No Clinically Relevant Pharmacokinetic and Safety Interactions of Ambrisentan in Combination With Tadalafil in Healthy Volunteers

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ABSTRACT: Ambrisentan is a nonsulfonamide, ET\(_\alpha\)-selective endothelin receptor antagonist (ERA) approved for the treatment of pulmonary arterial hypertension (PAH), and tadalafil is a phosphodiesterase type 5 (PDE-5) inhibitor under investigation for treatment of PAH. Due to the potential combination use, the pharmacokinetic (PK) interactions between these two drugs were assessed in a crossover study in 26 healthy adults. Single-dose PK of ambrisentan (10 mg) and its metabolite, 4-hydroxymethyl ambrisentan, were determined in the absence and presence of multiple doses of tadalafil (40 mg QD). Similarly, single-dose PK of tadalafil (40 mg) were evaluated in the absence and presence of multiple doses of ambrisentan (10 mg QD). In the presence of tadalafil, ambrisentan maximum plasma concentration (C\(_{\text{max}}\)) was similar (105.0% [90% CI: 95.9–115.0%]) and systemic exposure (AUC\(_{0–\infty}\)) was slightly decreased (87.5% [84.0–91.2%]), compared with ambrisentan alone. Similar changes were observed with 4-hydroxymethyl ambrisentan. Tadalafil C\(_{\text{max}}\) (100.6% [94.4–107.1%]) and AUC\(_{0–\infty}\) (100.2% [92.6–108.4%]) showed no difference in the absence and presence of ambrisentan. The safety profile of the drugs combined was similar to that of either drug alone. No dose adjustments should be necessary when these drugs are coadministered. These results are in contrast to previous reports that the sulfonamide-based ERA bosentan can cause marked decreases in the exposure of tadalafil. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 98:4962–4974, 2009

Keywords: ambrisentan; tadalafil; endothelin receptor antagonist (ERA); phosphodiesterase type 5 (PDE-5) inhibitor; pharmacokinetics; pulmonary arterial hypertension (PAH)

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a rare, life-threatening disease characterized by a progressive increase in pulmonary vascular resistance. Therapies approved for PAH target one of the three main pathways involved in the pathogenesis of PAH—the prostacyclin, nitric oxide (NO), and endothelin (ET-1) pathways.\(^1\)\(^–\)\(^3\) Combining drug products with different mechanisms of action is an evolving strategy for the treatment of PAH.\(^3\)\(^,\)\(^4\)

Sildenafil citrate, which is widely marketed as Viagra\(^\text{R}\) for erectile dysfunction, is the first phosphodiesterase type 5 (PDE-5) inhibitor approved for the treatment of PAH that targets the NO pathway. Through inhibition of PDE-5, sildenafil increases cytoplasmic cGMP concentrations in the smooth muscle cells and enhances NO-mediated vasodilation of the vasculature. As a globally available PAH therapy, sildenafil
Tadalafil is administered orally three times daily (TID). Tadalafil is another PDE-5 inhibitor commonly prescribed for erectile dysfunction (Cialis) that is currently under investigation for the treatment of PAH. Tadalafil is also orally administered, but has the advantage of a longer half-life, allowing for once-daily (QD) administration. Data from a Phase 3 placebo-controlled PAH study, which examined tadalafil doses that ranged from 2.5 to 40 mg QD, is currently under regulatory review.

Endothelin receptor antagonists (ERAs), which target the phospholipase-C-dependent endothelin pathway, are another class of drugs for the treatment of PAH. ET-1 is the primary member of a family of potent vasoconstrictor peptides, which are known to play an essential role in mammalian cardiovascular physiology. Two receptor subtypes, endothelin receptor type A (ET\textsubscript{A}) and endothelin receptor type B (ET\textsubscript{B}), mediate the effects of ET-1. Bosentan (Tracleer) is a twice-daily, orally active ET\textsubscript{A}/ET\textsubscript{B}-selective ERA, and sitaxsentan (Thelin) is an ET\textsubscript{A}-selective ERA administered once daily. Both bosentan and sitaxsentan are sulfonamide-based ERAs metabolized by the cytochrome P450 (CYP) enzyme system. Ambrisentan (Letairis, Volibris) is an oral, once daily, propanoic acid-based, ET\textsubscript{A}-selective ERA approved for the treatment of PAH.

The ease of administration makes a PDE-5 inhibitor and an ERA a likely pair for initial combination therapy. However, as with any concomitant treatment, the risk of adverse drug–drug interactions must be carefully considered. Tadalafil is rapidly absorbed with a maximum plasma concentration achieved at a median of 2 h; the rate and extent of absorption are not influenced by food. Approximately 94% of tadalafil is protein-bound. Similar to sildenafil, tadalafil is metabolized by the CYP pathway and in particular by CYP3A4. Tadalafil is converted to a catechol metabolite, which then undergoes extensive methylation and glucuronidation to form methylcatechol and methylcatechol glucuronide, the major circulating metabolite. These metabolites are not expected to be pharmacologically active at observed metabolite concentrations.

Excretion is predominantly as metabolites, mainly in the feces.

Ambrisentan is also rapidly absorbed into the systemic circulation ($t_{\text{max}} \sim 2$ h), and is highly plasma protein bound with a low distribution into red blood cells. Concentrations decline over time in a multi-exponential manner, and elimination is predominantly by nonrenal pathways. Both $C_{\text{max}}$ and $AUC_{0-\infty}$ increase proportionally over the therapeutic range of the drug, and pharmacokinetics (PK) were similar in a fed and fasted state. In contrast to the sulfonamide-based ERAs, ambrisentan is primarily metabolized by hepatic glucuronidation through several uridine diphospho-glucuronosyltransferase (UGT) enzymes.

To a minimal extent, ambrisentan also undergoes oxidative metabolism by the CYP pathway to form the metabolite, 4-hydroxymethyl ambrisentan. In addition, in vitro and in vivo studies demonstrated that ambrisentan does not inhibit or induce CYP enzymes; thus minimizing the risk for drug–drug interactions with other agents metabolized by CYP enzymes.

Due to the high clinical interest in a QD, orally administered combination of agents with non-overlapping mechanisms of action and metabolic pathways, the PK of ambrisentan and 4-hydroxymethyl ambrisentan in the presence of tadalafil, as well as the PK of tadalafil in the presence of ambrisentan, were evaluated. Furthermore, the safety and tolerability of the drug combination was assessed.

**METHODS**

**Caution:** the following chemicals are hazardous and were used in the analysis of ambrisentan, 4-hydroxymethyl ambrisentan and tadalafil plasma concentrations; toluene, trifluoroacetic acid, acetonitrile, and methanol. Animal studies have shown ERAs, including ambrisentan, to be teratogenic; therefore, administration is contraindicated in pregnancy. Ambrisentan should be handled appropriately to avoid exposure to women who are pregnant or breast-feeding.

**Subjects**

Men and women, age 18–55 years, with body mass index of 18.5–29.9, weight $\geq 50$ kg, and in good health were eligible for enrollment in this study. Women must have been surgically sterile or postmenopausal. Other key inclusion criteria included no tobacco use within 12 months prior to study; discontinuation of caffeine, alcohol, grapefruit, foods that interfere with CYP enzymes, and organic nitrates from 72 h before administration to 96 h after administration; no
blood or sperm donation throughout the study and 30 days after the last dose; normal cardiovascular function; serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations ≤1× the upper limit of normal; platelet count ≥100 × 10^9/L; normal coagulation parameters; negative results for HIV, hepatitis B and C; and negative results for drug and alcohol use at screening and at baseline.

Subjects were excluded from participation in the study if they had a history of asthma or significant allergies; had significant blood loss ≤30 days before administration; had received prescription, over the counter medications, or herbal remedies within 14 days of dosing until 96 h after last dose; had a history of angina, arrhythmia, hypotension or hypertension; had a family history of degenerative retinal disorders, sickle cell disease, or any bleeding disorder; had history of penile angulation or any other anatomical deformation of the penis; had a history of alcohol or illicit drug abuse or used tobacco in the last 12 months; had any clinical condition deemed unsuitable for study compliance; and/or had known hypersensitivity to ambrisentan or tadalafil. All volunteers provided written informed consent prior to enrollment in the study.

**Study Design**

Subjects were randomized to one of two treatment regimen sequences: ABC/DEF or DEF/ABC with a 7- to 10-day washout period between treatment periods (Fig. 1). Treatment A consisted of a single dose of 10 mg ambrisentan under fasted conditions on day 1, followed by a 72-h PK assessment of ambrisentan and its primary metabolite, 4-hydroxymethyl ambrisentan. Treatment B consisted of 40 mg (2 mg × 20 mg) tadalafil QD on days 4–8; tadalafil trough plasma concentrations were determined on days 6–9 prior to the daily tadalafil dose. Treatment C consisted of the coadministration of a single 10 mg dose of ambrisentan and 40 mg tadalafil on day 9, followed by a 72-h PK assessment of ambrisentan and metabolite. On days 10 and 11, tadalafil (40 mg QD) was continued. Subjects were discharged on day 12 of this period following the completion of study procedures and collection of the final PK blood sample.

*Figure 1.* Study design schematic. Treatment (A): single dose of ambrisentan 10 mg on day 1, and ambrisentan PK assessments on days 1–4. Treatment (B): 40 mg tadalafil QD on days 4–8 with tadalafil trough concentrations determined on days 6–9. Treatment (C): single 10 mg dose of ambrisentan (on day 9) plus 40 mg tadalafil QD on days 9–11, with ambrisentan PK assessment on days 9–12. Treatment (D): single dose of tadalafil 40 mg on day 1, with tadalafil PK assessments on days 1–5. Treatment (E): 10 mg ambrisentan QD on days 5–8, with ambrisentan trough concentrations determined on days 6–9. Treatment (F): single 40 mg dose of tadalafil (on day 9) plus 10 mg ambrisentan QD on days 9–12, with tadalafil PK assessment on days 9–13. ABS = ambrisentan; TDF = tadalafil; PK = pharmacokinetics; QD = once daily.
Treatment D consisted of a single dose of tadalafil 40 mg (2 mg x 20 mg) under fasted conditions on day 1, followed by a 96-h PK assessment. Treatment E consisted of 10 mg ambrisentan QD on days 5–8; ambrisentan and 4-hydroxymethyl ambrisentan trough plasma concentrations were determined on days 6–9 before the daily ambrisentan dose. Treatment F consisted of the coadministration of a single 40 mg dose of tadalafil and 10 mg ambrisentan on day 9, followed by a 96-h PK assessment of tadalafil. On days 10–12, ambrisentan (10 mg QD) was continued. Subjects were discharged on day 13 of this period following the completion of study procedures and collection of the final PK blood sample.

Treatment regimen ABC was 12-days in duration and DEF was 13 days, and subjects were confined to the clinical facility for the duration of each treatment regimen. A follow-up visit was performed 6–10 days after the last dose of study drug. Concomitant medications were prohibited with the exception of acetaminophen (≤3 doses at ≤1 g each). This study was approved by the Independent Investigational Review Board and was conducted in accordance with the Declaration of Helsinki and the International Conference of Harmonization guidelines.

Analytical Methods and Pharmacokinetic Assessments

On days 1 and 9 during regimen ABC, blood samples for ambrisentan and 4-hydroxymethyl ambrisentan PK were collected predose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48, and 72 h postdose. Blood samples were also collected at predose on days 6–9 to evaluate tadalafil trough plasma concentrations. On days 1 and 9 during regimen DEF, blood samples for tadalafil PK were collected at the time points mentioned above, as well as 96 h postdose. Blood samples were also collected at predose on days 6–9 to assess ambrisentan and 4-hydroxymethyl ambrisentan trough plasma concentrations.

Blood samples were collected in tubes containing K3-EDTA for tadalafil and sodium citrate for ambrisentan, immediately mixed by gentle inversion to prevent coagulation, and then placed on ice prior to centrifugation. Samples were centrifuged at 1500 x g for 15 min at 4°C ≤30 min after collection to separate plasma from cells. Plasma samples were stored at −20°C until analysis.

Plasma concentrations of ambrisentan, 4-hydroxymethyl ambrisentan, and tadalafil were measured by validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods at AMDEQuant (Colorado Springs, CO). Plasma concentrations of tadalafil were determined using tadalafil-methyl-d15 (Toronto Research Chemicals, Ontario, Canada) as an internal standard, calibration standards, and QC samples with each sample set. Tadalafil was extracted from 0.25 mL of plasma by precipitating proteins with equal volume of acetonitrile containing 0.05% trifluoroacetic acid. The supernatant was injected into a Perkin Elmer SCIEX API 3000 (Concord, Ontario, Canada) mass analyzer using a C18 HPLC column. Quantitation was carried out using the positive ionization mode with transition masses of 390.3 and 268.2. Tadalafil calibration curve ranged from 10 to 3000 ng/mL, with a lower limit of quantitation at 10 ng/mL. The precision of the assay was within ±15% as indicated by the percent coefficient of variation, and the accuracy was within ±15% of the actual concentration.

Plasma concentrations of ambrisentan and 4-hydroxymethyl ambrisentan were determined using a validated method that included an internal standard (BSF 127041, 2-[4,6-dimethoxy- pyrimidin-2-yl oxy]-3-phenoxy-3-phenyl-butyric acid), calibration standards, and QC samples. Ambrisentan and 4-hydroxymethyl ambrisentan were extracted from 0.1 mL plasma by liquid-liquid partition with acidified toluene. The organic phase was separated and evaporated under nitrogen. The residue was reconstituted in acetonitrile and injected into the LC-MS/MS system (PE SCIEX API 4000 QTrap, Concord) using a C18 HPLC column. Detection was by positive ionization mode with transition masses for ambrisentan equal to 379.2 and 125.2, and for 4-hydroxymethyl ambrisentan, 395.2 and 319.3. The calibration curve range was 1–400 ng/mL for ambrisentan and 0.2–100 ng/mL for 4-hydroxymethyl ambrisentan using a 0.1 mL of plasma sample aliquot. The precision of ambrisentan assay was ≤15% as indicated by the percent coefficient of variation, and the accuracy of the assay was within ±15% of the actual value for ambrisentan. The lower limit of quantitation for both ambrisentan and 4-hydroxymethyl ambrisentan as 0.5 ng/mL.

Pharmacokinetic analyses were performed by noncompartmental methods using WinNonlin-Pro Version 4.0.1 (Pharsight Corp, Palo Alto, CA). Plasma pharmacokinetic parameters included area under the plasma concentration-time
curve (AUC) from time 0 to infinity (AUC_{0→∞}) or last measurement (AUC_{0→last}), maximum plasma concentration (C_{max}), time to maximum concentration (t_{max}), and apparent terminal half-life (t_{1/2}), calculated as 0.693/\lambda_z, where \lambda_z is the apparent terminal elimination rate.

Safety Assessments

Adverse events (AEs) were monitored throughout the study. Electrocardiograms (ECG) were performed at screening, at the start of each treatment period, and at follow-up. Vital signs were measured at the start of each treatment period, at prespecified times throughout both treatment sequences, at discharge for each period, and at follow-up. Clinical laboratory parameters, including clinical chemistry, hematology, coagulation tests, and urinalysis were evaluated at the start of each period, at discharge for each period and at follow-up.

Statistical Analysis

The safety population consisted of all subjects who were treated with \geq 1 dose of either study drug. Safety data were summarized using descriptive methods, including N, mean, standard deviation (SD), median, minimum, and maximum for continuous measures, and counts and percent of subjects for categorical parameters. Descriptive statistics were used for demographics, baseline characteristics, ECGs, vital signs, and clinical laboratory tests.

The PK evaluable population for Treatment Regimen ABC consisted of all subjects who received study drug regimens A, B, and C and had sufficient plasma concentration data for assessment of ambrisentan C_{max} and AUC_{0→last} following Treatment A and Treatment C. The PK evaluable population for treatment regimen DEF consisted of all subjects who received study drug regimens D, E, and F and had sufficient plasma concentration data for assessment of tadalafil C_{max} and AUC_{0→last} following Treatment D and Treatment F. Trough plasma concentration analyses were conducted on these populations. Pharmacokinetic parameters (AUC_{0→∞}, AUC_{0→last}, and C_{max}) were natural log transformed for analysis and described by geometric mean ratios (GMRs) for the primary analysis. Point estimates and 2-sided 90% confidence intervals (CIs) were used to determine the effect of one study drug on the PK of the other study drug: if a CI was contained within 80–125%, it was concluded that no clinically relevant drug–drug interaction occurred. Steady-state assessments were conducted using analysis of variance (ANOVA). All statistical analyses were conducted using the SAS® software Version 8.2 (SAS Institute, Inc., Cary, NC).

RESULTS

Demographics and Baseline Characteristics

Twenty-six healthy volunteers were enrolled in the study and 23 (88.5%) completed both treatment regimens. Three subjects were discontinued prematurely from the study due to AEs of headache, myalgia, and anemia (described below). The majority of the subjects were male (73.1%). The mean age was 44.4 years ± 8.28 years, and the mean body weight was 76.4 ± 10.11 kg. The majority of subjects (84.6%) were White/Caucasian, and all 26 subjects had a Hispanic or Latino ethnic descent.

Pharmacokinetics

Ambrisentan and 4-Hydroxymethyl Ambrisentan PK Parameters

Following a single dose of ambrisentan alone (Treatment A), the PK of ambrisentan and 4-hydroxymethyl ambrisentan were assessed. After 4 days of QD dosing, tadalafil reached a steady state (Treatment B), after which, the PK of ambrisentan and metabolite in the presence of tadalafil at steady-state were assessed (Treatment C).

Ambrisentan was rapidly absorbed into the systemic circulation, with a time to peak plasma concentration (t_{max}) of 2.5 h in both the absence and presence of tadalafil (Fig. 2). Concentrations of ambrisentan remained above the lower limit of quantitation (LLQ) for all subjects until 48 h after dosing. The apparent terminal half-life (t_{1/2}) of ambrisentan was typical of that previously reported, and was not altered by the presence of tadalafil (Tab. 1). PK parameters, C_{max}, AUC_{0→last}, and AUC_{0→∞}, determined during ambrisentan plus tadalafil administration were similar to those determined during ambrisentan alone. The 90% CIs of the GMRs for all PK parameters were well

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within the prespecified no effect range (80–125%), indicating that ambrisentan PK was unaffected by tadalafil coadministration.

The primary oxidative metabolite of ambrisentan following oral administration is 4-hydroxymethyl ambrisentan formed via the CYP pathway. In addition to ambrisentan, the effect of tadalafil on the PK of this metabolite was also assessed in Treatment Regimen ABC. The maximum plasma concentrations of 4-hydroxymethyl

Table 1. Pharmacokinetics of Ambrisentan Following Administration in the Absence and Presence of Multiple Doses of Tadalafil (N = 23)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Arithmetic Mean ± SD</th>
<th>Geometric Mean&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Geometric Mean Ratio&lt;sup&gt;b&lt;/sup&gt; (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>ABS</td>
<td>838.3 ± 242.7</td>
<td>804.1</td>
<td>105.0 (95.9, 115.0)</td>
</tr>
<tr>
<td></td>
<td>ABS + TDF</td>
<td>898.5 ± 357.4</td>
<td>844.5</td>
<td></td>
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<tr>
<td>AUC&lt;sub&gt;0-last&lt;/sub&gt; (ng · h/mL)</td>
<td>ABS</td>
<td>6705.6 ± 1811.4</td>
<td>6468.6</td>
<td>88.4 (84.7, 92.4)</td>
</tr>
<tr>
<td></td>
<td>ABS + TDF</td>
<td>5879.8 ± 1387.0</td>
<td>5720.6</td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (ng · h/mL)</td>
<td>ABS</td>
<td>6976.5 ± 1870.3</td>
<td>6734.1</td>
<td>87.5 (84.0, 91.2)</td>
</tr>
<tr>
<td></td>
<td>ABS + TDF</td>
<td>6060.4 ± 1451.3</td>
<td>5893.5</td>
<td></td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>ABS</td>
<td>18.9 ± 7.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>ABS + TDF</td>
<td>15.1 ± 6.7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>ABS</td>
<td>2.5 (1.0, 8.0)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>ABS + TDF</td>
<td>2.5 (1.0, 3.0)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ABS, ambrisentan; TDF, tadalafil; SD, standard deviation; CI, confidence interval; C<sub>max</sub>, maximum plasma concentration; AUC<sub>0-last</sub>, area under the plasma concentration-time curve from time 0 to last measurable concentration; AUC<sub>0-∞</sub>, area under the plasma concentration-time curve from time 0 to infinity; t<sub>max</sub>, time to maximum plasma concentration; t<sub>1/2</sub>, apparent terminal half-life; ND, not done.

<sup>a</sup>Least squares geometric mean.

<sup>b</sup>(ABS + TDF)/ABS.
ambrisentan occurred later than parent ambrisentan, at a median of 10 h after dosing (Fig. 3). The 4-hydroxymethyl ambrisentan metabolite was observed to have a $C_{\text{max}} < 1\%$ and exposures of approximately 4% of those determined for the ambrisentan parent (Tabs. 1 and 2).

Concentration-time profiles of 4-hydroxymethyl ambrisentan in the absence and presence of tadalafl did not differ considerably (Fig. 3). Mean peak plasma concentrations were similar, and although the median time to reach $C_{\text{max}}$ occurred earlier in the presence of tadalafl (6.0 vs. 10.0 h), the $t_{\text{max}}$ range was quite variable for both PK assessments of the metabolite. The mean $t_{1/2}$ of the metabolite (33.8 ± 28.2 h) was greater compared to ambrisentan parent (19 h), and tadalafl did not affect the metabolite half-life (24.0 ± 14.3 h) beyond the inter-subject variability observed. In the context of this anticipated inter-subject variability, the $t_{\text{max}}$ and $t_{1/2}$ values were comparable for ambrisentan alone and in the presence of tadalafl. Overall, there was little effect on the PK of the metabolite in the presence of tadalafl at steady-state concentrations (Tab. 2). Only the GMR 90% CI lower limit of $AUC_{0-\infty}$ (74.1%) was outside the no effect range (80–125%); whereas, the upper limit was within the range (98.61%, Tab. 2). Because $AUC_{0-\infty}$ was extrapolated by >20% for half of the subjects evaluated, this parameter should be considered with caution.

**Tadalafil PK Parameters**

Following a single dose of tadalafl alone (Treatment D), the PK of tadalafl alone were assessed. After 4 days of multiple QD dosing, ambrisentan reached a steady state (Treatment E), after which, the PK of tadalafl in the presence of ambrisentan at steady-state were assessed (Treatment F).

The PK profile of tadalafl in the presence of ambrisentan at steady-state concentrations was nearly superimposable compared to tadalafl administered alone (Fig. 4). Peak plasma concentrations occurred approximately 2.5 h following tadalafl or tadalafl/ambrisentan administration, and for most subjects, tadalafl concentrations remained above LLQ until 96 h after dosing. The apparent terminal half-life ($t_{1/2}$) of tadalafl was 27 h in the absence or presence of ambrisentan (Tab. 3). The GMRs for $C_{\text{max}}$, $AUC_{0-\text{last}}$, and $AUC_{0-\infty}$ were approximately 100%, and all 90% CIs were within the prespecified no effect range. Based on these results, tadalafl PK is unaffected by ambrisentan coadministration.

**Safety and Tolerability**

Coadministration of ambrisentan and tadalafl was generally safe and well tolerated. Table 4 presents the most frequently reported AEs by Treatment Period. Headache was the most common AE experienced during this study, with
the majority of headaches reported during tadala
taf administration. Similarly, myalgia and back pain
were reported frequently following tadala
taf administration. Five subjects experienced tachy-
cardia during multiple QD dosing of ambrisentan;
three subjects experienced this event during the
multiple QD dosing period with tadala
taf, whereas
only one subject experienced tachycardia after
coadministration of the two drugs. Although
decreases in hemoglobin and/or hematocrit were
observed in some subjects, only one subject
experienced a decrease that was considered
clinically significant; this event was reported as
an AE of anemia which led to study discontinua-
tion (see below). No other shift in clinical
laboratory values was considered clinically sig-
ificant. A thorough monitoring of systolic and
diastolic blood pressure showed decreases during
treatment with either study drug alone or in
combination; however, there were no apparent
additive or synergistic effects on mean blood
pressure when ambrisentan and tadala
taf were
coadministered.

No death or other serious AE was reported. As
stated earlier, three subjects discontinued the
study prematurely due to AEs. One subject
experienced a severe headache after receiving
two doses of tadala
taf during Treatment B.
Another subject experienced severe myalgia,
along with mild headache, muscle fatigue, and
sinus tachycardia, following one dose of tadala
taf during Treatment B; the subject received a second
dose of tadala
taf but continued to experience
myalgia and muscle fatigue, and withdrew con-
sent before receiving the third dose. A third
subject, with normal hemoglobin values at screen-
ing, developed a hemoglobin decrease (slightly
below the lower limit of normal) at baseline prior
to the first dose of study drug. Laboratory tests
were evaluated on the last day of ambrisentan QD
dosing in Treatment F, and the subject showed
further decreases in hemoglobin (−2 g/dL from
baseline), reported as mild anemia. Because
hemoglobin remained low after the wash-out
period (−1.6 g/dL), the subject was discontinued
by the Investigator prior to starting Treatment
ABC. The subject was followed until hemoglobin
levels returned to the baseline value approxi-
mately 4 weeks after the last dose of study drug.
The Investigator felt the event was unrelated to
study treatment.

**DISCUSSION**

The development of various therapies with
different mechanisms of action has permitted
tremendous advances in the treatment of PAH.
Simultaneously combating more than one of the
intracellular targets involved in the pathogenesis

**Table 2.** Pharmacokinetics of 4-Hydroxymethyl Ambrisentan Following Administration of Ambrisentan in the
Absence and Presence of Multiple Doses of Tadala
taf, N = 23

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Arithmetic Mean ± SD</th>
<th>Geometric Meana</th>
<th>Geometric Mean Ratiob (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>ABS</td>
<td>7.2 ± 2.7</td>
<td>6.8</td>
<td>105.8 (96.4, 116.0)</td>
</tr>
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<td></td>
<td>ABS + TDF</td>
<td>7.6 ± 2.8</td>
<td></td>
<td>7.1</td>
</tr>
<tr>
<td>AUC0−last (ng·h/mL)</td>
<td>ABS</td>
<td>220.4 ± 106.1</td>
<td>195.9</td>
<td>93.3 (83.6, 104.1)</td>
</tr>
<tr>
<td></td>
<td>ABS + TDF</td>
<td>199.6 ± 83.8</td>
<td></td>
<td>182.7</td>
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<tr>
<td>AUC0−∞ (ng·h/mL)</td>
<td>ABS</td>
<td>308.3 ± 155.8</td>
<td>269.2</td>
<td>85.5 (74.1, 98.6)</td>
</tr>
<tr>
<td></td>
<td>ABS + TDFc</td>
<td>250.9 ± 100.0</td>
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<td>230.1</td>
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<tr>
<td>t1/2 (h)</td>
<td>ABS</td>
<td>33.8 ± 28.2</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td></td>
<td>ABS + TDFc</td>
<td>24.0 ± 14.3</td>
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</tr>
</tbody>
</table>

ABS, ambrisentan; TDF, tadala
taf; SD, standard deviation; CI, confidence interval; Cmax, maximum plasma concentration; AUC0−last, area under the plasma concentration-time curve from time 0 to last measurable concentration; AUC0−∞, area under the plasma concentration-time curve from time 0 to infinity; tmax, time to maximum plasma concentration; t1/2, apparent terminal half-life; ND, not done.

aLeast squares geometric mean.
b(ABS + TDF)/ABS.
cN = 21. λz could not be determined for two subjects; therefore, AUC0−∞ and t1/2 could not be determined.
**Figure 4.** Plasma concentration-time profile of tadalafl following a single dose of tadalafl alone (day 1) and a single dose of tadalafl when coadministered with ambrisentan at steady-state concentrations (day 9). Data are means ± standard deviations. Inset shows the same data over the first 24 h. ABS = ambrisentan; TDF = tadalafl.

**Table 3.** Pharmacokinetics of Tadalafl Following Administration in the Absence and Presence of Multiple Doses of Ambrisentan, N = 24

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Arithmetic Mean ± SD</th>
<th>Geometric Mean&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Geometric Mean Ratio&lt;sup&gt;b&lt;/sup&gt; (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>TDF</td>
<td>596.0 ± 131.0</td>
<td>581.1</td>
<td>100.6 (94.4, 107.1)</td>
</tr>
<tr>
<td></td>
<td>ABS + TDF</td>
<td>596.4 ± 129.2</td>
<td>584.3</td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-last&lt;/sub&gt; (ng·h/mL)</td>
<td>TDF</td>
<td>18579.9 ± 6025.7</td>
<td>17648.6</td>
<td>99.9 (92.9, 107.5)</td>
</tr>
<tr>
<td></td>
<td>ABS + TDF</td>
<td>18658.6 ± 6379.0</td>
<td>17635.6</td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (ng·h/mL)</td>
<td>TDF</td>
<td>21206.7 ± 8820.1</td>
<td>19634.7</td>
<td>100.2 (92.6, 108.4)</td>
</tr>
<tr>
<td></td>
<td>ABS + TDF</td>
<td>21217.8 ± 8559.6</td>
<td>19669.7</td>
<td></td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>TDF</td>
<td>27.4 ± 9.6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>ABS + TDF</td>
<td>27.4 ± 7.9</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Median (Min, Max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>TDF</td>
<td>2.50 (1.5, 4.0)</td>
</tr>
<tr>
<td></td>
<td>ABS + TDF</td>
<td>2.75 (1.5, 6.0)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Least squares geometric mean.

<sup>b</sup>(ABS + TDF)/ABS.

ABS, ambrisentan; TDF, tadalafl; SD, standard deviation; CI, confidence interval; C<sub>max</sub>, maximum plasma concentration; AUC<sub>0-last</sub>, area under the plasma concentration-time curve from time 0 to last measurable concentration; AUC<sub>0-∞</sub>, area under the plasma concentration-time curve from time 0 to infinity; t<sub>max</sub>, time to maximum plasma concentration; t<sub>1/2</sub>, apparent terminal half-life; ND, not done.
of this disease is an ever-increasing strategy that is supported by several exploratory studies which have shown synergistic benefits. 12–15

The current study was conducted to assess the interaction between tadalafil, a PDE-5 inhibitor, and ambrisentan, an ETₐ-selective ERA. The results demonstrate no clinically relevant pharmacologic interactions on ambrisentan or its primary metabolite, 4-hydroxymethyl ambrisentan, when coadministered with tadalafil. Similarly, the single-dose pharmacokinetics of tadalafil did not change in the presence of ambrisentan. The two drugs given alone or in combination were generally well-tolerated.

The single-dose PK of 10 mg ambrisentan alone observed in this study were similar to previous observations, where ambrisentan is rapidly absorbed with maximum plasma concentrations occurring approximately 2.5 h after dosing and an apparent half-life of ~15 h. 9,16 Tadalafil, at steady-state concentrations, had a minimal effect on the PK of ambrisentan. The Cₘₐₓ of ambrisentan was similar when administered alone or in the presence of tadalafil, and although ambrisentan exposure (AUC) was slightly decreased in the presence of tadalafil, 90% CIs for ambrisentan PK parameters (Cₘₐₓ, AUC₀₋ₐₙₙ, and AUC₀₋∞) were all within the 80–125% no effect range. 10 Although no formal statistical comparisons between treatments were performed for ambrisentan tₘₐₓ and t₁/₂, these parameters were similar when ambrisentan was administered alone or in combination with tadalafil.

Exposures of the 4-hydroxymethyl metabolite of ambrisentan were low at approximately 4% of parent ambrisentan and with a delayed tₘₐₓ relative to parent drug. The binding affinity of this metabolite for the human ETₐ receptor is approximately 60-fold less than that of ambrisentan; 11 therefore, at concentrations observed in the plasma, 4-hydroxymethyl ambrisentan is not expected to contribute to the pharmacological activity of ambrisentan. Nevertheless, this metabolite is formed via the CYP enzyme pathway, lending to a potential interaction with tadalafil, another CYP-metabolized compound. The results presented here show that, similar to parent ambrisentan, the PK of 4-hydroxymethyl ambrisentan was unaffected by multiple doses of tadalafil. The PK parameters, Cₘₐₓ and AUC₀₋ₐₙₙ, were within the no effect range, and these results are consistent with the fact that tadalafil does not affect CYP metabolism. 7

Following a single oral dose of 40 mg tadalafil, peak plasma concentrations occurred at 2.50–2.75 h. The median t₁/₂ was similar both when tadalafil was administered alone and in the presence of ambrisentan. These results are largely

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**Table 4. Summary of Adverse Events**

<table>
<thead>
<tr>
<th>Parameter, n (%)</th>
<th>Treatment Regimen ABC</th>
<th>Treatment Regimen DEF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABS Alone (N = 25)</td>
<td>TDF Alone (N = 25)</td>
</tr>
<tr>
<td>Subjects with at least 1 AE</td>
<td>0 (0.0)</td>
<td>18 (72.0)</td>
</tr>
<tr>
<td>Back pain</td>
<td>0 (0.0)</td>
<td>6 (24.0)</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>0 (0.0)</td>
<td>4 (16.0)</td>
</tr>
<tr>
<td>Headache</td>
<td>0 (0.0)</td>
<td>6 (24.0)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>0 (0.0)</td>
<td>3 (12.0)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>0 (0.0)</td>
<td>6 (24.0)</td>
</tr>
<tr>
<td>Nasal congestion</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Nausea</td>
<td>0 (0.0)</td>
<td>2 (8.0)</td>
</tr>
<tr>
<td>Pain</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Tachycardia*</td>
<td>0 (0.0)</td>
<td>3 (12.0)</td>
</tr>
</tbody>
</table>

ABS, ambrisentan; TDF, tadalafil AEs reported by >1 subject.

*Includes events of sinus tachycardia and tachycardia.

During treatment regimen ABC, an AE with a start date on days 1–3, days 4–8, or days 9–11 was counted in the “Ambrisentan Alone,” “Tadalafil Alone,” or “Ambrisentan and Tadalafil” treatment group, respectively. During treatment regimen DEF, an AE with a start date on days 1–4, days 5–8, or days 9–12 was counted in the “Tadalafil Alone,” “Ambrisentan Alone,” or “Tadalafil and Ambrisentan” treatment group, respectively. An AE with a start date on or after the last day of the subject’s last treatment (Treatment C or Treatment F) through the follow-up visit (if after the second treatment period) or the check-in visit for the second treatment period (including washout) was counted in the “Ambrisentan and Tadalafil” (i.e., Treatment C) or “Tadalafil and Ambrisentan” (i.e., Treatment F) treatment group, respectively, for that treatment regimen sequence.

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consistent with the known PK of tadalafl in healthy male subjects demonstrating low systemic clearance and a prolonged elimination half-life. Although the $t_{1/2}$ observed in this study alone and in the presence of ambrisentan is longer than that previously reported (17.5 h), the reasons for this discrepancy are unknown; the relatively small sample size and single-dose assessment of the current study limits the conclusions that can be drawn regarding this potential difference. In the presence of ambrisentan at steady-state concentrations, the PK of tadalafl was unchanged compared to administration alone, and all GMR 90% CIs for $C_{\text{max}}$ and AUC were within the no effect range.

The results of this study are consistent with recent reports of no clinically relevant interactions when ambrisentan was coadministered with either sildenafil or warfarin, both CYP substrates. This report is particularly well-timed and clinically relevant, as safety and efficacy data for tadalafl are currently under regulatory review for the treatment of PAH. As ambrisentan is already indicated for this disease, the oral, once-daily combination of this PDE-5 inhibitor and ET-A-selective ERA could become the first-choice combination therapy. The primary metabolic pathway of ambrisentan by glucuronidation, rather than CYP-mediated mechanisms, gives this ERA an important distinction among the ERAs. Results from an open-label, randomized study in healthy adult males demonstrated that when coadministered with bosentan, tadalafl $C_{\text{max}}$ and AUC decreased by 27% and 42%, respectively, consistent with the CYP-inducing properties of bosentan. When coadministered with sildenafil, there was a substantial increase in bosentan $C_{\text{max}}$ (42%) and AUC (50%); whereas, tadalafl coadministration had less effect on the exposure of bosentan with a 19.5% and 12.6% increase in $C_{\text{max}}$ and AUC, respectively. The combination of tadalafl and bosentan, compared to each drug alone, appeared to increase the incidence of some AEs, and although larger well-controlled studies in PAH patients are required to fully evaluate the safety profile, careful monitoring of safe and efficacious doses appears necessary.

The safety evaluation of ambrisentan and tadalafl coadministration performed in the current study showed that the combination was safe and well tolerated. The types and frequencies of AEs observed in this study are in line with previous reports for both ambrisentan and tadalafl. Overall, the most common AEs observed with either drug or with the combination were headache, back pain, myalgia, and tachycardia. Tachycardia has not been reported to any appreciable extent in large, placebo-controlled studies of ambrisentan in PAH patients; therefore, it is unlikely to be clinically relevant and may be due to the small number of subjects in this trial. The event of mild anemia that led to one subject’s premature discontinuation of the study was considered unrelated to treatment, which is reasonable given the observed decrease prior to dosing and continued low hemoglobin well after the last dose of study drug. However, it should be noted that decreases in hemoglobin and hematocrit are known ERA class effects; these decreases may be observed soon after initiation of treatment and remain constant with continued therapy. Decreases in systolic and diastolic blood pressure were observed in this study, consistent with the vasodilatory properties of the study drugs. The changes were not considered clinically significant and did not require dose changes, interruptions, or discontinuations. Importantly, there were no apparent additive or synergistic effects on blood pressure when ambrisentan and tadalafl were coadministered. No patients on ambrisentan experienced peripheral edema or elevations in aminotransferases, two AEs associated with ERA administration.

Limitations of this study include the generally small sample size consisting of healthy subjects and the short duration of treatment. A larger study involving PAH patients will be needed to determine whether the safety results observed in healthy subjects are similar to PAH patients. Compared to healthy subjects, increases in exposure have been observed for ambrisentan in PAH patients and for tadalafl in subjects with renal insufficiency. This phenomenon is expected to be observed and unchanged when ambrisentan and tadalafl are coadministered in PAH patients.

In conclusion, the single-dose pharmacokinetics of the ambrisentan and its metabolite, 4-hydroxymethyl ambrisentan were similar in the presence or absence of steady-state concentrations of tadalafl. Similarly, steady-state concentrations of ambrisentan had no effect on the single-dose pharmacokinetics of tadalafl. No significant safety concerns were observed in this study that would preclude this coadministration. These data indicate that no dose adjustment of ambrisentan or tadalafl should be necessary with this combination therapy.
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REFERENCES

