95% CI, and statistical significance were calculated for each parameter. Differences calculated for log transformed parameters were reported as ratios. The direction and magnitude of any PD parameter contrast that was statistically significant ($p< 0.05$, 2-sided) was noted. If the corresponding CI was within 80-125% this was interpreted to indicate no qualitative difference. Residuals of these models were examined to determine whether a natural log transformation was appropriate for each coagulation laboratory. Shapiro-Wilk statistics and box plots were used.

Determination of Sample Size

The within subject INR CV was projected to be 6%. Calculations using a paired difference t-statistic evaluated at the 5% significance level (2-sided) or a CI about the individual log-transformed Day 13 to Day 1 INR ratio, showed that with 18 subjects completing, this study had greater than 80% power to detect a 6% difference between he INR at Day 13 and 1.

Results

Demographics

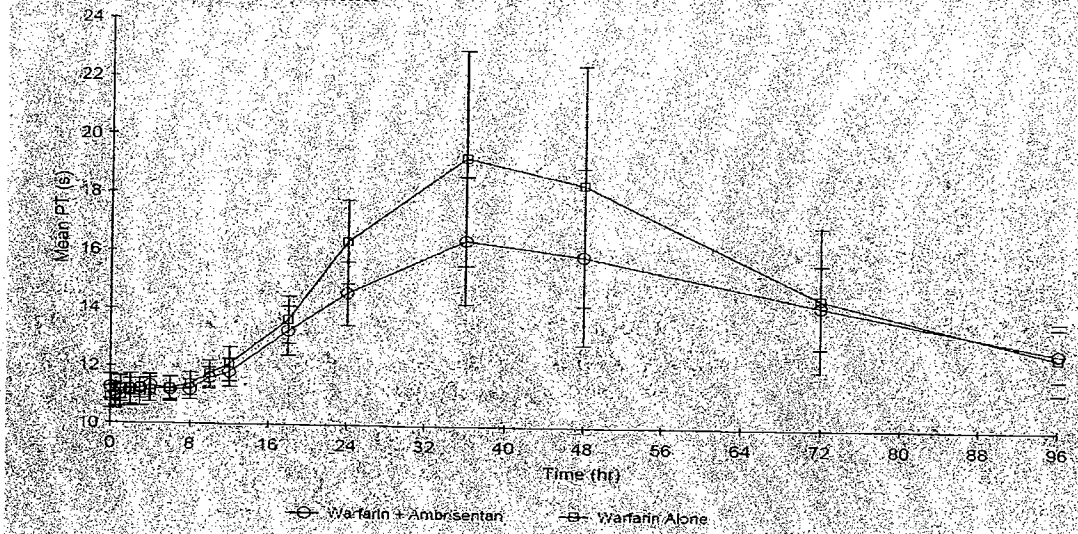
Twenty-two (22) subjects enrolled and completed the study. There were 19 males and 3 females of mean age 38.8 y and and weight 79.1 kg.

Impact of Ambrisentan on Warfarin

Pharmacodynamics

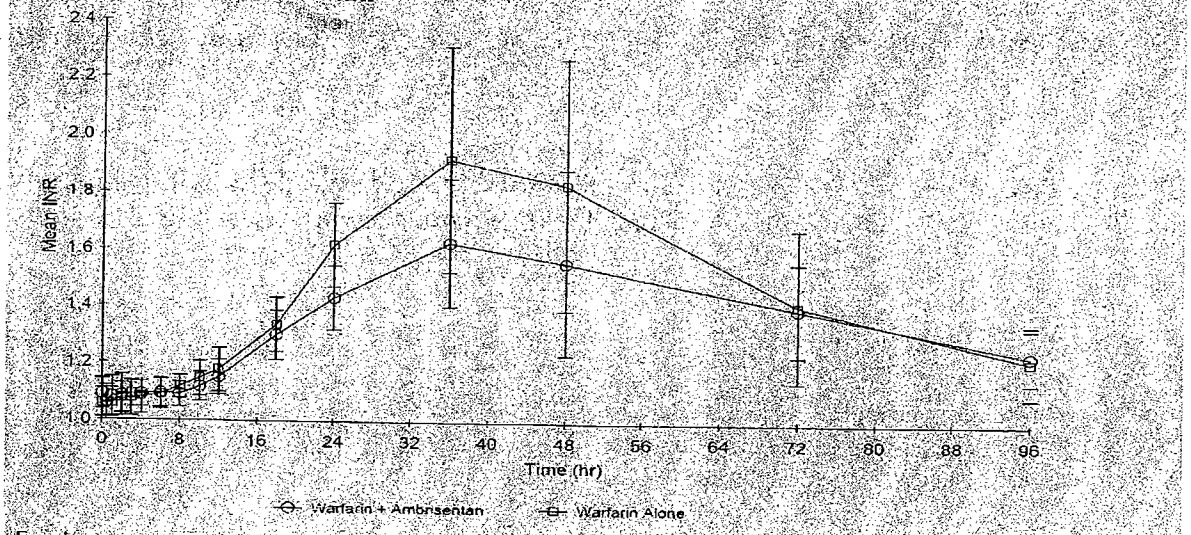
The mean Effect Profiles of PT and INR after a single dose of warfarin in the presence and absence of ambrisentan are shown in Figures 11.1 and 11.2:

Figure 11.1 Mean Effect-Time Profiles of PT Following a Single Oral Dose of 25 mg Racemic Warfarin Alone and in the Presence of Multiple Doses of Ambrisentan



Error bars represent standard deviations of the mean
Source: Summary Figure 14.4.7

Figure 11.2 Mean Effect-Time Profiles of INR Following a Single Oral Dose of 25 mg Racemic Warfarin Alone and in the Presence of Multiple Doses of Ambrisentan



Error bars represent standard deviations of the mean
Source: Summary Figure 14.4.6

The respectively derived parameters are listed in Tables 11.2 and 11.3:

Appears This Way
On Original

Table 11.2 Comparison of PT Pharmacodynamic Parameters Following a Single Dose of Warfarin Alone (Day 1) and in the Presence of Multiple Doses of Ambrisentan (Day 13)

PD Parameter	Treatment	n	Arithmetic Mean (SD)	Geometric Mean	Geometric Mean Ratio and 95% CI (Day 13/Day 1)
C_{max} (s)	warfarin (Day 1)	22	19.48 (3.952)	19.14	
	ambrisentan + warfarin (Day 13)	22	16.62 (2.888)	16.41	85.76 (83.43, 88.15)
$AUEC_{0-tau}$ (s*hr)	warfarin (Day 1)	22	1461.57 (198.045)	1450.19	
	ambrisentan + warfarin (Day 13)	22	1359.13 (128.087)	1353.72	93.35 (91.32, 95.42)
t_{max} (hr)[a]	warfarin (Day 1)	22	36.00 (36.00-48.02)	NA	
	ambrisentan + warfarin (Day 13)	22	36.00 (23.92-47.92)	NA	p = 0.999[b]

[a] For t_{max}, median (minimum, maximum) values are reported
[b] p-value of Wilcoxon signed rank test of hypothesis that median pair-wise day 13 - day 1 difference = 0
n = number of subjects included in the calculation of arithmetic and geometric mean
NA = not calculated
Source: Summary Tables 14.2.6 and 14.2.10

Table 11.3 Comparison of INR Pharmacodynamic Parameters Following a Single Dose of Warfarin Alone (Day 1) and in the Presence of Multiple Doses of Ambrisentan (Day 13)

PD Parameter	Treatment	n	Arithmetic Mean (SD)	Geometric Mean	Geometric Mean Ratio and 95% CI (Day 13/Day 1)
C_{max} (s)	warfarin (Day 1)	22	1.94 (0.423)	1.90	
	ambrisentan + warfarin (Day 13)	22	1.65 (0.305)	1.62	85.26 (82.40, 88.22)
$AUEC_{0-tau}$ (s*hr)	warfarin (Day 1)	22	143.86 (21.113)	142.56	
	ambrisentan + warfarin (Day 13)	22	133.23 (13.611)	132.61	93.02 (90.83, 95.26)
t_{max} (hr)[a]	warfarin (Day 1)	22	36.00 (24.00, 48.02)	NA	
	ambrisentan + warfarin (Day 13)	22	36.00 (18.00, 47.92)	NA	p = 0.078[b]

[a] For t_{max}, median (minimum, maximum) values are reported
[b] p-value of Wilcoxon signed rank test of hypothesis that median pair-wise day 13 - day 1 difference = 0
n = number of subjects included in the calculation of arithmetic and geometric mean
NA = not calculated
Source: Summary Tables 14.2.6 and 14.2.10

The respective geometric mean ratios (Day13/Day1) for Lmax of PT and INR for warfarin ambrisentan were 86% and 85 % and the respective 95% CI did not include 100%. The respective geometric mean ratios (Day13/Day 1 for AUC0-τ,ss of PT and INR were identical, 93% and 93% . These findings indicated that the anticoagulatory peak and average effects of warfarin in the presence of ambrisentan tended to be smaller. However, the respective 90% CI were within the predefined 80%-125% bioequivalence boundaries. The respective values for tmax of the maximum anti-coagulatory effect of warfarin in the presence and absence of ambrisentan were similar.

A linear plot of the mean plasma concentration time profiles of the S- and R-warfarin enantiomers in the presence and absence of ambrisentan are shown in Figures 11.3 and 11.4:

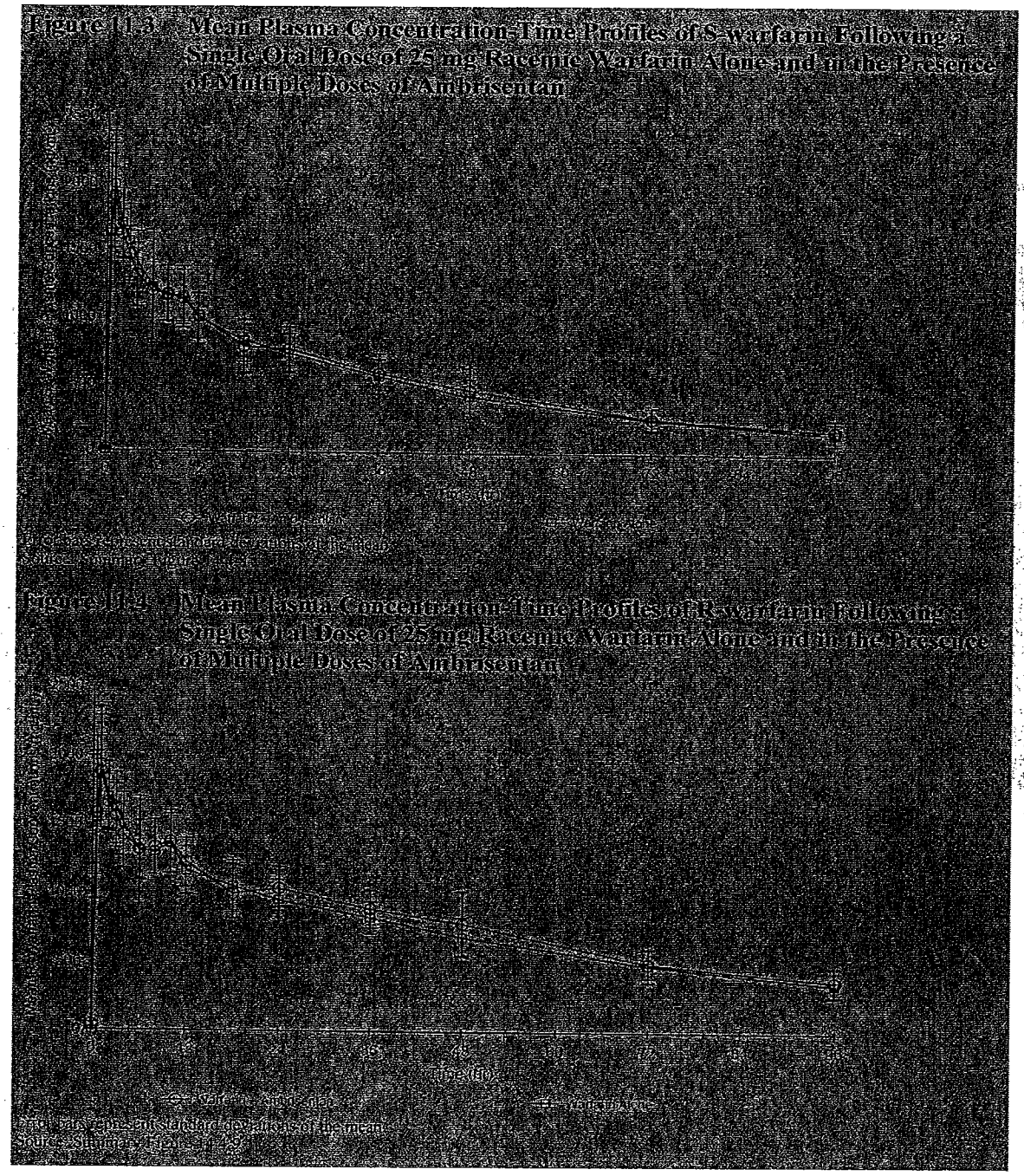
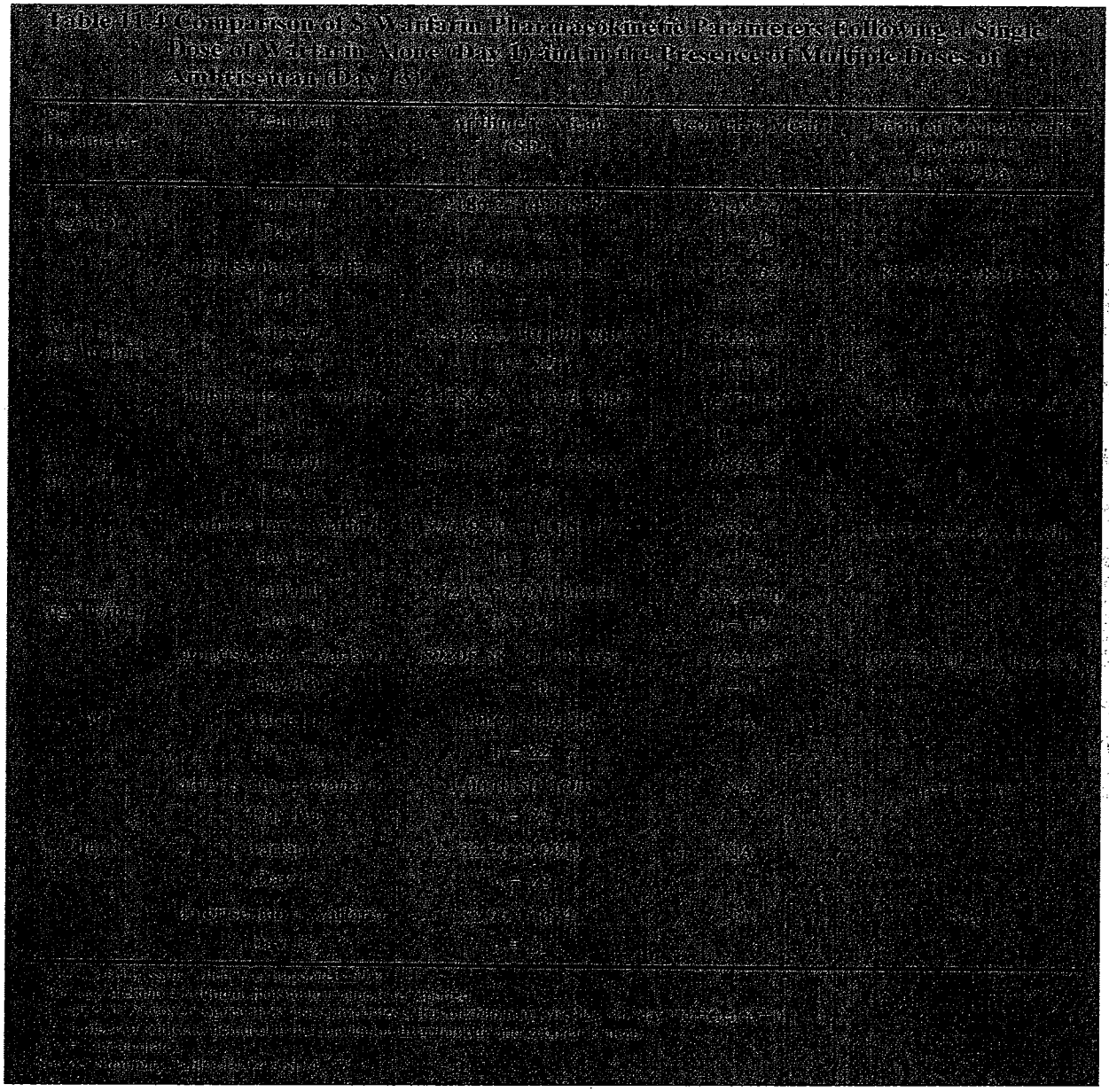


Figure 11.3 Mean Plasma Concentration Time Profiles of S-warfarin Following a Single Oral Dose of 25 mg Racemic Warfarin Alone and in the Presence of Multiple Doses of Ambrisentan

Figure 11.4 Mean Plasma Concentration Time Profiles of R-warfarin Following a Single Oral Dose of 25 mg Racemic Warfarin Alone and in the Presence of Multiple Doses of Ambrisentan

The derived parameters for S- and R-warfarin are listed in Tables 11.4 and 11.5:



Best Possible Copy

Table 11-5 Comparison of R-Warfarin Pharmacokinetic Parameters Following a Single Dose of Warfarin Alone (Day 1) and in the Presence of Multiple Doses of Ambrisentan (Day 13)

PK Parameter	Treatment	Arithmetic Mean (SD)	Geometric Mean	Geometric Mean Ratio and 90% CI (Day 13/Day 1)
C_{max} (ng/mL)	warfarin (Day 1)	2133.64 (437.786) n = 22	2091.49 n = 22	
	ambrisentan + warfarin (Day 13)	1947.05 (378.950) n = 22	1916.31 n = 22	91.62 (86.16, 97.44)
AUC_{0-tau} (ng*hr/mL)	warfarin (Day 1)	73322.17 (10618.175) n = 22	72533.90 n = 22	
	ambrisentan + warfarin (Day 13)	76825.83 (10229.377) n = 22	75913.44 n = 22	104.66 (101.72, 107.68)
AUC_{0-inf} (ng*hr/mL)	warfarin (Day 1)	100157.21 (18795.496) n = 21	97610.33 n = 20	
	ambrisentan + warfarin (Day 13)	103801.95 (23060.356) n = 21	101974.49 n = 20	104.47 (100.72, 108.36)
AUC_{0-inf} (ng*hr/mL)	warfarin (Day 1)	81497.67 (13185.496) n = 5	77885.59 n = 4	
	ambrisentan + warfarin (Day 13)	83954.47 (11735.228) n = 5	83785.32 n = 4	107.57 (97.64, 118.52)
t_{max} (hr)	warfarin (Day 1)	1.00 (0.50, 6.00) n = 22	NA	
	ambrisentan + warfarin (Day 13)	1.00 (0.50, 3.00) n = 22	NA	p = 0.810
$t_{1/2}$ (hr)	warfarin (Day 1)	50.19 (13.160) n = 21	NA	
	ambrisentan + warfarin (Day 13)	48.38 (9.982) n = 21	NA	NA

AUC = if missing when % extrapolated >20%
Stats = median (minimum, maximum) values are reported
p-value of Wilcoxon signed-rank test of hypothesis that median pair-wise day 13 - day 1 difference = 0
n = number of subjects included in the calculation of arithmetic and geometric mean
NA = not calculated
Source: Summary Tables 14.2.4 and 14.2.7

The respective mean plasma profiles and derived mean Cmax-, tmax- and AUC-values of the S- and R- enantiomers of warfarin in the presence and absence of ambrisentan were similar. The geometric mean ratios (Day 13/Day1) of Cmax for S- and R-warfarin were 89% and 92%, respectively, in accordance with the geometric mean ratio (Day 13/Day 1) of 86% and 85% for Lmax of PT and INR, respectively. The 90% CI of the geometric mean ratio (Day 13/Day1) for Cmax and AUC were within the 80% -125% bioequivalence limits. The respective values for tmax and t1/2 of the warfarin enantiomers in the presence and absence of ambrisentan were similar.

Impact of Warfarin on Ambrisentan

The trough plasma concentrations of ambrisentan on Days 10 through 13 are listed in Table 11.6:

Table 11.6 Summary of Predose (Trough) Ambrisentan Plasma Concentrations Obtained on Days 10-13

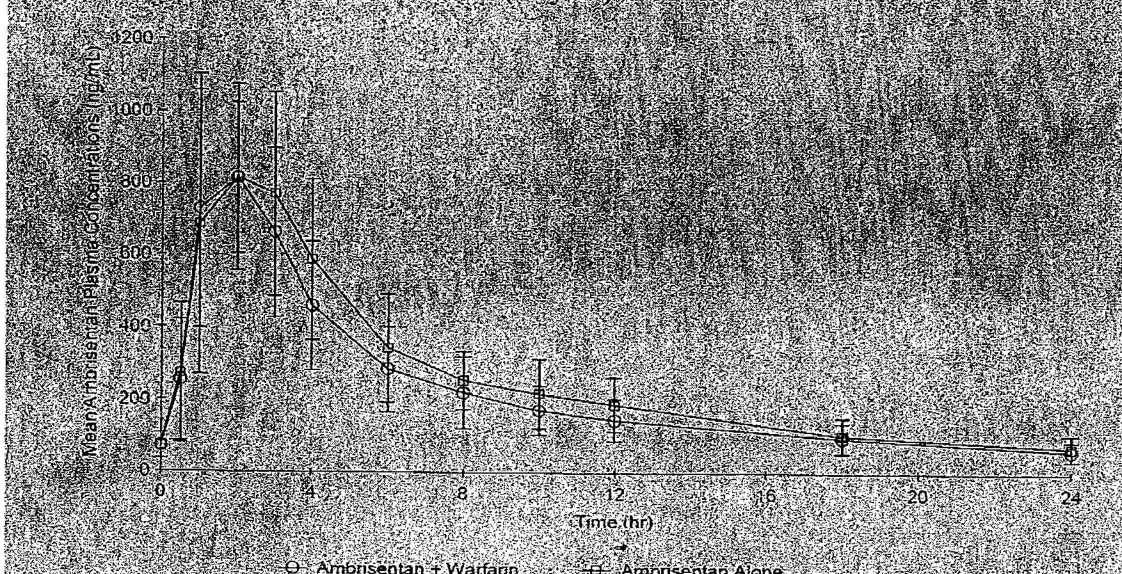
Ambrisentan Plasma Concentration (ng/mL)	Scheduled Interval			
	Day 10	Day 11	Day 12	Day 13
Mean (SD) N = 22	69.12 (28.44)	74.80 (33.92)	72.16 (35.41)	72.72 (36.71)
Median (Min, Max)	60.0 (17.4, 127.0)	61.4 (21.4, 157.0)	63.3 (21.1, 147.0)	63.0 (22.5, 161.0)

Source: Summary Table [illegible]

The trough plasma concentrations of ambrisentan in the absence of warfarin on Days 10 through 13 were similar. The results of the analysis of variance indicated that the trough concentrations met the pre-specified 80% equivalence criterion indicating that on Day 10 of a qd regimen with 10 mg ambrisentan steady-state is reached.

Linear plots of the mean plasma concentrations of ambrisentan in the presence and absence of warfarin and derived parameters are shown in Figure 11.5 and Table 11.7:

Figure 11.5 Mean Plasma Concentration-Time Profiles of Ambrisentan Following Multiple Doses of Ambrisentan Alone (Day 12) and in the Presence of a Single Dose of Warfarin (Day 13)



Error bars represent standard deviations of the mean
Source: Summary Figure 14.4.4

Table 11.7 Comparison of Ambrisentan Pharmacokinetic Parameters Following Multiple Doses of Ambrisentan Alone (Day 12) and in the Presence of a Single Dose of Warfarin (Day 13)

PK Parameter	Treatment	Arithmetic Mean (SD)	Geometric Mean	Geometric Mean Ratio and 90% CI (Day 13/Day 12)
$C_{max,ss}$ (ng/mL)	Ambrisentan (Day 12)	988.59 (278.348) n = 22	947.73 n = 22	
	ambrisentan + warfarin (Day 13)	944.05 (177.778) n = 22	928.31 n = 22	97.95 (87.62, 109.50)
$C_{min,ss}$ (ng/mL)	ambrisentan (Day 12)	68.37 (35.134) n = 22	60.97 n = 22	
	ambrisentan + warfarin (Day 13)	60.53 (25.770) n = 22	55.28 n = 22	90.67 (85.47, 96.18)
$AUC_{0-\tau,ss}$ (ng*hr/mL)	ambrisentan (Day 12)	6384.18 (1999.652) n = 21	6106.20 n = 21	
	ambrisentan + warfarin (Day 13)	5515.59 (1609.623) n = 22	5357.92 n = 21	87.75 (83.85, 91.82)
$t_{max,ss}$ (hr)[a]	ambrisentan (Day 12)	2.00 (1.00-3.00) n = 22	NA	
	ambrisentan + warfarin (Day 13)	2.00 (1.00-3.00) n = 22	NA	p = 0.957[b]
$t_{1/2}$ (hr)	ambrisentan (Day 12)	8.33 (1.945) n = 21	NA	
	ambrisentan + warfarin (Day 13)	10.50 (4.589) n = 22	NA	NA

[a] For $t_{max,ss}$, median (minimum, maximum) values are reported
[b] p-value of Wilcoxon signed rank test of hypothesis that median pair-wise day 13 - day 12 difference = 0
n = number of subjects included in the calculation of arithmetic and geometric mean, respectively
NA = not calculated
Source: Summary Tables 14.2.5 and 14.2.8

The Cmax,ss, Cmin,ss and AUC0-τ,ss values of ambrisentan tended to be lower in the presence of warfarin. However, the 90% CI of geometric mean ratio (Day13/Day12) were within the confines of the 80%-125% equivalence limits suggesting no clinically relevant impact of warfarin on the exposure to ambrisentan.

Conclusions

The PD parameters of warfarin and the PK parameters of the warfarin enantiomers in the presence and absence of ambrisentan are comparable. The PK parameters of ambrisentan at steady state in the presence and absence of warfarin are comparable. The results of the

interaction study in healthy subjects indicate that co-administering warfarin (single dose of 25 mg) and ambrisentan (10 mg qd) does not impact the PD or PK of warfarin or the PK of ambrisentan.

Comments

1. Given the difference in Cl/F between healthy subjects and patients with PAH a dose of ambrisentan of 20 mg would have been more appropriate.
2. Information on the PT and IRN assays was not provided in the report
3. The plasma concentrations of the S- and R-enantiomers were followed for 96 h, a short time interval given the t1/2 of 50 h. As a result the extrapolated parts of AUC0-00 exceeded 20% in most cases which is considerable

Study Report: AMB-105 "A Phase I, Open-Label, Randomized, 2-Period, Crossover Study to Assess the Pharmacokinetics of Ambrisentan Alone and in the Presence of Multiple Doses of Sildenafil, Alone and in the Presence of Multiple Doses of Ambrisentan in Healthy Adult Volunteers"

Study Investigator and Site:

[redacted]

Objectives

Primary

To assess the effects of multiple doses of sildenafil on the pharmacokinetics (PK) of a single dose of ambrisentan
To assess the effect of multiple doses of ambrisentan on the PK of a single dose of sildenafil

Secondary

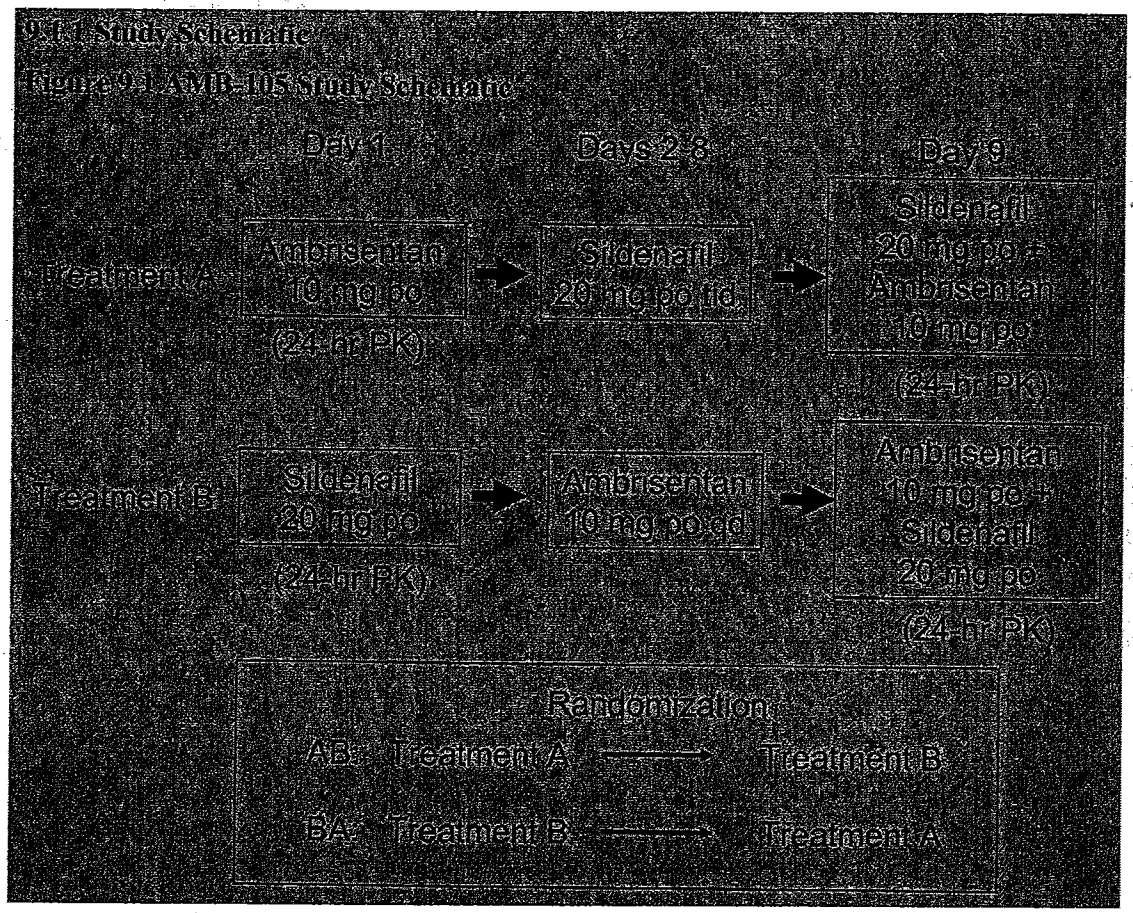
To examine the safety and tolerability of a single dose of ambrisentan in the presence of multiple doses of sildenafil and of a single dose of sildenafil in the presence of multiple doses of ambrisentan

Formulations

Ambrisentan was provided by Myogen, Inc., as 10 mg film-coated immediate release tablets. The [redacted] pink tablets were manufactured by [redacted]. The Packager's [redacted] Lot No. was L0001848 [redacted] Article/Material number 17102293; [redacted] Lot No. 280200A0).

Design

This is an open label, randomized, 2-period cross-over, single-center study with Treatments A and B as shown in the scheme below:



Twenty subjects were to be enrolled to ensure that 16 complete the study. The subjects were admitted to the clinic the night prior to dosing of each treatment period, and remained confined in the unit until completion of the clinical assessment on Day 10 of each treatment period. Between the last dosing in the first treatment period and the first dosing of the second treatment period a 6 day wash-out period was maintained. Following an overnight fast of at least 10 h, subjects were administered the study drug with 240 mL of water. No food was allowed for 4 h (Days 1 and 9) or 2 h (Days 2-8) after dosing. Concomitant medications, with the exception of up to 3 doses of acetaminophen at 1 g each or less, were prohibited throughout the study. In addition no foods or substances known to interfere with cytochrome P450 isozymes were to be consumed, including tobacco, caffeine, alcohol, and grapefruit containing products.

The scheduled activities during the study are tabulated in the below flow chart:

Table 9.1 Schedule of Assessments and Procedures

	Screening	In-House Phase of Study for Treatment Periods 1 and 2										Discharge	Follow-Up
Study Day	-21 to -1	-1	1	2	3	4	5	6	7	8	9	10	15 to 19
Signed informed consent	X												
Inclusion/exclusion criteria	X												
Medical history	X	X											
Concomitant medication review	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X	X										X	X
12-Lead ECG	X												X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X
Height and weight	X												
HIV test and hepatitis B and C	X												
Hematology, serum chemistry, and coagulation tests	X											X	X
Urinalysis	X											X	X
Urine drug screen & alcohol screen	X	X											
Admission to study unit		X											
Treatment A													
Ambrisentan dosing			X	X							X		
Sildenafil dosing				X	X	X	X	X	X	X	X		
Treatment B													
Sildenafil dosing			X								X		
Ambrisentan dosing			X	X	X	X	X	X	X	X	X		
Blood samples for PK analysis			X	X				X	X	X	X	X	
Adverse event monitoring			X	X	X	X	X	X	X	X	X	X	X

[1] Interim history from screening visit.
[2] Abbreviated physical examination.
[3] Vital signs (sitting blood pressure, temperature, respiration rate, and pulse) at screening, day -1, and at the following times relative to dosing: days 1 and 9 at predose, then at 1, 2, 4, and 12 h on day 2, days 2 through 8 at predose and at 4 hours postdose; day 10 at 24 hours after the day 9 dose. Also, blood pressure only at 1 hour post-morning dose on days 3 through 8.
[4] Standard clinical laboratory test panel (for details see Section 9.3.5.2).
[5] Must be negative for subject to receive study drug on day 1.
[6] 6-day washout between treatments.
[7] Blood collection times relative to the morning dosing on days 1 and 9: predose (within 30 minutes prior to each dose), then 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, and 24 hours. On days 6, 7, and 8: pre-morning dose.
[8] Samples analyzed for ambrisentan and/or for sildenafil, where appropriate.
[9] Adverse events query on days 1 through 10 when vital signs are obtained.

Pharmacokinetic Profiling

Blood samples for the determination of ambrisentan, sildenafil and N-desmethyl-sildenafil were collected at the following times during each treatment period:

Days 1 and 9: Pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, and 24 h post dose.
Days 6 through 8: Pre-dose (morning)

Bioassay

The plasma concentrations of ambrisentan were measured by a validated LC/MS/MS method with an LLOQ of [redacted] The plasma concentrations of sildenafil and N-desmethysildenafil were measured by a validated LC/MS/MS with an LLOQ of [redacted] for both.

PK Data Analysis

The following parameters were determined by non-compartmental methods using WinNonlin™ (WinNonlin Professional Network Edition, Version 4.0.1, Pharsight Corp., Palo Alto, CA): Cmax, tmax, AUC0-tllast, AUC0-00, λz, t1/2 CL/F and Vz/F.

The pre-dose concentrations of ambrisentan or sildenafil obtained on Days 6-8 of Treatment A or B were used to test whether steady-state is reached.

Statistical Analysis

Statistical inference was based on mixed-model analysis of variance (ANOVA) with effects of treatment sequence, random subject within sequence, and day. Ambrisentan Cmax, AUC0-tlast and AUC0-00 of Treatment A were natural log transformed and compared between Day 9 and 1. Sildenafil and N-desmethyl-sildenafil Cmax, AUC0-tlast and AUC0-00 of Treatment B were natural log transformed and compared between Days 9 and 1.The geometric mean ratios were used for primary analysis. Point estimates and 90% CI were reported for relative bioavailability ratios for assessment of the effect of multiple doses of sildenafil on single doses of ambrisentan and multiple doses of ambrisentan on a single dose of sildenafil. CIs were assessed relative to the 80%-125 bioequivalence limits. To evaluate a possible impact of co-administered drug on tmax of ambrisentan or sildenafil or desmethyl-sildenafil the Wilcoxon signed rank test was used to test the Day 9-Day 1 difference.

Attainment of steady-state concentrations of ambrisentan in Treatment B and sildenafil in Treatment B used the pre-dose concentrations obtained on Days 6-8. The following ANOVA model was used: ln (concentration) = subject +day, where subject is a random effect while day is a fixed effect. The following contrasts were examined Day 6 vs Days 7 and 8, and Day 7 vs Day 8. The lower bounds of the 90% CI about these contrasts were compared to the lower 80% bioequivalence limit.

Results

Twenty subjects were enrolled and 19 completed the study. There were 19 males and 1 female of average age 36.1 y and weight 79.1 kg. Subject 01-03, who was randomized to the BA treatment sequence experienced a headache on Day 3 of the first period and withdrew consent. Nineteen subjects were available for PK analysis.

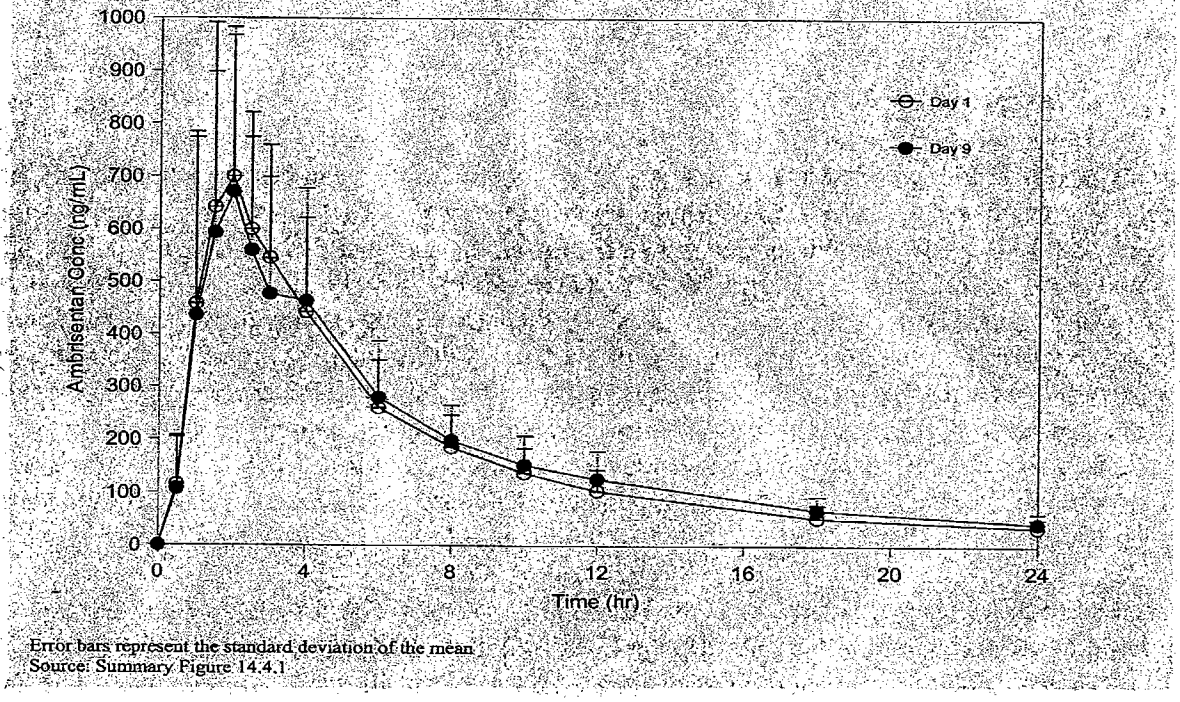
Only 1 blood sample was collected on Day 9 pre-dose instead of 2 (one sample for the drug administered in a single dose and the other sample for the drug administered in multiple doses Treatment A: Ambrisentan is the single dose drug and sildenafil the multiple dose drug).

PK

Impact of Sildenafil on Ambrisentan

The mean plasma concentrations of ambrisentan and derived parameters in the presence and absence of sildenafil are shown in Figure 11.1 and Table 11.2, respectively:

Figure 11.1 Mean Plasma Concentrations of Ambrisentan When Administered Alone (Day 1) or in the Presence of Sildenafil (Day 9)



Error bars represent the standard deviation of the mean
Source: Summary Figure 14.4.1

Appears This Way
On Original

Table 11.2 Comparison of Ambrisentan Pharmacokinetic Parameters When Administered Alone (Day 1) or in the Presence of Multiple Doses of Sildenafil (Day 9)

PK Parameter	Treatment	Arithmetic Mean (SD)	Geometric Mean	Geometric Mean Ratio and 90% CI (Day 9/Day 1)
C_{max} (ng/mL)	ambrisentan (Day 1)	863 (210.15) n = 19	839.5 n = 19	
	ambrisentan + sildenafil (Day 9)	849.3 (267.40) n = 19	808.4 n = 19	96.29 (86.04, 107.77)
$AUC_{0-tlast}$ (ng*h/mL)	ambrisentan (Day 1)	4254.9 (1270.88) n = 19	4029.0 n = 19	
	ambrisentan + sildenafil (Day 9)	4478.0 (1229.94) n = 19	4324.3 n = 19	106.01 (100.62, 111.69)
$AUC_{0-\infty}$ (ng*h/mL)	ambrisentan (Day 1)	4652.8 (1541.69) n = 16	4414.0 n = 16	
	ambrisentan + sildenafil (Day 9)	4970.5 (1427.02) n = 16	4790.5 n = 16	108.51 (102.60, 114.76)
t_{max} (hr)	ambrisentan (Day 1)	2.0 (1.00-4.00) n = 19	NA	
	ambrisentan + sildenafil (Day 9)	2.0 (1.00-4.00) n = 19	NA	P = 0.237
$t_{1/2(\beta)}$	ambrisentan (Day 1)	7.2 (1.08) n = 16	NA	
	ambrisentan + sildenafil (Day 9)	7.7 (0.94) n = 19	NA	NA

Source: Summary Table 14.2.9 and Summary Table 14.2.9.1

For t_{max}, median (minimum, maximum) is reported and p-value of Wilcoxon signed rank test of hypothesis that median ratio = 1 is reported.

Day 9 / day 1 difference = 0 is tested.

n = number of subjects included in the calculation of arithmetic mean value.

NA = not calculated.

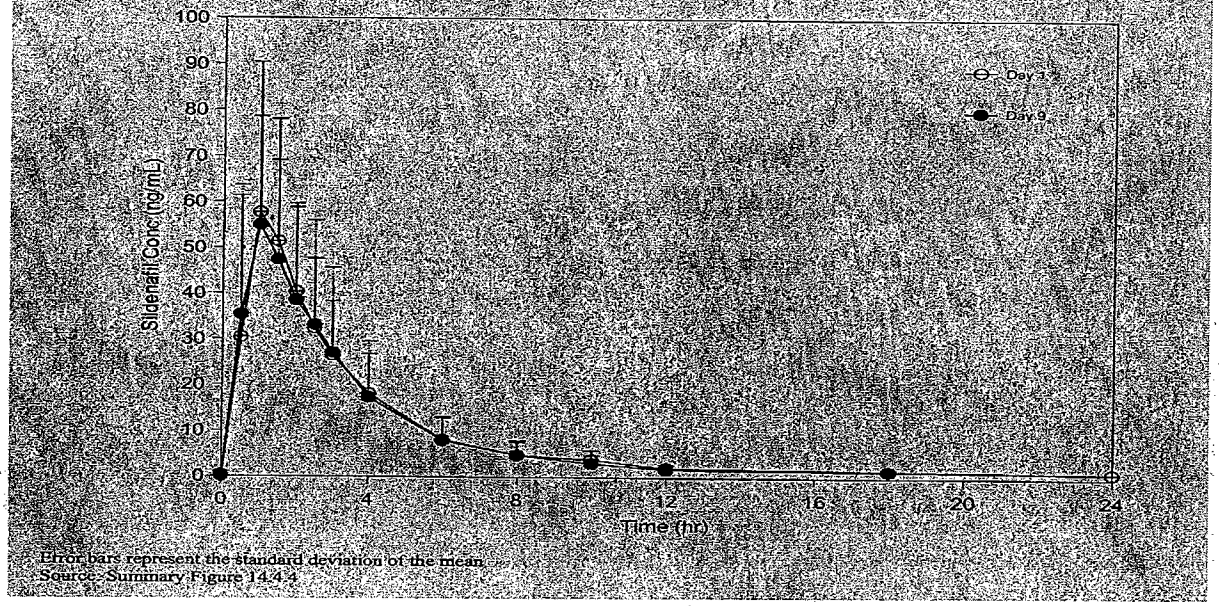
The mean plasma concentration profiles of ambrisentan in the presence and absence of sildenafil are similar and the respective 90% CI of the geometric mean ratios Day 9/Day1 for Cmax, AUC0-tlast and AUC0-00 are all within the 80% -125% bioequivalence limits, indicating no clinically relevant impact of sildenafil co-administration on ambrisentan exposure. The tmax of ambrisentan was also not affected by the presence of sildenafil. The inter-subject variation of Cmax, and AUC0-00 of ambrisentan was 10.9% and 31.3%, respectively, and the corresponding values for intra-subject variation were 20.2 and 9.1%, respectively.

Impact of Ambrisentan on Sildenafil

The mean plasma concentrations of sildenafil and derived parameters in the presence and absence of ambrisentan are shown in Figure 11.2 and Table 11.3, respectively:

Appears This Way
On Original

Figure 11.2 Mean Plasma Concentrations of Sildenafil When Administered Alone (Day 1) or in the Presence of Ambrisentan (Day 9)



Error bars represent the standard deviation of the mean
Source: Summary Figure 14.4.4

Appears This Way
On Original

Table 11.3 Comparison of Sildenafil Pharmacokinetic Parameters When Administered Alone (Day 1) or in the Presence of Multiple Doses of Ambrisentan (Day 9)

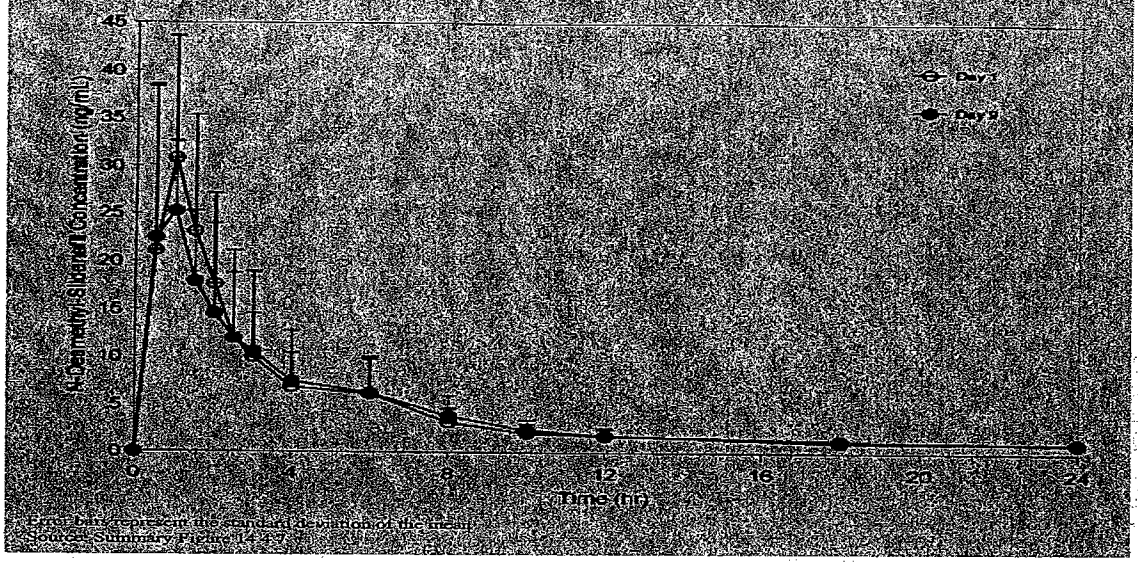
PK Parameter	Treatment	Arithmetic Mean (SD)	Geometric Mean	Geometric Mean Ratio and 90% CI (Day9/Day1)
C_{max} (ng/mL)	sildenafil (Day 1)	59.6 (31.39) n = 19	52.5 n = 19	
	ambrisentan + sildenafil (Day 9)	63.9 (24.00) n = 19	59.5 n = 19	113.40 (99.64,129.06)
$AUC_{0-tlast}$ (ng*h/mL)	sildenafil (Day 1)	181.8 (100.21) n = 19	158.1 n = 19	
	ambrisentan + sildenafil (Day 9)	180.8 (90.26) n = 19	158.6 n = 19	100.36 (91.16,110.48)
$AUC_{0-\infty}$ (ng*h/mL)	sildenafil (Day 1)	175.5 (94.44) n = 15	167.8 n = 15	
	ambrisentan + sildenafil (Day 9)	190.4 (94.58) n = 17	165.5 n = 15	98.65 (87.70,110.97)
t_{max} (hr)[1]	sildenafil (Day 1)	1.0 (1.00-2.00) n = 19	NA	
	ambrisentan + sildenafil (Day 9)	1.0 (0.50-2.00) n = 19	NA	p = 0.369
$t_{1/2}$ (hr)	sildenafil (Day 1)	2.0 (0.56) n = 15	NA	
	ambrisentan + sildenafil (Day 9)	2.1 (0.66) n = 17	NA	NA

Sources: Summary Table 14.2.7 and Summary Table 14.2.10
[1] For t_{max}, median (minimum, maximum) values are reported and p-value of Wilcoxon signed rank test of hypothesis that median pair-wise day 9 - day 1 difference = 0
n = number of subjects included in the calculation of mean values
NA = not calculated

The 90% CI of the geometric mean ratios (Day 9/Day1) of sildenafil in the presence and absence of ambrisentan for Cmax exceeded the upper limit of 125% of the bioequivalence criterion (129. 06 %). The point estimate was 113.4% indicating an impact of ambrisentan on peak exposure to sildenafil. The 90% CI for AUC0-tlast were within the 80%-125% bioequivalence limits. The tmax of sildenafil in the presence and absence of ambrisentan was similar. The mean t1/2 of the apparent terminal disposition phase of sildenafil in the presence and absence of ambrisentan were similar, 2.1 and 2.0 h, respectively.

The mean plasma concentrations of N-desmethylsildenafil and derived parameters in the presence and absence of ambrisentan are shown in Figure 11.3 and Table 11.4, respectively:

Figure 11.3 Mean Plasma Concentration–Time Profiles of N-Desmethyl-Sildenafil Following a Single Oral Dose of Sildenafil Alone (Day 1) or in the Presence of Ambrisentan (Day 9)



Error Bars represent the standard deviation of the mean
Source: Summary Figure 14.2.7

Table 11.4 Comparison of N-Desmethyl-Sildenafil Pharmacokinetic Parameters Following a Single Oral Dose of Sildenafil Alone (Day 1) or in the Presence of Multiple Doses of Ambrisentan (Day 9)

PK Parameter	Treatment	Arithmetic Mean (SD)	Geometric Mean	Geometric Mean Ratio and 90% CI (Day9/Day1)
C_{max} (ng/mL)	sildenafil (Day 1)	34.1 (14.90) n = 19	31.2 n = 19	
	ambrisentan + sildenafil (Day 9)	32.5 (10.25) n = 19	31.1 n = 19	99.64 (87.21, 113.84)
$AUC_{0-\infty}$ (ng•h/mL)	sildenafil (Day 1)	98.3 (45.25) n = 19	90.1 n = 19	
	ambrisentan + sildenafil (Day 9)	91.6 (40.60) n = 19	83.3 n = 19	192.44 (85.12, 100.39)
AUC_{0-t} (ng•h/mL)	sildenafil (Day 1)	113.9 (48.53) n = 14	107.9 n = 13	
	ambrisentan + sildenafil (Day 9)	117.1 (38.53) n = 13	108.4 n = 13	100.50 (91.17, 110.78)
t_{max} (hr)	sildenafil (Day 1)	1.0 (0.50-2.00) n = 19	NA	
	ambrisentan + sildenafil (Day 9)	1.0 (0.50-2.58) n = 19	NA	p = 0.063
$t_{1/2}$ (hr)	sildenafil (Day 1)	4.4 (4.16) n = 14	NA	
	ambrisentan + sildenafil (Day 9)	5.7 (7.90) n = 13	NA	NA

Sources: Summary Table 14.2.8 and Summary Table 14.2.10
For t_{max}, median (minimum, maximum) values are reported and p-value of Wilcoxon signed rank test of hypothesis that median pair-wise day 9 – day 1 difference = 0
n = number of subjects included in the calculation of mean values
NA = not calculated

The 90% CI of the geometric mean ratios (Day 9/Day1) for Cmax and AUC0-tlast were confined within the 80-125% bioequivalence criteria indicating that the presence of ambrisentan did not impact the exposure to N-desmethylsildenafil. The values for tmax and t1/2 of the sildenafil metabolite were also similar in the presence and absence of ambrisentan.

The geometric mean ratios of the trough concentrations of sildenafil on Days 6-8 were about 88% and 99%, respectively, and exceeded the pre-specified lower bound of 80% for attainment of steady state by Day 6.

The corresponding geometric mean ratios of the trough concentrations of N-desmethyl-sildenalfil on Days 6-8 was about 98% ad 100%, indicating attainment of steady state by Day 6.

The corresponding geometric mean ratios of the trough concentrations of ambrisentan on Days 6-8 were about 103% and 117%, indicating that ambrisentan achieved state-state by Day 6.

Conclusions

The study used the recommended dose regimen for sildenafil for the indication arterial pulmonary hypertension and the proposed regimen with the highest dose of ambrisentan for the same indication. However, it ought to be noted that the exposure to ambrisentan in healthy subjects may be smaller than in patients with pulmonary hypertension, because the clearance in the former population may be greater than in the latter. Multiple dose administration of sildenafil did not impact single dose exposure to ambrisentan. Multiple dose administration of ambrisentan decreased the peak exposure, but not the average dose interval exposure of sildenafil after single dose administration. Co-administration of ambrisentan had no impact on the exposure to N-desmethyl-sildenafil.

The ambrisentan induced small increase in peak exposure to sildenafil is not likely to be clinically relevant in patients with pulmonary arterial hypertension. Considerably higher doses of 100 mg sildenafil qd have been tolerated in patients treated for erectile dysfunction.

The negative results of this interaction study should not be extrapolated to the erectile dysfunction indication for sildenafil.

Comments

1. The estimates for t1/2 of the apparent terminal disposition phase for ambrisentan were estimated from an interval of about the same length as the t1/2 to be measured. It is not surprising that the computed value of 7 h underestimates the true value of t1/2 of about 15 h substantially. Estimates of t1/2 and λz dependent parameters including AUC0-00, CL/F and Vz/F are also biased.

2. The apparent terminal half life of sildenafil of 2 h estimated in this study is considerably smaller than the 4 h usually reported for the drug.

Study Report: AMB-220" A Phase 2, Randomized, Double-Blind, Dose-Controlled, Dose-Ranging Multi-Center Study of Ambrisentan (BSF 208075) Evaluating Exercise Capacity in Patients with Moderate to Severe Pulmonary Arterial Hypertension"

Study Investigator and Site: 21 Principal Investigators at 21 Sites in 6 countries (US, Germany, France, Belgium, Italy, Australia)

The review of the report concentrated on the PK of ambrisentan and effects on biomarkers including the plasma concentrations of endothelin-1 (ET-1), cardiac troponin (cTnT) and natriuretic B-type peptide (BNP)

Objectives

Primary

To examine the effect of ambrisentan on improvement in exercise capacity in subjects with moderate to severe pulmonary arterial hypertension, and to identify the minimally effective dose and define dose-response relationship for improvement in exercise capacity

Secondary

To evaluate:

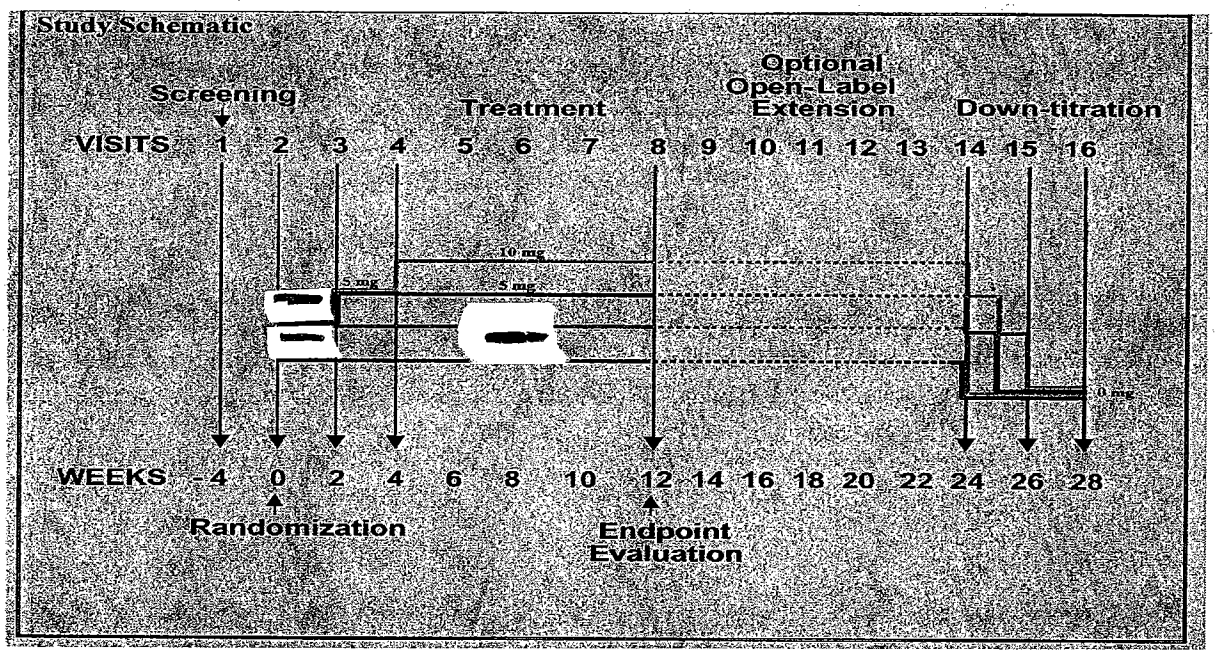
- The safety and tolerability of ambrisentan over the planned dose range and duration of exposure
- The effect of ambrisentan on the Subject Global Assessment (SGA), World Health organization (WHO) functional classification, and a composite of clinical outcomes
- The effect of ambrisentan on dyspnea as scored by the Borg Dyspnea index (BDI) immediately following the 6-minute walk test (6MWT)
- The effect of ambrisentan on cardiopulmonary hemodynamics in a selected subset of patients with moderate to severe PAH
- The first dose and steady-state plasma PK of ambrisentan in a selected subset of subjects with moderate to severe PAH
- The effect of ambrisentan on plasma endothelin-1 (ET-1) levels in a selected subset of subjects with moderate to severe PAH

Formulations

The study drug for oral administration was supplied as [redacted], pink, film-coated, immediate release tablets that were identical in appearance and size. Four strengths of study drug were used containing 0 (placebo), [redacted] and 5 mg of ambrisentan. Two tablets were combined to create daily doses [redacted] 5 and 10 mg of ambrisentan. The Lot No. were 0 mg (placebo) = L0001850, [redacted] = L0001852, [redacted] = L10001849, 5 mg = L0001851.

Design

This was a double-blind, dose-controlled, parallel group study with 4 periods: a 4 week screening period was followed by a 12-week blinded treatment period, an optional open-label extension period, and a 4 week down titration period. Eligible subject were randomized to receive [redacted] 5 or 10 mg ambrisentan orally qd in the blinded treatment period as shown in the study schematic:



Subjects randomized to [redacted] received the same dose throughout the 12 week blinded treatment period. Subjects in the two other dose groups began treatment with [redacted] qd for 2 weeks. Then their dose was increased to 5 mg for an additional 2 weeks. After 2 weeks at 5 mg, subjects randomized to the 10 mg dose level underwent a final up-titration. After reaching the randomized dose level, subjects received their assigned dose throughout the blinded treatment period. In the event that subjects did not tolerate the study drug, dose adjustment was permitted during the treatment period. Upon completion of the 12 week blinded treatment period, subjects either completed a 4 week down titration period or entered the optional open-label 12-week period without down-titration. The dose of ambrisentan of the subjects participating in the open-label extension part of the study was optimized based on the patient's response and tolerance.

Endpoints

The primary efficacy endpoint was the change from baseline in 6MWD evaluated after 12 weeks of treatment.

The secondary endpoints included a change from baseline (week 0) after 12 weeks of treatment in SGA, WHO functional class, composite clinical outcome for worsening PAH (time to clinical worsening), where the composite included death, all-cause hospitalizations, doubling of the dose of diuretics after randomization, and study withdrawal because of a need for the addition of other PAH therapeutic agents, BDI measured immediately following exercise, cardiopulmonary hemodynamics in a selected subset of patients

Other secondary endpoints included safety and tolerability of ambrisentan over the planned dose range and duration of exposure

First dose and steady-state PK in a selected subset of subjects with moderate or severe PAH, the effect of ambrisentan on plasma ET-1, B-type natriuretic hormone (BNP) and cardiac troponin T concentrations (cTnT) in a selected subset of subjects with moderate or severe PAH

The 6MWD was performed at screening, prior to and 4, 8 and 12 weeks after initiation of the double-blind treatment phase.

SGA was determined at screening, prior to and 2, 4, 8, and 12 weeks of the double-blind treatment period. A visual analog scale assessment based on the question "how do you feel today" was used. The response was measured by Myogen staff using standardized rulers.

The WHO functional status was measured at the same times as the SGA and a classification was used as described below:

Table 9.2 World Health Organization Classification of Functional Status of Patients with Pulmonary Hypertension

Class	Description
I	Patients with pulmonary hypertension in whom there is no limitation of usual physical activity; ordinary physical activity does not cause increased dyspnea, fatigue, chest pain, or presyncope.
II	Patients with pulmonary hypertension who have mild limitation of physical activity. There is no discomfort at rest, but normal physical activity causes increased dyspnea, fatigue, chest pain, or presyncope.
III	Patients with pulmonary hypertension who have a marked limitation of physical activity. There is no discomfort at rest, but less than ordinary activity causes increased dyspnea, fatigue, chest pain, and presyncope.
IV	Patients with pulmonary hypertension who are unable to perform any physical activity at rest and who may have signs of right ventricular failure. Dyspnea and/or fatigue may be present at rest, and symptoms are increased by almost any physical activity.

Time to Clinical Worsening was determined from the AE-, Concomitant Medication-, and Premature Discontinuation- CRFs.

The following hemodynamic measurement were obtained: HR, systemic blood pressure (systolic, diastolic, mean), pulmonary arterial pressure (systolic, diastolic, mean), right atrial pressure, RAP, pulmonary capillary wedge pressure (PCWP) or left ventricular end-diastolic pressure, LVEDP, (via separate left heart catheter), cardiac output (Fick method or thermodilution), arterial and mixed venous 02 saturation, cardiac index, stroke volume, pulmonary vascular resistance, PVR, was measured from mPAP, cardiac index and RAP. The hemodynamic parameters were measured prior to initiation and 12 weeks after initiation of the double-blind treatment period.

Changes in the plasma concentrations of ET-1, cTnT, BNP have been associated with changes in the status of PAH. Blood samples for the determination of theses biomarkers were collected in the subset of patients participating in the PK sub-study. Blood samples for the determination of ET-1 levels were collected at the week 0 and 12 visits pre-dose, and 2 h and 24 h post-dose. BNP and cTnT was measured in the same blood sample.

PK Profiling

All subjects of the subset started at an initial daily dose of [redacted] or [redacted]. Blood samples for the determination of the plasma concentrations of ambrisentan were collected at the week 0 and 12 visits at the following times: Pre-dose and 0.5, 1, 2, 3, 4, 6 and 24 h post-dose.

Bioassay

The plasma concentrations of ambrisentan were measured at [redacted] using a validated LC/MS/MS method with a LLOQ of [redacted].

PK Data Analysis

The following parameters were determined based on non-compartmental methods using WinNonlin 3.2, Pharsight Corporation, Mountain View, CA) with the exception of the accumulation ratio which was calculated using SAS:
Cmax, tmax, AUC0-tlast, AUC(0-24), AUC0-00, λz, t1/2, Cmax,ss, Cmin,ss, tmax,ss, AUC0-tlast,ss, AUC0-τ,ss, Cave,ss, λz,ss, t1/2,ss, and the accumulation factors nR_{Cmax} and nR_{AUC},

where nRCmax = Cmax,ss/Cmax • Dose, visit 2/Dose,visit 8 and

$nRAUC = AUC_{ss,0-\tau} / AUC_{0-24}$ • Dose, visit 2/Dose,visit 8

In subjects with qd dosing τ was 24 h and in subjects with bid dosing τ=12 h for the measurements of AUC0-τ. However, nRAUC was not calculated for patients receiving the drug bid. In determining λz it was at the discretion of the data analyst to use only 2 time points in the apparent terminal log linear phase to determine λz. Although not pre-specified in the statistical analysis plan for the PK subset analysis nor outlined in a protocol amendment, if it was suspected that a morning dose of ambrisentan had been taken prior to the 24 h post dose time point (high ambrisentan concentrations), the result was excluded and set to missing.

PD Profiling

Blood samples for the determination of ET-1, cTnT and BNP were collected at the same visits and same times as the blood samples for the determination of ambrisentan's plasma concentrations.

Bioassay for Endothelin-1

The plasma concentrations of ET-1 were measured by [redacted] using an Elisa kit manufactured by [redacted]. The limit of detection for the ET-1 analysis was reported to be [redacted].

PD Data Analysis

If treatment related and/or dose-dependent changes in baselineET-1 concentrations were found exploratory PD modeling using WinNonlin 3.2 (Pharsight Corp., Mountain View, CA) was to be performed to correlate plasma concentrations of ambrisentan and ET-1.

Results

The ITT population included 64 patients 10 males and 54 females of mean age 51.4 y and weight 73.0 kg.

PK and PD

Twenty subjects participated in the PK sub-study. All subjects received ambrisentan by a qd regimen. Two subjects in the PK population (subjects 22-001 (2.5mg) and 13-005 (10 mg)) discontinued prior to week 12, and did not provide steady-state PK information. Another subject 13-009 although completing the 12 week treatment period had also missing steady-state information. Four data sets were excluded as the pre-dose concentrations were similar to the Cmax value of the preceding day. The 4 excluded concentrations were for subjects 13-009 and 17-001 after a single dose and subjects 002-005 and 13-001 at steady-state.

The mean plasma concentrations of ambrisentan after a single dose and at steady-state and derived parameters are shown Figures 11.9 and 10 and Table 11.8:

Figure 11.9 Mean Single-Dose Ambrisentan Plasma Concentrations versus Time – Linear and Logarithmic Scales (Population: PK)

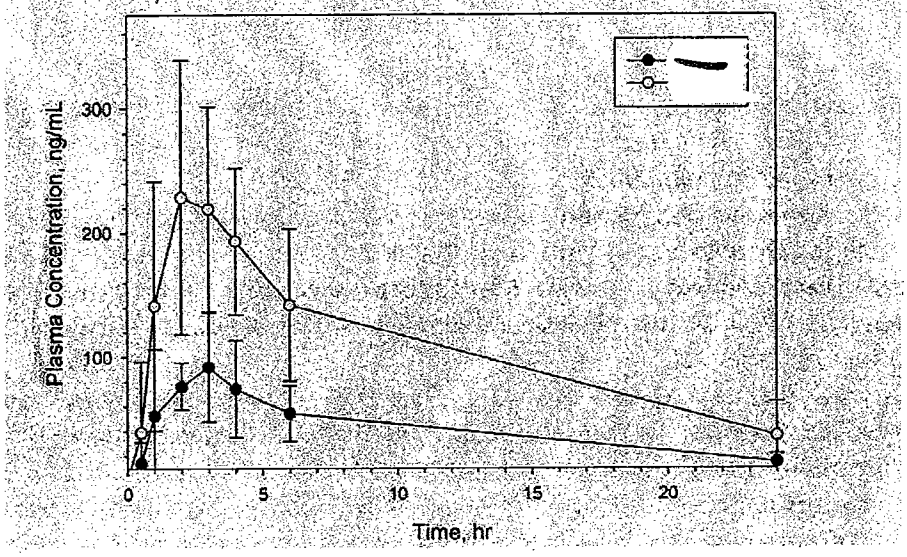
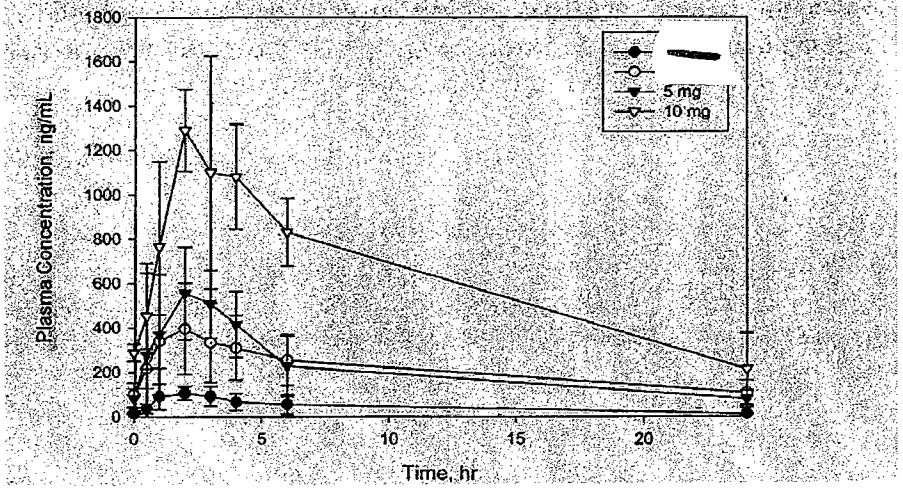


Figure 11.10 Mean Steady-State Ambrisentan Plasma Concentrations vs. Time – Linear and Logarithmic Scales (Population: PK)



Appears This Way
On Original