A Phase I Study to Show the Relative Bioavailability and Bioequivalence of Fixed-Dose Combinations of Ambrisentan and Tadalafil in Healthy Subjects

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ABSTRACT

Purpose: Pulmonary arterial hypertension (PAH) is a life-threatening disease that typically causes shortness of breath and exercise intolerance. Combination therapy with ambrisentan and tadalafil has proven to be more effective at preventing clinical failure events in patients with PAH than either drug alone. The aim of this study was to evaluate the bioequivalence of an ambrisentan/tadalafil fixed-dose combination (FDC) compared with co-administration of the 2 monotherapies.

Methods: This 3-part, randomized, single-dose, open-label crossover study was conducted in healthy volunteers. The first part of the study consisted of a 5-way crossover that compared the relative bioavailability of 4 FDC formulations (10-mg ambrisentan + 40-mg tadalafil) with co-administered reference monotherapies. One formulation was selected and its relative bioavailability was assessed when produced in 3 different granulation sizes during the second part of the study. In the third part of the study, the bioequivalence of the candidate FDC with the reference monotherapies was evaluated for the 10-mg/40-mg dose strength, in addition to 2 other dose strengths (5 mg/20 mg and 5 mg/40 mg). For all parts of the study, blood samples were taken at regular intervals after each dose, ambrisentan and tadalafil concentrations determined, and pharmacokinetic (PK) parameters (C_{max}, $AUC_{0-\infty}$, and AUC_{0-t}) obtained. Test/reference ratios of the geometric means of PK parameters were used to evaluate bioequivalence. Safety and tolerability were assessed by recording adverse events and monitoring vital signs, ECGs, and clinical laboratory data.

Findings: Of the 174 subjects screened for eligibility, 112 were allocated to a randomized treatment sequence across all study parts, and 100 completed their full assigned treatments. All 4 FDC formulations tested during part 1 of the study yielded PK parameters similar those of the reference treatments. In part 2, granulation size was found to not affect the relative bioavailability of the selected formulation. In part 3, the selected FDC was found to be bioequivalent to co-administration of the monotherapies in both the fasted and fed states. The FDC was also found to be bioequivalent to the reference treatments at the 2 additional dose strengths. All but one of the adverse events was mild to moderate in intensity, and no serious adverse events were reported.

Implications: An ambrisentan/tadalafil FDC was bioequivalent to concurrently administered monotherapies and therefore represents a viable alternative treatment to co-administration. Use of an FDC is likely to be associated with reduced costs and

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Key Words: ambrisentan, bioavailability, bioequivalence, pulmonary arterial hypertension, tadalafil.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a rare and progressive disease characterized by elevated pulmonary arterial pressure and blood flow resistance, which ultimately leads to right ventricular failure and death.[1] Patients with PAH typically experience increasing shortness of breath and a reduction in exercise capacity, although symptoms can also include fatigue, angina, and syncope.[1,2] The prevalence of PAH has been estimated to be between 7 and 52 cases per 1 million,[3–7] and the disease occurs with greater incidence in female subjects and the elderly.[8] A number of other risk factors have been identified, including HIV infection.[9] Prognosis after a PAH diagnosis is often poor, with 3-year survival rates of 52%–72% having been reported,[4,10] and there is currently no cure.

Endothelin-1 (ET-1) is a small peptide produced by vascular endothelial cells that acts as a strong vasoconstrictor.[11] ET-1 induces vasoconstriction by binding to the ET_A receptor, found predominantly on vascular smooth muscle cells. ET-1 plasma levels are elevated in patients with pulmonary hypertension,[12,13] and the ET-1 pathway has become a major target in the clinical management of PAH. Ambrisentan is a selective ET_A antagonist that has shown effectiveness for the treatment of PAH. Two concurrent, double-blind, Phase III trials found that patients with PAH who were administered ambrisentan for 12 weeks had a significantly higher exercise capacity than those who received placebo.[14] After these successful trials, the US Food and Drug Administration (FDA) approved ambrisentan for the treatment of PAH (World Health Organization [WHO] group 1) in adult patients of WHO functional class II to III in 2007.[15]

The nitric oxide (NO) pathway is an alternative target for PAH intervention. NO is produced in vascular endothelial cells; from there it diffuses to adjacent vascular smooth muscle cells to activate cyclic guanosine monophosphate and induce vasodilation.[16] Disruptions to the NO pathway are associated with PAH, and expression of NO synthase, the enzyme responsible for NO production, is often reduced in patients with pulmonary hypertension.[17] Phosphodiesterase type 5 (PDE-5) is an inactivator of cyclic guanosine monophosphate and a promoter of vasoconstriction, making PDE-5 a therapeutic target for PAH. Three PDE-5 inhibitors have been investigated to date as potential PAH treatments: sildenafil, tadalafil, and vardenafil.[18] Tadalafil has been found to improve the exercise capacity of patients with PAH and reduce rates of clinical worsening,[19] and the drug was approved for PAH (WHO group 1) by the FDA in 2009.[20]

The association of PAH with multiple, distinct intracellular signaling pathways has allowed for combination therapy as a potential treatment option. The progressive and severe nature of the disease is a further justification for this approach, and concurrent treatment with 2 drugs that target different pathways could produce additive effects and improved clinical outcomes. It has been shown that ambrisentan and tadalafil have no clinically relevant pharmacokinetic (PK) or safety interactions.[15,21] The AMBITION (Ambrisentan and Tadalafil in Patients with Pulmonary Arterial Hypertension) trial evaluated the efficacy of initial open-label ambrisentan and tadalafil combination therapy.[22] Patients receiving combination therapy had fewer clinical failures, a superior clinical response, and improved exercise tolerability compared with those administered monotherapies. The combination therapy had a safety profile similar to that of the monotherapies; thus, there is a clear benefit to treating patients with PAH with ambrisentan and tadalafil simultaneously. As such, ambrisentan and tadalafil are recommended as the first-choice initial combination therapy for PAH in the most recent European Society of Cardiology/European Respiratory Society guidelines.[18] Furthermore, more recent data suggest that there may be a survival advantage over the monotherapies of concurrent ambrisentan and tadalafil treatment in patients newly diagnosed with PAH.[23]

Patients who are prescribed both ambrisentan and tadalafil must currently take 2 separate pills. In addition, patients with PAH are often prescribed many concomitant drugs, such as anticoagulants and diuretics, resulting in a complex dosing regimen. Therefore, a fixed-dose combination (FDC) containing both ambrisentan and tadalafil could be beneficial. Ambrisentan and tadalafil are each taken once

daily and have shown effectiveness for PAH when co-administered. In addition, there would likely be benefits in terms of convenience, reduced costs, a simplified dosing regimen, and improved patient compliance. The aim of the present study was to evaluate the bioavailability and bioequivalence of various candidate ambrisentan and tadalafil FDCs compared with co-administration of the individual monotherapies.

SUBJECTS AND METHODS

Study Objectives and Design

This 3-part, single-center, Phase I, single-dose, randomized, open-label, crossover, combined bioavailability and bioequivalence study in healthy volunteers (clinicaltrials.gov identifier NCT02688387) was conducted between March 2016 and August 2017. Within each study part, subjects were planned to receive all possible treatments, with a Williams design to determine the treatment sequences. Subjects were screened for eligibility within 31 days of their first dosing session, and they returned for a follow-up visit within 7−14 days of their final dosing session.

Part 1 of the study was a 5-way crossover that characterized the relative bioavailability of 4 candidate FDC formulations (10-mg ambrisentan + 40-mg tadalafil) and the concurrently administered reference tablets. These dose strengths were selected because they represent the maximum approved doses of ambrisentan and tadalafil. The 4 formulations differed in sodium laurilsulfate (SLS) content and weight: F1, 840-mg/2-mg SLS; F2, 840-mg/4-mg SLS; F3, 560-mg/2-mg SLS; and F4, 560-mg/4-mg SLS. F1 and F2 were formulated by combining the monotherapy formulations with varying amounts of SLS. F3 and F4 were formulated by high-shear wet granulation of both drug substances to reduce the total tablet weight. Doses were taken in a fasted state, with a washout period of ≥7 days between each dose. The primary end points of Part 1 were plasma PK parameters (C_{max}, $AUC_{0-\infty}$, and AUC_{0-t}) of ambrisentan and tadalafil after administration of the 4 FDCs and the reference treatment. Based on these end points, a single candidate FDC formulation was taken forward to Part 2.

Part 2 had 4 dosing sessions and compared the relative bioavailability of 3 different FDC granulation sizes (10-mg ambrisentan + 40-mg tadalafil) versus the co-administered reference treatments. Granulation FG1 to FG3 are different variants of F3 in which the amount of water added during high-shear wet granulation was varied from 19% w/w, 22% w/w (target, F3), and 25% w/w, respectively. The primary end points of Part 2 were the same as Part 1, with these parameters used to select a single FDC granulation size for further assessment in Part 3.

Part 3 of the study was a powered study for the purpose of testing bioequivalence and was split into 2 subparts, each containing 4 dosing sessions. Part 3A assessed whether the candidate FDC brought forward from Part 2 is bioequivalent to the reference in both fasted and fed states. The fed arms of Part 3A used a standard high-fat breakfast.[24] Part 3B evaluated the bioequivalence of the candidate FDC at 2 additional dose strengths (5-mg ambrisentan + 40-mg tadalafil and 5-mg ambrisentan + 20-mg tadalafil) with concurrently administered equivalent reference tablets, in a fasted state. The same PK parameters used in Parts 1 and 2 (C_{max}, $AUC_{0-\infty}$, and AUC_{0-t}) were measured and used to assess bioequivalence.

A secondary objective of all 3 study parts was to monitor the safety and tolerability of the FDC compared with the reference treatments. The secondary end points were vital signs, ECG, clinical laboratory safety data (hematology, clinical chemistry, and urinalysis), and adverse events (AEs). Vital signs were measured in a semi-supine position after 5 min of rest and included temperature, systolic and diastolic blood pressures, pulse rate, and respiratory rate. Triplicate 12-lead ECGs were also measured in a semi-supine position after 5 min of rest. For AEs, information relating to their duration, severity, causality, and outcomes were recorded by the investigator or site staff.

Study Population

Healthy male or female volunteers of nonreproductive potential and 18−60 years of age were eligible for participation. A body mass index in the range 18−30 kg/m^2 was required, and a minimum body weight requirement of 50 kg for male subjects and 45 kg for female subjects was set. Key exclusion criteria included the use of prescription or nonprescription drugs within 7 days or 5 half-lives (whichever was longer) before the first dose, a history of regular alcohol consumption within the previous 6 months, and the smoking of >5 cigarettes per week. A full list of eligibility criteria is given in Supplemental Table I (see the online version at https://doi.org/10.1016/j.clinthera.2019.04.007).

All parts of the study were performed in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Good Clinical Practice, and any relevant country-specific requirements. Investigators complied with Good Clinical Practice guidelines, and subjects were required to provide written informed consent before involvement. The human biological samples were sourced ethically, and their research use was in accord with the terms of the informed consents. Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

PK Assessments

For each study part, PK samples were taken 0, 0.5, 1, 1.5, 2, 2.5, 4, 8, 12, 18, 24, 36, 48, and 72 h after every dose. For ambrisentan, 2.7 mL of blood was collected into sodium citrate tubes, and for tadalafil, 2.0 mL of blood was collected into dipotassium-EDTA tubes. Samples were centrifuged to plasma within 1 h of collection and the centrifuge refrigerated at 4 °C for 10 min at 1500*g*. Plasma was transferred to a 1.8 mL Nunc screw cap cryovial (Thermo Fisher Scientific, Waltham, Massachusetts), frozen, and stored immediately at −80 °C.

Sample analysis was performed by using 2 separate validated analytical methods, using protein precipitation followed by HPLC-MS/MS showing acceptable sensitivity, selectivity, precision, and accuracy for the quantitation of ambrisentan or tadalafil (in the presence of the other), and linearity over the range of 1−2000 ng/mL. The lower limit of quantification was 1 ng/mL using a 25 μL aliquot of sodium citrate or EDTA sample for ambrisentan and tadalafil, respectively. Details of the sample analysis procedure are found in the Supplemental Methods (see the online version at https://doi.org/10.1016/j.clinthera.2019.04.007). Quality control (QC) samples, containing ambrisentan and tadalafil at 3 different concentrations stored with the study samples, were analyzed with each batch of samples against separately prepared calibration standards. The PK analysis results were considered acceptable if no more than one third of the QC results deviated from the nominal concentration by more than 15%, and if at least 50% of the results from each QC concentration were within 15% of nominal. The %CV for QC samples ranged from 7.5% to 10.0% and 4.5%−25.0% for the ambrisentan and tadalafil plasma samples, respectively. The run-to-run mean bias values for QC samples ranged from −1.8% to 4.1% for the ambrisentan plasma samples and −2.4% to 2.1% for tadalafil plasma samples.

PK Statistical Analysis

SAS version 9.4 (SAS Institute, Inc, Cary, North Carolina) was used for all statistical analysis. For Parts 1 and 2 of the study, sample size assumptions were based on previously reported estimates of within-subject ambrisentan and tadalafil %CV for C_{max} and $AUC_{0-\infty}$.[25,26] The largest within-subject % CV was estimated to be 22%, which corresponds to an SD of 0.217 and a 90% CI half-width of 12.5% of the point estimate, based on a sample size of 20. For Part 3, the largest within-subject %CV was estimated at 22.4%, corresponding to an SD of 0.221. Thus, assuming that the actual ratio of the geometric means for ambrisentan C_{max} is 0.97, a sample size of 26 evaluable subjects was estimated to have 90% power to demonstrate bioequivalence. Assuming a 20% dropout rate, the planned number of subjects to be enrolled for both Parts 3A and 3B was 32.

The PK parameters (C_{max}, $AUC_{0-\infty}$, and AUC_{0-t}) were determined from the plasma concentration−time data for each treatment by using WinNonlin version 6.3 (Certara, Princeton, New Jersey). Summary statistics for ambrisentan and tadalafil plasma concentrations were calculated for all 3 study parts. C_{max}, $AUC_{0-\infty}$, and AUC_{0-t} were analyzed after $\log_e$ transformation by using a mixed effects model, fitting terms for treatment, period, and sequence as fixed effects, and subject as a random effect. The Kenward and Roger degrees of freedom approach was used. This model was used to provide point estimates for adjusted means on the $\log_e$ scale, mean differences between treatments, and 90% CIs. Back-transformation was performed to obtain adjusted geometric means and the corresponding 90% CIs. Subjects were excluded from any statistical analysis if their predose ambrisentan or tadalafil concentration was >5% of C_{max}.[24]

In Parts 1 and 2, statistical tests were not performed, with descriptive statistics instead used to select the candidate formulation and granulation size to progress to Part 3. For Parts 3A and 3B, bioequivalence was assessed for each primary PK variable. Test and reference treatments were

considered to be bioequivalent if the 90% CIs fell entirely within the range of 0.80−1.25, in accordance with the European Medicines Agency guidance on the investigation of bioequivalence.[24]

Several populations were used for analysis. The enrolled population comprised those who provided informed consent. The per-protocol population consisted of all subjects randomized to treatment who received at least a single dose of treatment and complied with the protocol. All subjects who received at least 1 treatment dose were included in the safety population and used for safety assessments. The PK concentration population included all subjects who had at least 1 PK sample obtained and analyzed. PK parameter populations were defined as the subjects who contributed data toward each parameter. The complete treatment/reference PK parameter populations comprised subjects within the PK parameter population who completed all assigned treatments in Part 3A or 3B.

RESULTS

Study Population

In total, 174 volunteers were assessed for eligibility (enrolled population), with 59 of these failing the screen and an additional 3 being unused reserve subjects. A total of 112 subjects were assigned to a treatment order across all study parts (per-protocol population). This approach resulted in 100 complete sets of treatment data. A summary of subject participation within each of the 3 study parts, including reasons for withdrawals, is given in Figure 1. Demographic characteristics of subjects, including age, sex, race, height, body mass index, and weight, are shown in Table I. Demographic characteristics were similar throughout all study parts, and only 1 of the 112 subjects who received a treatment dose was female.

Part 1: Relative Bioavailability of 4 Candidate FDCs

For Part 1 of the study, in which the bioavailability of 4 candidate FDCs (F1−F4) and the reference monotherapies was assessed, 26 subjects provided PK parameter data for at least 1 treatment and analyte (representing the per-protocol, safety, PK concentration, and PK parameter populations). Box plots were obtained from post hoc analyses and show the distribution of PK parameters (C_{max}, $AUC_{0-\infty}$, and AUC_{0-t}) after a single dose of the 5 treatments; they are shown in Figure 2 for ambrisentan and Figure 3 for tadalafil. A summary of ambrisentan and tadalafil PK plasma parameters is given in Table II. All 4 candidate FDCs yielded PK parameter values similar to those of the reference monotherapies. Formulation F3, however, was selected based on having more favorable pharmaceutical manufacturing qualities for development.

Part 2: Relative Bioavailability of the Candidate FDC in 3 Granulation Sizes

In Part 2, the relative bioavailability of the FDC F3 in 3 different granulation sizes was assessed. The per-protocol, safety, PK concentration, and PK parameter populations for this study part consisted of 21 subjects. One subject had a predose tadalafil concentration that was >5% of C_{max} (5.78%) and was excluded from any statistical analysis. Table III shows the PK parameters for the 3 granulation sizes, and reference treatments and box plots showing the distribution of PK parameters are given in Figure 2 for ambrisentan and Figure 3 for tadalafil. All 3 granulation sizes (FG1−FG3) produced very similar bioavailability PK parameters for both ambrisentan and tadalafil. Furthermore, these plasma exposures were in line with those of the reference monotherapies taken concurrently. Granulation size FG1 was selected for further development based upon considerations relating to the potential manufacturing qualities of each.

Part 3A: Bioequivalence of the Candidate FDC With Monotherapies

Study Part 3A aimed to establish whether the candidate FDC from Part 2 (FG1) was bioequivalent to 10-mg ambrisentan + 40-mg tadalafil monotherapies taken concurrently in the fasted and fed states. Thirty-two subjects provided data on PK parameters that were subsequently used for bioequivalence statistical analysis and thus represented the complete test/reference PK parameter population for Part 3A. No subject had a predose concentration of either drug >5% of C_{max}. Although there was no regulatory requirement,[24] this study part was conducted under both fasted and fed conditions. Historical results have shown no food effect for the individual entities[15,20]; however, the peak concentration of ambrisentan was reduced by 27% in a fed state compared with fasted state, although systemic plasma exposure levels of ambrisentan and

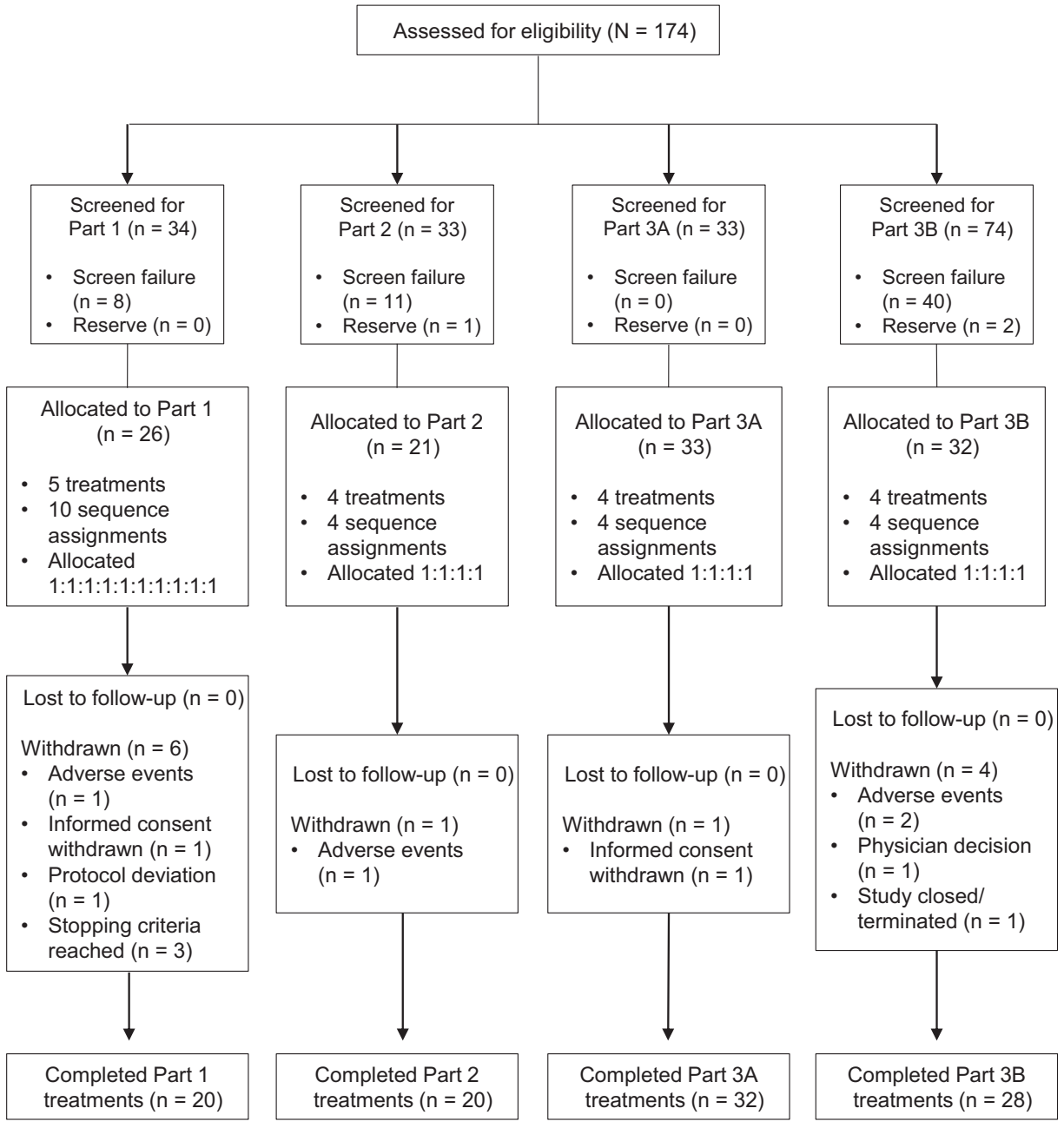

Figure 1. A Consolidated Standards of Reporting Trials flow diagram of study participation.

tadalafil were similar between states (Table IV). The presence of food more than doubled the median T_{max} of ambrisentan for both the FDC and reference treatments compared with the fasted state. Similarly, the median T_{max} of tadalafil was >3-fold higher in the fed state than in the fasted state. Generally, there was a greater variability in T_{max} in the fed state than in the fasted state for both drugs.

Plasma–time concentration profiles of ambrisentan and tadalafil after administration of the candidate FDC and co-administered monotherapies are shown in the Supplemental Figure (see the online version at

Table I. Demographic characteristics of subjects allocated to a treatment.

Characteristic	Part 1 (n = 26)	Part 2 (n = 21)	Part 3A (n = 33)	Part 3B (n = 32)	Total (N = 112)
Age, mean (SD), y	37.7 (11.68)	36.8 (12.07)	37.3 (10.36)	30.9 (12.05)	35.5 (11.71)
Sex, no. (%)					
Female	0	0	0	1 (3)	1 (<1)
Male	26 (100)	21 (100)	33 (100)	31 (97)	111 (>99)
Height, mean (SD), cm	179.1 (6.33)	177.3 (5.75)	178.7 (6.84)	176.9 (7.88)	178.0 (6.83)
Weight, mean (SD), kg	77.93 (9.663)	75.76 (9.172)	77.82 (10.796)	76.47 (10.285)	77.07 (10.005)
Body mass index, mean (SD), kg/m^2	24.26 (2.385)	24.06 (2.465)	24.37 (3.125)	24.46 (3.112)	24.31 (2.813)
Ethnicity, no. (%)					
Hispanic or Latino	2 (8)	1 (5)	1 (3)	1 (3)	5 (4)
Not Hispanic or Latino	24 (92)	20 (95)	32 (97)	31 (97)	107 (96)
Race, no. (%)					
Asian—Central/South Asian heritage	0	0	2 (6)	0	2 (2)
Asian—South East Asian heritage	0	0	1 (3)	0	1 (<1)
Black or African American	3 (12)	1 (5)	5 (15)	8 (25)	17 (15)
White—Arabic/North African heritage	0	0	0	1 (3)	1 (<1)
White—white/Caucasian/ European heritage	23 (88)	20 (95)	25 (76)	22 (69)	90 (80)
Multiple	0	0	0	1 (3)	1 (<1)

https://doi.org/10.1016/j.clinthera.2019.04.007). Test/reference ratios for all ambrisentan PK parameters were close to unity, and 90% CIs were within the 0.80 to 1.25 allowable range for bioequivalence (Table IV). Within-subject variability for fed-state ambrisentan AUC_{0-t} and C_{max} parameters were 7.8% and 23.7%, respectively. Within-subject variability for fasted state ambrisentan AUC_{0-t} and C_{max} parameters were 7.4% and 25.8%. These values are inside the normal range of intrasubject variability observed for ambrisentan, indicating no gross inadequacies in terms of sample size. Box plots for the PK parameters derived during Part 3A are shown in Figures 2 and 3 for ambrisentan and tadalafil.

Similarly, all tadalafil PK parameter test/reference ratios were close to unity, with 90% CIs falling within the range of 0.80−1.25 (Table IV). Within-subject variability for fed state tadalafil AUC_{0-t} and C_{max} parameters were 8.7% and 13.6%, respectively. Within-subject variability for fasted state tadalafil AUC_{0-t} and C_{max} parameters were 13.4% and 12.7%. These values are inside the normal range of intrasubject variability observed for tadalafil, indicating no gross inadequacies in terms of sample size. Together, the data in Table IV indicate that the candidate ambrisentan and tadalafil FDC formulation is bioequivalent to the reference monotherapies in both the fasted and fed states.

Part 3B: Assessing Bioequivalence at 2 Additional Dose Strengths

Although 10 mg and 40 mg are the maximum approved doses for ambrisentan and tadalafil, other FDC dose strengths would be required in practice to allow for safe up-titration and down-titration. Study Part 3B evaluated the bioequivalence of the candidate FDC formulation at 2 additional dose strengths, 5 mg/40 mg and 5 mg/20 mg. Bioequivalence testing

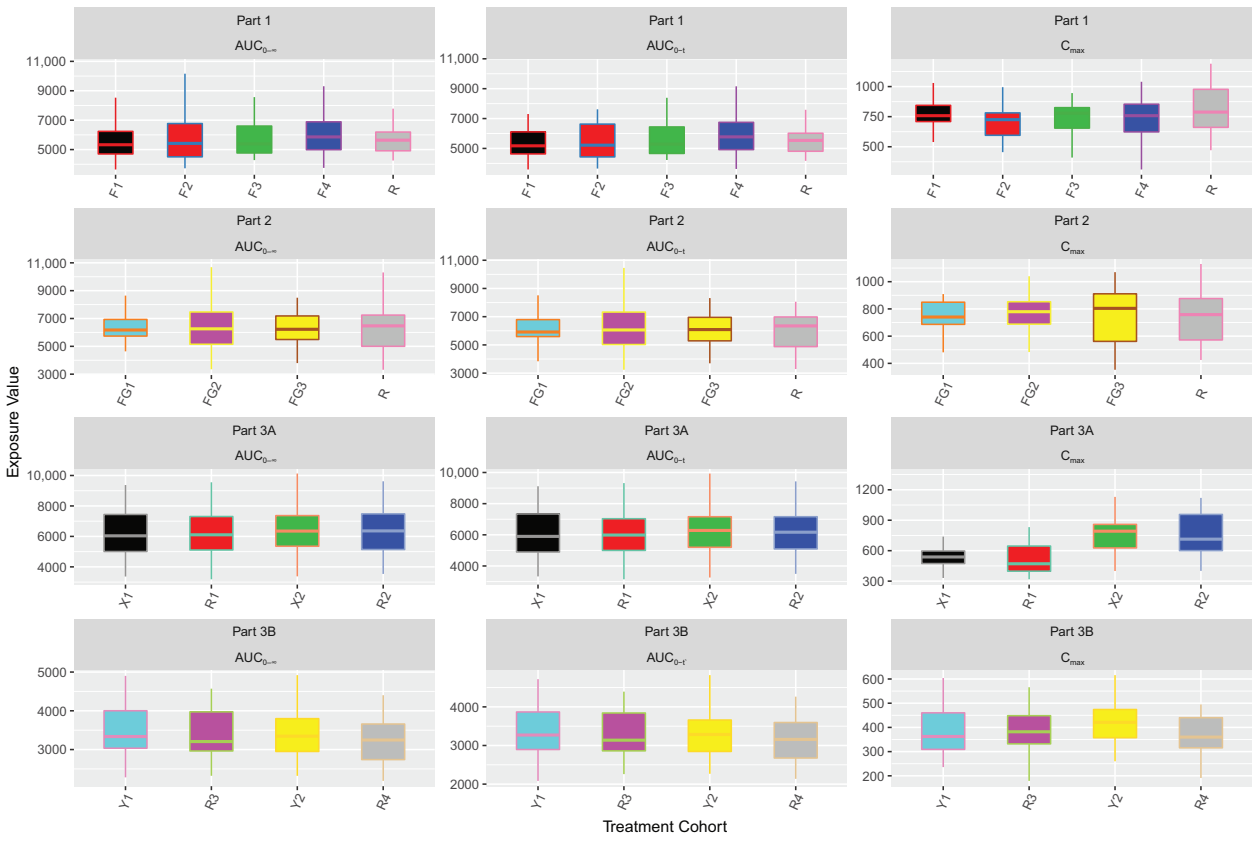

Figure 2. Box plots of ambrisentan pharmacokinetic parameters (C_{max}, $AUC_{0-\infty}$, and AUC_{0-t}) for all study parts. These plots were generated from a post hoc analysis. R refers to the reference for each study part. F1 to F4 refers to the formulations 1 to 4 (10 mg/40 mg) from Part 1. FG1 to FG3 refers to the granule size formulations 1 to 3 (10 mg/40 mg) from Part 2. X1 and X2 refer to the selected fixed-dose combination (FDC) formulation (10 mg + 40 mg) tested under fed and fasted states, respectively. Y1 and Y2 refer to the selected FDC at dose strengths of 5 mg/40 mg and 5 mg/20 mg.

was performed for the 5-mg/20-mg formulation due to conflicting data on the dose proportionality of tadalafil.[25] Although the 5-mg/40-mg formulation met regulatory requirements for pursuing a bioequivalence waiver, the formulation was tested for bioequivalence due to a commercial obligation.

One subject provided a sample for which the ambrisentan and tadalafil AUCs were <5% of the population geometric means and was excluded from relevant bioequivalence statistical analysis. No subjects had a predose concentration of either drug >5% of C_{max}.

Table V lists the ambrisentan and tadalafil PK parameter means at both additional dose strengths. Exposure levels of ambrisentan were expectedly similar across all four Part 3B treatments, owing to the 5-mg ambrisentan dose contained within each. At each of the 2 dose strengths tested, the candidate FDC and monotherapies provided very similar tadalafil plasma exposure levels. Test/reference ratios for ambrisentan and tadalafil PK parameters at both dose strengths were close to unity. All 90% CIs were within the 0.80 to 1.25 bioequivalence range, suggesting that the candidate FDC is bioequivalent to co-administration at each of the 2 additional dose strengths. Box plots for the PK parameters derived during Part 3A are shown in Figures 2 and 3 for ambrisentan and tadalafil.

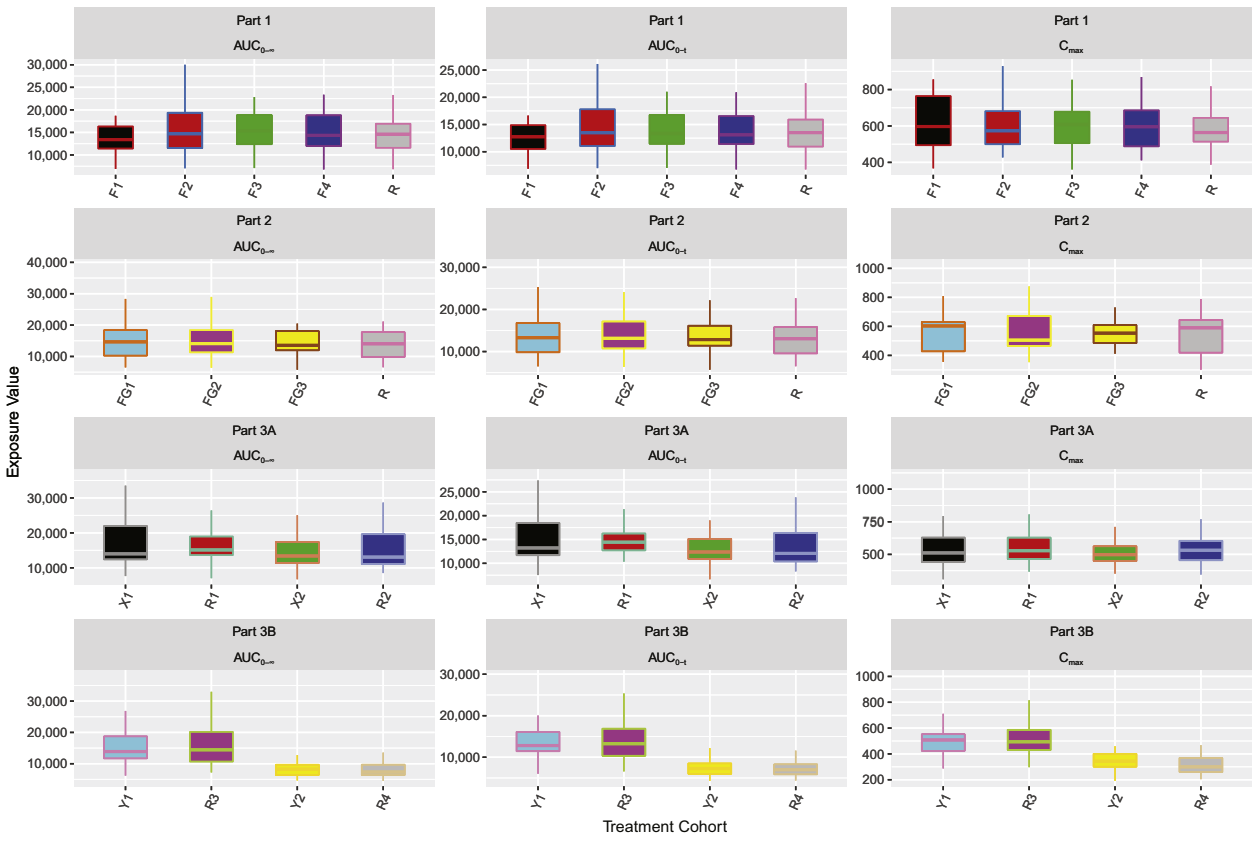

Figure 3. Box plots of tadalafil pharmacokinetic parameters (C_{max}, $AUC_{0-\infty}$, and AUC_{0-t}) for all study parts. These plots were generated from a post hoc analysis. R refers to the reference for each study part. F1 to F4 refers to the formulations 1 to 4 (10 mg/40 mg) from Part 1. FG1 to FG3 refers to the granule size formulations 1 to 3 (10 mg/40 mg) from Part 2. X1 and X2 refer to the selected fixed-dose combination (FDC) formulation (10 mg + 40 mg) tested under fed and fasted states, respectively. Y1 and Y2 refer to the selected FDC at dose strengths of 5 mg/40 mg and 5 mg/20 mg.

Within-subject variability for ambrisentan AUC_{0-t} and C_{max} parameters were 5.0% and 18.8% at the 5-mg/40-mg dose strength, and 7.8% and 21.7% at the 5-mg/20-mg dose strength, respectively. Within-subject variability for ambrisentan AUC_{0-t} and C_{max} parameters were 16.2% and 17.3% at the 5-mg/40-mg dose strength, and 8.9% and 16.2% at the 5-mg/20-mg dose strength. These values are inside the normal range of intrasubject variability observed for ambrisentan, indicating no gross inadequacies in terms of sample size.

Safety Results

A total of 99 subjects (88%) reported an AE across all study parts. Overall, the most common AEs were headaches (reported by 82% of subjects), nasal congestion (22%), back pain (22%), nausea (21%), and musculoskeletal pain (13%). Supplemental Table II (given in the online version at https://doi.org/10.1016/j.clinthera.2019.04.007) contains a breakdown of AEs according to study part. A total of 97 patients (87%) had an AE that was considered to be drug related. The most common drug-related AEs were headaches (reported by 80% of subjects), nasal congestion (22%), back pain (20%), and nausea (18%). A breakdown of drug-related AEs according to study part can be found in Supplemental Table II (see the online version at https://doi.org/10.1016/j.clinthera.2019.04.007). In Part 3B, drug-related AEs occurred at higher rates at

Table II. Pharmacokinetic parameters for ambrisentan and tadalafil after a single dose of the 4 candidate fixed-dose combination (FDC) formulations and concurrently taken reference monotherapies. Values are presented as geometric mean (%CV) unless otherwise stated.

Parameter	FDC Formulation				Reference
	F1 (840-mg/2-mg SLS)	F2 (840-mg/4-mg SLS)	F3 (560-mg/2-mg SLS)	F4 (560-mg/4-mg SLS)	
	n = 21	n = 23	n = 23	n = 22	n = 21
Ambrisentan					
$AUC_{0-\infty}$, ng · h/mL	5566 (23.8)	5556 (25.9)	5789 (25.8)	6008 (23.7)	5747 (20.8)
AUC_{0-t}, ng · h/mL	5418 (22.9)	5435 (25.3)	5656 (25.5)	5858 (22.9)	5606 (20.2)
C_{max}, ng/mL	766 (18.2)	685 (20.5)	739 (22.1)	723 (27.7)	756 (28.9)
T_{max}, median (range), h	1.00 (0.50—8.23)	1.50 (0.53—4.00)	1.50 (0.52—4.00)	1.50 (0.57—4.05)	1.50 (0.50—4.00)
$t_{1/2}$, h	16.74 (18.2)	15.25 (19.2)	15.76 (17.3)	15.25 (20.0)	16.08 (17.9)
Tadalafil					
$AUC_{0-\infty}$, ng · h/mL	13,408 (41.3)	14,416 (41.8)	14,545 (38.4)	14,418 (37.8)	13,956 (36.2)
AUC_{0-t}, ng · h/mL	12,470 (37.1)	13,308 (37.4)	13,377 (34.8)	13,239 (33.4)	12,805 (32.2)
C_{max}, ng/mL	581 (27.6)	596 (22.3)	588 (24.5)	590 (21.4)	568 (18.0)
T_{max}, median (range), h	1.50 (0.50—8.23)	2.00 (0.50—8.03)	2.00 (0.50—8.00)	2.00 (0.50—8.00)	1.50 (0.50—4.00)
$t_{1/2}$, h	17.08 (30.4)	17.76 (33.3)	18.38 (33.3)	18.48 (33.0)	18.04 (30.4)

SLS = sodium laurilsulfate.

Table III. Pharmacokinetic parameters for ambrisentan and tadalafil after a single dose of the 4 candidate fixed-dose combination (FDC) formulations and concurrently taken reference monotherapies. Values are given as geometric mean (%CV) unless stated otherwise.

Parameter	3 Granulation Sizes of FDC F3			Reference
	FG1	FG2	FG3	
Ambrisentan	n = 20	n = 20	n = 20	n = 21
$AUC_{0-\infty}$, ng · h/mL	6179 (27.2)	6189 (29.5)	6201 (25.7)	6029 (29.6)
AUC_{0-t}, ng · h/mL	6017 (27.1)	6052 (29.6)	6015 (25.9)	5894 (29.4)
C_{max}, ng/mL	721 (21.1)	749 (22.9)	726 (31.4)	711 (28.2)
T_{max}, median (range), h	1.50 (0.50–4.00)	1.50 (0.50–4.00)	1.51 (1.00–8.00)	1.50 (0.50–4.03)
$t_{1/2}$, h	15.76 (33.3)	15.39 (18.6)	16.60 (34.4)	15.19 (24.5)
Tadalafil	n = 20	n = 20	n = 20	n = 20
$AUC_{0-\infty}$, ng · h/mL	14,444 (44.8)	14,502 (40.5)	14,457 (40.5)	14,007 (46.7)
AUC_{0-t}, ng · h/mL	13,149 (37.5)	13,305 (35.2)	13,275 (33.3)	12,833 (39.6)
C_{max}, ng/mL	562 (28.4)	551 (28.6)	553 (20.5)	538 (27.6)
T_{max}, median (range), h	1.75 (0.50–8.08)	2.00 (0.50–8.00)	2.00 (0.50–12.10)	2.02 (0.50–8.02)
$t_{1/2}$, h	18.63 (33.2)	18.22 (33.2)	17.98 (32.6)	18.58 (30.6)

the 5-mg/40-mg dose strength than the 5-mg/20-mg treatments. All AEs reported during Parts 1, 2, and 3A were judged to be of mild or moderate intensity. A single drug-related AE of priapism in Part 3B was considered to be severe and led to the withdrawal of the subject. In addition, 1 subject was withdrawn from Part 1 due to a drug-independent musculoskeletal pain AE, 1 subject was withdrawn from Part 2 due to drug-related increased erections, and 1 subject was withdrawn from Part 3B due to a drug-independent scrotal abscess. No deaths or serious AEs were reported during the study.

AEs occurred at similar rates for the FDC and co-administered treatments. During each study part, AEs occurred at the following rates: 60% for FDC versus 57% for reference treatments in Part 1, 63% versus 62% in Part 2, 69% versus 75% in Part 3A, and 62% versus 63% in Part 3B.

Several subjects had changes from baseline in their chemistry and hematology values that were potentially clinically important. One subject had increased potassium levels 48 h after dosing, 3 subjects had low lymphocyte levels, 4 subjects had low neutrophil levels, 4 subjects had low leukocyte levels, and 1 subject had low platelet levels. None of these changes were clinically significant, and none was reported as an AE. Four subjects had abnormalities in their QTc corrected according to Bazett's or Fridericia's formula, defined as an increase of >60 ms from baseline, but these were not reported as an AE. A mean increase in heart rate of 10–17 beats/min and a mean decrease in diastolic and systolic blood pressures of ≤8 mm Hg was observed 12 h' postdose in all study parts, irrespective of treatment or dose. Alterations of this magnitude are not unusual based on the mechanism of action of ambrisentan and tadalafil.

DISCUSSION

Combination therapy is an attractive option for PAH treatment due to the involvement of various intracellular signaling pathways. Ambrisentan and tadalafil target 2 different pathways and have been shown to be effective when co-administered for PAH.[22] As such, they are currently the most strongly recommended initial combination therapy.[18] An ambrisentan and tadalafil FDC may provide benefits in terms of compliance and cost. This study aimed to assess whether a candidate FDC containing 10-mg ambrisentan +40-mg tadalafil is bioequivalent to co-administered, equivalent-dose monotherapies. The relative bioavailability of several FDC formulations was initially tested, with all providing exposures of ambrisentan and tadalafil similar to those of the reference tablets. The selected FDC formulation was reportedly bioequivalent to the corresponding co-

Table IV. Pharmacokinetic parameters and statistical comparisons after administration of the candidate fixed-dose combination (FDC) and reference monotherapies under fed and fasted states.

Parameter	Fed		Fasted	
	Candidate FDC (X1)	Reference (R1)	Candidate FDC (X2)	Reference (R2)
	n = 32	n = 32	n = 33	n = 32
Ambrisentan				
$AUC_{0-\infty}$, ng · h/mL*	6076 (26.1)	5899 (27.7)	6243 (25.5)	6156 (24.4)
Ratio (90% CI)†	1.03 (0.997–1.065)		1.014 (0.982–1.047)	
AUC_{0-t}, ng · h/mL*	5927 (25.7)	5767 (27.4)	6099 (25.2)	6013 (24.0)
Ratio (90% CI)†	1.028 (0.994–1.062)		1.014 (0.983–1.047)	
C_{max}, ng/mL*	550 (30.9)	516 (33.4)	754 (24.5)	728 (29.8)
Ratio (90% CI)†	1.067 (0.966–1.178)		1.035 (0.929–1.153)	
T_{max}, median (range), h*	4.00 (1.00–12.00)	4.00 (1.00–12.15)	1.50 (0.50–4.00)	2.00 (1.00–4.00)
$t_{1/2}$, h	15.65 (16.7)	14.66 (13.7)	15.42 (15.9)	15.63 (16.6)
Tadalafil				
$AUC_{0-\infty}$, ng · h/mL*	16,087 (41.5)	16,596 (37.8)	14,842 (40.7)	14,613 (41.5)
Ratio (90% CI)†	0.969 (0.931–1.009)		1.016 (0.955–1.08)	
AUC_{0-t}, ng · h/mL*	14,367 (33.7)	14,946 (31.1)	13,274 (32.9)	13,114 (32.6)
Ratio (90% CI)†	0.961 (0.927–0.997)		1.012 (0.956–1.072)	
C_{max}, ng/mL*	525 (26.5)	534 (19.5)	503 (24.3)	521 (21.1)
Ratio (90% CI)†	0.984 (0.929–1.042)		0.966 (0.915–1.019)	
T_{max}, median (range), h*	8.00 (0.50–12.02)	8.00 (1.50–12.15)	2.50 (0.50–12.02)	2.26 (0.50–8.03)
$t_{1/2}$, h	20.09 (34.4)	19.29 (33.4)	19.85 (34.2)	19.86 (36.8)

* Data are presented as geometric mean (%CV) unless stated otherwise.
† Data represent adjusted geometric mean ratio (test/reference) and the 90% CI calculated from a sample size of n = 32. X1, X2, R1, and R2 refer to the treatments found in Figures 2 and 3.

administered monotherapies in both the fed and fasted states. Furthermore, the FDC was found to be bioequivalent to the reference monotherapies at 2 additional dose strengths. The production of the FDC at various dose strengths would allow for dose titration. In addition, we observed that 4 different formulations (F1–F4) and 3 different granulation sizes (FG1–FG3) had very similar ambrisentan and tadalafil PK profiles. This outcome is likely due to the formulations and granule sizes having no impact upon tablet hardness, disintegration time, or dissolution, leaving the PK parameters unaffected.

The ambrisentan and tadalafil PK exposures obtained in this study after administration of the FDC or monotherapies are consistent with exposure data from previous studies.[21,25] The absence of a PK interaction between ambrisentan and tadalafil has been previously reported,[21] with this study further showing that such interactions do not occur when administered as an FDC. Tadalafil is metabolized through the cytochrome P450 (CYP) pathway, particularly by CYP3A4,[25] whereas ambrisentan is metabolized through glucuronidation by several uridine diphospho-glucuronosyltransferase enzymes.[21,27] Because ambrisentan has been shown to have no impact on the CYP pathway,[27] the drug is unlikely to interact with tadalafil metabolism. Furthermore, ambrisentan has been previously reported to have no clinically relevant interactions with sildenafil and warfarin,[28,29] which are also CYP substrates. Together with these previous data, our study highlights the potential for ambrisentan and tadalafil to be effectively administered as an FDC.

Table V. Pharmacokinetic parameters and statistical comparisons after administration of the candidate fixed-dose combination (FDC) and reference monotherapies at 5 mg/40 mg and 5 mg/20 mg of ambrisentan and tadalafil.

Parameter	5-mg Ambrisentan + 40 mg Tadalafil, Fasted		5-mg Ambrisentan + 20-mg Tadalafil, Fasted	
	Candidate FDC (Y1)	Reference (R3)	Candidate FDC (Y2)	Reference (R4)
	n = 30	n = 31	n = 31	n = 30
Ambrisentan				
$AUC_{0-\infty}$, ng · h/mL*	3398 (19.7)	3286 (21.1)	3320 (20.8)	3206 (20.6)
Ratio (90% CI)†	1.039 (1.017–1.062)		1.035 (1.001–1.071)	
AUC_{0-t}, ng · h/mL*	3297 (19.8)	3198 (20.6)	3228 (20.8)	3116 (20.3)
Ratio (90% CI)†	1.037 (1.014–1.060)		1.035 (1.000–1.071)	
C_{max}, ng/mL*	376 (23.0)	376 (25.6)	402 (25.0)	359 (24.5)
Ratio (90% CI)†	1.015 (0.935–1.103)		1.118 (1.017–1.229)	
T_{max}, median (range), h*	1.77 (1.00–4.03)	2.00 (1.00–8.00)	1.50 (1.00–8.00)	1.76 (1.00–8.00)
$t_{1/2}$, h	16.39 (21.6)	16.15 (18.7)	16.42 (17.1)	16.00 (19.7)
Tadalafil				
$AUC_{0-\infty}$, ng · h/mL*	14,724 (39.7)	15,107 (40.9)	8021 (32.4)	7749 (32.6)
Ratio (90% CI)†	0.981 (0.911–1.056)		1.036 (0.991–1.083)	
AUC_{0-t}, ng · h/mL*	13,120 (31.0)	13,433 (35.3)	7302 (26.9)	7077 (27.4)
Ratio (90% CI)†	0.982 (0.915–1.055)		1.032 (0.993–1.073)	
C_{max}, ng/mL*	488 (19.7	505 (27.8)	341 (23.5)	304 (22.5)
Ratio (90% CI)†	0.976 (0.904–1.053)		1.120 (1.043–1.202)	
T_{max}, median (range), h*	2.50 (0.50–8.00)	2.50 (0.50–8.00)	1.50 (0.50–8.00)	2.01 (0.50–8.00)
$t_{1/2}$, h	20.27 (34.3)	20.34 (34.4)	19.01 (31.1)	18.95 (30.8)

* Data presented as geometric mean (%CV) unless stated otherwise.
† Data represent adjusted geometric mean ratio (test/reference) and the 90% CI calculated from a sample size of n = 30. Y1, Y2, R3, and R4 refer to the treatments found in Figures 2 and 3.

Although the food effect analysis was not part of the scope of the study, the results showed that food had a negligible impact on overall tadalafil and ambrisentan absorption, for either the FDC or co-administration of monotherapies. This finding is consistent with previous studies on the individual component drugs, in which the negligible impact of food on the absorption of 2.5, 5, and 10 mg of ambrisentan and 20 mg of tadalafil have been reported.[25,30] However, peak absorption of ambrisentan was lower in the presence of food, reflecting changes to the absorption kinetics. Interestingly, in this study, the presence of food increased the time taken for ambrisentan and tadalafil to reach their maximum concentration by at least 2-fold when provided as either an FDC or co-administered monotherapies. This finding contrasts with the findings of Forgue et al,[25] who reported that food had little impact on the T_{max} of tadalafil.

The FDC had a consistent safety profile with the monotherapies, with no deaths or serious AEs reported. All AEs were mild or moderate in intensity, aside from one event of priapism that was judged to be severe. AEs occurred at similar rates after administration of the FDC or co-administration, suggesting that the safety of the FDC is consistent with the known safety profiles of the co-administered

monotherapies. Although there was a study-wide mean increase in heart rate of 10−17 beats/min, this finding was not considered to be unusual considering the mechanisms of action of ambrisentan and tadalafil, and an increased heart rate is a known side effect of tadalafil.[31]

CONCLUSIONS

This study showed that the proposed ambrisentan and tadalafil FDC formulation is bioequivalent to concurrently administered monotherapies, under both fed and fasted states in healthy volunteers. The safety and tolerability of the FDC are consistent with those of the individual monotherapies. The FDC is therefore a viable alternative to combination therapy with individual agents.

CONFLICTS OF INTEREST

Drs. Okour, Chen, Port, Berni, Khindri, Schneider, and Tenero were employees of GlaxoSmithKline at the time of the study. Drs. Okour, Chen, Port, Khindri, Schneider, and Tenero hold shares in GlaxoSmithKline. Dr. Puri is a salaried employee of Hammersmith Medicines Research Ltd who was contracted by GlaxoSmithKline. The authors have indicated that they have no other conflicts of interest regarding the content of this article.

GlaxoSmithKline was involved in the study design, analysis, and interpretation of data.

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M. Okour, G. Chen, A. Berni, S. Khindri, A. Puri, and D. Tenero contributed to the conception and design of the study. M. Okour, G. Chen, K. Port, A. Berni, S. Khindri, I. Schneider, and D. Tenero contributed to the analysis and interpretation of the data. A. Puri contributed to the acquisition of the data.

APPENDIX. SUPPLEMENTARY METHODS

Plasma sample analysis

Ambrisentan was extracted from 25 μL of human plasma containing sodium citrate by protein precipitation with acetonitrile:methanol (50:50) containing an isotopically labelled internal standard ($[^2H_{10}]$-AMB). Plasma extracts (25 μL) were injected onto a Synergy Fusion-RP column (Phenomenex, 50 × 2 mm, 4 μm) fitted with a KrudKatcher guard column (Phenomenex, 0.5 μm), maintained at 40 °C using a Waters Acquity UPLC. The mobile phase consisted of water-formic acid (100:0.1, v/v) (solvent A) and acetonitrile:methanol:formic acid (50:50:1, v/v/v) (solvent B). A flow rate of 0.8 mL/min was maintained for the entire run. The following 2.2-min linear gradient was used: start at 55% B, maintain for 0.15 min, increase to 85% B over 1.35 min, increase to 95% B over 0.2 min, and maintain at 95% B for 0.5 min. The column was re-equilibrated after each injection. Detection was performed by positive ion MS/MS using a TurboIonSpray interface on an API 5000 mass spectrometer (Applied Biosystems/MDS Sciex) with multiple reaction monitoring (*m/z* 379.2−125.2 for ambrisentan and *m/z* 389.1−125.1 for $[^2H_{10}]$-AMB). Tadalafil was extracted from 25 μL of human plasma containing sodium EDTA by protein precipitation with acetonitrile containing an isotopically labelled internal standard. Plasma extracts were injected onto an Acquity BEH Phenyl column (Waters, 50 × 2.1 mm, 1.7 μm), maintained at 40 °C. The mobile phase consisted of water:acetic acid (100:1, v/v) (solvent A) and acetonitrile:methanol:acetic acid (50:50:1, v/v/v) (Solvent B). A flow rate of 0.6 mL/min was maintained for the entire run. The following 1.8-min linear gradient was used: start at 40% B, maintain for 0.1 min, increase to 70% B over 1.4 min, increase to 98% B over 0.1 min, and maintain at 98% B for 0.2 min. The column was re-equilibrated after each injection. Detection was performed by positive ion MS/MS using a TurboIonSpray interface on an API 4000 mass spectrometer (Applied Biosystems/MDS Sciex) with multiple reaction monitoring (*m/z* 390.1−268.1 for tadalafil and *m/z* 393.1−271.1 for $[^2H_3]$- TAD).

Table S1.

Inclusion Criteria	• Healthy volunteers between 18 and 60 years of age (inclusive); • Body weight ≥50 kg (110 lbs) for men and ≥45 kg (99 lbs) for women and body mass index (BMI) within the range 18−30 kg/m2 (inclusive); • Males or Females of non-reproductive potential; • Capable of giving signed informed consent.
Exclusion Criteria	• Blood pressure <100/55 mm Hg; • Haemoglobin below normal range: <133 g/L for males and <114 g/L for females; • Alanine aminotransferase and bilirubin >1.5xupper limit of normal (ULN) (isolated bilirubin >1.5xULN was acceptable if bilirubin was fractionated and direct bilirubin <35%); • Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones) • QTc >450 msec; • Use of prescription or non-prescription drugs, including vitamins, herbal and dietary supplements (including St John's Wort) within 7 days (or 14 days if the drug was a potential enzyme inducer) or 5 half-lives (whichever was longer) prior to the first dose of study medication; • History of regular alcohol consumption within 6 months of the study; • Smoking more than 5 cigarettes per week and subjects had to abstain from smoking for a 24 h period prior to dose and any time whilst in the clinical unit. • History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy; • Presence of hepatitis B surface antigen (HBsAg), positive hepatitis C antibody test result at Screening or within 3 months prior to first dose of study treatment; • A positive test for Human Immunodeficiency Virus (HIV) antibody; • Exposure to more than four new chemical entities within 12 months prior to the first dosing day. • A positive pre-study drug/alcohol screen. • Where participation in the study would result in donation of blood or blood products in excess of 500 mL within previous 3 months • The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 3 months, 5 half-lives or twice the duration

Table S2.

Preferred Term [n (%)]	Part 1 n=26	Part 2 n=21	Part 3A n=33	Part 3B n=32	Total n=112
Total number of subjects with Adverse Events	22 (85)	19 (90)	32 (97)	26 (81)	99 (88)
Headache	21 (81)	18 (86)	31 (94)	22 (69)	92 (82)
Nasal congestion	6 (23)	7 (33)	8 (24)	4 (13)	25 (22)
Back pain	3 (12)	4 (19)	10 (30)	8 (25)	25 (22)
Nausea	3 (12)	5 (24)	10 (30)	5 (16)	23 (21)
Musculoskeletal pain	4 (15)	6 (29)	0	3 (9)	13 (12)
Pain in extremity	1 (4)	0	9 (27)	0	10 (9)
Total number of subjects with drug-related Adverse Events	20 (77)	19 (90)	32 (97)	26 (81)	97 (87)
Headache	19 (73)	18 (86)	31 (94)	22 (69)	90 (80)
Nasal congestion	6 (23)	7 (33)	8 (24)	4 (13)	25 (22)
Back pain	1 (4)	3 (14)	10 (30)	8 (25)	22 (20)
Nausea	2 (8)	5 (24)	9 (27)	5 (16)	21 (19)
Musculoskeletal pain	2 (8)	6 (29)	1 (3)	3 (9)	12 (11)
Pain in extremity	1 (4)	0	8 (24)	2 (6)	11 (10)

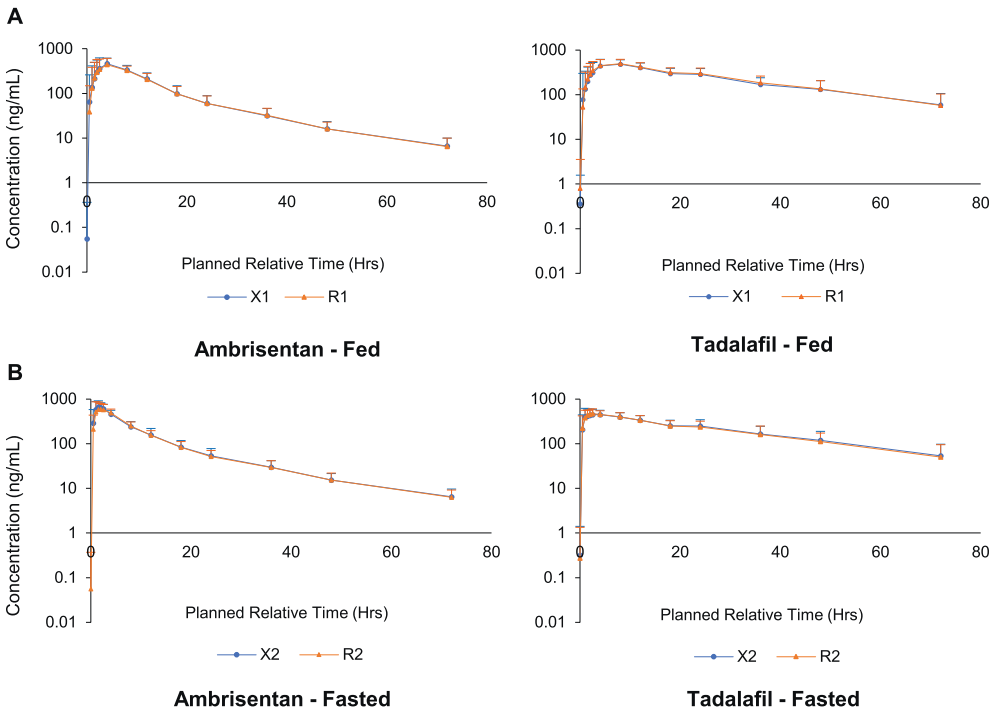

Figure S1.

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