

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-081

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

CLINICAL PHARMACOLOGY REVIEW

Division of Clinical Pharmacology I

NDA 22081

Submission Date: December 13, 2006

Type: NDA, Priority, Orphan Drug Status

Brand Name: Letairis

Generic Name: Ambrisentan

Dosage Strength: 5 mg and 10 mg tablets

Sponsor: Gilead Pharmaceuticals, Inc., Westminster, CO

Indication: Treatment of arterial pulmonary hypertension (PAH)

Reviewing Division: Division of Cardiovascular and Renal Products, HFD-110

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Table of Content

1. Executive Summary.....	1
1.1 Recommendation.....	6
2. Question Based Review.....	7
3. Label Recommendation.....	33
4. Individual Study Reports.....	53
5. Biopharmaceutics.....	188
6. QTcIRT Report.....	194

1. EXECUTIVE SUMMARY

The submission contained 18 Clinical Pharmacology study reports. Ten (10) reports related to in vitro studies with human tissues, 7 reports dealt with the PK (2 stage approach)-characteristics of ambrisentan and 1 report with POP-PK and PK-PD analyses. Included in the study reports were 9 assay reports. All 27 reports were reviewed.

Ambrisentan is a propanoic acid-type, non-sulfonamide, selective endothelin type ET_A receptor antagonist. Ambrisentan is a single enantiomer. Two receptor subtypes, ET_A and ET_B mediate the effects of endothelin-1 (ET-1), an auto- and paracrine peptide, in vascular smooth muscle and endothelium. The primary actions of ET-1 mediated by ET_A are vasoconstriction and cell proliferation. The predominant actions of ET-1 mediated by ET_B are vasodilation, antiproliferation, and ET-1 clearance. In PAH the levels of ET-1 are increased and correlate with increased right atrial pressure and disease severity.

Ambrisentan is indicated for the treatment of PAH (WHO Group I) in adults to improve exercise capacity, delay clinical worsening, [REDACTED]. The proposed dose regimen is 5 mg qd with or without food. If necessary the dose can be increased to 10 mg qd.

Clinical Pharmacology Studies

The sponsor characterized the pharmacokinetics of ambrisentan by in vitro studies with human tissues investigating the protein binding of ambrisentan, ambrisentan as substrate of CYPs and UGTs and transporters as well as its potential as inhibitor of enzymes and transporters. The sponsor performed in vivo single and multiple ascending dose studies, investigated the mass balance, the impact on the QT/QTc interval, the potential for an interaction when co-administered with food, warfarin or sildenafil and the bioequivalence of to be marketed and clinical service formulations of ambrisentan. The sponsor measured the pharmacodynamic effects of ambrisentan's on blood pressure and heart rate as well as ET-1 plasma levels in healthy subjects.

In the target population the sponsor studied the pharmacokinetics of ambrisentan in the Phase 2 dose ranging study using a rich sampling/traditional approach and in the pivotal Phase 3 studies in subgroups using a sparse sampling/POP-PK approach. The sponsor in a preliminary POPPK and POPPK-PD analysis investigated the pharmacokinetic (healthy subjects and patients) and pharmacodynamic information (patients) obtained.

Pivotal Efficacy and Safety Studies

The sponsor demonstrated efficacy and safety of ambrisentan in two placebo controlled, randomized double-blind studies of 12 week duration that used the same design, but different dose regimens: ARIES-1 (AMB-320) used 5 mg and 10 mg qd and ARIES-2 (AMB-321) 2.5 mg and 5 mg qd. Most patients were females of Caucasian origin (77%) with idiopathic PAH (64%) or PAH associated with connective tissue disease (32%). Most patients were of WHO functional Class II (38%) and III (55%). Ambrisentan or placebo was added to current therapy including a combination of anticoagulants, diuretics, calcium channel blockers, or digoxin, but not epoprostenol, treprostinil, iloprost, bosentan, or sildenafil. The primary endpoint was the 6 minute walking distance (6MWD). Time to clinical worsening, WHO functional class, dyspnea (Borg Dyspnea Index, BDI), and SF-36® Health Survey were the secondary endpoints assessed. The endpoints were evaluated relative to baseline and placebo. In addition hemodynamic parameters mean pulmonary arterial pressure (mPAP), pulmonary vascular resistance (PVR) and pulmonary capillary wedge pressure (PCWP) at baseline and after 12 weeks of treatment were measured. The study protocols did not specify the time of measuring the endpoints relative to drug intake. LFT values were closely monitored throughout the duration of the studies.

Salient Clinical Pharmacology Findings

The salient pharmacokinetic findings include that ambrisentan exhibits dose proportional kinetics, is rapidly absorbed with peak concentrations at 2 h, subject to intestinal extrusion (P-gp), highly plasma protein bound, and eliminated predominantly by non-renal routes. The relative contributions of metabolism and biliary excretion to ambrisentan's elimination from the

body are not known. Oral and renal clearances are small, 34 mL/min (38 mL/min by population PK) and 0.7 mL/min, respectively, in healthy subjects. The apparent terminal half-life is 15 h, however the effective half life is much shorter. When given every 24 h the accumulation of ambrisentan is 1.1. At steady-state the trough concentrations are only 15% of the peak concentrations in patients. The bioavailability of ambrisentan is not affected by food intake. In PAH patients oral clearance is reduced to 19 mL/min. The difference in CL/f between healthy subjects and patients may be disease related.

In vitro studies with human liver tissues indicate that ambrisentan is metabolized by CYPs 3A4 and 2C19, and UGT1A9S, 1A3S and 2B7S. Ambrisentan inhibits CYPs 2A6 and 2C8, 2C9 and UGTs 1A1, 1A6, 1A9 and 2B7 by 10-30%, but only at concentrations that exceed those reached under clinical conditions by a factor of ≥ 30 . Ambrisentan appears not to impact NTCP, OATP or BSEP. However, ambrisentan could be a substrate of OATP.

Co-administration of sildenafil in healthy volunteers appears not to impact the PK of ambrisentan and ambrisentan does not affect the PK of sildenafil and N-desmethyl-sildenafil. Co-administered ambrisentan appears not to impact the pharmacokinetics of the warfarin enantiomers, and does not affect prothrombin time (PT) or International Normalization Ratio (INR).

The clinical service formulations and to be marketed tablets of 5 mg and 10 mg strength are bioequivalent, but inspection of the site performing the study revealed failure to select and randomly retain reserve drug samples from the study drugs received. Therefore the study was deemed not to be acceptable.

The thorough QT/QTc study performed in healthy volunteers receiving a single supra-therapeutic dose of 40 mg ambrisentan was positive. The time matched and baseline adjusted mean difference in QTc at tmax between drug and placebo was 8 ms and the one sided 95% upper bound was 12 ms exceeding the regulatory threshold of 10 ms. No significant drug effect on QT/QTc was observed in the healthy volunteers receiving a dose regimen of 10 mg qd.

Ambrisentan decreased diastolic blood pressure and increased heart rate after 5 mg, 7.5 mg and 10 mg qd in healthy subjects. The hemodynamic effects were not dose dependent over that narrow dose range. Relative to baseline the ET-1 serum levels increased at the two measured time points 2 h and 10 h after multiple qd doses of 5 mg, 7.5 and 10 mg ambrisentan. These results indicate that ambrisentan exhibits in healthy subjects pharmacological effects that have a rapid onset and last for several hours after administration.

Salient Efficacy, Pharmacodynamic and Safety Findings in PAH Patients

The pivotal trials showed that ambrisentan at dose levels of 2.5 mg, 5 mg and 10 mg qd increased significantly and dose dependently 6MWD in the PAH population after 12 weeks of treatment. A significant increase in 6MWD was observed already 4 weeks after initiation of the treatment with ambrisentan and the improvement of 6MWD continued up to the 12th week of treatment at the 10 mg qd level. The 10 mg dose of ambrisentan resulted in a greater improvement for some patient groups. Ambrisentan significantly delayed clinical worsening of

PAH and significantly improved BDI compared to placebo. A significant improvement was found in the physical functional scale of the SF-36® Health Survey. Twelve week treatments with 2.5 mg, 5 mg or 10 mg ambrisentan qd increased cardiac index and decreased mPAP, PVR and PCWP significantly after 12 weeks compared to baseline. BNP serum concentrations were also decreased significantly after 12 weeks of treatment compared to baseline.

Information on onset and offset of the drug effects during the dose interval are not available. The protocol of the pivotal studies did not prescribe when the 6MWD and BDI tests were to be performed relative to the time of drug intake.

In the pivotal studies no patient developed an increase in ALT or AST > 3 • ULN compared to 2.3 % in the placebo groups. The incidence of ALT or AST increases in all clinical studies conducted with ambrisentan was 0.8% and the long term incidence was comparable to that in the patients receiving placebo. There was no dependence of AEs, SAEs or a combined set of preferred terms related to peripheral edema.

The review of the Clinical Pharmacology part of the submission indicated the following deficiencies:

Bioequivalence

Based on the data reported in the bioequivalence study AMB-103 the proposed marketed formulations (5 mg and 10 mg) are bioequivalent relative to the corresponding service formulations used in the pivotal studies. However, the inspection by the Division of Scientific Investigations (Memo March 29, 2007) showed that the clinical site [REDACTED] failed to randomly select and retain reserve drug samples so that the authenticity of the formulations used in the study is not assured. The inspection of the analytical site, [REDACTED] indicated that the accuracy of the ambrisentan concentrations measured in samples that were diluted is not assured, because dilution QC samples appear not to have been used. In conclusion, bioequivalence of to be marketed 5 mg and 10 mg formulations and corresponding clinical service formulations has not been demonstrated. The study was deemed to be deficient.

Metabolites and Metabolism

Ambrisentan is eliminated from the body mainly by non-renal pathways. The relative contributions of metabolism and biliary excretion to the elimination of systemically available ambrisentan are unknown. There is a lack of information on quantity and identity of the formed metabolites in plasma and excreta. In vitro experiments examining a possible metabolism of ambrisentan by intestinal contents were not performed. Quantitative methods for the determination of the metabolites in biological fluids (plasma, urine and feces) using radio-labeled or non-labeled methods were not developed. The plasma concentrations of three metabolites were estimated using a semi-quantitative method. The respective recoveries of total radioactivity assignable to the identified individual metabolites in urine and feces were not determined. The presence of not yet defined metabolites in plasma and excreta cannot be

excluded. In conclusion information on the mass balance of ambrisentan with quantified recoveries of parent drug and major metabolites in plasma and excreta is missing. Based on the available data a quantitative assignment of enzymes to generated metabolites is not possible.

The relevance of the known polymorphisms of CYP2C19 and UGTs 1A9S, 1A3S and 2B7S and of the transporters P-gp and OATP for the inter-subject variability of ambrisentan's pharmacokinetics has not been determined.

Interaction Liability

The interaction liability of ambrisentan has not been adequately investigated. Based on the available limited mass balance information it can be estimated that from 22.6% to 87.5 % of an administered dose of ambrisentan could be metabolized. Thus, clinically relevant metabolic interactions caused by metabolic inhibitors and inducers of ambrisentan cannot be ruled out. The main metabolite in plasma appears to be 4-hydroxymethyl ambrisentan. Based on the results of the in vitro studies with human liver tissues, involvement of CYPs 3A and 2C19 in addition to UGTs is probable. In vivo interaction studies exploring the impact of strong CYP 3A and 2C19 inhibitors and inducers have not been conducted. In vitro ambrisentan has been shown to be a substrate of P-gp and probably OATP. In vivo studies examining the impact of strong inhibitors and inducers of P-gp and of strong inhibitors of OATP have not been conducted.

In the two interaction studies performed by the sponsor with co-administration of ambrisentan and warfarin or sildenafil in healthy volunteers, a 10 mg dose of ambrisentan was administered, corresponding to the highest dose recommended for the treatment of patients with PAH. However, the oral clearance of ambrisentan in patients with PAH is about 50% of that in healthy volunteers. Thus, the exposure to ambrisentan in healthy volunteers receiving 10 mg ambrisentan is equivalent to that in patients receiving 5 mg ambrisentan. Ambrisentan 20 mg qd would have been more a more appropriate dose regimen to achieve in the interaction studies an exposure in healthy subjects equivalent to that in PAH patients receiving 10 mg qd.

In conclusion the interaction potential of ambrisentan has not been sufficiently characterized.

Adequacy of the Tested Dose Range and the Proposed 24 h Dose Interval

Reviewer's assessment: The highest multiple dose regimen tested in healthy subjects and PAH patients is 10 mg qd. The maximum tolerated dose has not been determined in either population. Ten (10) mg qd is the highest recommended therapeutic dose of ambrisentan. The adequacy of the proposed dose regimen with a dose interval of 24 h has not been demonstrated. The time interval between drug intake and performance of the 6MWD- and the BDI-testing was not pre-specified in the clinical study protocols. Performance of these tests at trough concentrations of the drug could have demonstrated that the qd dose regimen is appropriate for ambrisentan. It appears that the sponsor derived the dose interval of 24 h from the apparent terminal half life of ambrisentan estimated to be about 15 h. However, at steady-state the trough concentrations of the drug are about 15% of the peak concentrations. These results indicate that the effective pharmacokinetic half- life of the drug is substantially smaller than 15 h. Thus, the plasma

concentrations attained in the second part of the 24 h dose interval may not be effective. A therapeutic plasma concentration range for ambrisentan has not been defined.

PM reviewer's assessment: Yes, the dose and dosing regimen selected by the sponsor are consistent with the known relationship between dose-response for both effectiveness and safety. Even though the durability of QD regimen was questioned due to the lack of information about the timing of 6-minute walk measurement, it is not believed to be a major issue based on the time course of 6-minute walk (see Figure 2, 2.2.4.1 of QBR). Even though PK steady state is reached by day 6, the effect steady state on 6-minute walk is not reached even after weeks, suggesting that instantaneous ambrisentan concentration is not directly linked to the effectiveness. Therefore, despite the fluctuation of ambrisentan concentration at steady state, 6-minute walk distance is not expected to change in a similar pattern within a day, which is consistent with the observations for other drugs in the same class for the same indication.

Validation of Assays for Ambrisentan and R- and S-Warfarin

Only the plasma samples of the PK sub-studies of the two pivotal trials were assayed using a fully validated LC-MS/MS assay with inclusion of dilution QC samples when the concentrations exceeded the ULOQ. In the 6 Clinical Pharmacology studies in humans plasma concentrations > 500 ng/mL were measured by employing a dilution procedure apparently without the use of dilution QC samples. Therefore, accuracy and precision of ambrisentan concentrations > 500 ng/mL (C_{max}) is not guaranteed in these studies.

In the interaction study with warfarin the plasma concentrations of the warfarin enantiomers > 100 ng/mL were measured using a dilution procedure, apparently without using dilution QC samples. Therefore, the accuracy and precision of the enantiomer concentrations > 100 ng/mL (C_{max}) is not guaranteed.

1.1 RECOMMENDATION

From a Clinical Pharmacology viewpoint the submission is deficient because the authenticity of the formulations used in the bioequivalence study is not assured.

The sponsor is advised to resolve the above identified issues by:

- Demonstrating bioequivalence of the 5 mg and 10 mg commercial and clinical service formulations
- Exploring the interaction potential of ambrisentan in humans when co-administered with drugs known to be strong inhibitors of OATP and P-gp such as cyclosporine A and rifampin. In vitro studies indicate that ambrisentan is a substrate of P-gp and a probable substrate of OATP.

- Exploring the interaction potential of ambrisentan in humans when co-administered with strong inhibitors of CYP3A (e.g. ketoconazole) and CYP 2C19 (e.g. omeprazole). The CYP 450 catalyzed metabolism of ambrisentan is likely to exceed 20% of the administered dose in humans.
- Testing an increased dose range of ambrisentan and determining the adequacy of the 24 h dose interval by comparing q 12 h and q 24 h dose regimens (*Reviewer's assessment*)
- Exploring the impact of hepatic impairment (Child-Pugh Criteria) on the exposure to ambrisentan
- Exploring of the impact of severe renal impairment on the exposure to ambrisentan
- Validating the ambrisentan assay by inclusion of dilution QC samples

The sponsor is advised to resolve the above identified issues.

The briefing took place April, 27, 2007. In attendence were Drs. Stockbridge, Lesko, Lazor, Mehta, Rahman, Huang, Marciniak, Wang, Tornoe, Yasuda, Kim, Garnett, Dodapaneni, Bhattaram, Uppoor, Fadiran, R. Kumi, K. Kumi, Lau, Gobburu, Ong, Zhu, Zheng, Wu, Lau, Marroum, Hinderling

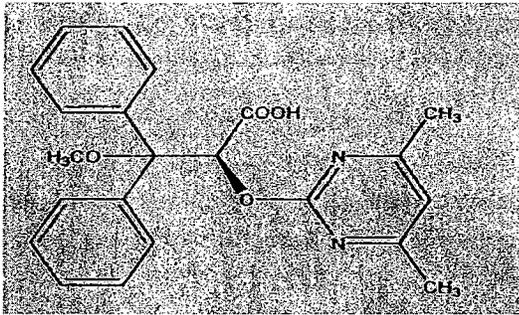
2. QUESTION BASED REVIEW

2.1 General attributes of the drug

2.1.1 What are the highlights of the chemistry and physico-chemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?

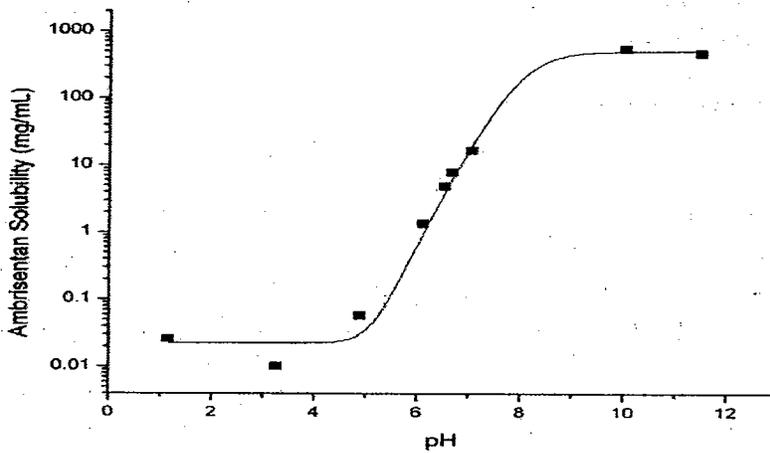
Ambrisentan is a propanoic acid-type, non-sulfonamide, specific ET_A receptor antagonist. It is a single enantiomer [(+)-(2S)-2-[(4,6-dimethylpyrimidin-2-yl)oxy]-3-methoxy-3,3-diphenylpropanoic acid] with a pKa of 4.0 and has the following structure:

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Ambrisentan's molecular weight is 378.42. Ambrisentan is practically insoluble in water at low pH. The solubility increases with increasing pH as shown below:

Figure 1 Ambrisentan Solubility Profile in pH Buffered Aqueous Solutions



Ambrisentan is available as 5 mg and 10 mg film-coated, non-scored tablets. The composition of to be marketed formulations is shown below:

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Table 2 Composition of Ambrisentan Tablets

Component	Function	Quality Standard	5 mg Tablet (mg/tablet)	10 mg Tablet (mg/tablet)
Core Tablet				
Ambrisentan	Active Ingredient	In-house	5 ¹	10 ¹
Croscarmellose Sodium				
Lactose Monohydrate				
Magnesium Stearate				
Microcrystalline Cellulose				
Total Tablet Weight			147.0	147.0

The quantity used is adjusted on the basis of purity (e.g., drug content factor) of each batch of ambrisentan with a concomitant decrease in lactose monohydrate.
See Table 3 for composition of [redacted] film coating material.
Film coated to a target weight gain [redacted] using an aqueous suspension of [redacted].
¹Removed during the manufacturing process.

The composition of the film coating materials is shown below:

Table 3 Composition of Film Coating Materials for Ambrisentan Tablets, 5 mg and 10 mg

Component	Function	Quality Standard
FD&C Red #40 Aluminum Lake	Colorant	21 CFR 74.340 21 CFR 74.1340
Lecithin		
Polyethylene Glycol		
Polyvinyl Alcohol		
Talc		
Titanium Dioxide		

2.1.2 What are the proposed mechanism(s) of action and therapeutic indication(s)?

Ambrisentan is a specific ET_A-receptor antagonist with high affinity (K_i= 0.011 nM) and selectivity for ET_A vs ET_B receptor (> 4000 fold): The primary actions of the endogenous ligand, ET-1, via ET_A are vasoconstriction and cell proliferation. In patients with PAH, plasma and lung tissue concentrations of ET-1 are up to 10 fold increased and correlate with increased right atrial

pressure and disease severity. These findings suggest that ET-1 may play a critical role in the pathogenesis and progression of PAH.

The therapeutic indication of ambrisentan is treatment of PAH (WHO Group 1) to improve exercise capacity, delay clinical worsening ~~_____~~.

2.1 General clinical pharmacology

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

The single ascending dose tolerability study (EE-001) investigated ambrisentan in healthy volunteers over a dose range of 1 mg to 100 mg. The multiple ascending dose study (EE-002) investigated ambrisentan 5 mg, 7.5 mg and 10 mg qd for 10 days. A single dose study using radiolabeled ambrisentan studied the mass balance of the drug. Single and multiple dose studies evaluated the plasma concentration time profiles of ambrisentan, and the dose-exposure relationship for blood pressure, heart rate and ET-1 levels. Single and multiple dose studies in healthy volunteers investigated the mutual impact of co-administered ambrisentan and warfarin or sildenafil. A randomized, positive controlled, parallel study in healthy subjects investigated the impact of ambrisentan on the QT/QTc interval. An additional study in healthy subjects studied the impact of food on the bioavailability of ambrisentan.

The dose ranging study AMB 220 (design: double-blind, uncontrolled, randomized, parallel) using doses of 1, 2.5, 5 or 10 mg qd for 24 weeks (weeks 1-12: blinded, fixed dose, weeks 13-24: unblinded, dose adjustment) determined ambrisentan levels, ET-1 and BNP plasma concentrations, hemodynamic parameters including mPAP, pPVR and PCWP and the clinical endpoint 6MWD. The pivotal Phase 3 studies AMB 320 and 321 (design double-blind, placebo controlled, randomized, parallel) used doses of 5 and 10 mg qd or 2.5 and 5 mg for 12 weeks and determined hemodynamic parameters mPAP, PVR, PCWP and the clinical endpoints 6MWD (primary clinical endpoint), BDI, SF36® Health Survey (self reporting, multi item scale measuring 8 health concepts), WHO functional class and time to clinical worsening.

2.2.2 What is the basis for selecting the response endpoints (i.e. clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics PD)) and how are they measured in clinical pharmacology and clinical studies?

ET-1 and BNP plasma concentrations as well as mPAP, PVR and PCWP are known to be elevated in PAH patients. The 6MWD, and BDI as well as the functional WHO classification and SF-36® Health Survey are abnormal in PAH patients.

2.2.3 Are the active moieties in the plasma (or other biological fluids) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

The sponsor measured the plasma concentrations of the parent drug and characterized the PK characteristics of the parent drug in healthy volunteers and PAH patients sufficiently. In vitro

studies indicated that the ET_A- affinity of the 4-hydroxymethyl metabolite is 30 - 60 times smaller than that of the parent drug. The ET_A affinity of the 2 other identified metabolites, ambrisentan glucuronide and 4-hydroxymethyl ambrisentan glucuronide are unknown. It appears that the main activity in plasma is related to the parent drug.

2.2.4 Exposure-response

2.2.4.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy? What is time of onset and offset of the desirable pharmacological response or clinical endpoint?

The maximum tolerated dose after multiple dose administration of ambrisentan was not determined by the sponsor. The highest dose administered to patients is 10 mg qd which is also the highest therapeutic dose.

In healthy subjects a decrease in blood pressure and an increased heart rate were observed quickly after ambrisentan administration, indicating a rapid onset of action on biomarkers. The impact of ambrisentan on the ET-1 levels in healthy volunteers was notable 2 h after administration (at C_{max} of parent drug) and exceeded baseline still 10 h after ambrisentan dosing. In PAH patients the cardiac index increased and mPAP, PVR and PCWP decreased significantly compared to baseline after a 12 week treatment with ambrisentan in doses of 1-10 mg qd. BNP concentrations also decreased significantly compared to baseline after 12 weeks of treatment with ambrisentan. However, a significant impact of ambrisentan on ET-1 levels in PAH patients was not evident. The relationship between the time of measuring these variables and the time of drug intake is not known so that the swiftness of onset and offset of the clinically relevant effects of ambrisentan are not known.

In a dose ranging Phase 2 study (AMB-220) in PAH patients, no dose-dependence was observed in 6MWD change from baseline at week 12 with doses of 1 mg, 2.5 mg, 5 mg and 10 mg of ambrisentan (Figure 1). In the 2 pivotal trials (studies AMB-320 and AMB-321), however, dose-response was demonstrated within each trial (Figures 2 and 3). A placebo-controlled parallel design was used in both pivotal trials with 5 mg and 10 mg studied in AMB-320 and 2.5 mg and 5 mg studied in AMB-321 (n=54-63 per group). Given the small sample size (n=13 to 19 per group) and the titration scheme in study AMB-220 (Figure 4), the results from the pivotal trials are more reliable. The noticeable difference between the dose-response curves from the two pivotal trials could be due to the geographic regions of the investigative sites because AMB-320 was conducted primarily in the United States and AMB-321 was conducted primarily in Europe.

Figure 1: Dose-Response for Ambrisentan in Study AMB-220

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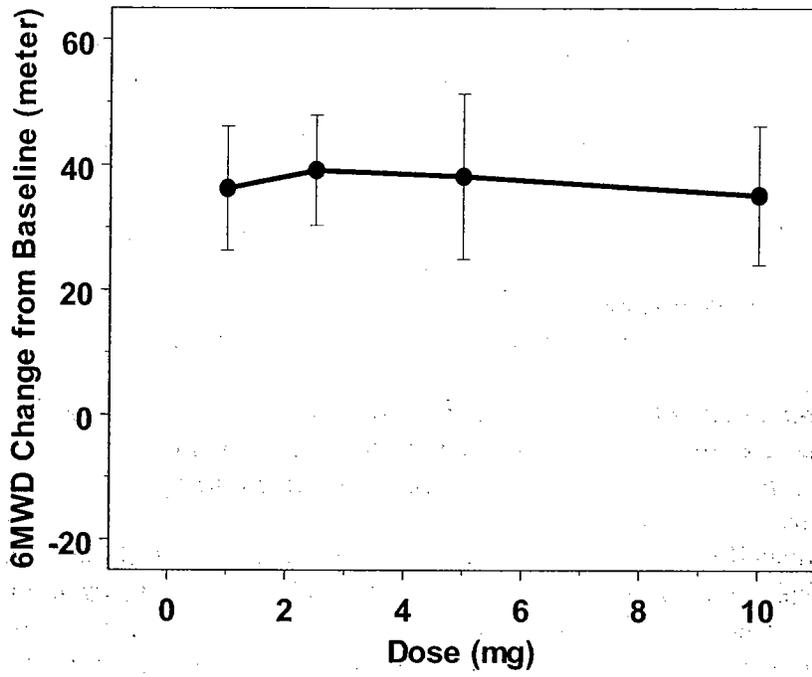
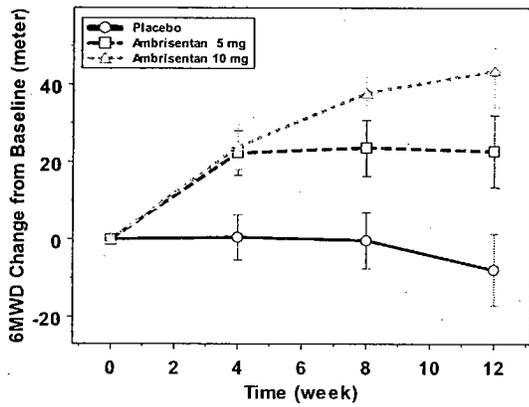


Figure 2: Time Course of 6-Minute Walk Distance Change in Studies AMB-320 and AMB-321

Study AMB-320



Study AMB-321

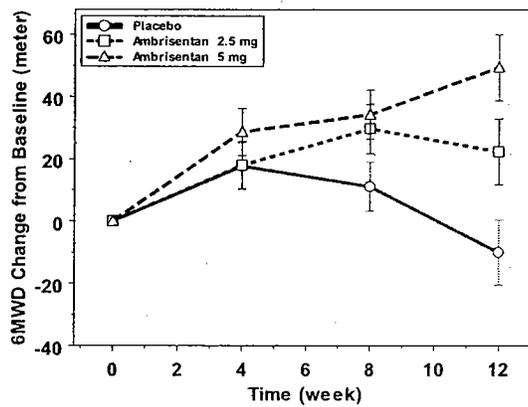


Figure 3: Dose-Response for Ambrisentan in Studies AMB-320 and AMB-321

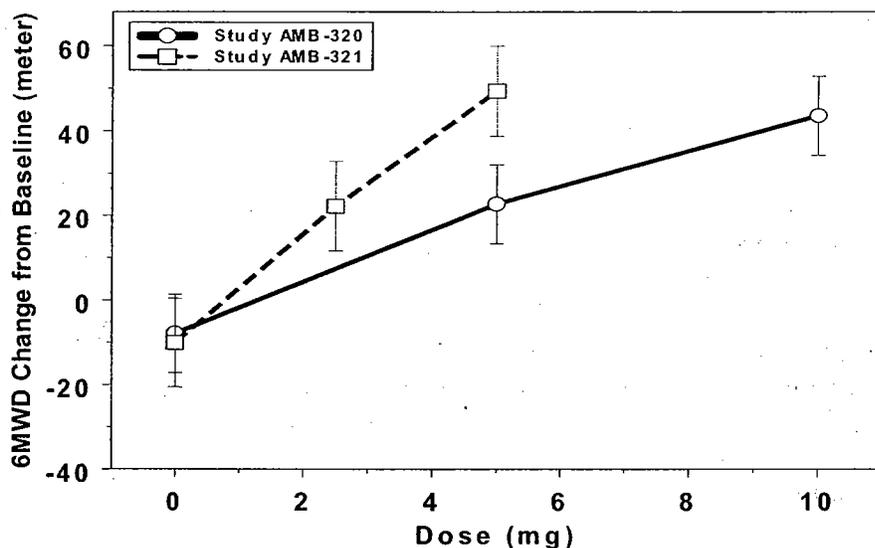
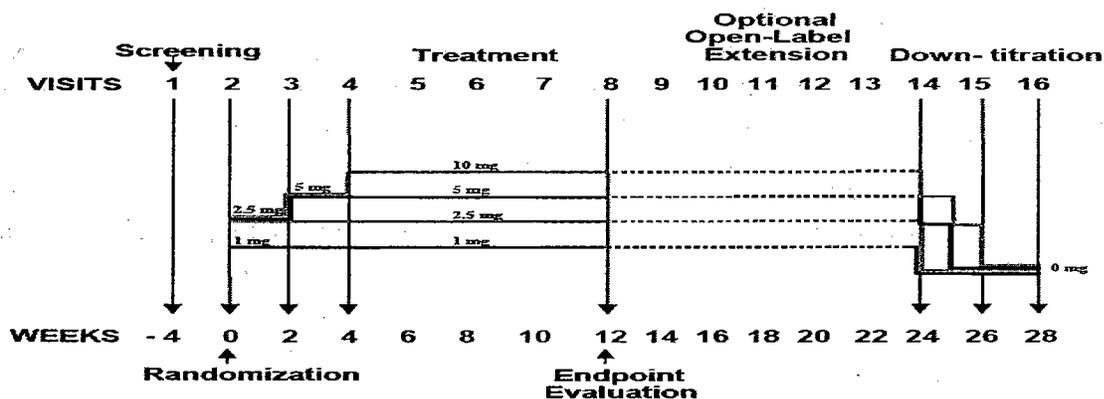


Figure 4: Study Schematic for Study AMB-220



2.2.4.2 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for safety?

In the combined analysis (AMB-320/321), the incidence of some common adverse events, such as peripheral edema, headache, dizziness, nasal congestion, and cough, appeared to increase with increased ambrisentan dose (Table 1). A preliminary population pharmacokinetic analysis (including data from Phase 1, 2, and 3 studies), however, did not reveal a relationship between ambrisentan exposure (analyzed as steady-state area under the plasma concentration-time curve and steady-state maximum plasma concentration) and the incidence of AEs, SAEs, or a combined set of preferred terms related to peripheral edema. This could be due to the unreliable exposure estimates (e.g. Cmax) and the loss of sensitivity with many combined AEs.

Even though dose-dependent liver toxicity was reported for sulfonamide-class endothelin receptor blockers (bosentan and sitaxsentan), data for ambrisentan did not show notable liver toxicity within the studied dose range during the 12-week period in AMB-320 and AMB-321 as shown in Tables 2 and 3:

Table 1: Common Adverse Events in Placebo or Combined Ambrisentan Treatment Groups (AMB-320/321) Population

Treatment group	Placebo (n = 132)	2.5 mg ambrisentan (n = 64)	5 mg ambrisentan (n = 130)	10 mg ambrisentan (n = 67)
Adverse event, n (%)				
Peripheral edema	14 (10.6)	2 (3.1)	24 (18.5)	19 (28.4)
Headache	18 (13.6)	5 (7.8)	20 (15.4)	13 (19.4)
Dizziness	13 (9.8)	3 (4.7)	9 (6.9)	6 (9.0)
Nasal congestion	2 (1.5)	1 (1.6)	7 (5.4)	7 (10.4)
Cough	8 (6.1)	2 (3.1)	7 (5.4)	5 (7.5)

Table 2: Summary of Serum Aminotransferases Relative to the Upper Limit of Normal for Study AMB-320

Treatment group	Placebo (n = 65)	5 mg ambrisentan (n = 65)	10 mg ambrisentan (n = 67)
ALT >3.0xULN	2 (3.0)	0 (0.0)	0 (0.0)
AST >3.0xULN	1 (1.5)	0 (0.0)	0 (0.0)

Table 3: Summary of Serum Aminotransferases Relative to the Upper Limit of Normal for Study AMB-321

Treatment group	Placebo (n = 64)	2.5 mg ambrisentan (n = 63)	5 mg ambrisentan (n = 63)
ALT >3.0xULN	1 (1.6)	0 (0.0)	0 (0.0)
AST >3.0xULN	1 (1.6)	0 (0.0)	0 (0.0)

2.2.4.3 Does the drug prolong the QT/QTc interval?

The thorough QT/QTc study performed in healthy volunteers receiving a single supra-therapeutic dose of 40 mg ambrisentan was positive. The time matched and baseline adjusted mean difference in QTc at tmax between drug and placebo was 8 ms and the one sided 95% upper bound was 12 ms exceeding the regulatory threshold of 10 ms. No significant drug effect

on QT/QTc was observed in healthy subjects receiving a dose regimen of 10 mg qd (Please refer to 6. QTcIRT Report).

2.2.4.4 Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

Reviewer's assessment: The evaluations of the 6MWD and BDI and other endpoints in the patients were not scheduled at trough. One must assume that most of the evaluations occurred in the early part of the 24 h dose interval. A therapeutic concentration range for ambrisentan has not been determined. Thus, it is unknown for how long effective ambrisentan concentrations are maintained during the 24 h dose interval. This constitutes an unresolved issue.

It appears that the sponsor's rationale for the qd regimen proposed for ambrisentan is derived from the terminal disposition half life of 15 h. However, the mean trough concentration of ambrisentan at steady state is about 15 % of the peak concentrations indicating that ambrisentan's disposition is multi-phasic with a mean effective PK half-life significantly smaller than 15 h.

PM Reviewer's assessment: Yes, the dose and dosing regimen selected by the sponsor are consistent with the known relationship between dose-response for both effectiveness and safety. Even though the durability of QD regimen was questioned due to the lack of information about the timing of 6-minute walk measurement, it is not believed to be a major issue based on the time course of 6-minute walk (see Figure 2, 2.2.4.1). Even though PK steady state is reached by day 6, the effect steady state on 6-minute walk is not reached even after weeks, suggesting that instantaneous ambrisentan concentration is not directly linked to the effectiveness. Therefore, despite the fluctuation of ambrisentan concentration at steady state, 6-minute walk distance is not expected to change in a similar pattern within a day, which is consistent with the observations for other drugs in the same class for the same indication.

2.2.5 What are the PK characteristics of the drug and its major metabolite(s)

2.2.5.1 What are the single and multiple dose PK parameters?

The PK of ambrisentan after single and multiple doses in the clinical dose range are dose proportional in healthy volunteers and patients. The drug is rapidly absorbed with t_{max} of 2 h in healthy subjects and patients. The apparent terminal $t_{1/2}$ is about 15 h, but the effective half-life is significantly smaller in healthy subjects and patients. The mean CL/F by traditional PK methods is 34 ml/min and the mean CLR is 0.7 mL/min in healthy subjects. The accumulation of ambrisentan after multiple doses administered qd is non-significant (accumulation factor=1.1) in healthy subjects and PAH patients.

2.2.5.2 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

Preliminary population PK analysis showed that CL/F of ambrisentan for a typical healthy subject and a typical PAH patient is 38 mL/min and 19 mL/min, respectively. The exact causes for the observed difference in ambrisentan exposure and CL/F between healthy subjects and PAH patients are unclear. It is possible that the presence of PAH and/or other co-existing disease may reduce the clearance of ambrisentan in patients. In contrast to AUC the C_{max} values in PAH patients were only about 30 % higher than in healthy subjects.

The major PK parameters of ambrisentan in healthy subjects and PAH patients are summarized in the below table:

PK Parameters of Ambrisentan in Healthy Subjects and PAH Patients

Subjects	t _{max} h	C _{max} ^a ng/mL	CL/F mL/min	CLR mL/min	t _{1/2z} h	Fluct. ^b	Accum. ^c	% Variability	
								Inters.	Intras.
Healthy	1-2	950	34, 38 ^d	0.7	15	0.06	1.1	33 ^d	11 ^e
PAH	2-3	1200	19 ^d	NR	14	0.15	1.3	33 ^d	NR

^a Geometric means at 10 mg qd dose level ^b Fluctuation = C_{min,ss}/C_{max,ss} ^c Accumulation = (AUC_{0-τ})_{ss}/(AUC_{0-τ})_{Day1} ^d POP PK, all other values by traditional PK ^e AUC NR=Not reported

The major metabolites of ambrisentan were not quantified after either single or multiple dose administration of ambrisentan in healthy subjects or patients. In vitro the 4- hydroxymethyl-metabolite exhibited 30 - 60 fold lower affinities for the ET_A receptor compared to the parent drug.

2.2.5.3 What are the characteristics of drug absorption (possible transporters and pH impact)

Absolute and relative bioavailability of ambrisentan have not been determined. The drug is rapidly absorbed.

In vitro ambrisentan was found to be a substrate of P-gp.

Ambrisentan is an acid of pK_a 4.0. Its water solubility in acid conditions is negligible and better under more alkaline conditions.

2.2.5.4 What are the characteristics of drug distribution (incl. plasma protein binding)

The mean plasma protein binding of ambrisentan is 98.6%, concentration independent over the clinically relevant range, and similar in healthy male and females. As expected for a drug with extensive plasma protein binding the red cell partitioning in whole blood is minimal with ambrisentan.

2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination?

The below table summarizes the results of the mass balance study performed by the sponsor:

Mean Recoveries of Total Radioactivity, Radioactivity Associated with Ambrisentan and Radioactivity not Associated with Ambrisentan

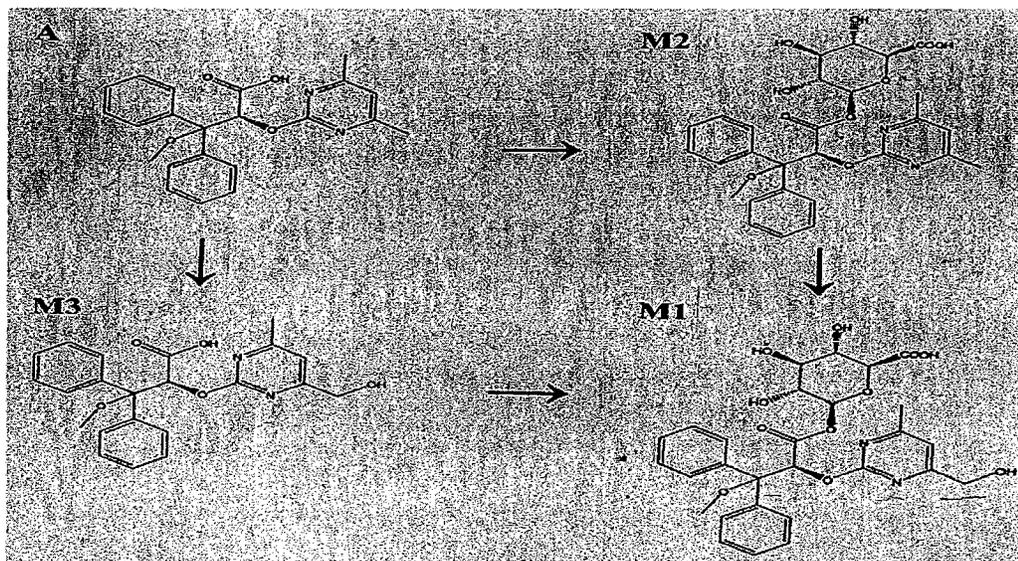
Mean Recovery in % of Dose			
Matrix	Total Radioactivity	Ambrisentan	Non-Ambrisentan
Feces	65.73	36.49	29.24
Urine	22.58	3.35	19.23
Total	88.31	39.84	48.47

Most of the radioactivity after administration of labeled ambrisentan is excreted in the feces. < 5% of the dose is excreted as unchanged ambrisentan in urine. Thus, non-renal elimination by metabolism and/or biliary excretion is the main route of elimination for systemically available ambrisentan. The respective fractions of the total radioactivity excreted in feces and urine assignable to parent drug and metabolites as well as a possible metabolism of ambrisentan by intestinal contents were not determined by the sponsor. The relative contributions of metabolism and biliary excretion to the elimination of ambrisentan are unknown.

2.2.5.6 What are the characteristics of drug metabolism? (extraction ratio, metabolic scheme, enzymes responsible, fractional clearances)

In vitro studies with human hepatic tissues suggest that CYPs 3A and 2C19 and UGTs 1A9S, 1A3S and 2B7S are involved in the metabolism of ambrisentan.

In vivo the metabolites 4-hydroxymethyl ambrisentan (M3), ambrisentan glucuronide (M2) and 4-hydroxymethyl ambrisentan (M1) have been identified in plasma in humans suggesting the following metabolic scheme:



Based on the available data one must assume that CYP3A and 2C19 are involved in the formation of M3 (4-hydroxymethyl ambrisentan) and UGTs 1A9S, 1A3S and 2B7S in the formation of M2 (ambrisentan glucuronide) and M1 (4-hydroxymethylambrisentan-glucuronide).

Mean CL/F of ambrisentan in the clinically effective dose range is 38 mL/min for a typical healthy subject and 19 mL/min for a typical PAH patient indicating a low hepatic extraction efficiency for ambrisentan.

Fractional metabolic clearances cannot be estimated because the metabolites were not quantitated in plasma or excreta.

2.2.5.7 What are the characteristics of drug excretion

After oral administration of labeled ambrisentan the largest fraction of radioactivity and unchanged ambrisentan were recovered in the feces. The urinary excretion of total radioactivity and unchanged ambrisentan was 22.58% and 3.35%, respectively, of the dose. The renal clearance of ambrisentan is about 0.7 mL/min in healthy subjects which equals roughly the predicted glomerular clearance if the extensive protein binding of the drug is considered. However tubular secretion and consecutive tubular re-absorption of ambrisentan cannot be excluded. Ambrisentan is highly lipophilic.

2.2.5.8 Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

The PK of ambrisentan are dose proportionate within the clinically relevant dose range in healthy subjects and patients. Dose proportionality of the PK of the metabolites cannot be assessed because the metabolites were not measured after either single or multiple dose administration of ambrisentan.

2.2.5.9 How do the PK parameters change with time following chronic dosing?

There is no evidence of self-induction of ambrisentan. Mean CL/F and CLR were identical on Days 1 and 10 of treatments with 5 mg, 7.5 mg and 10 mg ambrisentan in healthy subjects as shown in the below table:

Arithmetic Mean Oral and Renal Clearance on Days 1 and 10 of Treatments with 5, 7.5 or 10 mg qd of Ambrisentan

Dose Level, mg qd	CL/F, mL/min		CLR, mL/min	
	Day 1	Day 10	Day 1	Day 10
5	36	34	0.8	0.9
7.5	35	33	0.6	0.5
10	35	36	0.9	0.8

The data on 6 β -hydroxycortisol excretion in urine, a surrogate for CYP3A activity, from the ascending multiple dose study of ambrisentan, were inconclusive. The existence of a circadian rhythm in the PK of ambrisentan cannot be excluded because drug administration was always in the morning. The mean ratio of AUC₀₋₂₄(Day 10) to AUC₀₋₂₄ (Day 1) was 1.1 indicating a small degree of accumulation of ambrisentan with the qd regimen.

2.2.5.10 What is the inter- and inter-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

The inter-subject variability of CL/F was estimated to be 33% based on a population PK analysis including multiple PK studies with both healthy volunteers and patients. The intra-subject variability for C_{max} and AUC was 22.2% and 11.4%, respectively, estimated by traditional PK analysis.

2.3. Intrinsic factors

2.3.1 What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

PAH patients had a significantly lower apparent clearance of ambrisentan than healthy subjects. Increased subject weight was found to increase the apparent clearance of ambrisentan. Levels of creatinine clearance, alkaline phosphatase, and total bilirubin were also found to be related to the apparent clearance of ambrisentan. The exposure difference caused by these covariates, however, was not significant enough to result in a difference in response based on the safety and efficacy of ambrisentan as observed in the Phase 3 studies.

In vitro studies with human liver tissues indicated that ambrisentan can be metabolized by CYPs 3A4, 2C19 and UGTs 1A9, 1A3S and 2B7S. Except for CYP3A4, all of these enzymes have been shown to exert polymorphism. In vitro studies suggest that ambrisentan is a substrate of P-gp and OATP. Both transporters are subject to polymorphism. In none of the in vitro or in vivo studies performed by the sponsor were geno-or phenotype determined. Thus, the potential impact of enzyme-and transporter polymorphism for the exposure to ambrisentan is not known.

2.3.2 Based on what is known about exposure-response relationships, what dosage regimen adjustments, if any, are recommended for each subgroup listed below?

No dose adjustment is recommended for elderly, females versus males, race or patients with mild or moderate renal impairment. No recommendations can be made for patients with severe renal impairment, hepatic impairment or pediatric populations, because the PK of ambrisentan have not been investigated in these populations.

2.3.2 Based on what is known about exposure-response relationships and their variability and the groups studied, healthy volunteers vs. patients vs. specific populations (examples shown below), what dosage adjustments, if any, are recommended for each of these groups? If dosage regimen adjustments are not based upon exposure-response relationships, describe the alternative basis for the recommendation.

2.3.2.1 Elderly (see Study of Drugs likely to be used in the Elderly,

No dose adjustment is necessary

2.3.2.2 Pediatric patients. Also what is status of pediatric studies and/or any pediatric plan for study?

Safety, efficacy and PK of ambrisentan in pediatric patients have not been investigated.

2.3.2.3 Gender

No dose adjustment is necessary

2.3.2.4 Race, in particular differences in exposure and or response in Caucasians, African Americans, and/or Asians

No dose adjustment is necessary

2.3.2.5 Renal Impairment

A full experimental study in patients with different degrees of renal impairment has not been conducted. An analysis of the impact of renal impairment on exposure was performed using a population PK approach with Phase 3 data from patients with CLcr values ranging from 20-150 mL/min (n=167 normal, n=114 mild renal impairment, n=29 moderate renal impairment, n=5 severe renal impairment). The preliminary population analysis showed that the oral clearance of ambrisentan for a patient with a creatinine clearance of 30 mL/min is estimated to be 24% lower than that for a patient with a creatinine clearance of 88 mL/min. The resulting increase in exposure (32 %) is not considered to be clinically relevant and an adjustment of the dose of ambrisentan in patients with mild or moderate renal impairment is not necessary. The 5 subjects with severe renal impairment had CLcr values in the range 20-29 mL/min and did not represent the entire CLcr range found in this patient group. A definitive determination of the impact of CLcr on exposure and a dosing recommendation in this patient group cannot be made.

2.3.2.6 Hepatic Impairment

A full experimental study in patients with different degrees of hepatic impairment was not performed. An analysis of the possible impact of the covariates ALP and bilirubin (n=34 patients

with increased values) on exposure was performed using a population PK approach with data obtained in the phase 3 trials. The preliminary population PK analysis appeared not to indicate that ALP or bilirubin is a clinically relevant covariate. However, the 34 patients with elevated ALP and bilirubin values were not classified in accordance with Child-Pugh criteria. Thus, the impact of mild, moderate or severe hepatic impairment according to Child-Pugh on the disposition of ambrisentan is unknown and dose recommendations for patients with different degrees of hepatic impairment cannot be given.

2.3.2.7 What pharmacogenetic information is there in the application and is it important or not

The submission did not contain pharmacogenetic information.

2.3.2.7 What pregnancy and lactation use information is there in the application? What other human factors are important to understanding the drug's efficacy and safety?

The submission did not contain pregnancy or lactation use information on ambrisentan.

2.4. Extrinsic Factors

2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or-response and what is the impact of any differences in exposure on response?

The sponsor performed 2 drug-drug interaction studies with ambrisentan and warfarin or sildenafil in healthy volunteers. The results of the first study indicated that co-administration of ambrisentan 10 mg qd at steady-state and a single dose of 25 mg warfarin had no impact on PT and INR. Also, the exposure to S- or R-warfarin appeared not to have been affected by ambrisentan. Similarly, co-administration of a single dose of warfarin 25 mg and ambrisentan 10 mg appeared not to have an impact on the exposure of ambrisentan. In the preliminary population PK analysis warfarin use was determined to be an additional significant covariate on CL/F. Ambrisentan CL/F was estimated to be on average 8.0% higher when warfarin was used concurrently. However, the magnitude of the difference is not significant enough to result in difference in safety and effectiveness.

The results of the second interaction study indicated that co-administration of a single dose of 10 mg ambrisentan with 20 mg sildenafil tid at steady-state appeared not to impact the exposure to ambrisentan. Similarly, co-administration of a single dose of 20 mg sildenafil together with ambrisentan 10 mg qd at steady-state had no impact on the exposure to sildenafil and its main metabolite N-desmethyl sildenafil.

The doses used in the interaction studies for sildenafil and warfarin in healthy subjects correspond to the maximum recommended doses for the indications of interest in PAH patients. However, because of the reduced oral clearance in PAH patients the dose of 10 mg ambrisentan in healthy volunteers is predicted to produce an exposure in PAH patients that is equivalent to

that of a 5 mg dose. Thus, a 20 mg dose of ambrisentan would have been more adequate for the interaction studies in healthy volunteers.

The submission did not contain information on the possible impact of herbal products, diet smoking and alcohol use on the response. No recommendations can be made regarding these extrinsic factors.

2.4.2 Drug-drug interactions

2.4.2.1 Is there an in vitro basis to suspect in vivo drug-drug interactions?

In vitro data (Caco2 cells) indicate that ambrisentan is a substrate of P-gp. Experiments with human liver tissues suggest that ambrisentan is a substrate of CYPs 3A4 and 2C19 and UGTs 1A9S, 2B7S, 1A3S. In vitro data point to the possibility that ambrisentan is a substrate of OATP.

In vitro data suggest that ambrisentan can inhibit CYPs 2A6, 2C8 and 2C9 and UGTs but only at concentrations that exceed the clinical range >30 times. However, it should be noted that ambrisentan in these experiments was not pre-incubated.

2.4.2.2 Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?

Ambrisentan is a substrate of CYPs 3A4 and 2C19. CYP 2C19 is polymorphic. Ambrisentan is also a substrate of the polymorphic UGTs 1A9S, 2B7S, 1A3S. Therefore, the pharmacokinetics of ambrisentan could be impacted by genetic differences.

2.4.2.3 Is the drug an inhibitor and/or an inducer of CYP enzymes?

In vitro ambrisentan inhibits CYPs 2A6, 2C8 and 2C9, but only at concentrations that exceed the therapeutic range by > 30 fold. 6 β -hydroxycortisol excretion data (surrogate for CYP3A activity) in the presence and absence of ambrisentan are inconclusive. No other induction studies were performed with ambrisentan.

2.4.2.4 Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?

Caco2-cell data indicate that ambrisentan is a substrate but not an inhibitor of P-gp.

2.4.2.5 Are there other metabolic/transporter pathways that may be important?

Other acidic endothelin receptor antagonists have been shown in vitro to be substrates of hepatic uptake and/or hepatic extrusion transporters. Using sandwich-cultured human hepatocytes ambrisentan has been shown to possibly be a substrate of OATP.

2.4.2.6 Does the label specify co-administration of another drug (e.g. combination therapy in oncology) and, if so, has the interaction potential between these drugs been evaluated?

No

2.4.2.7 What other co-medications are likely to be administered to the target population?

Other drugs indicated for the treatment of PAH including epoprostenol, treprostinil, iloprost, bosentan, sildenafil. In addition, PAH patients are treated with anticoagulants, diuretics and calcium channel blockers or digoxin.

In vivo interaction studies showed that ambrisentan does not alter the pharmacodynamics (PT, INR) of co-administered warfarin. Ambrisentan appears not to impact importantly the pharmacokinetics of the R- and S-enantiomers of warfarin. Also, co-administered warfarin appears not to impact importantly the exposure to ambrisentan. Ambrisentan does not impact the PK of sildenafil or its metabolite and sildenafil appears not to alter the exposure to ambrisentan importantly.

Thus, of the many likely drugs co-administered with ambrisentan, the interaction potential was investigated in vivo with just two, warfarin and sildenafil and in vitro with one, digoxin.

2.4.2.8 Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

No.

2.4.2.9 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

Ambrisentan administration induced significant changes in hemodynamic parameters such as cardiac index, mPAP, PVR and PCWP in patients as well as a decrease in mean systemic diastolic blood pressure and an increase in heart rate in healthy subjects. Co-administration of ambrisentan together with other drugs impacting blood pressure and/or heart rate may result in additive effects.

2.4.2.10 Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?

The identified metabolites in plasma and excreta were not quantified after single or multiple dose administration of ambrisentan. There may be relevant quantities of unidentified metabolites circulating in the biological fluids. The lack of information from the mass balance study is an unresolved issue.

The relative contributions of CYPs 3A4 and 2C19 and the UGTs to the metabolism of ambrisentan are unknown. Studies with strong CYP 3A- and 2C19 inhibitors or inducers have not been performed. Ambrisentan is a substrate of P-gp and OATP. In vivo studies with strong inhibitors of OATP and strong inducers or inhibitors of P-gp have not been performed. Thus, the interaction liability of ambrisentan has not been determined adequately which constitutes an unresolved issue.

2.4.3 What issues related to dose, dose regimens, or administration are unresolved and represent significant omissions?

The tested dose range of ambrisentan is narrow in healthy subjects and in PAH patients. The highest dose (10 mg qd) tested in PAH patients is the highest recommended therapeutic dose. The adequacy of the 24 h dose interval proposed for ambrisentan has not been demonstrated and remains an unresolved issue.

2.5 General biopharmaceutics

2.5.1 Based on the biopharmaceutics classification system (BCS) principles, in what class is this drug and formulation? What solubility, permeability, and dissolution data support this classification?

The water solubility of ambrisentan is low. Ambrisentan's permeability/absolute bioavailability is undetermined. Ambrisentan is a substrate of P-gp.

2.5.2 What is the relative bioavailability of the proposed to-be-marketed formulation to the pivotal clinical trial?

Based on the data reported in study AMB-103 the proposed marketed formulations (5 mg and 10 mg) are bioequivalent to the corresponding service formulations used in the pivotal studies. However, the inspection by the Division of Scientific Investigations (Memo March 29, 2007) showed that the clinical site _____ failed to randomly select and retain reserve drug samples so that the authenticity of the formulations used in the study is not assured. The inspection of the analytical site, _____ indicated that the accuracy of the concentrations measured in samples that were diluted is not assured in 39 subjects. Dilution QC samples have not been processed along with samples with unknown concentrations. Also, inaccurate QC samples in 2 runs were not rejected.

Thus, the bioequivalence study of the proposed marketed formulations and clinical service formulations is deemed to be unacceptable.

2.5.2.1.1 What data support or do not support a waiver of in vivo BE data?

- *BCS classification system*

- *Formulation ingredient information*
- *Dissolution profiles*
- *Others*

Ambrisentan is a low solubility drug. Absolute bioavailability and permeability of ambrisentan are unknown. Ambrisentan is a substrate of P-gp.

The active and passive ingredients of the commercial and clinical service formulations both at the 5 mg and 10 mg levels are identical. The 5 mg and 10 mg formulations are proportionally similar. However, the commercial formulations differ from the clinical service formulations in ~~_____~~. Dissolution testing performed in the media 0.1 n HCl, pH 3.0, pH 5.0 and pH 6.8 showed that the pH 5.0 medium was best in discriminating the dissolution performance of the commercial tablets. At pH 5.0 the commercial 5 mg tablet ~~_____~~ and one of the two commercial 10 mg tablets ~~_____~~, ~~_____~~ failed the F2 test, whereas the other 10 mg commercial tablet ~~_____~~ passed the F2-test. ~~_____~~ may impact the dissolution behavior of ambrisentan at pH 5.0. Systematic studies are required to fully understand the role of these factors for the dissolution process. The available information does not support granting a biowaiver.

2.5.2.2 What are the safety or efficacy issues, if any, for BE studies that fail to meet the 90% CI using equivalence limits of 80-125%?

The authenticity of the 5 mg and 10 mg commercial and clinical service formulations used in the bioequivalence study is not known and thus the safety and efficacy of ambrisentan when administered as the 5 mg or 10 mg commercial formulations is not assured.

2.5.2.3 If the formulations do not meet the standard criteria for bioequivalence, what clinical pharmacology and/or clinical safety and efficacy data support the approval of the to be marketed product?

None

2.5.3 What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

Food has no clinically relevant impact on Cmax or AUC of ambrisentan.

2.5.4 When would a fed BE study be appropriate and was one conducted (Refer to Appendix 3-Table 1, When to Request a Fasted BE Study)

NA

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2.5.5 How do the dissolution conditions and specifications ensure in vivo performance and quality of the product?

See 2.5.2.1.1

2.5.6 If different strength formulations are not bioequivalent based on standard criteria, what clinical safety and efficacy data support the approval of the various strengths of the to be marketed product?

Bioequivalence of the 5 mg and 10 mg strength commercial tablets has not been demonstrated.

2.5.7 If the NDA is for a modified release formulation of an approved immediate release product without supportive safety and efficacy studies, what dosing regimen changes are necessary, if any, in the presence or absence of PK-PD relationship?

NA

2.5.8 If unapproved products or altered approved products were used as active controls, how is BE to the approved product demonstrated? What is the basis for using either in vitro or in vivo data to evaluate BE?

NA

2.5.9 What other significant, unresolved issues related to in vitro dissolution or in vivo BA and BE need to be addressed?

None

2.6 Analytical section

This section should address issues related to the analytical and bioanalytical methods used to support the clinical pharmacology and biopharmaceutics studies

2.6.1 How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

Ambrisentan is the most active and probably most abundant compound in the circulation. The metabolites were not quantitated in the biological fluids after single or multiple dose administration of ambrisentan.

2.6.2 Which metabolites have been selected for analysis and why?

None.

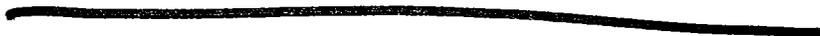
2.6.3 For all moieties measured, is free, bound, or total measured? What is the basis for that decision, if any, and is it appropriate?

The total (bound + unbound) concentration of ambrisentan was measured. The plasma protein binding of ambrisentan is constant over the clinically significant concentrations range and in healthy subjects no gender specific difference was noted. Thus, since the ratio of unbound (=active moiety) to total ambrisentan is constant measurement of the total plasma concentration in plasma is justifiable.

2.6.4 What bioanalytical methods are used to assess concentrations?

Closely related LC/MS/MS assays were used to measure ambrisentan in plasma and urine. For the determination of sildenafil, N-desmethyilsildenafil, S- and R-warfarin also LC/MS/MS methods were used.

2.6.4.1 What is the range of the standard curve? How does it relate to the requirements for clinical studies? What curve fitting techniques are used?



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Deliberative Process

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4. INDIVIDUAL STUDY REPORTS

Study MPF/DDK 9912: Binding of BSF208075 to Plasma Proteins and Distribution of BSF 208075 between Erythrocytes and Plasma in Rats, Rabbits, Dogs and Humans (In Vitro)

Study Investigator and Study Site:

[Redacted]

Objectives

To investigate the plasma protein binding and the distribution between erythrocytes and plasma

Methods

[Redacted]

Each experiment with individual plasma was carried out in duplicate. The experiments with albumin and α 1-acid glycoprotein were carried out in quinduplicate.

Results

The results on the plasma protein binding of BSF 208075 are shown in Table 1:

Table 1 Binding of BSF 208075 in different concentrations to human, rat, rabbit and dog plasma proteins (mean and SD of 5 individuals)

Species	BSF 208075 bound (%)							
	200 [ng/ml]		2 000 [ng/ml]		20 000 [ng/ml]		200 000 [ng/ml]	
	mean	SD	mean	SD	mean	SD	mean	SD
Human male	98.8	0.13	98.9	0.08	98.8	0.10		
Human female	98.8	0.04	98.8	0.06	98.7	0.05		

The results indicate that the average plasma protein binding of BSF 208075 in healthy male and female subjects is concentration independent 98.8% and 98.8%, respectively, and identical.

The binding of BSF 208075 to albumin and α 1-acid glycoprotein is shown in Table 2:

Table 2 Binding of BSF 208075 in different concentrations to human plasma albumin (50.0 g/l) and human α 1-acid glycoprotein (0.9 g/l) Mean and SD of 5 replicates

Human plasma proteins	BSF 208075 bound (%)					
	200 [ng/ml]		2 000 [ng/ml]		20 000 [ng/ml]	
	mean	SD	mean	SD	mean	SD
Albumin	96.6	0.12	96.5	0.03	96.5	0.06
α 1-acid glycoprotein	15.0	1.39	15.8	1.38	11.8	0.27

The results show that the plasma protein binding of BSF 208075 is mainly due to the binding to albumin.

The distribution between erythrocytes and plasma was investigated in heparinized blood from 5 female and 5 male healthy subjects. ¹⁴C- BSF 208075 was used. The experiments were conducted at 37 ° C over a period of 30 minutes. The concentrations of BSF 208075 were measured by [redacted] in plasma and erythrocytes [redacted] separation. The initial concentration of BSF 208075 in blood was 2000 ng/mL. The ratio of the concentrations in red cells and plasma was calculated from $C_{red\ cells}/C_{plasma} = C_{blood}/(C_{plasma} \cdot Ht) - 1/Ht + 1$. Hematocrit was measured using a [redacted].

The results are shown in Table 3:

Species	Ratio C(blood)/C(plasma)		Ratio C(erythrocyte)/C(plasma)		Fraction of BSF 208075 in plasma	
	mean	SD	mean	SD	mean	SD
Human male	0.565	0.020	0.075	0.005	93.8	0.6
Human female	0.609	0.038	0.087	0.016	93.9	0.5

The mean ratios of the concentration in red cells and plasma of 0.075 and 0.087 in males and females, respectively, indicate that more than 90% of the drug resides within the plasma compartment and less than 10% in the red cells. This is not surprising given that slightly more than 1 % of BSF 208075 is unbound in plasma and available for diffusion into the red cells. The calculated ratio of the concentrations in red cells and plasma water is 6.52 and 6.96 in males and females, respectively, indicating binding of BSF 208075 to constituents of the red cells.

Conclusion

The plasma protein binding of BSF 208075 is high (98.8%), identical in healthy male and females and concentration independent. The acidic compound is mainly bound to albumin. The red cell partitioning of BSF 220875 in human blood is very small due to the high plasma protein binding.

Comments

1. The report does not provide evidence that equilibration is reached 5 hour after spiking the plasma side with drug.

2. Dextran was used to decrease the osmotic water shift during the 5 hour dialysis. —


3. The report ought to indicate how many runs were performed in determining the red cell to plasma partitioning of BSF 208075.

Study MPR/PKD 0105: Plasma Protein Binding Interactions between BSF 208075 and Warfarin (In Vitro)

Study Investigator and Study Site:

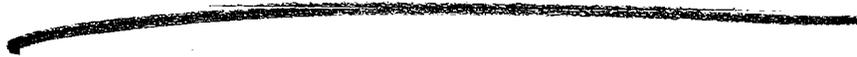

Objectives

To investigate whether BSF 208075 and warfarin mutually affect each others plasma protein binding

Methods

14

14



Results

The results are shown in Table 1:

Table 1 Effect of warfarin on the human plasma protein binding of BSF 208075 – mean and SD of n = 5 individuals

Sex	Concentration of warfarin [µg/ml]	Binding of BSF 208075 [%]			
		10 µg/ml		20 µg/ml	
		Mean	SD	Mean	SD
Male	0	98.9	0.08	98.9	0.10
	10	98.8	0.09	98.8	0.10
Female	0	98.8	0.08	98.8	0.11
	10	98.8	0.10	98.7	0.10

The results indicate no impact of warfarin on the plasma protein binding of BSF 208075.

The impact of BSF 208075 concentrations of 0, 10 and 20 µg/mL on the plasma protein binding of warfarin in a concentration of 10 µg/mL is shown in Table 2:

Table 2 Effect of BSF 208075 on the human plasma protein binding of warfarin (10 µg/ml) – mean and SD of n = 5 individuals

Sex	Concentration of BSF 208075 [µg/ml]	Binding of warfarin [%]	
		10 µg/ml	
		Mean	SD
Male	0	98.8	0.09
	10	98.8	0.09
	20	98.7	0.11
Female	0	98.8	0.04
	10	98.7	0.07
	20	98.7	0.06

The results indicate that BSF 208075 has no impact on the plasma protein binding of warfarin.

Conclusion

The plasma protein binding of BSF 208075 in high concentrations is not affected by warfarin and vice versa.

Comment

1. The report does not provide evidence that equilibration is reached 5 hour after spiking the plasma side with drug.

2. Dextran was used to decrease the osmotic water shift during the 5 hour dialysis. In determining the drug concentration in the buffer compartment the concentrations were not corrected for the volume occupied by dextran.

Study Report 4MYOGP1S1: Bidirectional Caco-2 Permeability of Ambrisentan

Study Investigator and Site: _____

Objectives

To determine the bidirectional permeability of ambrisentan in Caco-2 cells

Methods

1.2 Certification

Percent recovery was obtained from:

Results

All cell monolayers passed the post-experimental ~~_____~~ monolayer integrity test.

The results of the bidirectional apparent permeability and recovery of ambrisentan are shown in Table 1.1:

Table 1.1 Recovery and Apparent Permeability (10^{-6} cm/s) of Ambrisentan

Test Article Identification	Percent Recovery ^(C)			P_{app} ^(D) Blank	P_{app} A→B			P_{app} B→A			$\frac{P_{app} B \rightarrow A}{P_{app} A \rightarrow B}$ Ratio ^(E)	Absorption Potential ^(A)	Significant Efflux ^(F)
	Blank	A→B	B→A		Rep. 1	Rep. 2	Avg.	Rep. 1	Rep. 2	Avg.			
Ambrisentan	92	81	84	33.3	_____	_____	0.57	_____	_____	3.37	5.9	Medium	Yes

(A) Absorption Potential Classification:
 P_{app} (A-to-B) $> 1.0 \times 10^{-6}$ cm/s High
 P_{app} (A-to-B) $\geq 0.5 \times 10^{-6}$ cm/s; P_{app} $< 1.0 \times 10^{-6}$ cm/s Medium
 P_{app} (A-to-B) $< 0.5 \times 10^{-6}$ cm/s Low

(B) Efflux considered significant if
 P_{app} (B-to-A) $\geq 1.0 \times 10^{-6}$ cm/s and Ratio P_{app} (B-to-A) / P_{app} (A-to-B) ≥ 3.0

(C) Low recoveries caused by non-specific binding, etc. can affect the measured permeability.

(D) A low rate of diffusion ($< 20 \times 10^{-6}$ cm/s) through the cell-free membrane indicates a lack of free diffusion, which may affect the measured permeability.

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The data indicate a significant efflux for ambrisentan similar to that for digoxin. The efflux of ambrisentan is significantly larger than the influx.

Conclusion

The data indicate that ambrisentan is a substrate for an extrusion transporter (P-gp).

Comment

1. The report does not indicate what transporters are expressed in the Caco-2 cells used.
2. The report does not specify the acceptance criteria regarding recovery.
3. Information on LLOQ, accuracy and precision of the assay used were not provided.

Study Report 4MYOGP2: Bidirectional Caco-2 Permeability of Ambrisentan in the Presence and Absence of Cyclosporine

Study Investigator and Site: 

Objective

To determine the bidirectional permeability of ambrisentan in presence and absence of cyclosporine A. Cyclosporine is a known inhibitor of P-glycoprotein.

Methods



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1.2 Certification

[Redacted]

[Redacted]

[Redacted]

[Redacted]

Percent recovery was obtained from:

[Redacted]

Results

The results are shown in Table 1.1:

Table 1.1 Recovery and Apparent Permeability (10^{-6} cm/s) of Ambrisentan

Test Article Identification	Percent Recovery ^(c)		P_{app} A→B			P_{app} B→A			$\frac{P_{app} B \rightarrow A}{P_{app} A \rightarrow B}$ Ratio ^(b)	Absorption Potential ^(a)	Significant Efflux ^(b)
	A→B	B→A	Rep. 1	Rep. 2	Avg.	Rep. 1	Rep. 2	Avg.			
Ambrisentan	81	97	—	—	1.21	—	—	4.34	3.6	High	Yes
Ambrisentan + CSA	69	68	—	—	1.04	—	—	1.97	1.0	High	No

(A) Absorption Potential Classification
 P_{app} (A-to-B) $\geq 1.0 \times 10^{-6}$ cm/s High
 P_{app} (A-to-B) $\geq 0.5 \times 10^{-6}$ cm/s P_{app} $< 1.0 \times 10^{-6}$ cm/s Medium
 P_{app} (A-to-B) $< 0.5 \times 10^{-6}$ cm/s Low

(B) Efflux considered significant if:
 P_{app} (B-to-A) $\geq 1.0 \times 10^{-6}$ cm/s and Ratio $\frac{P_{app} (B-to-A)}{P_{app} (A-to-B)} \geq 3.0$

(C) Low recoveries caused by non-specific binding, etc. can affect the measured permeability.

There appears to be significant efflux of ambrisentan in the absence of cyclosporine. The efflux decreased significantly in the presence of cyclosporine. The results on influx and efflux of ambrisentan in the absence of cyclosporine agree with those in study 4MYOGP1S1.

Conclusion

The results suggest that ambrisentan is a substrate of an efflux transporter.

Comment

1. The report does not indicate what transporters are expressed in the Caco-2 cells used.
2. The report does not specify the acceptance criteria regarding recovery.
3. Information on LLOQ, accuracy and precision of the assay used were not provided.

Study Report 06DMM108: An in Vitro Investigation of the Inhibition by BSF 208075 of Xenobiotic Transport via Human P-glycoprotein, Heterologously Expressed in MDCKII Cells

Study Investigator and Study Site:

Objective

To determine whether BSF208075 inhibits transport of P-glycoprotein substrates in vitro

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 Draft Labeling

 Deliberative Process

Results

Transport rates and percentage of control transport rates of ^3H digoxin in the presence and absence of BSF 807025 are shown in Table 1:

Table 1 The Effect of BSF208075 on Human Pgp Mediated Transport of 30 nM [^3H]-Digoxin Using MDCKII-MDR1 Cells

Compound	Conc. (μM)	Digoxin transport rate (pmole/cm 2 /h)	SD	Digoxin transport rate (% control)	SD
BSF208075	0.3	1.3	0.3	85.2	19
	1	1.45	0.09	95.2	5.7
	3	1.56	0.31	103	20
	10	1.52	0.1	100	6.4
	30	1.67	0.15	110	9.6
	100	1.91	NA	125	NA
Digoxin Only Control (no inhibitor)	-	1.53	0.03	100	2
GF120918A	2	0.34	0.04	22.4	2.8

SD is standard deviation

NA is not applicable

The results indicate that the transport rate of ^3H digoxin in a concentration of 23.4 ng/mL in the presence of BSF 208075 concentrations ranging between 0.13 - 37.8 ng/mL is similar to that in the absence of BSF 208075. In contrast the strong P-gp inhibitor, GF 120918A, reduced the transport rate of ^3H -digoxin to about 22 % of the control value.

Conclusion

BSF 208075 is not an inhibitor of P-gp.

Comments

None