	NA

[a] NA: Value not available

[b] Normal range: 21.0 – 72.0 U/l

[c] Normal range: 17.0 – 59.0 U/l

↑ above normal range

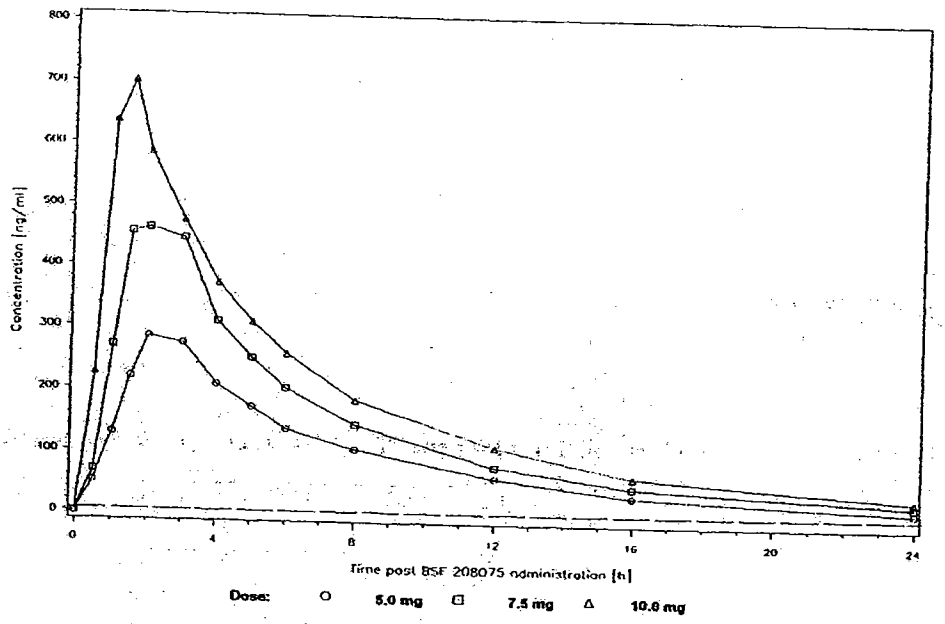
Data source: Appendix 2.8, Table 1.2.1.1-4 and Table 1.2.2.1-4

The pre-dose-, Day 7- and Day 10-means of SGPT and SGOT in the placebo group did not change with time. However, a trend for an increase of SGPT and SGOT during the same time period was noted in the subjects receiving drug. The results of the ANOVA performed on SGPT, SGOT and γ-GT showed no statistically significant dose effect. The individual SGPT and SGPT values in subjects experiencing elevations showed a time course as well which continued on Day 13, three days after the last drug administration. All of the SGPT and SGOT values were < 3● ULN.

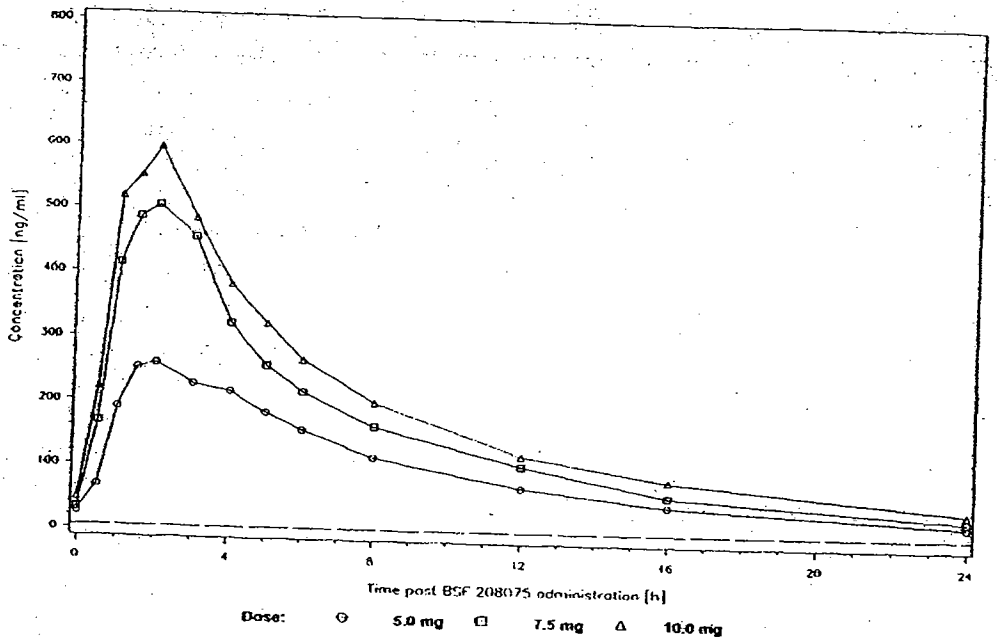
Pharmacokinetics

Linear plots of the geometric mean plasma concentration time profiles of BSF 208075 at the 3 dose levels of 5 mg, 7.5 mg and 10 mg qd for Day 1 and Day 10, respectively, are shown in the below figures :

Trial day: 1



Trial day: 10



The derived PK parameters for BSF 208075 on Days 1 and 10 are listed by dose regimens in Tables 5 and 6, respectively:

Table 5 Plasma pharmacokinetic parameters by dose - Day 1

Dose	Statistics	C_{max} [ng/ml]	t_{max} [h]	C_{min} [ng/ml]	AUC_{0-24} [ng h/ml]	AUC_{0-inf} [ng h/ml]
5 mg/day	Geom. Mean	348.84	1.80	16.59	2242.04	2400.24
N = 8	*Geom. SD*	*1.25*	*1.53*	*1.57*	*1.34*	*1.35*
7.5 mg/day	Geom. Mean	621.28	1.76	27.32[a]	3438.23	3682.09
N = 8	*Geom. SD*	*1.20*	*1.29*	*1.32*	*1.23*	*1.25*
10 mg/day	Geom. Mean	732.88	1.22	35.13	4401.67	4763.79
N = 8	*Geom. SD*	*1.20*	*1.24*	*1.27*	*1.14*	*1.14*
		$A_{e\,0-24}$ [ug]	$CL_{r\,0-24}$ [l/h]	CL/F [l/h]	K_{el} [1/h]	$t_{\frac{1}{2}}$ [h]
5 mg/day	Geom. Mean	102.02	0.0454	2.084	0.1093	6.347
N = 8	*Geom. SD*	*1.46*	*1.5295*	*1.348*	*1.1557*	*1.155*
7.5 mg/day	Geom. Mean	117.90	0.0344	2.036	0.1074	6.446
N = 8	*Geom. SD*	*1.58*	*1.6594*	*1.247*	*1.3648*	*1.364*
10 mg/day	Geom. Mean	227.60	0.0516	2.098	0.0996	6.957
N = 8	*Geom. SD*	*1.33*	*1.2821*	*1.137*	*1.1267*	*1.126*

[a] N = 7

Data source: Appendix 2.5, Tables 3.1.1-3.1.3

Table 6 Plasma pharmacokinetic parameters by dose - Day 10

Dose		C_{max} [ng/ml]	t_{max} [h]	C_{ss} [ng/ml]	$AUC_{216-240}$ [ng h/ml]	C_{av} [ng/ml]
5 mg/day	Geom. Mean	343.6	2.69	23.93	2499.77	104.14
N = 8	*Geom. SD*	*1.52*	*1.82*	*1.45*	*1.26*	*1.26*
7.5 mg/day	Geom. Mean	609.62	1.36	30.42	3861.35	160.9
N = 8	*Geom. SD*	*1.22*	*1.86*	*1.59*	*1.24*	*1.24*
10 mg/day	Geom. Mean	727.93	1.28	44.26	4655.47	193.97
N = 6	*Geom. SD*	*1.1*	*1.34*	*1.31*	*1.13*	*1.14*
		K_{el} [1/h]	$t_{\frac{1}{2}}$ [h]	$A_{e\,216-240}$ [ug]	CL/F [l/h]	CL_r [l/h]
5 mg/day	Geom. Mean	0.0512	13.58	124.68	2.0	0.05
N = 8	*Geom. SD*	*1.3511*	*1.35*	*1.48*	*1.25*	*1.2882*
7.5 mg/day	Geom. Mean	0.0419	16.49[a]	125.79	1.94[a]	0.0314[a]
N = 8	*Geom. SD*	*1.4258*	*1.42*	*1.45*	*1.24*	*1.3504*
10 mg/day	Geom. Mean	0.0455	15.24	217.5	2.14	0.0466
N = 6	*Geom. SD*	*1.3046*	*1.31*	*1.44*	*1.14*	*1.5025*

[a] N = 7

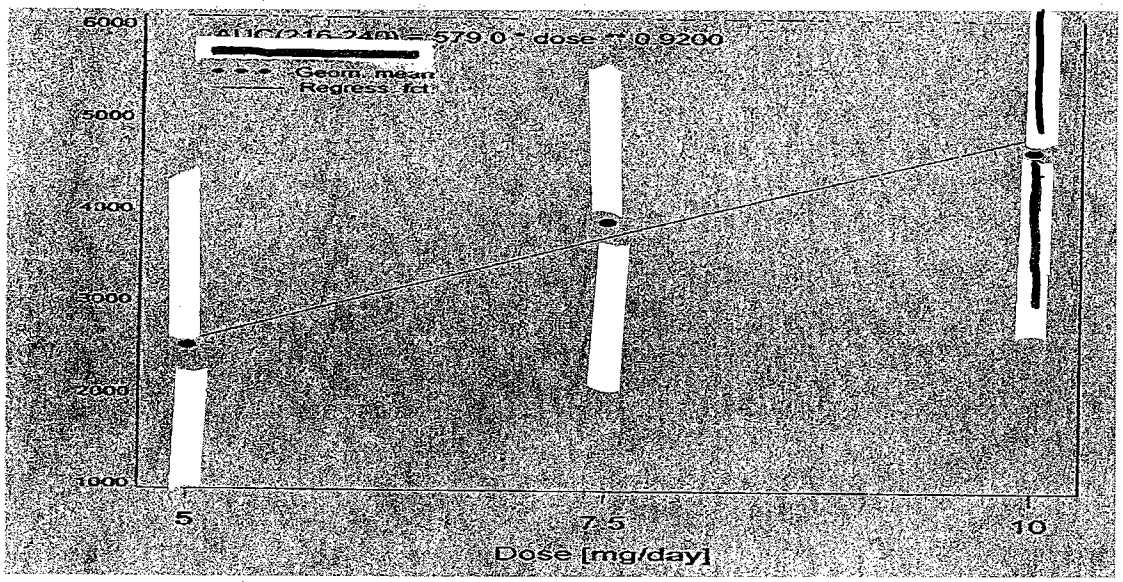
Data source: Appendix 2.5, Tables 3.2.1-3.2.3

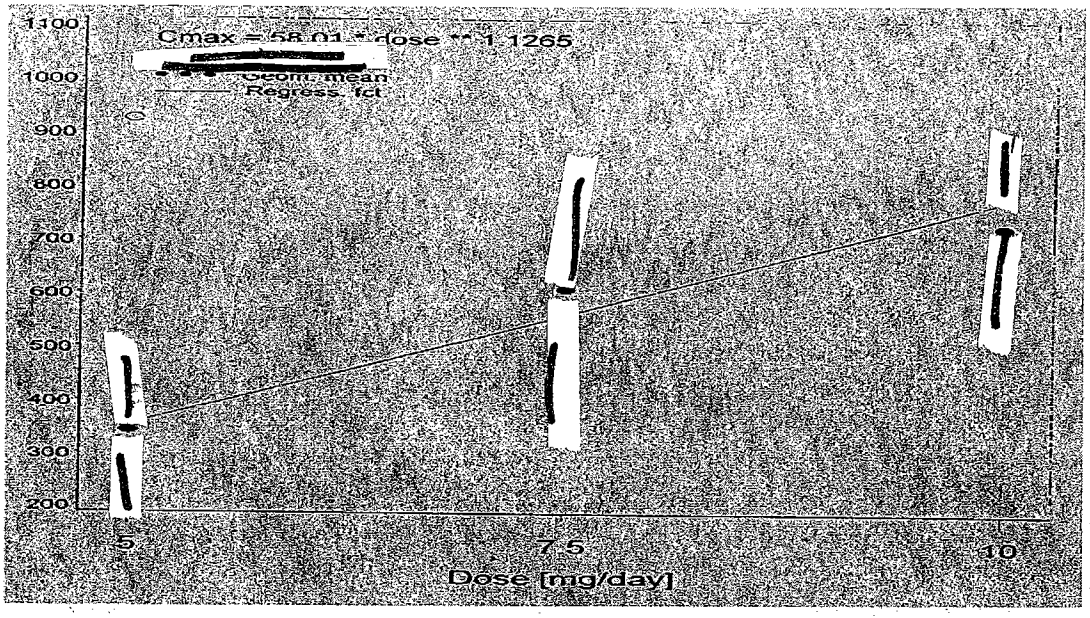
On Day 1 the geometric means of Cmax and AUC0-00 appear to be dose proportionate. The tmax values are similar at the 3 dose levels tested. Mean CLr is about 0.7 mL/min and indicative

of high plasma protein binding. Tubular secretion and subsequent effective tubular reabsorption of BSF 208075 cannot be excluded. The low value of CLr also indicates that the drug is eliminated mainly by non-renal routes.

On Day 10 the geometric means of Cmax and AUC0-24,ss are dose proportional and tmax is similar at the 3 dose levels. Mean tmax at steady-state ranges between 1.33 h and 2.44 h. The $t1/2\lambda z$ is about 15 h with little variance at the 3 dose levels. However, it should be noted that the plasma concentrations were only followed for 24 h after the last dose. Half life estimates obtained from data points collected over a period similar to the length of the estimated half life are to be interpreted with due caution. Derived parameters such as AUC0-00 may be biased as well. The geometric mean renal clearance of BSF 208075 is about 0.8 mL/min. A comparison of the Cmax- and AUC values on Day 1 and 10 indicates a small 1.1 fold accumulation. The mean CL/F at steady state is 34 mL/min, suggesting low extraction in the eliminating organs. The inter-subject variation for Cmax on Day 1 ranges between 16.6 % and 24.0% and on Day 10 between 9.8% and 59.1%. The inter-subject variability for AUC0-24 and AUC0-24,ss on Day 1 ranges between 14.2% and 27.9% and on Day 10 between 12.5% and 22.1%. The PTF values at steady-state vary between 303% and 358 % indicating that the PK of BSF 208075 exhibit multi-phasic disposition.

Linear regressions of log transformed AUC0-τ and Cmax on Day 10 are shown in below:

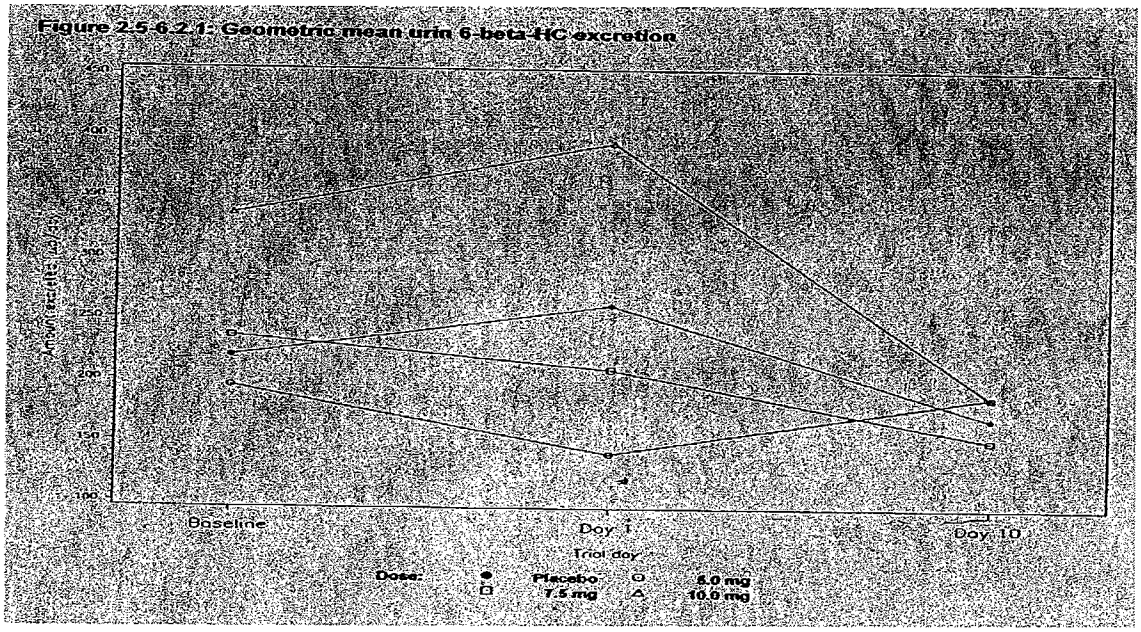




The respective slopes were statistically significantly different from zero suggesting dose related PK of BSF 208075. The PK of BSF 208075 appear to be dose proportionate.

Pharmacodynamics

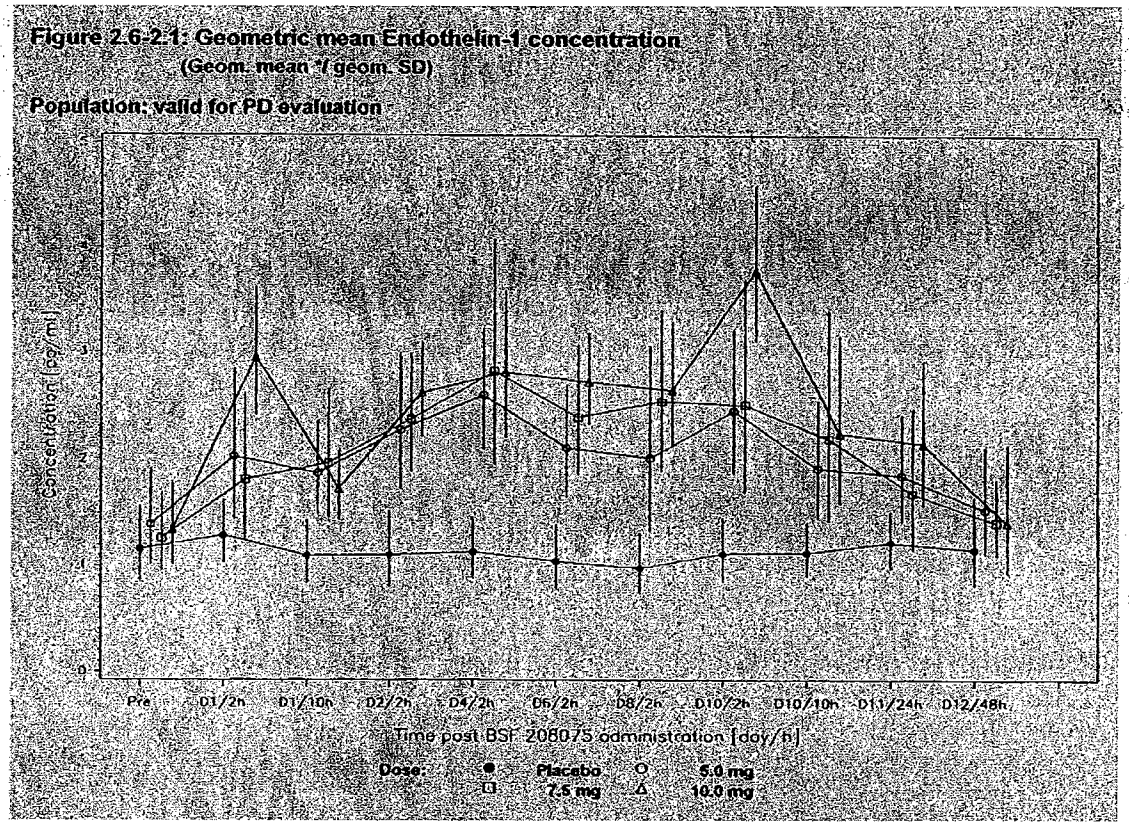
The geometric means of the amounts of β-hydroxycortisol excreted in 24 h in urine are depicted in the below Figure:



The interpretability of the data is limited by the difference in the baseline values of 6β-hydroxycortisol among the subjects in the placebo and drug receiving drugs. Also, there is substantial variability in the values of the placebo group. The data are inconclusive.

Endothelin-1

The time profile of the endothelin-1 plasma concentrations are shown in the below Figure:



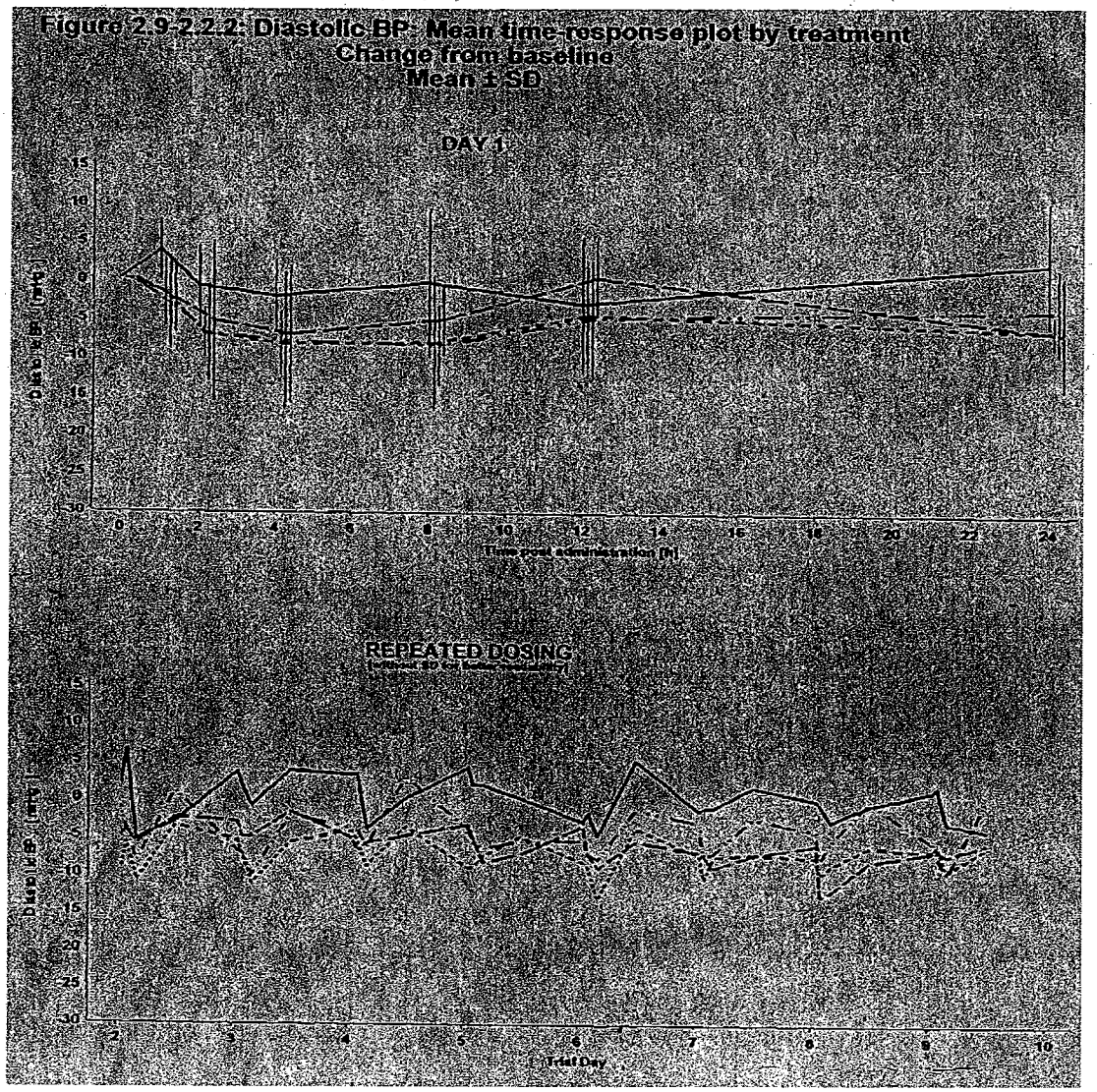
The placebo subtracted geometric mean concentrations of endothelin-1 (pg/mL) measured at 2 h and 10 h after BSF 208075 administration on Days 1 and 10 are listed in the below table (values computed by the Reviewer):

Dose, mg qd	Pre-dose	Day 1, 2 h pa	Day 1, 10 h pa	Day 10, 2 h pa	Day 10, 10 h pa
5	0.23	0.73	0.77	1.33	0.79
7.5	0.10	0.52	0.86	1.39	1.06
10	0.18	1.66	0.62	2.66	1.11

The data appear to indicate that the tested three dose regimens of BSF 208075 increase the plasma concentrations of endothelin-1 dose dependently. The concentrations at 2 h were consistently larger than those at 10 h after BSF 208075 administration on Days 1 and 10. Both the 2 h and 10 h values on Day 10 were greater than the corresponding values on Day 1 suggesting that the kinetics of the effect of BSF 208075 are possibly different from that of the drug. Collection of more blood samples would have allowed a better definition of the time profile of the drug's effect on endothelin-1.

Hemodynamic Parameters

There was no overt evidence for an effect of BSF 208075 on systolic blood pressure. However, the diastolic blood pressure tended consistently to decrease below pre-dose levels following administration of BSF 208075 on Days 1 through 10 as shown in the below figure:



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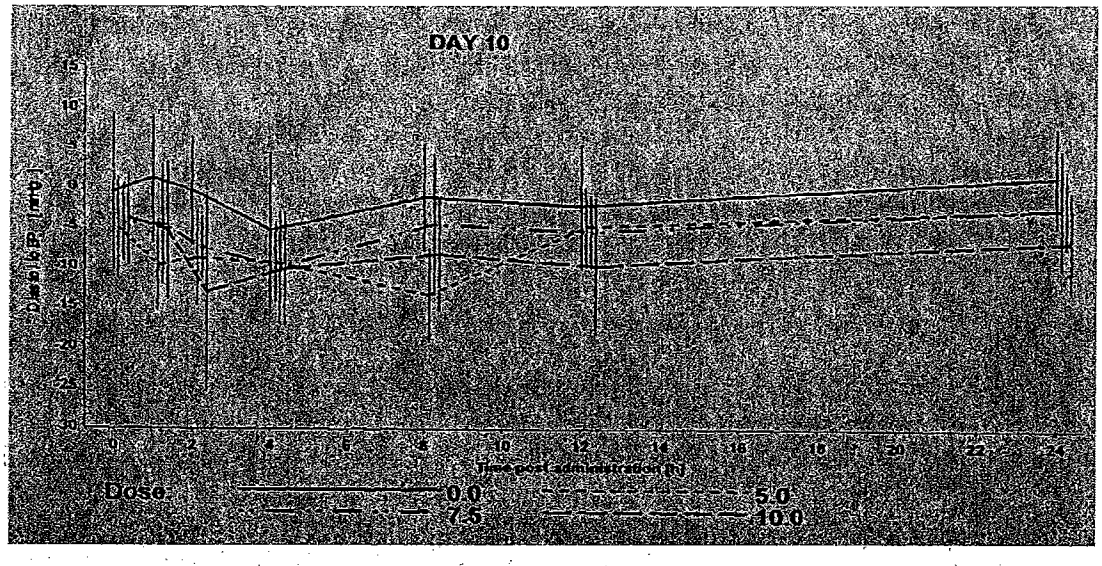

The apparent effect was not dose dependent. The BSF 208075 induced decrease in diastolic blood pressure was accompanied by a trend for an increase in heart rate as shown in the figure below:

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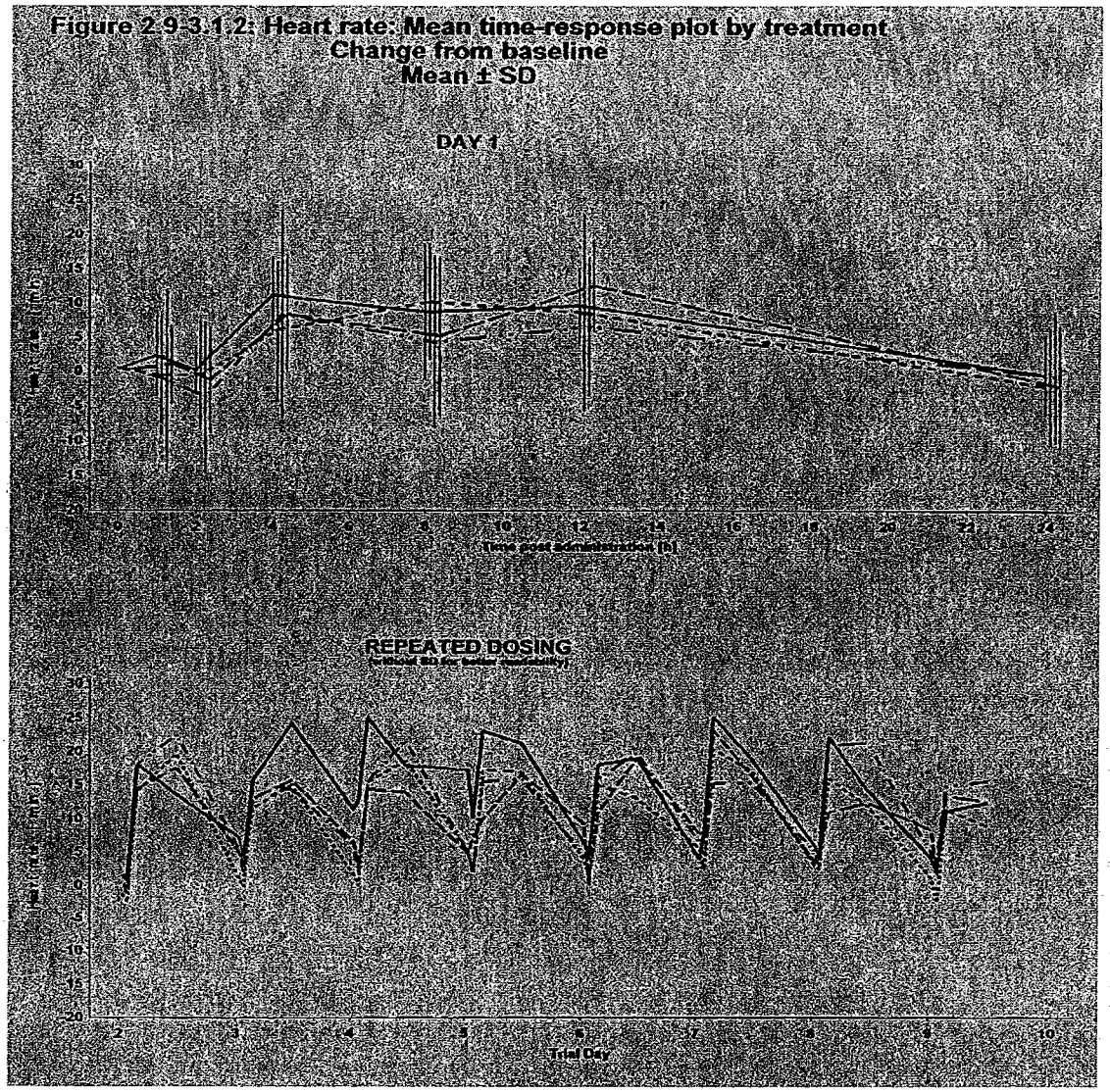

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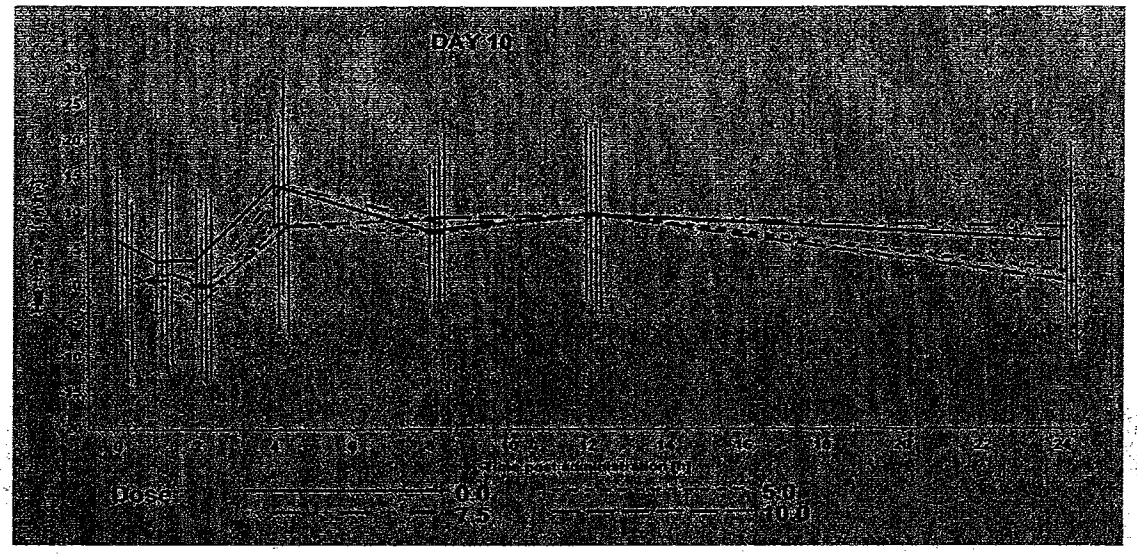


Conclusions

The dose levels tested were 5 mg qd, 7.5 mg qd and 10 mg qd. The PK of BSF 208075 are dose proportional. The drug is readily absorbed with mean Tmax ranging between 1.25 h and 2.44 h and eliminated with an apparent terminal t1/2 of about 15 h. The drug is eliminated predominantly by non-renal routes. Multiple qd dose regimens within the tested dose range result in minimal accumulation. The percent peak to trough fluctuation ranges between 305% to 358% which is large. The inter-subject variation for mean Cmax,ss ranges between 9.8% and 59.1% and for mean AUC0-24,ss between 12.5% and 22.1%. BSF 208075 at the dose levels tested induced significant elevations of endothelin-1 plasma concentrations indicating pharmacological activity of the drug. BSF 208075 induced a decrease in diastolic blood pressure accompanied by an increase in pulse rate. The sponsor did not define the maximum tolerated dose and the nature of dose limiting tolerability/toxicity. The amounts of 6β- hydroxycortisol in urine after multiple dose administration of BSF 208075 did not increase suggesting that the drug does not induce CYP 3A.

Comments

1. The sponsor did not define the maximum tolerated dose and the nature of the dose limiting tolerability/toxicity.
2. A more frequent sample collection would have allowed a better definition of the time course of the endothelin-1 plasma concentrations and hence of the endothelin receptor antagonistic effect of ambrisentan and its time duration.

3. Renal clearance is defined as CLr=Ae0-24/AUC0-24. CLr is not to be divided by F.
4. Accumulation should be estimated from AUC0-24,ss/AUC0-24
5. Attainment of steady-state was not statistically evaluated
6. The method used to measure the plasma concentrations of endothelin-1 is not described
7. The method used to measure 6 β-hydroxycortisol is not indicated
8. Female subjects should have been included in the study.

Study Report: AMB-107 "A Phase 1, Open-Label, Single-Dose Study to Evaluate the Absorption, Distribution, Metabolism, and Excretion (ADME) of $^{2}H/^{14}C$) Ambrisentan Blend in Healthy Adult Male Volunteers"

Study Site and Investigator:

[redacted]

Objectives

Primary

To evaluate the ADME of ^{2}H-labeled/^{14}C-radiolabeled ambrisentan blended with unlabeled ambrisentan by assessing the PK characteristics of radioactivity in whole blood, plasma, urine and feces

To assess the PK profile of parent compound ambrisentan in plasma, urine and feces

To establish the metabolic pattern of ambrisentan in plasma, urine and feces

Secondary

To examine the safety and tolerability of ambrisentan

In addition to the stated objectives of the protocol metabolic identification was also performed

Formulation

The [$^{2}H/^{14}C$] ambrisentan blend (Lot 60052,01 December 2005) was provided as a 10 mg dose of dry powder (total approximate radioactivity: 100 µCi) contained within a capsule. The lot number of the capsule was 65009, expiration date: 01 January 2006. The labeled ambrisentan blend and the capsule were provided by [redacted].

Design

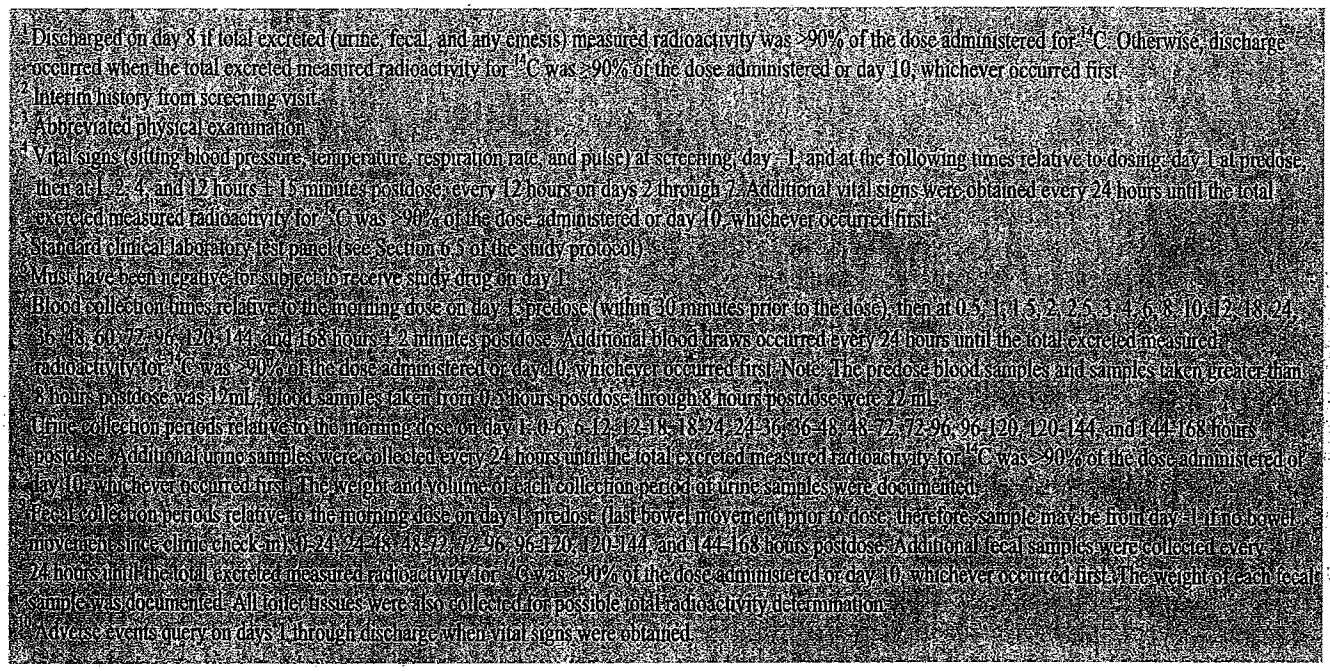
This was an open-label, single center study in which healthy male volunteers received a single 10 mg oral dose capsule of labeled ambrisentan blend to evaluate ADME characteristics. A total of 8 healthy male subjects in the age between 18 and 55 y were to be enrolled to ensure that 6 subjects completed the study. The 10 mg ambrisentan dose was administered to the overnight fasted subjects together with 240 mL water. No food was allowed for 4 h post-dose. The subjects remained confined to the unit for at least 8 days. If their total excreted ^{14}C-radioactivity on Day 8 was > 90% of the administered dose, then the subject was discharged; if not, subject remained in the unit until their total ^{14}C radioactivity was 90% of the dose administered or Day 10 was attained, which ever occurred first. The discharge from the clinic occurred on Day 8, 9 or 10.

The scheduled study activities are shown in the below schemes:

Table 9.1 Schedule of Assessments and Procedures

	Screening Visit	In-House Phase of Study for Treatment Periods 1 and 2								Discharge Day[1]	Follow-up[1]	
Study Day	-21 to -1	-1	1	2	3	4	5	6	7	8	9	10
Signed informed consent	X											
Inclusion/exclusion criteria	X											
Medical history	X	X[2]										
Concomitant medication review	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X[3]										X
12-Lead ECG	X											X
Vital signs[4]	X	X	X	X	X	X	X	X	X	X	X	X
Height and weight	X											
HIV test and hepatitis B and C serology	X											
Hematology, serum chemistry, LFT[5]	X											X
Urinalysis	X											X
Urine drug & alcohol screen	X[6]	X[2]										
Admission to study unit		X										
^{3}H, ^{14}C ambrisentan blend dosing[7]			X									
Blood samples for PK and radioactivity analyses[8]			X	X	X	X	X	X	X	X	X	X
Urine samples for PK and radioactivity analyses[8]			X	X	X	X	X	X	X	X	X	X
Fecal samples for PK and radioactivity analyses[8]			X	X	X	X	X	X	X	X	X	X
Adverse event monitoring[10]			X	X	X	X	X	X	X	X	X	X

Appears This Way
On Original



[1] Discharged on day 8 if total excreted (urine, fecal, and any emesis) measured radioactivity was >90% of the dose administered for ^{14}C. Otherwise, discharge occurred when the total excreted measured radioactivity for ^{14}C was >90% of the dose administered or day 10, whichever occurred first.
[2] Interim history from screening visit.
[3] Abbreviated physical examination.
[4] Vital signs (sitting blood pressure, temperature, respiration rate, and pulse) at screening, day -1, and at the following times relative to dosing: day 1 at predose, then at 1, 2, 4, and 12 hours ± 15 minutes postdose; every 12 hours on days 2 through 7. Additional vital signs were obtained every 24 hours until the total excreted measured radioactivity for ^{14}C was >90% of the dose administered or day 10, whichever occurred first.
[5] Standard clinical laboratory test panel (see Section 6.5 of the study protocol).
[6] Must have been negative for subject to receive study drug on day 1.
[7] Blood collection times relative to the morning dose on day 1: predose (within 30 minutes prior to the dose), then at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, 72, 96, 120, 144, and 168 hours ± 2 minutes postdose. Additional blood draws occurred every 24 hours until the total excreted measured radioactivity for ^{14}C was >90% of the dose administered or day 10, whichever occurred first. Note: The predose blood samples and samples taken greater than 8 hours postdose was 12 mL; blood samples taken from 0.5 hours postdose through 8 hours postdose were 22 mL.
[8] Urine collection periods relative to the morning dose on day 1: 0-6, 6-12, 12-18, 18-24, 24-36, 36-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours postdose. Additional urine samples were collected every 24 hours until the total excreted measured radioactivity for ^{14}C was >90% of the dose administered or day 10, whichever occurred first. The weight and volume of each collection period of urine samples were documented.
[9] Fecal collection periods relative to the morning dose on day 1: predose (last bowel movement prior to dose, therefore, sample may be from day -1 if no bowel movement since clinic check-in), 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours postdose. Additional fecal samples were collected every 24 hours until the total excreted measured radioactivity for ^{14}C was >90% of the dose administered or day 10, whichever occurred first. The weight of each fecal sample was documented. All toilet tissues were also collected for possible total radioactivity determination.
[10] Adverse events query on days 1 through discharge when vital signs were obtained.

Pharmacokinetic Profiling

Blood samples for the determination of radioactivity in whole blood and plasma and concentration of ambrisentan and identification of ambrisentan-related metabolites (plasma only) were collected at the following times:

Day 1: Pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, 24, 48, 72, 96, 120, 144, and 168 h post-dose. Additional 24 h blood samples were collected until the total measured radioactivity for ^{14}C in the excreta exceeded 90% of the administered radioactivity, or Day 10 was reached, whatever occurred first.

Urine samples for the analysis of radioactivity and concentrations of ambrisentan and identification of ambrisentan related metabolites were collected during the following intervals:

Pre-dose, 0-6 h, 6-12 h, 12-18 h, 18-24 h, 24-36 h, 36-48 h, 48-72 h, 72-96 h, 96-120 h, 120-144 h, and 144-168 h post-dose. Additional 24 h urine volumes were collected until the total measured radioactivity for ^{14}C in the excreta exceeded 90% of the administered radioactivity or Day 10 was reached whatever occurred first.

Fecal samples for the analysis of radioactivity, concentrations of ambrisentan, and identification of ambrisentan- related metabolites were collected during the following intervals: 0-24 h, 24-48 h, 48-72 h, 72-96 h, 96-120 h, 120-144 h and 144-168 h post-dose. Additional 24 h fecal samples were collected until the total measured radioactivity for ^{14}C in the excreta exceeded 90% of the