Original Manuscript

ACCP
AMERICAN COLLEGE OF CLINICAL PHARMACOLOGY
Advancing Clinical Care through Pharmacology

Pharmacokinetic Interaction Between Isavuconazole and a Fixed-Dose Combination of Lopinavir 400 mg/Ritonavir 100 mg in Healthy Subjects

Takao Yamazaki[1], Amit Desai[1], David Han[2], Kota Kato[3], Donna Kowalski[1], Shahzad Akhtar[1], Christopher Lademacher[1], Laura Kovanda[1], and Robert Townsend[1]

Abstract

This phase 1, open-label study evaluated the pharmacokinetic effects of coadministration of the antifungal agent, isavuconazole (administered as its water-soluble prodrug isavuconazonium sulfate), with the antiretroviral agent lopinavir/ritonavir in healthy adults. In part 1, 13 subjects were randomized to 2 arms to receive multiple doses of oral isavuconazole 100 mg either alone or with lopinavir/ritonavir 400/100 mg. In part 2, a different group of 55 subjects were randomized to 3 arms to receive multiple doses of oral isavuconazole 200 mg, either alone or with lopinavir/ritonavir 400/100 mg, or to receive oral lopinavir/ritonavir 400/100 mg alone. Mean area under the concentration-time curve (AUC) following the last dose (AUC_τ) and C_{max} of isavuconazole increased by 113% and 96% in part 1 and by 96% and 74% in part 2 in the presence vs absence of lopinavir/ritonavir, respectively. Mean AUC_τ and C_{max} of lopinavir were 27% and 23% lower, and mean AUC_τ and C_{max} of ritonavir were 31% and 33% lower in the presence vs absence of isavuconazole, respectively. Mild to moderate gastrointestinal disorders were the most common adverse events experienced. These findings indicate that coadministration of lopinavir/ritonavir with isavuconazole can decrease the exposure of lopinavir/ritonavir and increase the exposure of isavuconazole. Patients should be monitored for reduced antiviral efficacy if these agents are coadministered.

Despite advances in antiretroviral therapy, some patients with human immunodeficiency virus (HIV) infection are at an increased risk of invasive fungal infections due to immunosuppression, including infections caused by *Cryptococcus* spp, *Candida* spp, and *Aspergillus* spp.[1] This increased susceptibility to fungal infection raises the likelihood that antifungal agents and antiretroviral drugs may be administered concurrently in these patients. In order to avoid undesirable interactions, it is important to evaluate the effects of coadministration of these 2 classes of agents.

Isavuconazonium sulfate is a novel broad-spectrum triazole antifungal prodrug that was approved in 2015 by the US Food and Drug Administration for the primary treatment of adults with invasive aspergillosis and with invasive mucormycosis, and by the European Medicines Agency for the primary treatment of

adults with invasive aspergillosis and of adults with mucormycosis when amphotericin B is inappropriate,

[1] Astellas Pharma Global Development, Inc., Northbrook, IL, USA
[2] PAREXEL, Los Angeles, CA, USA
[3] Analysis & Pharmacokinetics Research Laboratories, Astellas Pharma Inc., Osaka, Japan

Corresponding Author:
Robert Townsend, PhD, Astellas Pharma Global Development, Inc., Global Clinical Pharmacology & Exploratory Development Science, 1 Astellas Way, Northbrook, IL 60062
(e-mail: Robert.Townsend@astellas.com)

based on the results of phase 3 clinical trials.[2,3] The active moiety of isavuconazonium sulfate, isavuconazole, is a sensitive substrate and moderate inhibitor of cytochrome P450 3A4 (CYP3A4) enzyme in humans.[4] In human liver microsomes, isavuconazole metabolism was most strongly correlated with CYP3A4/5 activity (testosterone/midazolam hydroxylation activity; $P < .001$ and r > 0.82 for both), more weakly correlated with the activities of CYP2B6 (S-mephenytoin demethylation; $P < .01$, r = 0.65) and CYP2C8 (paclitaxel hydroxylation; $P < .05$, r = 0.57) and not correlated with activities of other tested CYP isoenzymes (data on file). In CYP-expressing human liver microsomes, isavuconazole was most efficiently metabolized by CYP3A4 (33.8% remaining) or CYP3A5 (68.4% remaining) compared with CYP2B6, CYP2C8, or CYP3A7 (all >98% remaining). The inhibitory constant K_i of isavuconazole for CYP3A4 in human liver microsomes in vitro was 0.62 μmol/L using midazolam as a probe and 1.93 μmol/L with testosterone as a probe. In cultured human hepatocytes, isavuconazole also induces increases of mRNA and activity of CYP3A4 (6.4-fold and 3.4-fold, respectively) and CYP2B6 (11.4-fold and 13.4-fold, respectively). In vivo, isavuconazole is also a weak inducer of CYP2B6[5] and a weak inhibitor of uridine diphosphate glucuronosyltransferase (UGT) as well as the transporters P-glycoprotein (P-gp), organic cation transporters 1 and 2 (OCT 1 and OCT2), and multidrug and toxin extrusion protein 1 (MATE1).[6,7] In human liver microsomes in vitro, isavuconazole also has been shown to inhibit UGT (IC$_{50}$ for 17β-estradiol 3-glucuronidation [UGT1A1], 9.0 μmol/L; for propofol glucuronidation [UGT1A9], 19 μmol/L; for morphine 3-glucuronidation [UGT2B7], 44 μmol/L). Transport of substrates in monolayers of LLC-PK1 cells or human embryonic kidney (HEK293) cells transfected with transporter-expressing constructs has been used to show isavuconazole-mediated inhibition of P-gp (IC$_{50}$ 25.7 μmol/L using [^{3}H]digoxin substrate), OCT1 (IC$_{50}$ 3.74 μmol/L; K_i 1.74 μmol/L, with [^{14}C]tetraethylammonium bromide substrate), OCT2 (IC$_{50}$ 1.97 μmol/L, and K_i 0.69 μmol/L with [^{14}C]metformin substrate), and MATE1 (IC$_{50}$ 6.31 μmol/L with [^{14}C]metformin substrate); it does not appear to be a substrate of these transporters (data on file). Because the recommended clinical dosing regimen (200 mg 3 times daily for 2 days, then 200 mg daily) generally results in plasma concentrations <7 μg/mL (data on file; isavuconazole molecular weight 437.47 g/mol), values of IC$_{50}$ or $K_i \le 16$ μmol in vitro may suggest the greatest potential for clinical relevance.

Lopinavir (400 mg)/ritonavir (100 mg) is a coformulated fixed-dose antiviral medication approved for the treatment of HIV infection in adults and children. Lopinavir and ritonavir are substrates of CYP3A,[7] and the combined drug is a strong inhibitor of these isoenzymes.[8–10] Lopinavir and ritonavir are also inhibitors of P-gp[11,12] as well as organic anion-transporting polypeptides 1B1 (OATP1B1) and 1B3 (OATP1B3).[13] In addition, ritonavir has induction potential for UGT enzymes.[14] Given the potential for interaction between isavuconazole and lopinavir/ritonavir, this study examined the pharmacokinetic (PK) and safety effects of coadministration of multiple doses of oral isavuconazole with multiple doses of oral lopinavir/ritonavir in healthy subjects.

Methods

Study Design
The study was 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 in this study provided Institutional Review Board–approved written informed consent prior to initiation of any study-related procedures (Aspire IRB, LLC, Santee, California).

This was a 2-part, phase 1, open-label, multiple-dose, 3-arm parallel study (trial conducted June to October 2012; PAREXEL International, Los Angeles, California; ClinicalTrials.gov Identifier: NCT01660477) conducted to evaluate the PK and safety effects of coadministration of multiple doses of isavuconazole (administered as isavuconazonium sulfate; CRESEMBA® oral capsules; Astellas Pharma US, Inc., Northbrook, Illinois) with multiple doses of lopinavir/ritonavir (KALETRA® oral tablets; AbbVie Inc., North Chicago, Illinois). A 2-part study design was used to establish the PK and tolerability of concurrent use of a fixed-dose combination of lopinavir/ritonavir with half the clinical dose of isavuconazole (part 1) prior to evaluation of PK and safety effects of coadministration of lopinavir/ritonavir with the clinically targeted dose of isavuconazole (part 2). Healthy male and female subjects, aged 18 to 55 years, with a body weight of ≥45 kg, and a body mass index of 18 to 32 kg/m^2 were eligible for enrollment.

Dosing and Sampling Schedule
In this report, dosing information is expressed as the isavuconazole equivalent of the prodrug; eg, oral capsules each contained isavuconazonium sulfate 186 mg, equivalent to isavuconazole 100 mg. The clinically targeted dose is isavuconazonium sulfate 372 mg (equivalent to isavuconazole 200 mg) 3 times a day (TID) for 2 days as a loading dose, followed by 372 mg once daily (QD).

In part 1 of the study, subjects were screened between days –28 and –2. On day –1, 12 subjects were
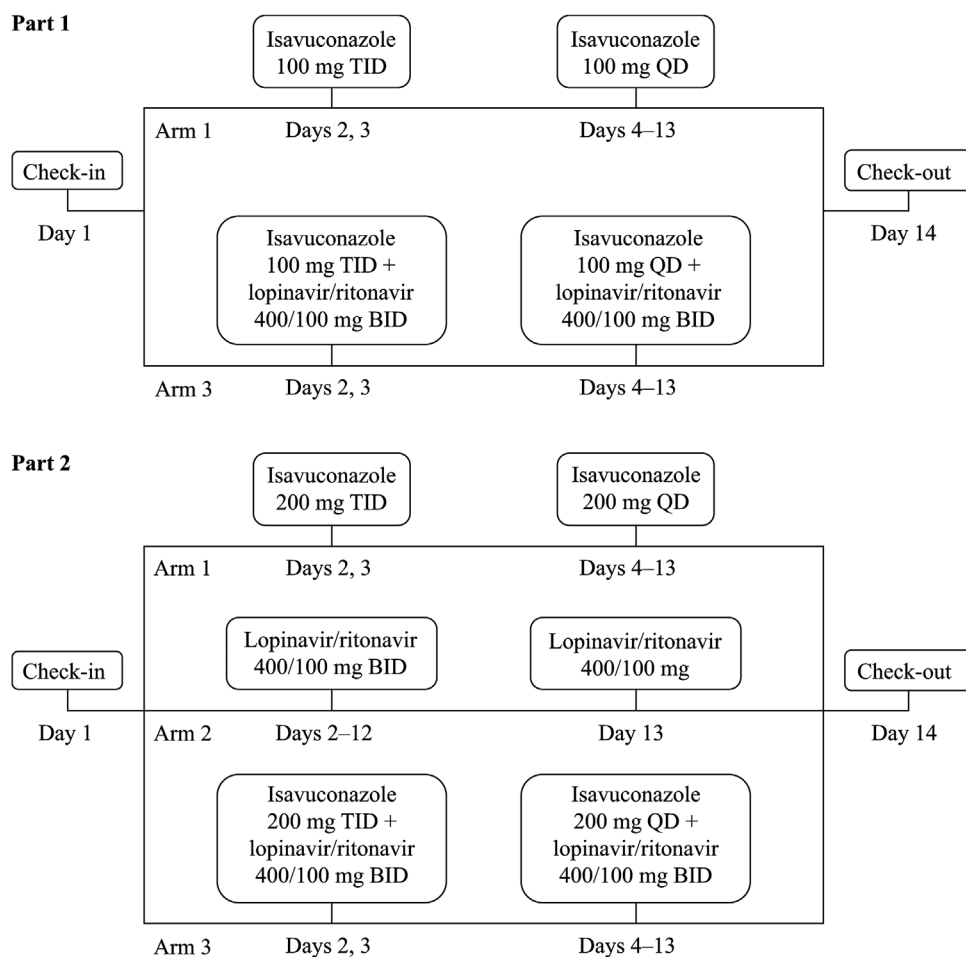
Part 1

Part 2

Figure 1. Study design. BID, twice daily; QD, once daily; TID, three times a day. Isavuconazole 100 mg and 200 mg were administered as isavuconazonium sulfate 186 mg and 372 mg, respectively.

randomized 1:1 to 2 study arms (Figure 1). Subjects in arm 1 received a loading-dose regimen of oral isavuconazole 100 mg TID (approximately 8 hours apart) on days 1 and 2, followed by oral isavuconazole 100 mg QD on days 3 to 13. Subjects in arm 3 received a loading-dose regimen of oral isavuconazole 100 mg TID plus lopinavir/ritonavir 400/100 mg twice daily (BID; approximately 12 hours apart) on days 1 and 2, followed by oral isavuconazole 100 mg QD plus oral lopinavir/ritonavir 400/100 mg BID (approximately 12 hours apart) on days 3 to 13. Subjects remained at the study center from day –1 until check-out on day 14, and returned to the center on day 20 (± 2 days) for a follow-up visit.

Part 2 of the study was conducted after it had been determined in part 1 that isavuconazole had an acceptable safety and tolerability profile and that the exposure of plasma isavuconazole was not more than 150% higher during coadministration of isavuconazole with lopinavir/ritonavir, compared with isavuconazole alone.

In part 2, subjects were screened between days –28 and –2, then randomized 1:1:1 to 3 study arms on day –1 (Figure 1). Subjects in arm 1 received a loading-dose regimen of oral isavuconazole 200 mg TID on days 1 and 2, followed by oral isavuconazole 200 mg QD on days 3 to 13. Subjects in arm 2 received oral lopinavir/ritonavir 400/100 mg BID on days 1 to 12 and a single dose of oral lopinavir/ritonavir 400/100 mg on day 13. In arm 3, subjects received a loading-dose regimen of oral isavuconazole 200 mg TID on days 1 and 2, followed by oral isavuconazole 200 mg QD on days 3 to 13. Subjects in this arm also received oral lopinavir/ritonavir 400/100 mg BID on days 1 to 13. Subjects remained in the study center from day –1 until checkout on day 14 and returned to the center on day 20 (±2 days) for a follow-up visit. In both parts of the study isavuconazole was administered immediately after administration of lopinavir/ritonavir on the days combination treatment was given. On days 1 to 12 of all arms, the study drugs were given under fed conditions, and on day 13 they were administered in the fasted state.

Blood samples were collected for PK assessment of isavuconazole on day 13 at predose and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 hours postdose in

parts 1 and 2. Samples were also collected for PK assessment of lopinavir and ritonavir on day 13 at predose and at 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hours postdose.

Pharmacokinetic Assessments

The PK parameters of lopinavir, ritonavir, and isavuconazole were assessed in plasma using validated liquid chromatography–tandem mass spectrometry methods (Pharmaceutical Product Development, Middleton, Wisconsin). The method for bioanalysis of isavuconazole is described elsewhere.[4] The interassay precisions (percentage coefficient of variation [CV]) of the 200, 400, 1000, 2500, and 8000 ng/mL isavuconazole quality control samples were 3.2%, 2.6%, 4.1%, 4.0%, and 2.9%, respectively whereas, the interassay accuracies (percentage relative error [RE]) were 0.3%, 2.9%, 5.5%, 1.6%, and 1.5%, respectively. For lopinavir and ritonavir, plasma samples (tripotassium EDTA; 0.1 mL) were combined with the internal standard (lopinavir-d_8/ritonavir-d_6) and subjected to protein precipitation with acetonitrile. The resulting supernatants were evaporated to dryness under a stream of nitrogen, followed by reconstitution of the residue with acetonitrile, ammonium acetate, and formic acid. Chromatographic separation was achieved using a Gemini C18 column (50 × 2.0 mm, 5 μm; Phenomenex, Torrance, California) with a gradient mobile phase consisting of ammonium formate, formic acid, acetonitrile, and water. A Quattro Micro mass spectrometer (Waters Corporation, Milford, Massachusetts) in positive-ion mode was used to assess the analytes. Multiple reaction monitoring transitions were m/z 629.5→447.2 for lopinavir, 721.1→296.1 for ritonavir, 637.6→447.2 for lopinavir-d_8, and 727.4→302.1 for ritonavir-d_6. The validated curve range was 10 to 10,000 ng/mL, and any samples measuring above the upper limit were diluted 2-fold prior to analysis. Interassay precisions of 30, 75, 300, 1200, and 7500 ng/mL lopinavir quality control samples were 4.6%, 3.6%, 3.9%, 4.5%, and 3.0%, respectively; whereas, the interassay accuracies were –8.2%, –0.1%, –1.3%, 0.0%, and –0.7%, respectively. Interassay precisions (%CV) of 30, 75, 300, 1200, and 7500 ng/mL ritonavir quality control samples were 3.7%, 3.7%, 2.4%, 1.8%, and 2.3%, respectively; whereas, the interassay accuracies (%RE) were –7.3%, 3.8%, 2.3%, 1.3%, and –3.6%, respectively. All study samples were analyzed within the established long-term stability limit (108 days at –25 ± 5°C).

The primary PK parameters of lopinavir and ritonavir calculated in part 2 were area under the concentration-time curve (AUC) following the last dose on day 13 (12 hours; AUC_τ) and maximum concentration (C_{max}). The primary plasma PK parameters

of isavuconazole calculated in parts 1 and 2 included AUC_τ on day 13 (24 hours) and C_{max}.

Safety Assessments

Treatment-emergent adverse events (TEAEs) were monitored throughout the study. Safety was also assessed by vital-sign measurements, 12-lead electrocardiograms, clinical laboratory testing (hematology, chemistry, and urinalysis), and physical examinations.

Statistics

Demographics, baseline characteristics, and TEAEs were summarized using descriptive statistics in all enrolled subjects who received ≥1 dose of a study drug. The PK parameters were assessed in all subjects who received ≥1 dose of a study drug and whose PK data were adequate for calculation of ≥1 PK parameter. Levels of analyte below the level of quantification were entered as 0 for calculations. To assess the effect of lopinavir/ritonavir on the PK of isavuconazole, and vice versa, the log-transformed plasma AUC_τ and C_{max} values were analyzed using an analysis of variance with treatment as a factor. The 90% confidence intervals (CIs) for isavuconazole PK parameters were constructed around the geometric least-squares mean ratio of lopinavir/ritonavir plus isavuconazole to isavuconazole alone. The 90%CIs for lopinavir/ritonavir PK parameters were constructed around the geometric least-squares mean ratio of lopinavir/ritonavir plus isavuconazole to lopinavir/ritonavir alone. Analyses were conducted using Phoenix® WinNonlin® version 6.0 (Certara USA, Inc, Princeton, New Jersey). All data processing, summarization, and analyses were conducted using SAS® Version 9.1 (Statistical Analysis Software, Cary, North Carolina).

Results

Subjects

Thirteen healthy subjects were enrolled, and 12 completed part 1 of the study (Table 1). One subject did not complete part 1 due to a treatment-emergent adverse event (TEAE). Fifty-five healthy subjects were enrolled, and 50 completed part 2 of the study (Table 1). Three subjects discontinued part 2 due to TEAEs, and 2 subjects discontinued due to a family emergency and moving away.

Pharmacokinetics

Because of the potential for a strong PK interaction between lopinavir/ritonavir and isavuconazole, part 1 of the study was first conducted to evaluate coadministration of lopinavir/ritonavir with 50% of the clinical dose of isavuconazole, ie, 100 mg TID followed by 100 mg QD (administered as isavuconazonium sulfate

Table 1. Demographics and Baseline Characteristics

Characteristic	Part 1 (N = 13)	Part 2 (N = 55)
Sex, n (%)		
Male	7 (53.8)	28 (50.9)
Female	6 (46.2)	27 (49.1)
Race, n (%)		
White	7 (53.8)	32 (58.2)
Black or African American	6 (46.2)	16 (29.1)
Other[a]	0	7 (12.7)
Ethnicity, n (%)		
Not Hispanic or Latino	8 (61.5)	40 (72.7)
Age, mean (SD), years	32.5 ± 8.7	29.0 ± 8.0
Weight, mean (SD), kg	74.1 ± 16.8	73.2 ± 13.6
BMI, mean (SD), kg/m^2	25.2 ± 3.7	25.1 ± 3.7

BMI, body mass index.
[a]"Other" category includes "Asian," "American Indian or Alaska Native," and "Other."

Table 3. Statistical Analysis of Isavuconazole Pharmacokinetic Parameters in Parts 1 and 2

	Geometric Least-Squares Mean Ratio, % (90%CI)	
Parameter	Part 1 (Isavuconazole 100 mg)	Part 2 (Isavuconazole 200 mg)
AUC_τ	213 (174, 262)	196 (164, 235)
C_{max}	196 (155, 248)	174 (146, 208)

AUC_τ, area under the plasma concentration-time curve following last dose on day 13 (24 hours); C_{max}, maximum plasma concentration; CI, confidence interval.

and 33% lower in the presence vs absence of isavuconazole, respectively (Tables 4 and 5; see Figure 2 for concentration-time profiles). Plasma trough concentrations of lopinavir and ritonavir are shown in Figure S1.

Safety

No deaths or serious TEAEs were experienced in either part of the study. In part 1, 2 (33.3%) subjects experienced TEAEs of headache (n = 1) and nausea (n = 1) during isavuconazole alone, and 1 (14.3%) subject experienced TEAEs of headache and myalgia during coadministration of isavuconazole with lopinavir/ritonavir. All TEAEs were of mild intensity and considered to be possibly or probably related to drug treatment. None of the TEAEs was considered sufficient to prevent progression to part 2 of the study.

In part 2, 11 (61.1%) subjects in the isavuconazole-alone arm, 14 (73.7%) subjects in the lopinavir/ritonavir-alone arm, and 13 (72.2%) subjects in the isavuconazole-plus-lopinavir/ritonavir arm, experienced TEAEs. The TEAEs were considered to be possibly or probably drug related in the majority of subjects in the isavuconazole-alone (n = 19; 86.4%), lopinavir/ritonavir-alone (n = 28; 73.7%), and

186 mg). Mean AUC_τ and C_{max} of isavuconazole were 113% and 96% higher in the presence vs absence of lopinavir/ritonavir, respectively (Tables 2 and 3; see Figure 2 for concentration-time profile).

Having established that lopinavir/ritonavir did not increase the exposure of isavuconazole in excess of the prespecified limit of 150% in part 1 of the study, coadministration of lopinavir/ritonavir with the clinical dose of isavuconazole, ie, 200 mg TID followed by 200 mg QD (administered as isavuconazonium sulfate 372 mg) was examined in part 2. Mean AUC_τ and C_{max} of isavuconazole were 96% and 74% higher in the presence vs absence of lopinavir/ritonavir, respectively (Tables 2 and 3; see Figure 2 for concentration-time profile). By contrast, mean AUC_τ and C_{max} of lopinavir were 27% and 23% lower in the presence vs absence of isavuconazole, respectively (Tables 4 and 5). Likewise, mean AUC_τ and C_{max} of ritonavir were 31%

Table 2. Plasma Pharmacokinetics of Isavuconazole in Parts 1 and 2

	Part 1 (Isavuconazole 100 mg)		Part 2 (Isavuconazole 200 mg)	
Parameter[a]	Isavuconazole Alone (n = 6)	Isavuconazole + Lopinavir/Ritonavir (n = 6)[b]	Isavuconazole Alone (n = 17)[c]	Isavuconazole + Lopinavir/Ritonavir (n = 16)[d]
AUC_τ, h·µg/mL	54.9 (13.0)	116.2 (19.9)	113.8 (37.3)	221.6 (63.7)
C_{max}, µg/mL	3.4 (0.8)	6.6 (1.5)	7.8 (2.4)	13.6 (3.9)
t_{max}, hours	3.0 (2.0-4.0)	3.0 (2.0-4.0)	3.0 (2.0-6.0)	3.0 (1.0-4.0)

AUC_τ, area under the plasma concentration-time curve following last dose on day 13 (24 hours); C_{max}, maximum plasma concentration; t_{max}, time to C_{max}; TEAE, treatment-emergent adverse event.
[a]AUC_τ and C_{max} values are expressed as mean (standard deviation); t_{max} is expressed as median (range).
[b]One subject withdrew consent and discontinued the study (day 3).
[c]One subject withdrew consent and discontinued the study (day 11).
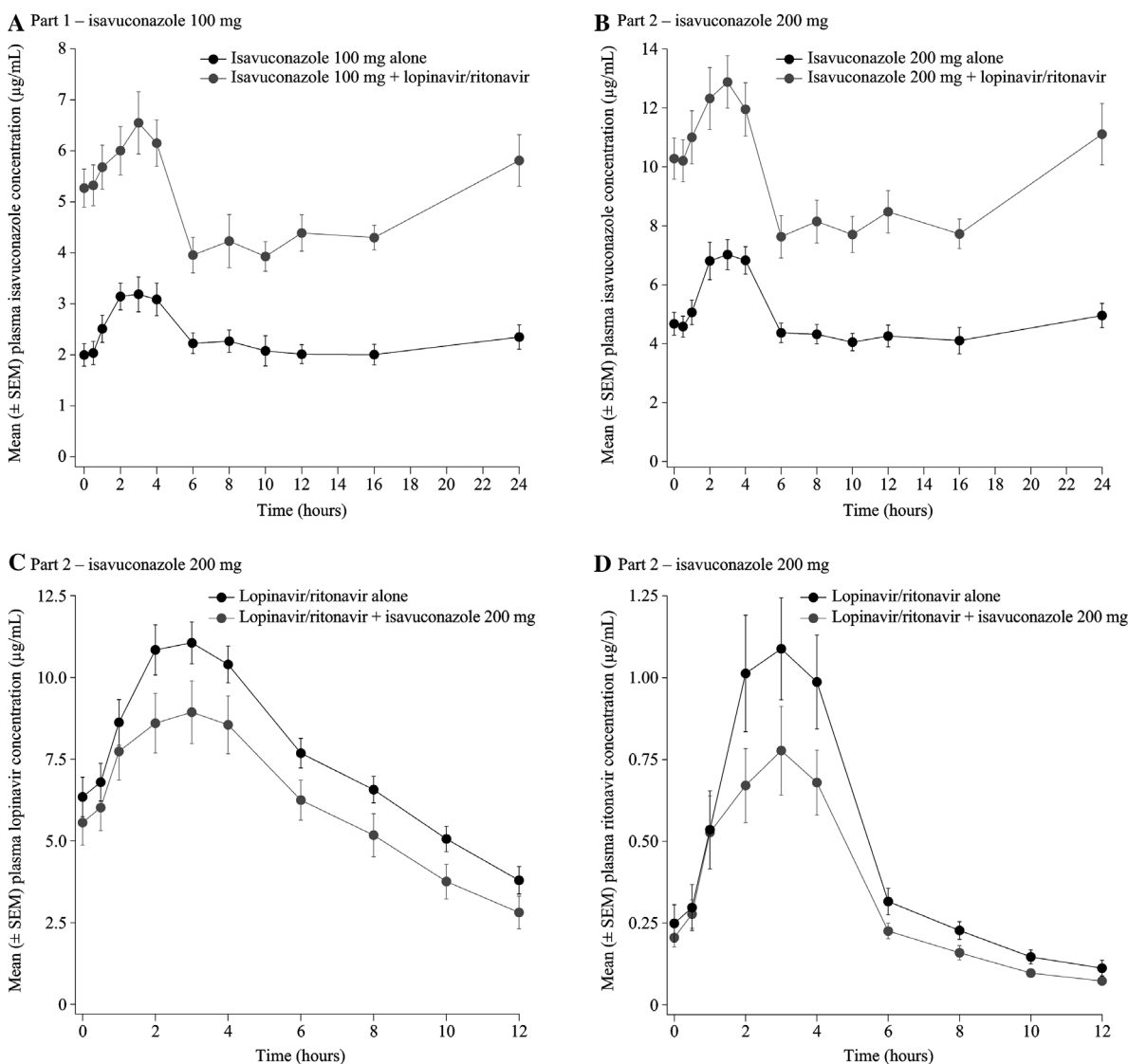[d]Two subjects discontinued the study due to TEAEs (days 2 and 3).

Figure 2. Mean plasma isavuconazole concentration-time profiles in the presence and absence of lopinavir/ritonavir in part 1 (A) and part 2 (B), and mean plasma lopinavir and ritonavir concentration-time profiles in the presence and absence of isavuconazole (C and D). SEM, standard error of the mean.

isavuconazole-plus-lopinavir/ritonavir (n = 52; 91.2%) arms. The most common TEAEs were those in the system organ class gastrointestinal disorders (Supplementary Table S1), which were experienced by more subjects during lopinavir/ritonavir alone (n = 12; 63.2%) and isavuconazole plus lopinavir/ritonavir coadministration (n = 11; 61.1%) than during isavuconazole alone (n = 3; 16.7%). Diarrhea, nausea, and abdominal pain were the most frequently experienced TEAEs in this class.

Three subjects in part 2 discontinued the study early due to TEAEs. One subject discontinued following mild, intermittent diarrhea during lopinavir/ritonavir alone administration on day 2. Another subject discontinued following an episode of mild nightmares

during isavuconazole plus lopinavir/ritonavir coadministration on day 3. A third subject discontinued following 2 episodes of moderate emesis during isavuconazole plus lopinavir/ritonavir coadministration on days 1 and 2. All TEAEs that led to study discontinuation resolved.

Discussion

This study evaluated the PK and safety effects of coadministration of the combined, fixed-dose antiviral agent lopinavir/ritonavir with isavuconazole in healthy human subjects. Coadministration with lopinavir/ritonavir significantly increased the exposure of isavuconazole, whereas the exposures of both

Table 4. Plasma Pharmacokinetics of Lopinavir and Ritonavir in Part 2

	Lopinavir		Ritonavir	
Parameter[a]	Lopinavir/Ritonavir Alone (n = 18)	Lopinavir/Ritonavir + Isavuconazole (n = 16)[b]	Lopinavir/Ritonavir Alone (n = 18)	Lopinavir/Ritonavir + Isavuconazole (n = 16)[b]
AUC_τ, h·μg/mL	91.0 (24.8)	73.3 (33.1)	5.5 (3.5)	4.0 (2.2)
C_{max}, μg/mL	11.4 (2.9)	9.2 (3.8)	1.2 (0.8)	0.8 (0.6)
t_{max}, hours	3.0 (2.0-4.1)	3.0 (0.0-4.0)	3.0 (2.0-4.0)	3.0 (0.0-4.0)

AUC_τ, area under the plasma concentration-time curve following last dose on day 13 (12 hours); C_{max}, maximum plasma concentration; t_{max}, time to C_{max}; TEAE, treatment-emergent adverse event.
[a]AUC_τ and C_{max} values are expressed as mean (standard deviation); t_{max} is expressed as median (range).
[b]Two subjects discontinued the study due to TEAEs (days 2 and 3).

Table 5. Statistical Analysis of Lopinavir and Ritonavir Pharmacokinetic Parameters in Part 2

	Geometric Least-Squares Mean Ratio, % (90%CI)	
Parameter	Lopinavir	Ritonavir
AUC_τ	73 (56, 96)	69 (48, 98)
C_{max}	77 (62, 95)	67 (46, 98)

AUC_τ, area under the plasma concentration-time curve following last dose on day 13 (12 hours); C_{max}, maximum plasma concentration; CI, confidence interval.

lopinavir and ritonavir were significantly decreased in the presence of isavuconazole.

The findings of this study support isavuconazole as a sensitive substrate of CYP3A4. Given that lopinavir/ritonavir is established as a strong inhibitor of CYP3A4,[7] it was surprising that exposure of isavuconazole was not increased by a greater degree than 2-fold. When isavuconazole and the strong CYP3A4 inhibitor ketoconazole are given together, the exposure of isavuconazole increases approximately 5-fold,[4] and it has been noted that ritonavir is a stronger inhibitor of CYP3A4 than ketoconazole.[8,9] The difference between these results may be at least in part owing to differences in study design. In the ketoconazole study, isavuconazole PK was assessed as a single added dose, whereas in the current study, subjects received multiple doses of both lopinavir/ritonavir and isavuconazole. It is also possible that the differences might be attributed to the fact that isavuconazole is also metabolized by CYP3A5 in vitro, and CYP3A5 has also been demonstrated to be inhibited by ketoconazole (albeit to a lesser extent[15–17]) but not by ritonavir. Therefore, it is possible that inhibition of isavuconazole metabolism by ritonavir was partially compensated by CYP3A5. However, another possibility is raised by the observation that, following continued exposure, ritonavir is known to mildly increase new CYP3A protein synthesis,[18] and isavucona-

zole itself has also demonstrated inductive potential for CYP3A4 in vitro (see above). Therefore, it is possible that, even though the net effect would be overall inhibition of CYP3A4, increases in CYP3A4 with prolonged exposure to both drugs might have permitted more CYP3A residual activity that could have attenuated greater increases in isavuconazole exposure.

Because the metabolism of both ritonavir and lopinavir are also mediated by CYP3A4, the possibility of coinduction of CYP3A4 by ritonavir and isavuconazole might also explain the reduced exposure of these drugs in this study. Ritonavir has been shown to induce metabolic enzymes,[14] as evidenced by the fact that predose concentrations of both lopinavir and ritonavir decline with time during multiple-dose therapy of the combination product prior to stabilization within 10 to 16 days (see KALETRA® package insert), and so the observed decrease in exposure may represent an increase in induction during coadministration of isavuconazole. In fact, reductions in lopinavir/ritonavir exposure have also been reported in a study of voriconazole plus low-dose ritonavir coadministration,[19] although potential long-term effects of voriconazole on induction of CYP3A4 have not been reported.

In addition to CYP3A4, lopinavir and ritonavir are inhibitors of the transporters P-gp, OATP1B1, and OATP1B3.[11–13] Ritonavir is also an inducer of UGT.[14] However, isavuconazole does not appear to be a substrate of these transporters in vitro (data on file) or in vivo,[6] and although isavuconazole undergoes secondary metabolism by UGT in vivo, it takes place only after metabolism by CYP3A4 and CYP3A5 (unpublished data). Together, these properties suggest that it is unlikely that interactions with P-gp, OATP1B1, OATP1B3, or UGT affected the metabolism of isavuconazole.

Few randomized trials have examined drug-drug interactions between the triazole antifungal medications and lopinavir/ritonavir. Because of the potential for increased levels of the antifungal agents, daily doses

of ketoconazole and itraconazole should not exceed 200 mg if coadministered with lopinavir/ritonavir (see KALETRA® package insert). By contrast, fluconazole PK is not expected to be impacted by coadministration with lopinavir/ritonavir. The PK effects of coadministration of voriconazole and posaconazole with lopinavir/ritonavir have not been evaluated. However, coadministration of lopinavir/ritonavir with voriconazole is not recommended due to disrupted voriconazole PK when given with ritonavir alone[19,20] ie, decreased voriconazole exposure possibly caused by induction of CYP2C9 and CYP2C19 activity by ritonavir.[19]

Overall, no unexpected safety concerns were reported in this study. There were no deaths or serious TEAEs, and discontinuations due to study treatments were rare. The majority of TEAEs were mild gastrointestinal disorders and had resolved by the end of the study.

In summary, the findings of this study suggest that clinical doses of isavuconazole may be safely coadministered with lopinavir/ritonavir. No modification of isavuconazole or lopinavir/ritonavir doses is recommended when the drugs are coadministered. Patients should be monitored for reduced antiviral efficacy to ensure adequate therapy. The results obtained in this study may be useful to predict interactions between isavuconazole and other clinically important antiretroviral medications. Currently recommended protease inhibitors for HIV therapy include darunavir/ritonavir and atazanavir/ritonavir.[21] Because each agent is boosted with the CYP3A4 inhibitor ritonavir, there is a possibility that coadministration with these agents may lead to increased exposure to isavuconazole.

Acknowledgments

Declaration of Conflicting Interests

Isavuconazole was codeveloped by Astellas Pharma Global Development, Inc. and Basilea Pharmaceutica International Ltd. T.Y., A.D., K.K., D.K., S.A., C.L., L.K., and R.T. are employees of Astellas Pharma Global Development, Inc. D.H. is an employee of PAREXEL who was contracted by Astellas Pharma Global Development, Inc. to perform the study.

References

1. Marukutira T, Huprikar S, Azie N, Quan SP, Meier-Kriesche HU, Horn DL. Clinical characteristics and outcomes in 303 HIV-infected patients with invasive fungal infections: data from the Prospective Antifungal Therapy Alliance registry, a multicenter, observational study. *HIV AIDS (Auckl)*. 2014;6:39-47.

2. Maertens JA, Raad II, Marr KA, et al. Isavuconazole versus voriconazole for primary treatment of invasive mould disease caused by *Aspergillus* and other filamentous fungi (SECURE): a phase 3, randomised-controlled, non-inferiority trial. *Lancet*. 2016;387:760-769.

3. Marty FM, Ostrosky-Zeichner L, Cornely OA, et al. Isavuconazole treatment for mucormycosis: a single-arm open-label trial and case-control analysis. *Lancet Infect Dis*. 2016;16:828-837.

4. Townsend R, Dietz AJ, Hale C, et al. Pharmacokinetic evaluation of CYP3A4-mediated drug-drug interactions of isavuconazole with rifampin, ketoconazole, midazolam, and ethinyl estradiol/norethindrone in healthy adults. *Clin Pharmacol Drug Dev*. 2017;6:44-53.

5. Yamazaki T, Desai A, Goldwater R, et al. Pharmacokinetic effects of isavuconazole co-administration with the cytochrome P450 enzyme substrates bupropion, repaglinide, caffeine, dextromethorphan, and methadone in healthy subjects. *Clin Pharmacol Drug Dev*. 2017;6: 54-65.

6. Yamazaki T, Desai A, Goldwater R, et al. Pharmacokinetic interactions between isavuconazole and the drug transporter substrates atorvastatin, digoxin, metformin, and methotrexate in healthy subjects. *Clin Pharmacol Drug Dev*. 2017;6:66-75.

7. Groll AH, Desai A, Han D, et al. Pharmacokinetic assessment of drug-drug interactions of isavuconazole with the immunosuppressants cyclosporine, mycophenolic acid, prednisolone, sirolimus, and tacrolimus in healthy adults. *Clin Pharmacol Drug Dev*. 2017;6: 76-85.

8. Greenblatt DJ, Harmatz JS. Ritonavir is the best alternative to ketoconazole as an index inhibitor of cytochrome P450-3A in drug-drug interaction studies. *Br J Clin Pharmacol*. 2015;80:342-350.

9. Greenblatt DJ. Evidence-based choice of ritonavir as index CYP3A inhibitor in drug-drug interaction studies. *J Clin Pharmacol*. 2016;56:152-156.

10. Weemhoff JL, von Moltke LL, Richert C, Hesse LM, Harmatz JS, Greenblatt DJ. Apparent mechanism-based inhibition of human CYP3A in-vitro by lopinavir. *J Pharm Pharmacol*. 2003;55:381-386.

11. Lee CG, Gottesman MM, Cardarelli CO, et al. HIV-1 protease inhibitors are substrates for the MDR1 multidrug transporter. *Biochemistry*. 1998;37:3594-3601.

12. Vishnuvardhan D, von Moltke LL, Richert C, Greenblatt DJ. Lopinavir: acute exposure inhibits P-glycoprotein;

extended exposure induces P-glycoprotein. *AIDS.* 2003;17:1092-1094.

13. Annaert P, Ye ZW, Stieger B, Augustijns P. Interaction of HIV protease inhibitors with OATP1B1, 1B3, and 2B1. *Xenobiotica.* 2010;40:163-176.

14. Foisy MM, Yakiwchuk EM, Hughes CA. Induction effects of ritonavir: implications for drug interactions. *Ann Pharmacother.* 2008;42:1048-1059.

15. Gibbs MA, Thummel KE, Shen DD, Kunze KL. Inhibition of cytochrome P-450 3A (CYP3A) in human intestinal and liver microsomes: comparison of K_i values and impact of CYP3A5 expression. *Drug Metab Dispos.* 1999;27:180-187.

16. Patki KC, Von Moltke LL, Greenblatt DJ. In vitro metabolism of midazolam, triazolam, nifedipine, and testosterone by human liver microsomes and recombinant cytochromes p450: role of cyp3a4 and cyp3a5. *Drug Metab Dispos.* 2003;31:938-944.

17. Allqvist A, Miura J, Bertilsson L, Mirghani RA. Inhibition of CYP3A4 and CYP3A5 catalyzed metabolism of alprazolam and quinine by ketoconazole as racemate and four different enantiomers. *Eur J Clin Pharmacol.* 2007;63:173-179.

18. Kirby BJ, Collier AC, Kharasch ED, Whittington D, Thummel KE, Unadkat JD. Complex drug interactions of HIV protease inhibitors 1: inactivation, induction, and inhibition of cytochrome P450 3A by ritonavir or nelfinavir. *Drug Metab Dispos.* 2011;39:1070-1078.

19. Liu P, Foster G, Gandelman K, et al. Steady-state pharmacokinetic and safety profiles of voriconazole and ritonavir in healthy male subjects. *Antimicrob Agents Chemother.* 2007;51:3617-3626.

20. Yakiwchuk EM, Foisy MM, Hughes CA. Complexity of interactions between voriconazole and antiretroviral agents. *Ann Pharmacother.* 2008;42:698-703.

21. Department of Health and Human Services. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. https://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf. Accessed April 8, 2015.

Supporting Information

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