

Two Phase I, Open-Label, Mass Balance Studies to Determine the Pharmacokinetics of ¹⁴C-Labeled Isavuconazonium Sulfate in Healthy Male Volunteers

Clinical Pharmacology
in Drug Development
2018, 7(2) 207–216

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Pharmacology in Drug Development

Published by Wiley Periodicals, Inc. on
behalf of The American College of

Clinical Pharmacology

DOI: 10.1002/cpdd.376

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Abstract

Isavuconazonium sulfate is the water-soluble prodrug of the active triazole isavuconazole. Two phase I studies were conducted to identify the metabolic profile and mass balance of isavuconazole and BAL8728 (inactive cleavage product). Seven subjects in study 1 (isavuconazole mass balance) received a single oral dose of [cyano-¹⁴C]isavuconazonium sulfate corresponding to 200 mg isavuconazole. Six subjects in study 2 (BAL8728 mass balance) received a single intravenous dose of [pyridinylmethyl-¹⁴C]isavuconazonium sulfate corresponding to 75 mg BAL8728. Pharmacokinetic parameters of radioactivity in whole blood and plasma and of isavuconazole and BAL8728 in plasma were assessed. Radioactivity ratio of blood/plasma, percentage of dose, and cumulative percentage of radioactive dose recovered in urine and feces for isavuconazole and BAL8728 were assessed. Metabolic profiling was carried out by high-performance liquid chromatography and mass spectrometry. Mean plasma isavuconazole pharmacokinetic parameters included apparent clearance (2.3 ± 0.7 L/h), apparent volume of distribution (301.8 ± 105.7 L), and terminal elimination half-life (99.9 ± 44.6 hours). In study 1, isavuconazole-derived radioactivity was recovered approximately equally in urine and feces (46.1% and 45.5%, respectively). In study 2, BAL8728-derived radioactivity was predominantly recovered in urine (96.0%). Isavuconazole (study 1) and M4 (cleavage metabolite of BAL8728; study 2) were the predominant circulating components of radioactivity in plasma.

Keywords

isavuconazole, mass balance, pharmacokinetics, phase I, safety

Isavuconazonium sulfate is a water-soluble prodrug designed to deliver the poorly soluble, active triazole isavuconazole to the systemic circulation. Isavuconazole is an inhibitor of the enzyme sterol-14- α -demethylase, which is essential for the biosynthesis of ergosterol in the fungus cell wall.^{1,2} It has potent activity in vitro against a wide range of clinically important molds, yeasts, and dimorphic fungi.^{3,4} Isavuconazonium sulfate is available in water-soluble oral and intravenous (IV) cyclodextrin-free formulations. Important properties of isavuconazole in vivo include the lack of an effect of food on absorption for the oral formulation, the lack of a potentially nephrotoxic solubilizing excipient for the IV formulation, the consistency of intersubject dose-exposure relationships,³ and comparatively favorable drug-drug interaction profiles.^{5–10} Isavuconazole has demonstrated noninferiority to voriconazole for the primary treatment of invasive

mold disease¹¹ and has shown successful outcomes in patients with mucormycosis in phase 3 trials.¹² It

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Submitted for publication 6 December 2016; accepted 6 June 2017.

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*At time of study.

was approved in 2015 by the US Food and Drug Administration for the treatment of adults with invasive aspergillosis and invasive mucormycosis and by the European Medicines Agency for treatment of adults with invasive aspergillosis and for mucormycosis in patients for whom amphotericin B is not appropriate.

Following IV infusion, the prodrug isavuconazonium sulfate is hydrolyzed rapidly by esterases in plasma to the active triazole isavuconazole and an inactive water-soluble cleavage product (BAL8728).^{13,14} During a 1-hour IV infusion of isavuconazonium sulfate, plasma levels of the prodrug remain fairly constant but quickly fall below detectable limits within 15 minutes postinfusion.¹³ Isavuconazole levels peak at the end of infusion, followed by a biphasic decline, reflecting mostly redistribution to tissues during the first phase, followed by a phase in which a slow elimination rate predominates. Isavuconazole has been observed to have a large apparent volume of distribution and a long terminal elimination half-life ($t_{1/2}$). Maximal plasma concentrations of the BAL8728 moiety also decline rapidly postinfusion, but the total exposure of BAL8728 is approximately 1.3% that of isavuconazole after IV administration (based on area under the plasma concentration-time curve [AUC] from the time of dosing to the last measurable concentration [AUC_{last}]).¹³

Following oral administration, nonenzymatic (chemical) hydrolysis of isavuconazonium sulfate takes place in the intestinal tract, where it is absorbed due to its high permeability.^{7,15} Both the prodrug and the BAL8728 cleavage product lack oral bioavailability, but isavuconazole is absorbed readily, and, relative to IV isavuconazonium sulfate (determined by isavuconazole AUC), bioavailability is 98%.^{7,13} The pharmacokinetic (PK) properties of isavuconazole are linear and dose proportional.¹³ *In vitro* metabolism studies using human liver microsomes have shown that isavuconazole is predominantly metabolized by the human cytochrome P450 isoenzymes 3A4 and 3A5.¹⁰

Although those studies have helped to clarify the PK parameters of isavuconazole and the inactive BAL8728 moiety, other details of the metabolic profile of isavuconazonium sulfate have not been reported previously. BAL8728 has no antifungal activity, but the possibility of off-target effects has not been excluded, and so understanding its disposition is useful for assessing the safety of isavuconazonium sulfate. Herein, we report the results of 2 phase 1 studies conducted to identify the routes of elimination and metabolic profile of isavuconazole and BAL8728 in human plasma, urine, and/or feces after a single dose of either oral or IV ¹⁴C-labeled isavuconazonium sulfate. The high bioavailability of isavuconazole with oral dosing allowed the study of its metabolism to be performed with oral administration

of isavuconazonium sulfate^{13,14} (study 1). However, BAL8728 is not readily detectable in plasma following oral dosing,^{13,14} and so assessment of its metabolism was performed following IV administration of the prodrug. Safety and tolerability of isavuconazonium sulfate also were assessed.

Methods

Study Design

Two phase 1, open-label, single-center, mass-balance studies were conducted in healthy males aged 18 to 55 years with a body weight ≥ 45 kg and a body mass index of 18 to 32 kg/m² (NCT01813461 and NCT02059590). Both studies were conducted at the Covance Clinical Research Unit, Madison, Wisconsin. These studies were conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, Good Clinical Practice, International Conference on Harmonisation guidelines, and applicable laws and regulations. All subjects provided written informed consent (approved by Independent Investigational Review Board Inc, Sunrise, Florida) prior to initiation of any study-related procedures.

Subjects

Screening of subjects was performed within the 28 days prior to administration of study drug (day 1). Subjects were eligible for study participation if the following criteria were met: general good health, no significant disease history in any major organ system, and no significant findings in clinical laboratory tests, vital signs, physical examination, and 12-lead electrocardiogram screening at day -1, and willingness and ability to comply with all study requirements.

Specific exclusion criteria included the following: history of a swallowing disorder, bowel obstruction, severe gastrointestinal disorders, major gastrointestinal surgery, actively bleeding hemorrhoids, or gastric/duodenal ulcer; and/or significant occupational, medicinal, or research study-related radiation exposure in the previous 12 months.

Dosing

Due to the high oral bioavailability of isavuconazole, profiling was carried out using orally administered [cyano-¹⁴C]isavuconazonium sulfate, labeled in the isavuconazole component of the drug (Figure 1a). However, because the oral bioavailability of BAL8728 is very low (<0.15%), profiling of BAL8728 (study 2) was achieved using IV-administered [pyridinylmethyl-¹⁴C]isavuconazonium sulfate, labeled in the BAL8728 prodrug moiety (Figure 1b). Study 1 (isavuconazole mass balance) included 7 subjects who received a single oral dose of 372.6 mg [cyano-¹⁴C]isavuconazonium

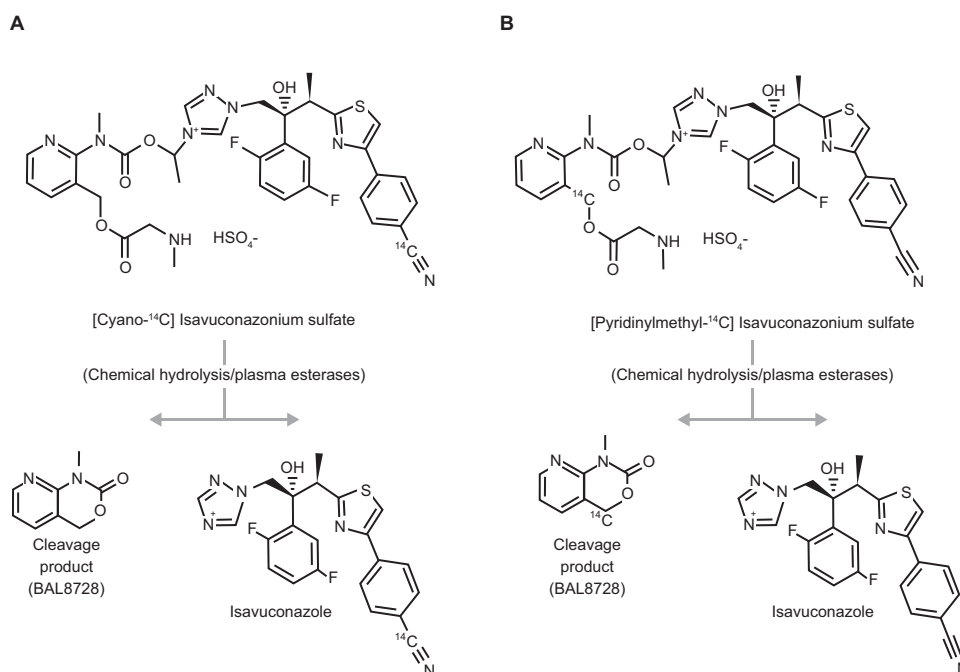


Figure 1. Radiolabeled isavuconazonium sulfate. Positions of radiolabels are indicated as ¹⁴C. [Cyano-¹⁴C]isavuconazonium sulfate is labeled in the isavuconazole part of the prodrug, whereas [pyridinylmethyl-¹⁴C]isavuconazonium sulfate is labeled in the BAL8728 part of the prodrug.

sulfate, corresponding to 200 mg isavuconazole. Study 2 (BAL8728 mass balance) included 6 subjects who received a single IV dose of 372.6 mg [pyridinylmethyl-¹⁴C]isavuconazonium sulfate, corresponding to 75 mg BAL8728. In both studies the study drug was administered after 10 hours of fasting, and subjects continued to fast for an additional 4 hours after dosing.

Radioanalysis Studies

Sample Collection and Preparation. Samples of residual dose vials, blood, plasma, urine, and feces from the Covance clinical research unit were uniquely identified to indicate origin and collection time (Supplementary Table S1) and were sent to Covance Laboratories (Madison, Wisconsin) for radioanalysis as described in detail in Supplementary Materials.

Pharmacokinetic Assessment. Pharmacokinetic assessment was carried out by Astellas Pharma Global Development Inc., Northbrook, Illinois. The PK parameters assessed for isavuconazole in plasma for study 1 included AUC from time of dosing extrapolated to infinity (AUC_{∞}) and AUC_{last} , maximum observed concentration (C_{max}), time to reach C_{max} (t_{max}), apparent clearance, apparent volume of distribution, and $t_{1/2}$. Parameters assessed for BAL8728 in plasma for study 2 included AUC_{∞} , AUC_{last} , C_{max} , t_{max} , total clearance, volume of distribution, and $t_{1/2}$. In study 2 isavuconazole AUC_{last} , C_{max} , and t_{max} also were assessed.

Pharmacokinetic parameters assessed in urine for both studies included amount excreted, percentage of dose excreted, and renal clearance. Radioactivity parameters for both studies included measurement of radioactivity in whole blood (AUC_{∞} , AUC_{last} , C_{max} , t_{max} , and $t_{1/2}$), in plasma (AUC_{∞} , AUC_{last} , C_{max} , t_{max} , and $t_{1/2}$), blood/plasma concentration ratio, percentage of dose, and cumulative percentage of dose of radioactivity recovered in urine and feces.

Metabolic Profiling Study

To clarify details regarding the metabolism of isavuconazonium sulfate metabolic profiling, studies were conducted as described in detail in the Supplementary Materials. A summary of the samples analyzed and pooled are included in Supplementary Table S1.

Safety Assessments

An adverse event was defined as any untoward medical occurrence in a subject administered a study drug that did not necessarily have a causal relationship with this treatment. From dosing of the study drug on day 1 through the end of the study, the incidence, nature, and severity of treatment-emergent adverse events (TEAEs) were monitored and assessed. Adverse events were coded and summarized using the Medical Dictionary for Regulatory Activities (MedDRA; Version 12.1). Additional safety parameters included

Table 1. Demographics and Baseline Characteristics

| Parameter | Isavuconazole (Study 1) (n = 7) | BAL8728 (Study 2) (n = 6) |
|-------------------------------------|------------------------------------|------------------------------|
| Sex, n (%) | | |
| Male | 7 (100) | 6 (100) |
| Race, n (%) | | |
| White | 4 (57.1) | 4 (66.7) |
| Black | 2 (28.6) | 1 (16.7) |
| Other | 1 (14.3) | 1 (16.7) |
| Ethnicity, n (%) | | |
| Not Hispanic or Latino | 7 (100) | 5 (83.3) |
| Hispanic or Latino | 0 | 1 (16.7) |
| Age [y], mean (SD) | 36.1 (11.6) | 28.8 (11.4) |
| Weight [kg], mean (SD) | 83.9 (11.5) | 98.2 (7.7) |
| Height [cm], mean (SD) | 180.4 (5.8) | 186.8 (4.8) |
| BMI [kg/m ²], mean (SD) | 25.7 (3.5) | 28.2 (2.5) |

BMI indicates body mass index (weight [kg]/height [m]²).

vital sign assessments, clinical laboratory measurements (hematology, biochemistry, and urinalysis), 12-lead electrocardiogram measurements, and physical examinations.

Statistical Analysis

The safety analysis set comprised all subjects who received the study drug. The PK analysis set comprised subjects in the safety analysis set for whom PK data were adequate for the calculation of at least 1 of the primary PK parameters. The safety population was used for summaries of demographic and baseline characteristics and for all safety and tolerability variables. Descriptive statistics were used to summarize continuous and categorical variables. Geometric mean and coefficient of variation also were calculated for PK data. Levels of analyte below the level of quantification were entered as 0 for calculations. SAS[®] version 9.1 or higher was used for all data processing and summarization. The PK parameters were derived by noncompartmental methods and calculated using Phoenix[®] WinNonlin[®] version 6.3 (Pharsight Corp, Mountain View, California).

Results

Demographics and Baseline Characteristics

Seven healthy male subjects were enrolled in study 1, and 6 healthy male subjects were enrolled in study 2 (Table 1).

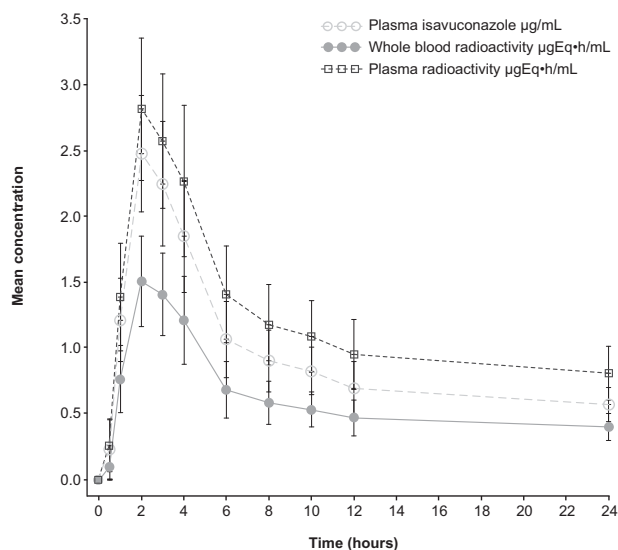


Figure 2. Mean (SD) plasma concentrations as well as plasma and whole-blood radioactivity concentrations for isavuconazole (normal-scale plot). The lower limit of quantification for isavuconazole for radioactivity in plasma was 82.0 ngEq/mL isavuconazole from pre-dose to 312-hours postdose, and 42.2 ngEq/mL isavuconazole from 360 to 576 hours postdose; and that for whole blood was 108 ngEq/mL isavuconazole.

Pharmacokinetics

Study 1: Isavuconazole Mass Balance. To assess the pharmacokinetics, absorption, metabolism, and excretion of isavuconazole and its metabolites, concentration-time profiles for plasma isavuconazole and the cyano-¹⁴C label (labeled on the active moiety) were examined following oral administration. The profiles of plasma isavuconazole as well as radioactivity associated with plasma and with whole blood demonstrated parallel rises to C_{max} , followed by biphasic declines (Figure 2). Mean cyano-¹⁴C concentrations in plasma were higher than isavuconazole concentrations in plasma, implying that the metabolites of isavuconazole were also present in plasma (Figure 2). The mean C_{max} of cyano-¹⁴C-derived radioactivity was higher in plasma (2.8 $\mu\text{g}\cdot\text{Eq}/\text{mL}$ isavuconazole) than whole blood (1.5 $\mu\text{g}\cdot\text{Eq}/\text{mL}$ isavuconazole), implying minimal association of isavuconazole with red blood cells. Mean concentrations for both peaked at 2 hours, coinciding with peak isavuconazole concentrations (Table 2; Figure 2). Levels of radioactivity in blood fell below the lower limit of quantification (LLOQ) by 216 hours postdose in 5 subjects and by 312 hours postdose for the remaining 2 subjects. Blood/plasma ratios ranged between 0.5 and 0.7.

For isavuconazole, mean C_{max} and AUC_{∞} were 2.5 $\mu\text{g}/\text{mL}$ and 96.2 $\mu\text{g}\cdot\text{h}/\text{mL}$, respectively, with mean $t_{1/2}$ of 99.9 hours (Table 3). Mean total radioactivity excretion was 191.0 $\text{mg}\cdot\text{Eq}$ isavuconazole, with

Table 2. Summary of Plasma and Whole-Blood Radioactivity Parameters of Isavuconazole (From Study 1) and BAL8728 (From Study 2)

| Parameter | Isavuconazole (Study 1) | | BAL8728 (Study 2) | |
|---|-------------------------|------------------------|-------------------|------------------------|
| | Plasma (n = 7) | Whole Blood (n = 7) | Plasma (n = 6) | Whole Blood (n = 6) |
| AUC_{∞} , $\mu\text{gEq}\cdot\text{h/mL}$ | | | | |
| Mean \pm SD | 171.5 \pm 44.0 | 96.6 \pm 21.9 | 15.4 \pm 3.4 | 9.0 \pm 2.3 |
| %CV | 25.6 | 22.7 | 22.2 | 25.3 |
| AUC_{last} , $\mu\text{gEq}\cdot\text{h/mL}$ | | | | |
| Mean \pm SD | 156.8 \pm 35.2 | 61.6 \pm 16.4 | 15.0 \pm 3.4 | 8.6 \pm 2.3 |
| %CV | 22.4 | 26.6 | 22.7 | 27.1 |
| C_{max} , $\mu\text{gEq/mL}$ | | | | |
| Mean \pm SD | 2.8 \pm 0.5 | 1.5 \pm 0.3 | 3.1 \pm 0.4 | 1.9 \pm 0.2 |
| %CV | 18.8 | 22.4 | 11.9 | 11.1 |
| t_{max} , h | | | | |
| Median (min-max) | 2.0 (2.0-3.0) | 2.0 (2.0-3.0) | 1.0 (1.0-1.3) | 1.0 (1.0-1.0) |
| $t_{1/2}$, h | | | | |
| Mean \pm SD | 141.9 \pm 41.8 | 160.5 \pm 64.5 | 5.7 \pm 1.1 | 5.1 \pm 2.0 |
| %CV | 29.4 | 40.2 | 19.9 | 38.3 |

%CV indicates coefficient of variation; AUC_{∞} , area under the plasma concentration-time curve extrapolated to infinity; AUC_{last} , area under the plasma concentration-time curve from the time of dosing to the last measurable concentration; C_{max} , maximum plasma concentration; t_{max} , time to C_{max} ; $t_{1/2}$, terminal half-life.

94.9 mg•Eq and 96.2 mg•Eq isavuconazole excreted on average in urine and feces, respectively (Table 4). Mean isavuconazole urinary amount excreted was 83.82 μg (0.04% of dose).

Over the 600-hour study period, total recovery of radioactivity ranged from 86.3% to 96.7%, with a mean of 91.6%. Most of the administered radioactivity (81.6%) was recovered in the first 312 hours postdose in both the urine and feces (Figure 3). Overall, a mean of 45.5% and 46.1% of the dose was recovered in urine and feces, respectively. All subjects but 1 had levels of radioactivity that fell below the LLOQ by 600 hours. The maximum mean amount excreted of [cyano- ^{14}C]isavuconazonium sulfate-derived radioactivity was observed in samples collected from 24 to 48 hours postdose for urine and 48 to 72 hours postdose for feces.

Study 2: BAL8728 Mass Balance. The pharmacokinetics, absorption, metabolism, and excretion of the BAL8728 moiety was assessed following IV administration of [pyridinylmethyl- ^{14}C]BAL8728-labeled isavuconazonium sulfate. At all time points [pyridinylmethyl- ^{14}C]BAL8728-derived mean plasma radioactivity concentrations were higher than those in whole blood (Supplementary Figure S1), indicating minimal association of radioactivity with red blood cells. Maximal mean concentrations of drug-derived radioactivity in plasma and whole blood of 3.1 and 1.9 $\mu\text{g}\cdot\text{Eq/mL}$ BAL8728, respectively, were observed at 1 hour postdose (Table 2). Levels of radioactivity fell below the LLOQ in whole blood in 2 subjects by

24 hours postdose and in the remaining 4 subjects by 48 hours postdose. In plasma, levels of radioactivity fell below the LLOQ in 5 subjects by 48 hours postdose and in the single remaining subject by 72 hours postdose. Blood/plasma concentration ratios ranged from 0.5 to 0.8 through 24 hours postdose.

For BAL8728, mean C_{max} and AUC_{last} in plasma were 0.7 $\mu\text{g/mL}$ and 1.1 $\mu\text{g}\cdot\text{h/mL}$, respectively, and mean $t_{1/2}$ was 1 hour (Table 3). Mean C_{max} and AUC_{last} of isavuconazole in plasma were 3.4 $\mu\text{g/mL}$ and 43.3 $\mu\text{g}\cdot\text{h/mL}$, respectively, with the C_{max} observed 1 hour postdose (Table 3).

Over the 168 hours of study, total recovery of radioactivity ranged from 93.8% to 101%, with a mean of 98.4%. Most of the administered radioactivity was recovered in the first 12 hours postdose (87.0%) in urine. Overall, 2.4% and 96.0% of the dose were recovered in feces and urine, respectively (Table 4; Supplementary Figure S2). The maximum mean concentrations of [pyridinylmethyl- ^{14}C]BAL8728-derived radioactivity were observed in samples collected from 0 to 4 hours postdose for urine and 48 to 72 hours postdose for feces.

Metabolite Analysis

Representative HPLC-radiochromatograms in plasma, urine, and/or feces samples are presented in Figure 4 and Supplementary Figure S3 for study 1 and study 2, respectively. A summary of the postulated metabolic pathways of isavuconazonium sulfate is provided in Figure 5.

Table 3. Summary of Plasma and Urine PK Parameters of Isavuconazole From Study 1 and of Plasma and Urine PK Parameters of BAL8728 and Isavuconazole From Study 2

| Parameter | Isavuconazole (Study 1) | | BAL8728 (Study 2) | | |
|--|-------------------------|------------------|-------------------|-------------------|-----------------|
| | Isavuconazole | | BAL8728 | | Isavuconazole |
| | Plasma (n = 7) | Urine (n = 7) | Plasma (n = 6) | Urine (n = 6) | Plasma (n = 6) |
| AUC_{∞} , $\mu\text{g}\cdot\text{h}/\text{mL}$ | | | | | |
| Mean \pm SD | 96.2 \pm 30.7 | ... | 1.1 \pm 0.1 | | ... |
| %CV | 31.9 | | 12.3 | | |
| AUC_{last} , $\mu\text{g}\cdot\text{h}/\text{mL}$ | | | | | |
| Mean \pm SD | 92.2 \pm 26.5 | ... | 1.1 \pm 0.1 | | 43.3 \pm 14.5 |
| %CV | 28.7 | | 12.5 | | 33.4 |
| C_{max} , $\mu\text{g}/\text{mL}$ | | | | | |
| Mean \pm SD | 2.5 \pm 0.4 | ... | 0.7 \pm 0.09 | | 3.4 \pm 0.6 |
| %CV | 17.8 | | 13.4 | | 17.4 |
| t_{max} , h | | | | | |
| Median (min-max) | 2.0 (2.0-3.0) | ... | 1.0 (0.8-1.0) | | 1.0 (1.0 - 1.0) |
| $t_{1/2}$, h | | | | | |
| Mean \pm SD | 99.9 \pm 44.6 | ... | 1.0 \pm 0.2 | | ... |
| %CV | 44.6 | | 16.1 | | |
| CL/F, L/h | | | | | |
| Mean \pm SD | 2.3 \pm 0.7 | ... | ... | | ... |
| %CV | 30.8 | | | | |
| CL, L/h | | | | | |
| Mean \pm SD | ... | ... | 69.9 \pm 8.4 | | ... |
| %CV | | | 12.0 | | |
| V_z/F , L | | | | | |
| Mean \pm SD | 301.8 \pm 105.7 | ... | ... | | ... |
| %CV | 35.0 | | | | |
| V_z , L | | | | | |
| Mean \pm SD | ... | ... | 104.2 \pm 12.2 | | ... |
| %CV | | | 11.7 | | |
| Ae, μg | | | | | |
| Mean \pm SD | ... | 83.82 \pm 36.2 | ... | 457.0 \pm 108.0 | ... |
| %CV | | 43.2 | | 23.6 | |
| Ae% | | | | | |
| Mean \pm SD | ... | 0.04 \pm 0.02 | ... | 0.61 \pm 0.14 | ... |
| %CV | | 43.2 | | 23.6 | |

%CV indicates coefficient of variation; Ae, amount excreted; Ae%, percentage of dose excreted; AUC_{∞} , area under the plasma concentration-time curve extrapolated to infinity; AUC_{last} , area under the plasma concentration-time curve from the time of dosing to the last measurable concentration; CL/F, oral clearance; CL, total clearance; C_{max} , maximum plasma concentration; ..., not applicable; PK, pharmacokinetic; t_{max} , time to C_{max} ; $t_{1/2}$, terminal half-life; V_z/F , volume of distribution at the terminal phase over bioavailability; V_z , volume of distribution at the terminal phase.

Sample collection for [pyridinylmethyl- ^{14}C]isavuconazonium study was optimized for the short half-life of BAL8728 and was not consistent with [cyano- ^{14}C]isavuconazonium sulfate study sample collection.

Study 1: Isavuconazole Mass Balance. After a single oral dose of [cyano- ^{14}C]isavuconazonium sulfate, isavuconazole made up 88% of the [^{14}C]AUC_{last} up to 144 hours postdose. The remaining plasma radioactivity was composed mainly of M1 (hydroxylated isavuconazole carbamoyl form). Trace levels of M2 (hydroxylated isavuconazole) and M3 (oxidative *N*-dealkylated form of isavuconazole) were also detected.

Radioactivity in urine was composed of 12 peaks, of which M11-M14 accounted for 50% of the total sample radioactivity (M11, *N*-acetylcysteine con-

jugate of hydroxylated 4-methylcarbamoylbenzene; M12, 2-[4-cyanophenyl]-2-hydroxyacetic acid; M13, *N*-acetylcysteine conjugate of dihydroxylated 4-methylcyanobenzene; M14, *O*-glucuronide of hydroxylated 4-[2-ethyl-1,3-thiazol-4-yl]carbamoylbenzene). The remaining radioactivity was composed of M8-M10, and some minor unidentified metabolites (M8, *O*-glucuronide of hydroxylated 4-methylcyanobenzene; M9, *O*-glucuronide of hydroxylated 4-methylcarbamoylbenzene; M10, *O*-glucuronide of hydroxylated 4-[2-hydroxyethyl]-

Table 4. Total Radioactive Dose Recovered in Urine and Feces

| Parameter | Isavuconazole (Study 1) | | | BAL8728 (Study 2) | | |
|-----------|-------------------------|-------------|-------------|-------------------|-----------|-------------|
| | Urine | Feces | Total | Urine | Feces | Total |
| Ae, mg•Eq | | | | | | |
| Mean ± SD | 94.9 ± 15.4 | 96.2 ± 13.2 | 191.0 ± 9.8 | 73.5 ± 3.5 | 1.8 ± 0.3 | 75.3 ± 3.6 |
| Ae% | | | | | | |
| Mean ± SD | 45.5 ± 7.4 | 46.1 ± 6.3 | 91.6 ± 4.7 | 96.0 ± 2.7 | 2.4 ± 0.4 | 98.4 ± 2.59 |

Ae indicates amount excreted; Ae%, percentage of dose excreted.

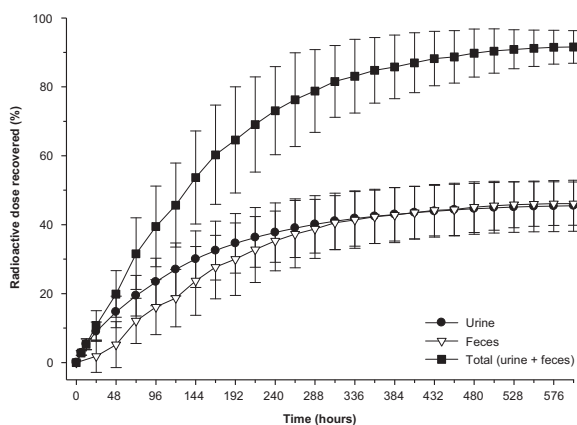


Figure 3. Mean (SD) cumulative percentage of radioactive dose recovered in urine and feces during isavuconazole mass balance study.

cyanobenzene). In urine, isavuconazole accounted for only 0.04% of the administered radioactive dose. However, in feces, isavuconazole was the predominant component of radioactivity (33%), corresponding to approximately 15% of the dose. In total, 18 additional peaks were detected from urine and feces, of which none accounted for >1% of the dose.

Study 2: BAL8728 Mass Balance. After a single IV dose of [pyridinylmethyl-¹⁴C]isavuconazonium sulfate, 2 metabolic peaks of BAL8728 and M4 (2 [methylamino]nicotinic acid; oxidative carbamate cleavage metabolite of BAL8728) were detected mainly in plasma, representing 8% and 83% of the [¹⁴C]AUC_{last} up to 24 hours postdose, respectively. The main metabolite recovered in urine was M4, accounting for 56% of the total radioactivity. M20 was present in urine and comprised 18% of the dose. Eleven additional metabolites were detected in urine, but none of these accounted for >3% of the dose.

Safety Assessments

In study 1, 2 subjects experienced a total of 11 TEAEs. Of these, 1 subject had diarrhea, which was possibly related to the study drug, and 1 subject had 5 TEAEs probably related to the study drug including headache,

edema, erythema on the neck and trunk, and pruritus of the face. In study 2, 1 subject experienced a total of 2 TEAEs: a single episode of mild paresthesia and a single episode of moderate back pain; neither was considered to be related to isavuconazole. No serious AEs, discontinuations due to an AE, or deaths were reported in either study.

Discussion

These studies provide important information on the clearance mechanisms, metabolism, and PK parameters of the active triazole isavuconazole and the inactive water-soluble cleavage product BAL8728 that have been useful to guide the clinical development of isavuconazonium sulfate. Following oral dosing, levels of isavuconazole peaked at 2 hours, whereas following IV dosing, levels of BAL8728 peaked at the end of infusion. Isavuconazole demonstrated low apparent clearance, large volume of distribution, and a long $t_{1/2}$. A number of minor metabolites were identified in addition to isavuconazole and BAL8728. Nevertheless, the major compound in plasma was isavuconazole, and no individual metabolite was observed with an AUC_{last} >10% for the total drug-related exposure. This is consistent with the interpretation that isavuconazole is the major metabolite of isavuconazonium sulfate. Isavuconazole-derived radioactivity was eliminated approximately equally via the feces and urine, whereas BAL8728-derived radioactivity was eliminated almost exclusively in urine. The mean renal excretion of unchanged isavuconazole was low (0.04%; range 0.01% to 0.07%). Radioactivity levels for both isavuconazole and BAL8728 were higher in plasma than in whole blood at all time points, suggesting minimal binding to or accumulation in erythrocytes and supporting the clinical meaningfulness of plasma clearance measurements. Taken together, these observations account for the fate of isavuconazonium sulfate in vivo.

The major form of urine radioactivity was M4 (56% of total dose), the oxidative carbamate cleavage metabolite of BAL8728. Renal elimination of intact BAL8728 was less than 1% of the total dose

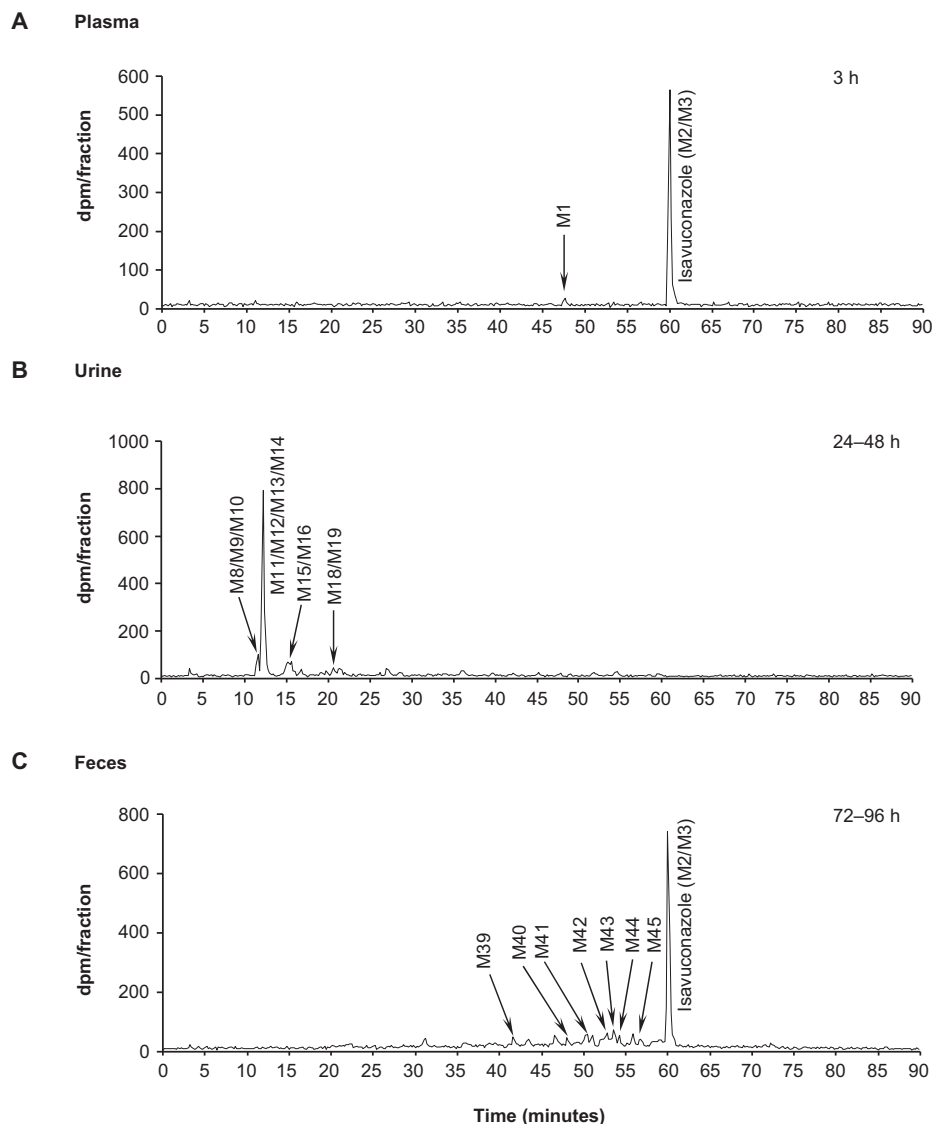


Figure 4. Representative HPLC-radiochromatograms in pooled plasma, urine, and feces after a single oral dose of [cyano- ^{14}C]isavuconazonium sulfate. HPLC indicates high-performance liquid chromatography.

administered. BAL8728 had a short $t_{1/2}$, and the majority of BAL8728-derived radioactivity was recovered in urine (96%) by 12 hours postdose, which suggests that BAL8728 and/or its metabolites are eliminated primarily by renal excretion.

Metabolism of isavuconazonium sulfate after oral or IV administration occurred primarily via nonenzymatic (chemical) cleavage of the prodrug in the gut or enzymatic cleavage by esterase in the plasma, which led to generation of isavuconazole. Based on the analysis of reaction products, it can be inferred that isavuconazole, a substrate of CYP3A4, was metabolized by oxidation and hydrolysis of the cyano group and oxidation of the carbamoyl form. After cleavage of the thiazole ring, additional metabolites were formed by oxidation and subsequent glucuronide and acetylcys-

teine conjugation, or hydrolysis of the cyano group, and oxidation of the carbamoyl form and glucuronide or acetylcysteine conjugation. Small amounts of M6 (thiazole ring-cleaved metabolite of isavuconazole) and M7 (carboxylic acid form of destriazole isavuconazole) were present in plasma.

Cleavage of the prodrug also generated BAL8728, which was metabolized predominantly to M4 and M20. BAL8728 was also metabolized by oxidation and subsequent glucuronide or cysteine and acetylcysteine conjugation. Levels of the cleavage product and its metabolites were far lower than levels of isavuconazole, suggesting that they are unlikely to have a major influence on efficacy or safety.

Pharmacokinetic parameters of isavuconazole measured in this study after a single oral dose or IV

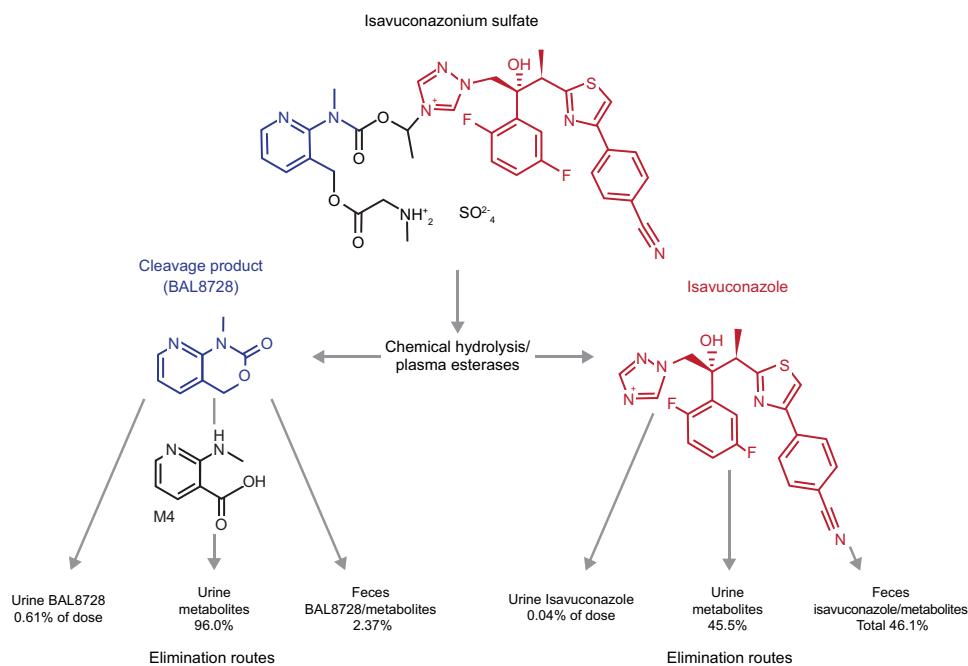


Figure 5. Summary of the postulated metabolic pathways of isavuconazonium sulfate. Following single doses of [cyano-¹⁴C]isavuconazonium and [pyridinylmethyl-¹⁴C]isavuconazonium in humans, a number of minor metabolites were identified in addition to the active moiety and the inactive cleavage product (BAL8728). Except for the active moiety isavuconazole, no individual metabolite was observed with an AUC > 10% of that of the parent compound. Only major metabolites are shown; sarcosine and acetaldehyde are also produced from prodrug cleavage (not shown). AUC, area under the plasma concentration-time curve.

infusion of the prodrug were comparable to those obtained in a previous isavuconazole phase 1 study.¹³ In the current study unchanged isavuconazole was eliminated predominately via feces (33% of total drug dose recovered), and less than 1% of the dose recovered in urine was unchanged isavuconazole. The negligible urinary excretion of unchanged isavuconazole may also explain why no alteration in isavuconazole PK is observed in patients with renal impairment, including patients with end-stage renal disease.¹⁶

Isavuconazole was safe and well tolerated in these studies. Of the 13 subjects in both studies, 3 experienced a total of 13 TEAEs that were considered mild or moderate, 6 of which were considered to be related to isavuconazole administration.

Taken together, these studies provide important information regarding the PK, metabolism, and elimination of isavuconazonium sulfate that has been used to help guide development of this agent for clinical use.

Acknowledgments

These studies were funded by Astellas Pharma, Inc. Isavuconazole has been codeveloped by Astellas Pharma Global Development, Inc and Basilea Pharmaceutica International Ltd. Medical writing support was provided by Barrie J. Anthony, PhD, a medical writer at Envision Scientific Solutions,

funded by Astellas Pharma, Inc. The authors are grateful for the contributions of the investigators and staff who conducted the clinical trials, and to the subjects who volunteered for these studies.

Declaration of Conflicting Interests

R.T., K.K., D.K., C.L., T.Y., and A.D. are employees of Astellas Pharma Global Development Inc. S.A. was an employee of Astellas Pharma Global Development, Inc, at the time of study. C.H. is an employee of Covance, which was contracted by Astellas Pharma Global Development, Inc to perform work related to the study.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website.