

Ambrisentan as film coated immediate-release tablets was provided by Myogen, Inc.

Test Commercial Formulations

They were manufactured by _____

- _____ ambrisentan _____ mg tablets for oral administration debossed _____ on one side and _____ on the other side, _____ Lot No. R0135002 (_____ Lot No. L0101702, expiration date December 2006), manufactured by _____ drug substance Batch 3109.F.04.4 having a _____ of _____
- Square, pale-pink ambrisentan 5 mg tablets for oral administration debossed _____ on one side and "5" on the other side, _____ Lot No. R0136002, _____ Lot No. L0101701, expiration date December 2006), manufactured by _____ drug substance Batch 3109.F.04.4 having a _____ of _____
- Oval, dark-pink ambrisentan 10mg tablets for oral administration debossed _____ on one side and "10" on the other side, _____ Lot No. R0137002, _____ Lot No. L0101699, expiration date December 2006), manufactured by _____ drug substance Batch 3109.F.04.4 having a _____ of _____
- Oval, dark-pink ambrisentan 10 mg tablets for oral administration debossed _____ on one side and "10" on the other side, _____ Lot No. R0137001 (_____ Lot No. L0101704, expiration date December 2006), manufactured by _____ drug substance Batch 3109.F.04.2 having a _____ of _____

Reference Clinical Formulations

They were manufactured by _____

- _____ pink, _____ ambrisentan _____ tablets for oral administration, _____ Lot No. 171022292, expiration date January 2007, _____ Lot No. 280200A0, expiration date January 2007), manufactured by _____ drug substance Batch L0003139 _____ Lot No. L0001849) having a _____ of _____
- _____ pink, _____ ambrisentan 5 mg tablets for oral administration _____ Lot No. 17102330, expiration date January 2007, _____ Lot No. 280200A0, expiration date January 2007), manufactured by _____ drug substance Batch L0003139 (_____ Lot No. L0001851) having a _____ of _____
- _____ pink, _____ ambrisentan 10 mg tablets for oral administration _____ Lot No. 17102393, expiration date January 2007, _____ Lot No. 280200A0, expiration date January 2007), manufactured by _____ drug substance Batch L0003139 _____ Lot No. L0001848) having a _____ of _____

The respective batch size of the [redacted] (R0135002), 5 mg (R0136002), 10 mg (R0137002) and 10 mg tablets (20137001) test tablets was not indicated, [redacted]. The respective batch size of the [redacted] (L0001849), 5 mg (L0001851) and 10 mg (10001848) reference tablets was not indicated, [redacted]. The batch size of the 5 mg and 10 mg formulations tested fulfilled the minimum size requirements.

Design

This was an open-label, randomized, 2 period (for [redacted] 5 mg tablets) or 3-period (for 10 mg tablets) crossover, single center study. A total of 65 healthy subjects; males and females (with no child bearing potential) in the age between 18 and 55 y, were to be enrolled to ensure that 60 subjects (20 subjects for each dose group) complete both treatment periods with the [redacted] and 5 mg tablets and the 3 treatment periods with the 10 mg tablets. Subjects were admitted to the Unit the night prior to dosing of each treatment period and remained confined in the Unit until Day 3 of each treatment period.

Between administrations of a single dose of study drug in each treatment period, there was a 6 day wash-out period. All subjects were to take the tablets after an overnight fast together with 240 mL water. Concomitant medications, with the exception of 3 doses of acetaminophen at 1 g each or less were prohibited for the duration of the study. In addition, no foods or substances known to interfere with cytochrome P450 isozymes could be consumed, including tobacco, alcohol, and grapefruit containing products.

A study schematic (treatments with test formulations are denoted with A ([redacted] 5mg) and A or B (10 mg) and treatments with the test formulations are denoted with R) and a schedule of activities is shown in Figure 9.1 and Table 9.1, respectively:

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Table 9.1 Schedule of Assessments

Study Day	Screening Visit	In-House Phase of Study for Treatment Periods			Clinic Discharge	Follow-Up 4±1 Days After Last Dose of Study Drug
	-21 to -1	1	1	2	3	6-8
Signed informed consent	X					
Inclusion/exclusion criteria	X					
Medical history	X	X				
Concomitant medication review	X	X	X	X	X	
Physical examination	X	X				X
12-Lead ECG	X					X
Vital signs	X	X	X	X	X	X
Height and weight						
HIV test and hepatitis B and C serology	X					
Hematology, serum chemistry, and coagulation tests	X					X
Urinalysis	X					X
Urine drug screen & alcohol screen	X	X				
Admission to study unit		X				
Randomization to Dose Group		X				
Randomization to Treatment Sequence		X				
Ambrisentan dosing			X	X		
Blood samples for PK analysis			X	X	X	
Adverse event monitoring			X	X	X	X
Study Discharge						X ¹¹

Interim history from screening visit.
 Abbreviated physical examination.
 Vital signs (sitting blood pressure, temperature, respiration rate, and pulse) at screening day -1 and at the following times relative to day 1 dosing: pre-dose (within 30 minutes prior to dosing), then at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, and 48 hours post-dose.
 Standard clinical laboratory test panel (see Section 9.5.5.2).
 Must have been negative for subject to receive study drug on day 1.
 6-day washout between treatments.
 Assignment to 1 of 3 dose groups: Dose Group 1: 5 mg ambrisentan; Dose Group 2: 5 mg ambrisentan; or Dose Group 3: 10 mg ambrisentan.
 Randomization to Treatment Sequence: RA, AR, RA, AR, BA, AB, BA, AB, or BA, AB after randomization to 1 of 3 parallel dose groups.
 Blood collection for the determination of ambrisentan plasma concentrations was at the following times relative to day 1 dosing: pre-dose (within 30 minutes prior to each dose), then at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, 24, 36, and 48 hours post-dose.
 Adverse events query after vital signs were obtained.
 Study discharge after all follow-up procedures and assessments were completed.

PK Profiling

Blood samples for the determination of ambrisentan plasma concentrations were collected at the following times after dosing on Day 1: Pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, 24 and 48 h.

PK Analysis

In Group 1 () 1 subject (01-106) withdrew consent during Period 2 due to refusal to use an IV catheter and another subject (01-121) was lost to follow-up.

In Group 2 (5 mg) 5 subjects were withdrawn due to noncompliance: Subject 01-205 after Period 2 for refusal to participate in end of study procedures, Subjects 01-212 and 01-213 for not returning for the Period 2 check-in, Subject 01-216 for not returning for the end of study procedures and Subject 01-217 for a positive drug screen at the Period 2 check-in.

In Group 3 (10 mg) 1 subject (01-322) was discontinued during Period 2 due to a streptococcal pharyngitis.

Data Sets Analyzed for PK

In Group 1 () 20 subjects were included in the analysis. Subject 01-106 was not considered evaluable for PK analysis as he did not have sufficient plasma data after Treatment A1.

In Group 2 (5 mg) 19 subjects were included in the analysis. Subjects 01-212, 01-213, and 01-217 were considered not evaluable because they only received 1 dose during period 1.

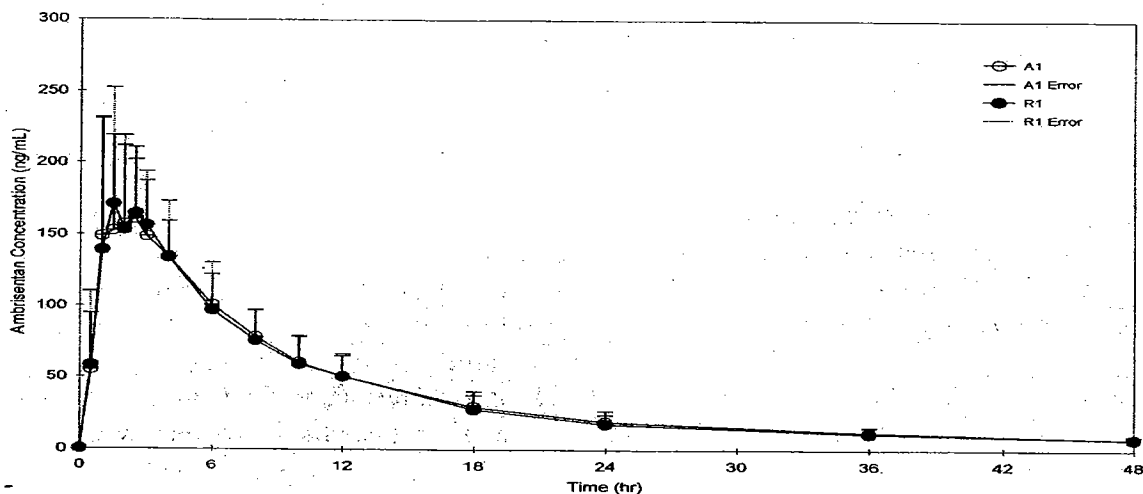
In Group 3 (10 mg) 20 subjects were included in the analysis. Subject 01-322 was considered not evaluable for PK analysis because he only received 1 dose during Period 1.

The analysis of variance analysis did not show evidence for sequence or period difference.

Mean plasma concentrations and derived measures of bioavailability/bioequivalence are shown for test and reference formulations of ambrisentan at the () dose level in Figures 11.1 and Table 11.2, respectively:

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Figure 11.1 Plasma Concentration-Time Profiles of Ambrisentan Following a Single Oral Dose of Ambrisentan from Test Product A1 (Test Drug A) or Reference Product R1 (Reference Drug) (Linear Scale)



Error bars represent SDs from the arithmetic mean
Source: Summary Figure 14.4.1

Table 11.2 Comparison of Ambrisentan Pharmacokinetic Parameters Following Treatment A1 (Test Drug A) and Treatment R1 (Reference Drug)

PK Parameter	Treatment	Arithmetic Mean (SD) n	Geometric Mean n	Geometric Mean Ratio and 90% CI (A1/R1)
C_{max} (ng/mL)	A1	203.5 (56.8) n = 20	199.1 n = 20	94.1 (83.4, 106.2)
	R1	217.1 (49.7) n = 20	211.5 n = 20	
AUC_{0-last} (ng*hr/mL)	A1	1833.3 (353.0) n = 20	1800.3 n = 20	104.5 (97.3, 112.3)
	R1	1762.5 (404.0) n = 20	1722.7 n = 20	
$AUC_{0-\infty}$ (ng*hr/mL) ^{1,2}	A1	2110.0 (362.0) n = 17	2096.3 n = 16	105.9 (98.8, 113.5)
	R1	2008.0 (376.3) n = 18	1980.0 n = 16	
t_{max} (hr) ³	A1	2.0 (1.0, 6.0) n = 20	NA	p = 0.27
	R1	1.5 (1.0, 6.0) n = 20	NA	
$t_{1/2}$ (hr) ¹	A1	16.2 (5.0) n = 17	NA	NA
	R1	15.0 (3.7) n = 18	NA	

Sources: Summary Table 14.2.4 and Summary Table 14.2.7

¹ $t_{1/2}$ and $AUC_{0-\infty}$ could not be determined for three subjects in Treatment A1 and two subjects in Treatment R1

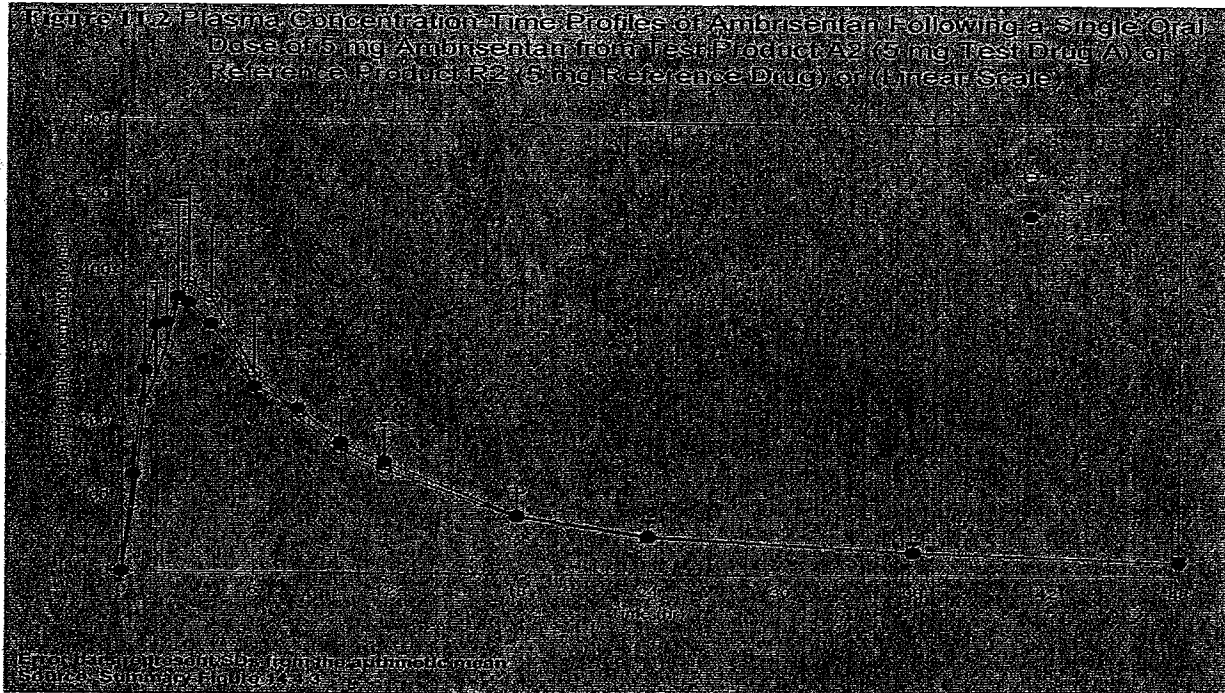
²Geometric means were based on mixed model ANOVA and only included subjects that had $AUC_{0-\infty}$ values in Treatments A1 and R1

³For t_{max} , median (minimum, maximum) values are reported and p-value of Wilcoxon signed rank test of hypothesis that median pair-wise T-R difference = 0

n = number of subjects included in the calculation of mean values

NA = not applicable/not calculated

Mean plasma concentrations and derived measures of bioavailability/bioequivalence are shown for test and reference formulations of ambrisentan at the 5 dose level in Figures 11.2 and Table 11.3, respectively:



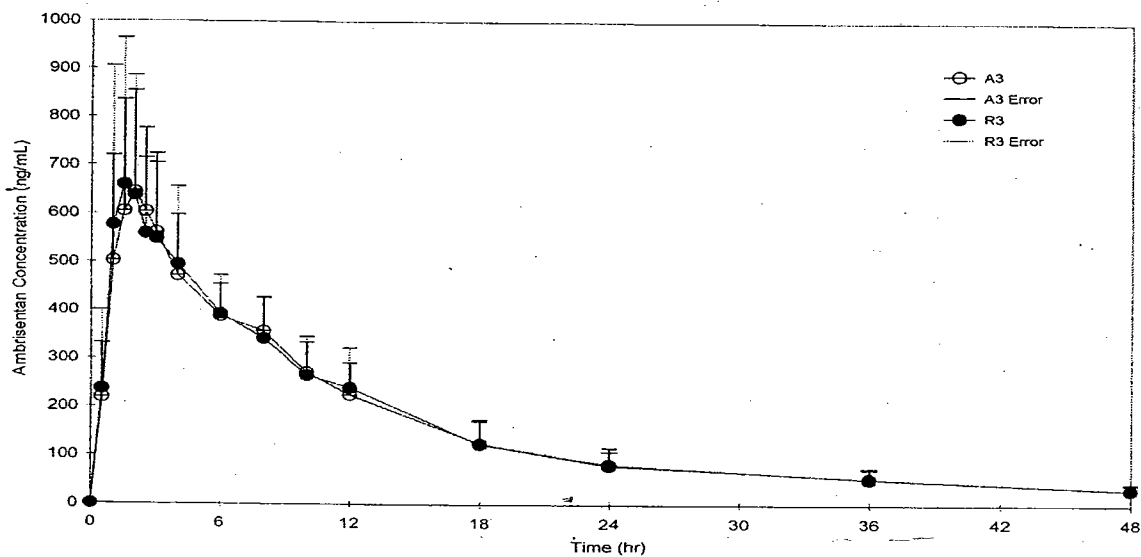
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Table 11.3 Comparison of Ambrisentan Pharmacokinetic Parameters Following Treatment A2 (Single Test Drug A) and Treatment R2 (Single Reference Drug)

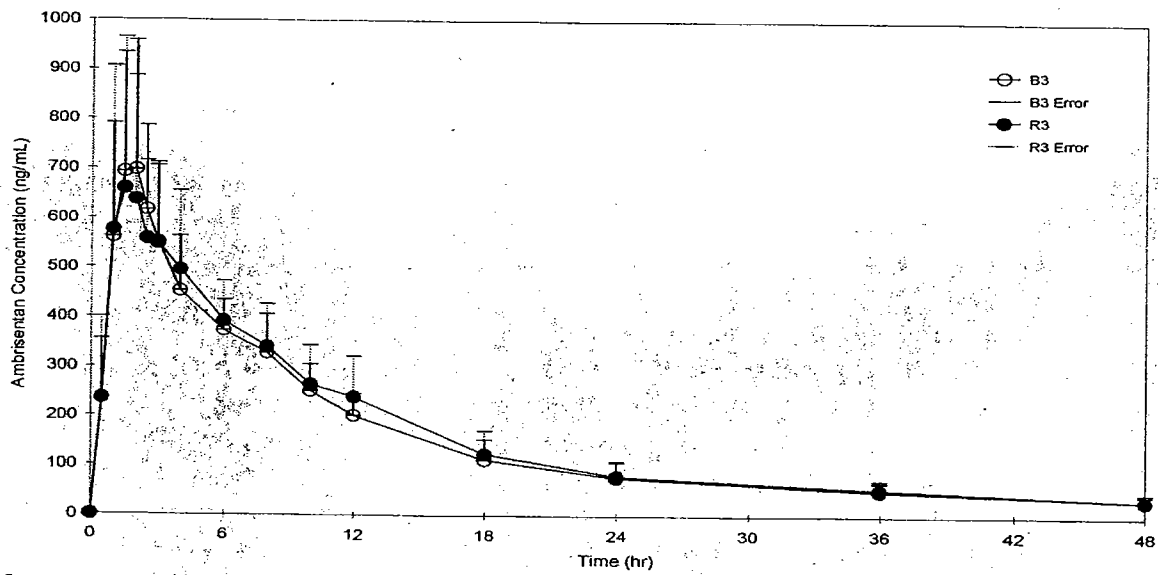
Parameter	Treatment	Arithmetic Mean (SD)	Geometric Mean (SD)	Geometric Coefficient of Variation (%)
C _{max} (ng/mL)	A2	650 (150)	650 (150)	23.1
	R2	650 (150)	650 (150)	23.1
AUC ₀₋₄₈ (ng·hr/mL)	A2	12000 (3000)	12000 (3000)	25.0
	R2	12000 (3000)	12000 (3000)	25.0

Figure 11.3 Plasma Concentration-Time Profiles of Ambrisentan Following a Single Oral Dose of 10 mg Ambrisentan from Test Product A3 (10 mg Test Drug A) versus Reference Product R3 (10 mg Reference Drug) (Linear Scale)



Error bars represent SDs from the arithmetic mean
Source: Summary Figure 14.4.5

Figure 11.4 Plasma Concentration-Time Profiles of Ambrisentan Following a Single Oral Dose of 10 mg Ambrisentan from Test Product B3 (10 mg Test Drug B) versus Reference Product R3 (10 mg Reference Drug) (Linear Scale)



Error bars represent SDs from the arithmetic mean
Source: Summary Figure 14.4.5

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Table 11.4 Comparison of Ambrisentan Pharmacokinetic Parameters Following Treatment A3 (10 mg Test Drug A) and B3 (10 mg Test Drug B) and Treatment R3 (10 mg Reference Drug)

PK Parameter	Treatment	Arithmetic Mean (SD) n	Geometric Mean n	Geometric Mean Ratio and 90% CI (A3/R3 or B3/R3)
C_{max} (ng/mL)	A3	721.2 (190.5) n = 21	697.1 n = 21	87.2 (79.3, 95.9)
	B3	803.0 (226.3) n = 21	783.6 n = 21	98.0 (89.1, 107.8)
	R3	838.8 (222.2) n = 21	799.7 n = 21	
AUC_{0-last} (ng*hr/mL)	A3	7573.7 (1674.7) n = 21	7381.7 n = 21	99.7 (95.5, 103.9)
	B3	7277.9 (1542.1) n = 21	7131.4 n = 21	96.3 (92.3, 100.4)
	R3	7643.0 (1651.8) n = 21	7407.5 n = 21	
$AUC_{0-\infty}$ (ng*hr/mL) ¹	A3	8419.9 (1911.2) n = 21	8195.3 n = 18	99.9 (95.4, 104.5)
	B3	7998.2 (1583.2) n = 18	7896.3 n = 18	96.2 (91.7, 101.0)
	R3	8621.4 (1870.7) n = 20	8207.6 n = 18	
t_{max} (hr) ²	A3	2.0 (1.0, 8.0) n = 21	NA	p = 0.39
	B3	1.5 (1.0, 4.0) n = 21	NA	p = 0.32
	R3	1.5 (1.0, 8.0) n = 21	NA	
$t_{1/2}$ (hr) ¹	A3	18.1 (4.3) n = 21	NA	NA
	B3	18.4 (3.8) n = 18	NA	NA
	R3	18.1 (4.3) n = 20	NA	

Sources: Summary Table 14.2.6 and Summary Table 14.2.9

¹ $t_{1/2}$ and $AUC_{0-\infty}$ could not be determined for three subjects in Treatment B3 and one subject in Treatment R3

²For t_{max} , median (minimum, maximum) values are reported and p-value of Wilcoxon signed rank test of hypothesis that median pair-wise T - R difference = 0

n = number of subjects included in the calculation of mean values

NA = not applicable/not calculated

Mean plasma concentrations and derived measures of bioavailability/bioequivalence are shown for test and reference formulations of ambrisentan at the 10 mg dose level in Figures 11.3 and 11.4 and Tables 11.4, respectively:

In all subjects and treatments ambrisentan was measurable in the first plasma sample (0.5 h) collected after administration. At the 10 mg level the plasma concentrations of ambrisentan could be followed up to 48 after administration in all 40 treatments. At the 5 mg dose level the plasma concentrations could be measured up to 48 h after administration in 37 of 38 treatments. At the

_____ level the plasma concentrations of ambrisentan were measurable for 48 h in 17 of the 40 treatments. The ratio of mean AUC_{0-tlast}/mean AUC_{0-∞} ranged between 0.87 to 0.91 indicating that the extrapolated part of AUC_{0-∞} is small.

The results indicate that the 90% CI for C_{max}, AUC_{0-tlast} and AUC_{0-∞} for the test formulations of _____ and 5 mg strength were bounded within the 80%-125% limits for bioequivalence. At the 10 mg dose level only the test formulation with a _____ fulfilled the criteria for bioequivalence with the reference formulation. In contrast, C_{max} of the commercial 10 mg tablet with the _____ failed to meet the lower bioequivalence boundary. There was no statistically significant difference between the t_{max} values of the test and reference formulation at the 3 dose levels. Mean t_{max} among the commercial formulations ranged between 1.5 h and 3.0 h and among the clinical formulations between 1.5 h and 2.5 h. The half life of the apparent terminal log linear phase of ambrisentan ranged between 15 h and 18 h.

The respective slopes for C_{max} and AUC for the A1, A2 and A3 test formulation treatments were 0.91 (95% CI: 0.78, 1.04) and 1.02 CI: 95% CI: 0.89, 1.15) indicating that the PK after single dose administration is dose proportionate.

The intra-subject variation of C_{max} and AUC ranged between 11.4 % and 21.8% and 8.1% and 10.7%, respectively.

Conclusions

The _____ and 5 mg (_____ commercial formulations are bioequivalent to the respective clinical service formulations. The commercial 10 mg formulation (_____ does not meet the bioequivalence criteria for C_{max}. In contrast, the commercial 10 mg formulation _____ meets the bioequivalence criteria.

The PK of ambrisentan after administration of the commercial formulations were dose proportional. The intra-subject variation for C_{max} and AUC ranged between 11.4% and 21.8% and 8.1% and 21.8%, respectively.

Comments

1. The criteria applied in determining the slope of the log linear terminal phase are not stated in the report. Also, the method used to compute AUC_{0-tlast} is not stated in the report.
2. Possible causes for the failure of the 10 mg commercial formulation of _____ to meet the bioequivalence criteria were not discussed in the report

Study Report: AMB-106" A Phase 1, Open-Label Study To Evaluate the Potential for a Pharmacodynamic and/or Pharmacokinetic Interaction of Ambrisentan with a Single Dose of Racemic Warfarin in Healthy Adult Volunteers"

Study Investigator and Site: _____

Objectives

Primary

To assess the effects of multiple doses of ambrisentan on the pharmacodynamics (PD) of a single dose of racemic warfarin, as determined by assessments of prothrombin time (PT) and International Normalized Ratio (INR)

Secondary

To assess the effect of multiple doses of ambrisentan on the pharmacokinetics (PK) of a single dose of warfarin (S- and R-enantiomers)

To assess the effect of a concomitant, single dose of warfarin on the PK of multiple doses of ambrisentan

To examine the safety and tolerability of ambrisentan in the presence of a single dose of warfarin

Formulations

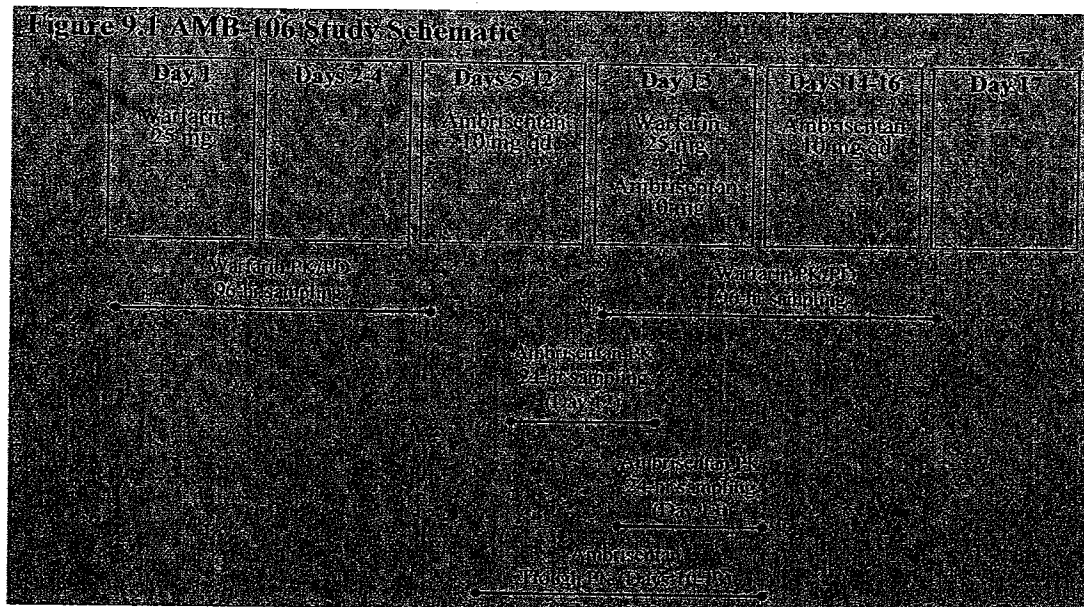
Ambrisentan was provided by Myogen, Inc., as 10 mg film-coated immediate release tablets.

The _____ pink, _____ tablets were manufactured by: _____
_____. Commercially available warfarin was provided to the clinical site as 5 mg tablets for oral administration, manufactured by _____ Lot No. ETD286A. The packager _____ Lot No. for the ambrisentan tablets was L0001848 (_____ article/material No. 17102293, _____ Lot No. 280100A0).

Design

This was an open-label, non-randomized, 2-period crossover, single center study as shown in the below study schematic:

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Twenty-two (22) subjects were to be enrolled to ensure that 18 subjects completed the study. On Day 1, subjects were administered a single 25 mg dose of warfarin. Prothrombin Time (PT) and International Normalization Ratio (INR) and the PK of the S- and R-enantiomers of warfarin were assessed at scheduled intervals for 96 h after the Day 1 warfarin dose. Subjects then received a once daily 10 mg of ambrisentan on Days 5 through 16. Blood samples were collected on Days 10 through 12 to assess attainment of steady-state. On Day 12, blood samples were taken over a 24 h period for PK assessment of ambrisentan. PT and INR and the PK profile of the warfarin enantiomers were assessed at scheduled intervals for 96 h after the Day 13 warfarin dose and the ambrisentan doses of Days 13 through 17.

Subjects were admitted to the unit the night before dosing and remained in the clinic until Day 17 or when their INR value was < 1.2, whichever was longer.

The scheduled activities of the study are shown in Table 9.1:

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The plasma concentration-time data of warfarin (S- and R- enantiomers) and ambrisentan were determined using non-compartmental methods. The software WinNonlin™ was used (inNonlin Professional Network Edition, Version 4.0.1, Pharsight Corp, Palo Alto, CA)

For the S- and R-enantiomers of warfarin the following parameters were determined: C_{max}, t_{max}, t_{lag}, AUC_{0-tlast}, AUC_{0-∞}, λ_z, t_{1/2}, CL/F and V_z/F.

For ambrisentan the following parameters were determined: C_{max,ss}, C_{min,ss}, C_{avg,ss}, t_{max,ss}, AUC_{0-τ,ss}, AUC_{0-tlast,ss}, AUC_{0-∞,ss}, λ_z, t_{1/2}, CL_{ss}/F (Dose/AUC_{0-τ,ss}) and V_{z,ss}/F (Dose/(λ_z • AUC_{0-τ,ss}))

PK Data Analysis

To assess the effect of multiple doses of ambrisentan on the PK of warfarin (S- and R- enantiomers), individual within-subject pair-wise differences were summarized for the natural logarithm of the S- and R- enantiomers C_{max}, AUC_{0-tlast} and AUC_{0-∞}. Geometric mean ratios of Day 13 to Day 1 were reported with 90% CI. The ratios and CIs were expressed as a percentage and compared to the standard criterion for bioequivalence (90% CI within 80-125%). The Wilcoxon signed rank test was used to determine the differences in t_{max} on Days 13 and 1.

To assess the possible impact of the presence of warfarin on the PK of ambrisentan the same statistical methods were used. The differences in the parameters C_{max,ss}, C_{min,ss} and AUC_{0-τ,ss} and t_{max} between Days 13 and 12 were computed.

To assess attainment of steady-state of ambrisentan on Days 10 through 13 the natural log transformed trough concentrations were subjected to an analysis of variance model that included a random subject effect and fixed day effect. The following contrasts were compared: Day 10 vs average of Days 10-13, Day 11 vs average of Days 12-13, and Day 12 vs Day 13. The lower bound of each 90% CI about these contrasts was compared to an 80% equivalence criterion. If for example the lower bound of the Day 10 vs Day 11 through 13 contrast was above 80%, then this supported an inference that ambrisentan steady-state was achieved by Day 10.

PD Profiling

Blood samples for the determination of PT and INR were collected after a single dose of warfarin in the presence and absence of ambrisentan at the same times as for the determination of the PK parameters on Days 1-5 and 13-17. L_{max} (maximum observed value of PT or INR) and the associated time, t_{max}, and AUE_{0-tlast} (area under the PT or INR time curve from time zero the last measurable concentration) using the linear trapezoidal rule were determined. Description and validation performance for PT and INR assays were not included in the study report.

PD Data Analysis

To assess the effect of multiple doses of ambrisentan on the PD of warfarin, individual within-subject pair-wise differences were summarized for the natural logarithm transformed L_{max} and AUE_{0-tlast} on Days 1 and 13 for PT and INR. Treatment contrasts (Day 13 vs Day 1), 2-sided