ARTICLE

Clinical Drug–Drug Interaction Potential of BFE1224, Prodrug of Antifungal Ravuconazole, Using Two Types of Cocktails in Healthy Subjects

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BFE1224, prodrug of ravuconazole, is a novel, once-daily, oral, triazole antifungal drug, and currently in development for the treatment of onychomycosis. The clinical drug–drug interaction (DDI) potential of BFE1224 with cytochrome P450 (CYP) and transporter was assessed by using two types of cocktails in healthy subjects in separate clinical studies. The CYP and transporter cocktails consisted of caffeine/tolbutamide/omeprazole/dextromethorphan/midazolam used in study 1 and digoxin/rosuvastatin used in study 2. In addition, repaglinide was separately administered to the same subjects in study 2. There were no major effects on the pharmacokinetics of CYP and transporter substrates, except for an approximate threefold increase in midazolam exposure after oral administration of BFE1224. The clinical DDIs of BFE1224 were mild for CYP3A and minor for other major CYPs (CYP1A2/2C8/2C9/2C19/2D6) as well as those of P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), organic anion transporting polypeptide (OATP) 1B1, and OATP1B3.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✔ Itraconazole and terbinafine are used worldwide as oral drugs for onychomycosis because of their high initial cure rates, although they have contraindications with many medicines, especially for itraconazole, owing to pharmacokinetic DDI. Therefore, new oral antifungals that can overcome DDI issues are required.

WHAT QUESTION DID THIS STUDY ADDRESS?

✔ This study addressed the question of potential clinical DDIs of a new triazole, BFE1224, by using novel CYP and transporter cocktails.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

✔ BFE1224 is considered a moderate inhibitor against CYP3A *in vivo*, but has no clinically relevant effects on CYP1A2, 2C8, 2C9, 2C19, and 2D6, or P-gp, BCRP, OATP1B1, and OATP1B3.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

✔ These two novel CYP or transporter cocktails are valuable and efficient tools for the investigation of CYP- or transporter-based DDIs and accelerate drug development.

Ravuconazole is a novel, broad spectrum, triazole antifungal agent, which inhibits CYP51 present in all yeasts and molds, and an active component of prodrug BFE1224, which is under investigation as an oral (p.o.) treatment drug for onychomycosis.[1,2] Since BFE1224 is almost completely converted to ravuconazole during oral absorption, BFE1224 is scarcely detected in plasma. Ravuconazole, an active component of BFE1224, reached maximum plasma concentration (C_{max}) at 2.5–3.3 hours after oral administration of BFE1224 followed by elimination with a half-life of 71–101 hours (unpublished data). Although two systemic antifungals, terbinafine and itraconazole, are currently available worldwide, their use is limited because of high recurrence rates, problematic adverse effects, or drug–drug interactions (DDIs).[3–17] BFE1224, as a new generation triazole drug, is expected to be a promising candidate to overcome the limitations of currently available oral therapies for onychomycosis, has broad antimicrobial coverage against almost all onychomycosis-causing agents, including dermatophytes, *Candida* spp., and several nondermatophytic molds.[18–24] In addition, clinical trials have shown that BFE1224 has high cure rates (unpublished data), no severe adverse effects, good tolerability, and a low DDI risk.[25]

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Table 1 Demographic and other baseline characteristics (safety population)

Characteristic		Study 1	Study 2
n		30	12
Gender n (%)	Male	22 (73.3%)	12 (100%)
	Female	8 (26.7%)	
Race n (%)	Black or African American	5 (16.7%)	
	White	25 (83.3%)	
	Asian (Japanese)		12 (100%)
Age (years)	Mean (SD)	35.7 (6.29)	27.5 (7.5)
	Median	36.5	24.5
	Range	22–45	21–42
Weight (kg)[a]	Mean (SD)	74.06 (9.93)	64.54 (4.72)
	Median	72.50	65.55
	Range	56.3–105.4	57.6–71.8
Height (cm)[a]	Mean (SD)	168.3 (9.32)	171.4 (4.02)
	Median	169.5	171.7
	Range	154–189	164.3–177.7
CYP2D6 phenotype n (%)	Extensive metabolizer	26 (86.7%)	n/a
	Intermediate metabolizer	1 (3.3%)	
	Poor metabolizer	1 (3.3%)	
	See interpretation[b]	1 (3.3%)	
	Missing[c]	1 (3.3%)	
CYP2C19 phenotype n (%)	Extensive metabolizer	30 (100.0%)	n/a
CYP2C9 phenotype n (%)	Extensive metabolizer	20 (66.7%)	n/a
	Intermediate metabolizer	10 (33.3%)	

n/a: not available; SD: standard deviation.
[a]Screening value.
[b]Despite repeated attempts, analysis for the single nucleotide polymorphism (SNP) 1846 G>A failed. Without this SNP, it was not possible to distinguish between two possible genotypes.
[c]Data were missing.

It has become very important to obtain *in vivo* information on DDIs from a safety aspect, as multiple drug therapy is commonly used in clinical practice. Although the *in vitro* evaluation to assess the metabolism- and transporter-mediated DDIs is established at a relatively early stage of drug development in many pharmaceutical companies, *in vivo* clinical evaluations are required for compounds that exceed the criteria set by regulatory agencies[26-28]; these drug interaction trials are costly and time-consuming.

CYP and transporter *in vitro* inhibition studies were conducted for only ravuconazole in accordance with the DDI guideline,[26] since BFE1224 is almost completely converted to ravuconazole before systemic exposure *in vivo*. As ravuconazole showed *in vitro* inhibitory activities against several CYP enzymes (IC_{50} values were as follows: CYP2C8/2C9/2C19/3A(testosterone)/3A(midazolam) = 2.69/1.51/7.49/2.28/1.07 μmole/L), as well as transporters, P-gp (IC_{50} = 7.12 μmole/L) and BCRP (IC_{50} = 1.14 μmole/L) (unpublished data), a cocktail approach was chosen for the evaluation of clinical DDI after oral administration of BFE1224. Ravuconazole was not an inhibitor for the other CYPs (CYP1A2/2B6/2D6) as well as transporters (organic anion transporter (OAT) 1/3**,** organic anion transporting polypeptide (OATP) 1B1/1B3, and multidrug and toxin extrusion (MATE) 1/2-K) (unpublished data). With regard to organic cation transporter (OCT) 2, ravuconazole was inhibited, with IC_{50} 2.80 μmole/L *in vitro*, which was lower than the criteria in the guideline to go to a clinical DDI study.

A number of cocktail methods have been previously reported.[29–32] In this report we investigated the effects of clinical CYP- and transporter-mediated DDIs by BFE1224 using two novel CYP and transporter cocktails, and evaluated their usefulness in drug development.

METHODS

Two clinical DDI studies, 1 and 2, were reviewed and approved by the Institutional Review Board (Independent Investigational Review Board Inc., Florida, USA and Souseikai, Fukuoka, Japan, respectively), and conducted in compliance with Good Clinical Practice by using probe drugs in two new cocktails, a Cooperstown W/T cocktail (a new CYP cocktail which modified the Cooperstown cocktail[29] with tolbutamide instead of warfarin) and a new transporter cocktail containing digoxin and rosuvastatin.

Clinical trial design

Study 1

This was an open-label, two-period study to determine the effect of BFE1224 on the metabolism of major CYP probe drugs in healthy subjects. It was conducted in two periods separated by at least a 3-day washout. On Day 1, after a minimum 10-hour fast, the subjects were orally administered a single dose of a Cooperstown W/T CYP cocktail (caffeine (200 mg), tolbutamide (500 mg), omeprazole (40 mg), dextromethorphan (60 mg), and midazolam (2 mg syrup)) with 240 mL water. On Day 2, midazolam (2 mg/100 mL) was infused intravenously (i.v.) over 30 minutes.

On Day 8, after at least a 10-hour fast, the subjects were administered BFE1224 alone for 9 days (loading dose: 400 mg twice daily on Days 8, 9, and 10, followed by maintenance dose of 200 mg once daily on Days 11–16) to achieve a steady state. On Day 15, after a 10-hour fast, BFE1224 and the probe drug cocktail were orally administered with 240 mL water. On Day 16, 2 hours after the administration of BFE1224, midazolam was administered via a 30-minute i.v. infusion.

The plasma samples for the pharmacokinetic (PK) analysis were collected as follows: i) for BFE1224 and ravuconazole, Days 8–14 (before dosing), Days 16–18 (0, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 18, 24 (Day 17), and 48 (Day 18) hours after dosing), Days 23, 30, 37 (after the last dose); ii) for probe drugs and their metabolites, Days 1 and 15 (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, and 48 hours after p.o.), Days 2 and 16 (0, 10, 20, 30, 35, 45 minutes, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, and 24 hours after the start of midazolam i.v.).

Study 2

This was an open-label, two-period study to determine the effects of orally administered BFE1224 on the PK of repaglinide, digoxin, and rosuvastatin in healthy subjects. On Day 1,

Table 2 Summary of bioanalytical assay performance for probe drugs and their metabolites as well as BFE1224 and ravuconazole

Drug	Extraction method used	Analyte	LLOQ (ng/mL)	ULOQ (ng/mL)	Between-run %CV	Maximum % deviation from nominal concentration	MS/MS conditions (m/z)
BFE1224	LLE	BFE1224	100	25,000	3.3–4.6	7.2	548.0→450.0
		Ravuconazole (study 1)	200	50,000	5.4–5.9	9.2	438.0→224.0
		Ravuconazole (study 2)	25	25,000	1.8–7.5	5.5	438.2→224.1
Caffeine	LLE	Caffeine	25.0	25,000	3.9–5.7	–2.9	194.9→137.8
		1,7-Dimethylxanthine	25.0	25,000	3.6–6.6	–2.6	180.9→123.6
Repaglinide	SPE	Repaglinide	0.005	5	4.5–10.9	2.1	453.4→230.2
Tolbutamide	LLE	Tolbutamide	100	10,0000	6.2–10.3	2.2	269.1→170.0
		Carboxytolbutamide	5.00	5,000	4.7–7.7	5.5	299.0→199.9
		Hydroxytolbutamide	2.50	2,500	3.9–11.0	–2.5	284.9→186.0
Omeprazole	LLE	Omeprazole	1.00	1,000	3.1–7.6	–2.6	346.0→198.1
		5-Hydroxyomeprazole	1.00	1,000	3.1–5.3	–6.5	361.8→213.9
Dextromethorphan	LLE	Dextromethorphan	0.0500	50.0	5.0–9.4	4.0	272.4→215.4
		Dextrorphan	0.800	800	4.7–7.9	3.1	258.4→199.3
Midazolam	LLE	Midazolam	0.100	100	3.8–5.3	–2.0	325.7→290.9
		1'-Hydroxymidazolam	0.100	100	4.0–6.5	4.6	341.7→323.7
Digoxin	SLE	Digoxin	0.05	25	1.8–6.2	2.3	798.5→651.4
Rosuvastatin	SPE	Rosuvastatin	0.05	100	0.9–7.0	4.0	482.0→258.0

LLE: liquid–liquid extraction, SPE: solid phase extraction, SLE: solid supported liquid–liquid extraction, LLOQ: lower limit of quantification, ULOQ: upper limit of quantification, CV: coefficient of variation.

after at least a 10-hour fast, the subjects were orally administered a single dose of the CYP2C8 probe drug, repaglinide (0.25 mg), with 200 mL water. On Day 2, the transporter cocktail, which consisted of digoxin (0.25 mg) and rosuvastatin (5 mg), was administered. On Day 5, the subjects were administered BFE1224 for 7 days at a dose of 400 mg/day under fasted conditions. Repaglinide and the transporter cocktail were administered under fasted conditions on Days 10 and 11, respectively.

The plasma samples for PK analysis were collected as follows: i) for ravuconazole, Day 5 (before dosing), Days 6–12 (24 (Day 6: 0 hour), 48, 72, 96, 120, 144, and 168 (Day 12: 0 hour) hours after the first dose); ii) for repaglinide, Days 1 and 10 (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, and 8 hours after p.o.), for digoxin and rosuvastatin, Days 2 and 11 (0, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, and 48 hours after p.o.).

Clinical trial participants

The demographics of the subjects and other baseline characteristics are summarized in **Table 1**.

Study 1

Thirty subjects (22–45 years old) were enrolled and entered Period I, and 29 subjects continued to enter Period II. The majority of the subjects were white (83.3%) and male (73.3%). All subjects were genotyped as extensive CYP2C19 metabolizers and the majority were extensive CYP2D6 (86.7%) and 2C9 (66.7%) metabolizers.

Study 2

Twelve male Japanese subjects (21–42 years old) were enrolled and all subjects completed the study.

Assays

The plasma concentrations of BFE1224, ravuconazole, and the probe drugs and their metabolites were extracted using liquid–liquid extraction, solid phase extraction, or solid-supported liquid–liquid extraction and then measured by validated assays using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

The information for the analyte, between-run precision (%CV), maximum % deviation from nominal concentration, lower limit of quantification (LLOQ), and upper limit of quantification (ULOQ) are summarized in **Table 2**.

Data analysis

PK data

A noncompartmental PK method was used to calculate the area under plasma concentration–time curve (AUC), maximum plasma concentration (C_{max}), time to reach C_{max} (t_{max}), and elimination half-life ($t_{1/2}$) of probe drugs and their metabolites as well as ravuconazole by using WinNonlin Professional v. 4.1 or higher. Additionally, to assess the interaction levels of each probe drug, 90% confidence intervals (CIs) for the ratio of the geometric means (GMR) between Period I and Period II for the AUC and C_{max} of the corresponding probe drug were calculated.

Safety data

The incidence of adverse events, vital signs, physical examination results, and changes from baseline laboratory values were summarized for all subjects enrolled in the studies and the appropriate descriptive statistics provided.

Genotyping

DNA was extracted from 3-mL blood sample collected at screening in study 1, and genotyping for CYP2C9/2C19/2D6

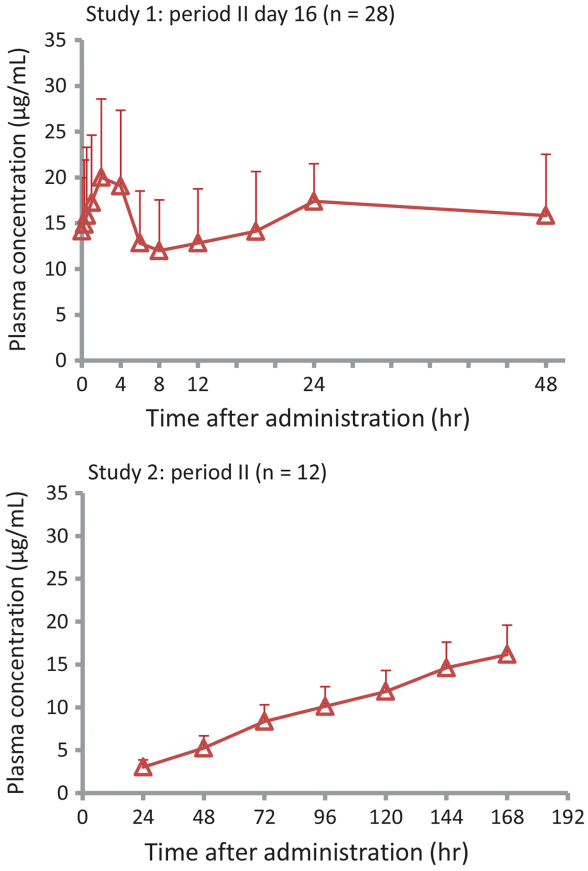

Figure 1 Mean plasma concentration–time profiles of ravuconazole after oral administration of BFE1224. Data are presented mean + SD ($n = 28$) and mean + SD ($n = 12$) in studies 1 and 2, respectively. △ Study 1, Period II on day 16: with BFE1224 (800/200 mg), Study 2, Period II: with BFE1224 (400 mg)

was performed on the TM bioscience/luminex universal array platform using primer extension chemistry. The genotyping was not conducted in study 2.

RESULTS

Safety

There were no serious adverse events or deaths in these two studies. Four (13.3%) and 24 (82.8%) subjects reported treatment-emergent adverse events (TEAEs) during Periods I (probe drug alone) and II (probe drug + BFE1224), respectively, in study 1. It should be noted, however, that the reporting period for Period II was 30 days compared with 8 days for Period I. The frequently (>10%) reported TEAEs were dizziness, headache, anxiety, liver function test abnormal, thrombin time abnormal, euphoric mood, and vomiting. Most TEAEs were mild in severity and nearly all were considered either possibly or probably related to study treatment.

On the other hand, in study 2 only one TEAE (8.3%, mild gastroenteritis) was reported in Period II and considered to be no relation to study treatment.

Plasma BFE1224 and ravuconazole concentrations

In study 1, both BFE1224 and ravuconazole in plasma were measured: BFE1224 was detected in only six samples in five subjects with 1/40–1/100 of ravuconazole minimum concentration (C_{min}), therefore PK analysis for BFE1224 was not conducted; ravuconazole reached t_{max} at 3.12 hours after dosing with a C_{max} of 24.07 μg/mL and C_{min} value of 11.93 μg/mL on day 16 (**Figure 1**). In study 2, only plasma ravuconazole was measured: the C_{min} values on Days 5 and 6 were 11.85 and 14.61 μg/mL, respectively (**Figure 1**). In both studies, ravuconazole concentrations exceeded the plasma ravuconazole concentration (10.84 μg/mL) observed at 100 mg (quaque die (q.d.), therapeutic dose) for consecutive once-daily administration for 12 weeks in phase II clinical trials (unpublished data).

Effects of BFE1224 oral administration on pharmacokinetics of major CYPs

The mean plasma concentration–time profiles and PK parameters of probe drugs and their metabolites are shown in **Figures 2 and 3** and **Table 3**, respectively.

Effect of BFE1224 on CYP1A2 (study 1)

The mean plasma concentration–time profiles of caffeine and its metabolite, 1,7-dimethylxanthine, in Period I were similar to those in Period II (**Figure 2**). The AUC and C_{max} for caffeine showed less than a 10% difference. The AUC and C_{max} for 1,7-dimethylxanthine decreased by ~25% and 20%, respectively, after coadministration of BFE1224. The 90% CI values of the GMR of both the AUC and C_{max} data for caffeine were between 0.8 and 1.25, which was indicative of bioequivalence between Periods I and II (**Table 4**). In contrast, the 90% CI values for the metabolites were slightly lower than this range (GMR (90% CI) AUC: 0.778 (0.739, 0.818); C_{max}: 0.811 (0.768, 0.857)). Although these metabolite data suggest that there is a small possibility that BFE1224 affects CPY1A2, this effect is not likely to be clinically relevant and does not meet the definitions of a weak effect in the US Food and Drug Administration (FDA) DDI guidance.[27]

Effect of BFE1224 on CYP2C8 (study 2)

The mean plasma concentration–time profiles and the PK parameters of repaglinide were similar in both periods (**Figure 2**, **Table 3**).

The 90% CI values for the GMR of the AUC and C_{max} of repaglinide were mostly between 0.8 and 1.25 (GMR (90% CI) AUC: 1.012 (0.903–1.134); C_{max}: 1.065 (0.878–1.292)) (**Table 4**), indicating that BFE1224 does not affect CYP2C8 activity.

Effect of BFE1224 on CYP2C9 (study 1)

The mean plasma concentration–time profiles of tolbutamide and its metabolites, carboxytolbutamide and hydroxytolbutamide, and their PK parameters were similar in both periods.

The 90% CI values for the ratios of the AUC and C_{max} were both between 0.8 and 1.25 for tolbutamide and for its metabolites, carboxytolbutamide and hydroxytolbutamide (**Table 4**). These data indicate that the AUC and C_{max} of tolbutamide and its metabolites could be considered equivalent with or without BFE1224 coadministration.

Collectively, the data suggest that BFE1224 does not affect CYP2C9 activity, as the disposition of tolbutamide and its metabolites are shown to be bioequivalent regardless of the presence or absence of BFE1224 coadministration.

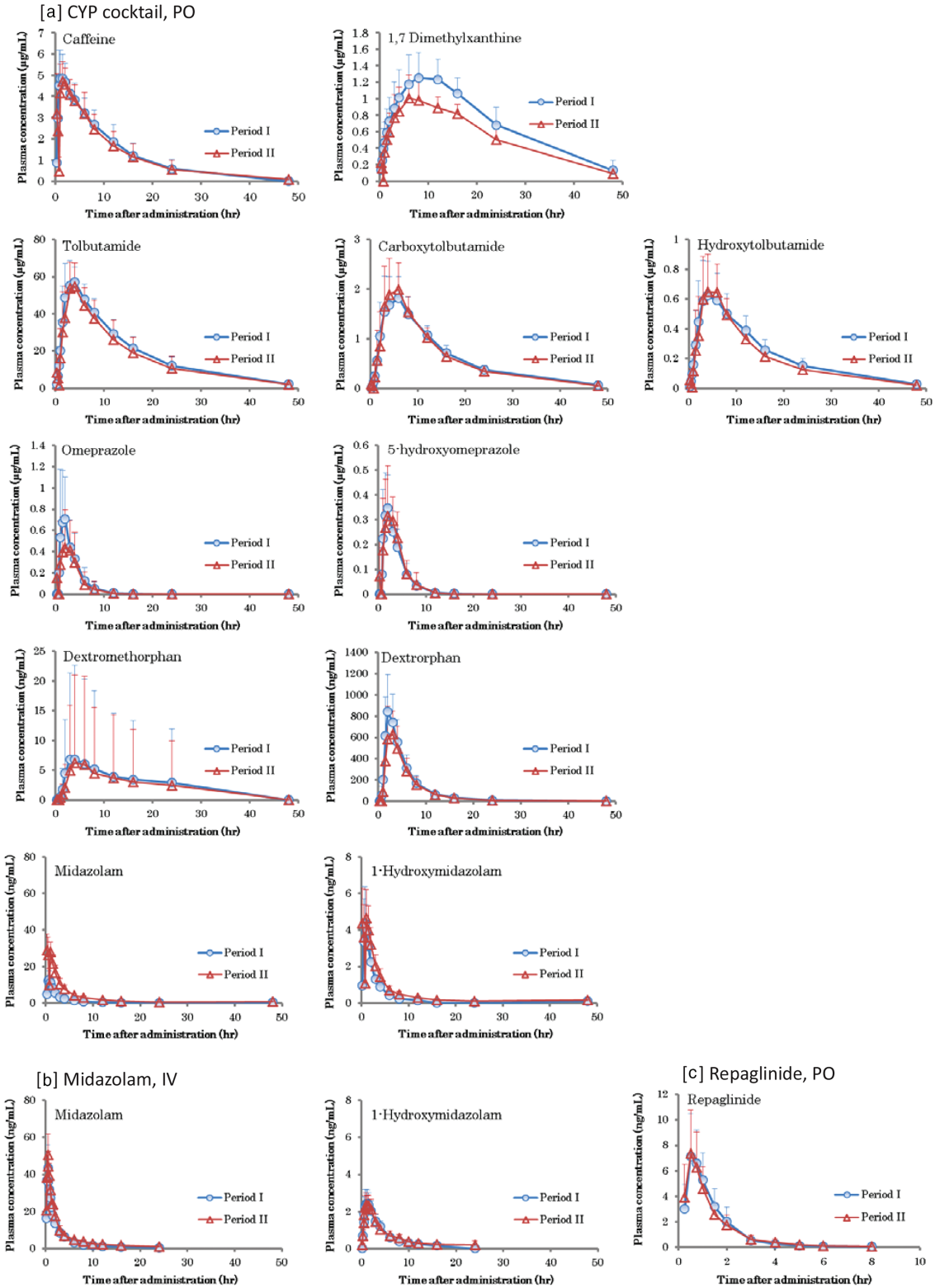

Figure 2 Mean plasma concentration–time profiles of CYP probe drugs and their metabolites after administration of CYP cocktail or repaglinide with or without coadministration of BFE1224. CYP: Cytochrome P450. (**a**) CYP cocktail, p.o. Data are presented mean + SD ($n = 27$–28). (**b**) Midazolam, i.v. Data are presented mean + SD ($n = 24$–28). (**c**) Repaglinide, p.o. Data are presented mean + SD ($n = 12$). ○Period I: cocktail or repaglinide alone, △Period II: cocktail + BFE1224 (800/200 mg) or repaglinide + BFE1224 (400 mg). CYP cocktail: caffeine, tolbutamide, omeprazole, dextromethorphan, and midazolam. Midazolam syrup and other probe drugs were orally administered on Days 1 and 15, and midazolam was intravenously infused on Days 2 and 16.

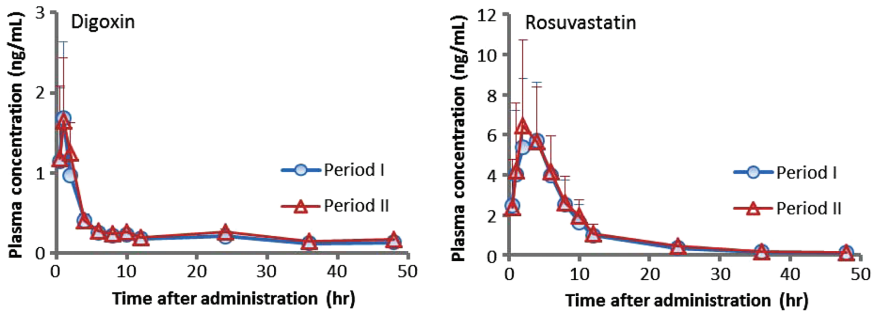

Figure 3 Mean plasma concentration–time profiles of transporter probe drugs after oral administration of transporter cocktail with or without coadministration of BFE1224. Data are presented mean + SD ($n = 12$). ○Period I: cocktail alone, △Period II: cocktail + BFE1224 (400 mg). Transporter cocktail: digoxin and rosuvastatin.

Effect of BFE1224 on CYP2C19 (study 1)

The mean plasma concentration–time profile of omeprazole was lower between 1 and 3 hours after BFE1224 administration than that of omeprazole alone (**Figure 2**). In contrast, the profile of the metabolite of omeprazole, 5-hydroxyomeprazole, was similar in both periods.

The AUC and C_{max} for omeprazole decreased slightly by BFE1224 coadministration (~20%–25% decrease), but values for 5-hydroxyomeprazole were similar in both periods (less than 5% difference, **Table 3**).

The 90% CI values for the ratio of the AUC and C_{max} in Periods II/I for omeprazole were both below the range of 0.8–1.25, but those for 5-hydroxyomeprazole were both between 0.8 and 1.25 (**Table 4**). However, this effect is unlikely to be clinically relevant, as it represents only a 20%–25% decrease in both AUC and C_{max}; this change in AUC value is below that defined for a CYP inducer in the FDA DDI guidance.[27]

Effect of BFE1224 on CYP2D6 (study1)

During Period II, the mean plasma concentrations of dextromethorphan were slightly lower at most time points after the first hour compared with that in Period I (**Figure 2**). The mean plasma concentrations of the metabolite dextrorphan were generally lower, between 1 and 6 hours of Period II than in Period I, but were thereafter generally similar in both periods and the t_{max} was slightly increased from 2 to 3 hours.

The AUC and C_{max} of dextromethorphan decreased by ~30% and 20%, respectively, after coadministration of BFE1224 (**Table 3**). There was a slight decrease in the AUC and C_{max} of dextrorphan during Period II (~15% and 20%, respectively).

The 90% CI values for the ratio of the AUC and C_{max} were below the range of 0.8–1.25 for dextromethorphan, whereas these PK parameters were equivalent for dextrorphan, suggesting that BFE1224 has no effect on the CYP2D6 metabolic pathway (**Table 4**).

Effect of BFE1224 on CYP3A (study 1)

Effect of BFE1224 on CYP3A following oral administration of midazolam

The mean plasma concentrations of midazolam were higher in the presence of BFE1224 (**Figure 2**). The difference was observed 15 minutes after the oral administration of midazolam and continued for 16 hours. The coadministration of BFE1224 had a small effect on the plasma concentrations of 1′-hydroxymidazolam.

After coadministration of BFE1224, the AUC and C_{max} of midazolam were ~3- and 2.4-fold higher, respectively (**Table 3**), and the oral clearance (CL/F) of midazolam decreased by over 65%, but the $t_{1/2}$ minimally increased. The AUC for 1′-hydroxymidazolam increased by over 50%, but the C_{max} was only minimally affected by BFE1224 coadministration. The 90% CI values for the ratio of the AUC and C_{max} in Periods II/I were well above the range of 0.8–1.25 for midazolam (**Table 4**). These data suggest that ravuconazole suppresses the metabolism of midazolam. For the metabolite 1′-hydroxymidazolam, the 90% CI for the ratio of the AUC was well above the range of 0.8–1.25 and the value for the C_{max} was slightly above that range, providing evidence that BFE1224 affects the metabolism of orally administered midazolam.

The FDA DDI guidance[27] indicates that based on the current results, BFE1224 can be considered a moderate inhibitor of CYP3A, as it meets the following criteria: two to fivefold increase in the AUC of midazolam and a decrease in the oral clearance of midazolam in excess of 50%.

Effect of BFE1224 on CYP3A after intravenous infusion of midazolam

The AUC of midazolam increased 1.4-fold by BFE1224 coadministration (**Table 3**). The C_{max} and $t_{1/2}$ of midazolam increased slightly, but the t_{max} remained relatively unchanged. The $t_{1/2}$ for 1′-hydroxymidazolam increased by ~30% by BFE1224 coadministration. The t_{max} and AUC of 1′-hydroxymidazolam slightly increased, but the C_{max} remained relatively unchanged. The 90% CI for the ratio of the AUC in Period II/I was slightly above the range of 0.8–1.25 for midazolam, but that of the C_{max} was mostly within the range of 0.8–1.25 (**Table 4**). For the metabolite, 1′-hydroxymidazolam, the 90% CI for the ratios of AUC and C_{max} were both within the range of 0.8–1.25. These data indicate that the influence of coadministration of BFE1224 on midazolam PK is marginal in the case of i.v. infusion.

Effects of BFE1224 oral administration on major transporters

Effect of BFE1224 on P-gp (study 2)

The PK profiles and parameters of digoxin were generally similar in both periods (**Figure 3**, **Table 3**). The 90% CI

Table 3 Pharmacokinetics parameters of probe drugs and their metabolites

Probe drug / metabolite	Period	C_{max} (μg/mL)	AUC_{inf} (μg. hr/mL)	$t_{1/2}$ (hr)	t_{max} (hr)	CL (L/hr)
Caffeine	I	5.48 (1.30)	57.46 (20.06)	6.96 (2.38)	1.00	n/a
	II	5.13 (0.98)	54.15 (20.29)	6.97 (2.46)	1.50	n/a
1,7-Dimethylxanthine	I	1.33 (0.25)	32.38 (6.60)	8.34 (2.55)	8.00	n/a
	II	1.07 (0.22)	24.31 (2.78)	8.39 (1.54)	6.00	n/a
Tolbutamide	I	61.28 (10.21)	878.9 (212.4)	8.75 (2.20)	3.00	n/a
	II	61.03 (10.61)	796.5 (280.7)	8.59 (2.48)	3.00	n/a
Carboxytolbutamide	I	1.91 (0.53)	28.0 (4.2)	8.59 (2.34)	6.00	n/a
	II	2.20 (0.65)	27.6 (3.1)	8.12 (2.25)	6.00	n/a
Hydroxytolbutamide	I	0.66 (0.22)	10.4 (2.2)	9.11 (2.46)	4.00	n/a
	II	0.72 (0.23)	9.5 (1.4)	8.61 (2.69)	4.00	n/a
Omeprazole	I	0.95 (0.48)	2.45 (1.55)	1.55 (0.50)	1.75	n/a
	II	0.74 (0.39)	1.80 (1.19)	1.24 (0.33)	2.00	n/a
5-Hydroxyomeprazole	I	0.43 (0.11)	1.30 (0.27)	1.63 (0.44)	1.75	n/a
	II	0.46 (0.15)	1.35 (0.26)	1.38 (0.30)	2.00	n/a
Probe drug / metabolite	**Period**	**C_{max} (ng/mL)**	**AUC_{inf} (ng. hr/mL)**	**$t_{1/2}$ (hr)**	**t_{max} (hr)**	**CL (L/hr)**
Dextromethorphan	I	7.35 (16.19)	47.0 (135.0)	6.19 (2.08)	3.00	n/a
	II	6.87 (15.67)	37.5 (111.6)	6.17 (2.38)	3.00	n/a
Dextrorphan	I	904.93 (331.33)	4513 (768)	5.13 (2.08)	2.00	n/a
	II	714.24 (250.87)	3675 (1031)	5.95 (3.33)	3.00	n/a
Midazolam, p.o.	I	14.22 (5.26)	38.5 (13.4)	5.75 (2.03)	0.75	n/a
	II	32.05 (6.10)	110.6 (25.2)	6.33 (1.44)	0.25	n/a
	I	4.72 (2.00)	11.3 (4.3)	3.17 (1.53)	0.75	n/a
1'-Hydroxymidazolam	II	5.09 (1.51)	17.8 (4.3)	5.92 (1.81)	0.50	n/a
Midazolam, i.v.	I	45.92 (12.54)	100.0 (23.4)	5.29 (1.86)	0.50	21.15 (5.30)
	II	52.52 (9.91)	134.6 (20.5)	5.81 (1.52)	0.50	15.17 (2.22)
	I	2.67 (0.80)	12.8 (3.3)	4.87 (2.19)	1.00	n/a
1'-Hydroxymidazolam	II	2.54 (0.60)	14.3 (3.3)	6.33 (1.76	1.25	n/a
Probe drug	**Period**	**C_{max} (ng/mL)**	**AUC_{last} (ng. hr/mL)**	**$t_{1/2}$ (hr)**	**t_{max} (hr)**	**CL (L/hr)**
Repaglinide	I	7.49 (3.08)	10.45 (4.26)	1.70 (1.07)	0.50	n/a
	II	7.84 (2.92)	10.23 (3.08)	1.44 (0.34)	0.50	n/a
Digoxin	I	1.96 (0.94)	11.66 (2.75)	45.96 (8.03)	1.00	n/a
	II	2.00 (0.61)	13.52 (2.03)	49.48 (11.07)	1.00	n/a
Rosuvastatin	I	6.01 (3.19)	54.18 (27.15)	12.63 (5.89)	4.00	n/a
	II	6.85 (4.01)	59.02 (26.07)	11.26 (3.00)	3.00	n/a

Values are expressed as arithmetic mean (SD) except t_{max} values, which are expressed as median.
n/a: not available; SD: standard deviation; AUC_{inf}: area under the plasma concentration–time curve from 0 hours to infinity; AUC_{last}: Area under the plasma concentration–time curve from 0 hours to the last measurable concentration; C_{max}: maximum plasma concentration; $t_{1/2}$: eElimination half-life; t_{max}: time to reach C_{max}; CL: clearance; p.o.: oral administration; i.v.: intravenous administration; CYP: cytochrome P450. Caffeine, tolbutamide, omeprazole, dextromethorphan, and midazolam were administered in Study 1 - Period I: Probe drugs alone; Period II: Probe drugs + BFE1224 (800/200 mg); repaglinide, digoxin, and rosuvastatin were administered in Study 2 - Period I: Probe drugs alone; Period II: Probe drugs + BFE1224 (400 mg).

values of the GMR of both the AUC and C_{max} data were slightly above the range of 0.8–1.25 (GMR (90% CI) AUC: 1.179 (1.074–1.293); C_{max}: 1.132 (0.827–1.551)) (**Table 4**). These data suggest the small possibility that BFE1224 coadministration may have an effect on P-gp, but it is not likely to be clinically relevant owing to the size of the effect.

Effect of BFE1224 on BCRP, OATP1B1, and OATP1B3 (study 2)

The PK profiles and parameters of rosuvastatin were generally similar in both periods (**Figure 3**, **Table 3**). The 90% CI values of the GMR of both the AUC and C_{max} data were slightly above the range of 0.8–1.25 (GMR (90% CI) AUC: 1.139 (1.016–1.277); C_{max}: 1.138 (1.000–1.296) (**Table 4**). Although these data suggest that there is a small possibility that coadministration of BFE1224 may affect BCRP (as well as OATP1B1 and 1B3, as ravuconazole inhibited neither OATP1B1 nor 1B3 *in vitro* (unpublished data)), this effect is not likely to be clinically relevant.

DISCUSSION

One of the key factors for the successful development of a new drug is a prompt and accurate assessment of its pharmacokinetic DDI potential in accordance with the DDI guidance/guideline from the Pharmaceuticals and Medical Devices Agency (PMDA), FDA, and European Medicines Agency (EMA), which contain highly detailed evaluation procedures.[26–28]

Table 4 GMR and 90% CI between period I (probe drug only) and period II (probe drug + BFE 1224) for AUC and C_{max} of intact and metabolites of probe drugs

CYP	Probe drug	AUC	C_{max}
Transporter	**Metabolite**	**GMR (90% CI)**	**GMR (90% CI)**
CYP1A2	Caffeine	0.920 (0.861, 0.982)	0.946 (0.899, 0.997)
	1,7-Dimethylxanthine	0.778 (0.739, 0.818)	0.811 (0.768, 0.857)
CYP2C9	Tolbutamide	0.879 (0.840, 0.921)	1.004 (0.966, 1.043)
	Carboxytolbutamide	1.005 (0.979, 1.033)	1.175 (1.120, 1.232)
	Hydroxytolbutamide	0.937 (0.905, 0.970)	1.129 (1.062, 1.201)
CYP2C19	Omeprazole	0.745 (0.685, 0.810)	0.780 (0.698, 0.872)
	5-Hydroxyomeprazole	1.041 (1.005, 1.077)	1.049 (0.959, 1.147)
CYP2D6	Dextromethorphan	0.719 (0.658, 0.786)	0.763 (0.670, 0.869)
	Dextrorphan	0.846 (0.816, 0.878)	0.830 (0.785, 0.877)
CYP3A	Midazolam (p.o.)	3.010 (2.667, 3.398)	2.384 (2.152, 2.641)
	1'-Hydroxymidazolam	1.640 (1.446, 1.860)	1.120 (0.986, 1.273)
	Midazolam (i.v.)	1.405 (1.292, 1.529)	1.201 (1.094, 1.318)
	1'-Hydroxymidazolam	1.117 (1.045, 1.195)	1.016 (0.918, 1.125)
CYP2C8	Repaglinide	1.012 (0.903, 1.134)	1.065 (0.878, 1.292)
P-gp	Digoxin	1.179 (1.074, 1.293)	1.132 (0.827, 1.551)
BCRP/OATP1B1, 1B3	Rosuvastatin	1.139 (1.016, 1.277)	1.138 (1.000, 1.296)

GMR: geometric mean ratio; CI: confidence interval; AUC: area under the plasma concentration–time curve from 0 hours to infinity for study 1 and; area under the plasma concentration–time curve from 0 hours to the last measurable concentration for study 2; C_{max}: maximum plasma concentration; p.o.: oral administration; i.v.: intravenous infusion; CYP: cytochrome P450. Caffeine, tolbutamide, omeprazole, dextromethorphan, and midazolam were administered in Study 1 (day 15 / day 1) - Period I: Probe drugs alone; Period II: Probe drugs + BFE1224 (800/200 mg). Repaglinide, digoxin, and rosuvastatin were administered in Study 2 (day 10 / day 1 for repaglinide, day 11 / day 2, for digoxin/rosuvastatin) - Period I: Probe drugs alone; Period II: Probe drugs + BFE1224 (400 mg).

Several previous reports suggested that probe drugs (caffeine, tolbutamide, omeprazole, dextromethorphan, and midazolam) in our Cooperstown W/T cocktail did not affect CYP2C9-mediated tolbutamide metabolism, although omeprazole was reported to increase the tolbutamide AUC by 10% after coadministration.[29,31,33–36] From the information, our Cooperstown W/T cocktail was considered suitable for use as a tool for the investigation of major CYP-based DDIs in clinical trials.

In the second study, we assessed the CYP2C8-mediated metabolism and P-gp- and BCRP/OATP1B1/OATP1B3-mediated transport through measurement of the exposure to repaglinide, digoxin, and rosuvastatin with or without BFE1224 coadministration. Repaglinide was administered on Days 1 and 10, which was followed by the administration of the transporter cocktail (digoxin and rosuvastatin) on Days 2 and 11, as repaglinide does not affect the PK of either digoxin or rosuvastatin owing to the rapid elimination of repaglinide ($t_{1/2}$ = 46.4 min[33]). The drug interaction between rosuvastatin and digoxin does not occur; rosuvastatin does not affect digoxin PK and several reports have indicated that rosuvastatin bioavailability is hardly affected by digoxin.[33,37–40] Supported by the results of our literature search, this transporter cocktail and dosing regimen allowed us to assess the DDI potential of ravuconazole against CYP2C8, P-gp, BCRP, OATP1B1, and OATP1B3, although this transporter cocktail has not been validated clinically. BCRP variants such as c.421C>A were reported to be elevated plasma concentrations of rosuvastatin,[41] which is recommended as an *in vivo* probe drug in the guideline. Ravuconazole showed *in vitro* inhibitory activity against BCRP, and it might be possible that ravuconazole also elevates plasma levels of rosuvastatin *in vivo*. However, the present study showed that plasma AUCs of rosuvastatin were almost the same regardless of the presence or absence of ravuconazole. Therefore, it is conceivable that DDI would not be clinically relevant.

The present studies showed that BFE1224, prodrug of ravuconazole, exerts no clinically relevant effects on the metabolic activities of CYP1A2, 2C8, 2C9, 2C19, and 2D6 *in vivo*, although CYP3A-mediated metabolism was moderately affected by coadministration of BFE1224. It is also reported that AUC values of nelfinavir (16.2% lower)[42] and simvastatin (fourfold higher)[43] were affected. .

After the oral administration of midazolam, the AUC and C_{max} of midazolam markedly increased (AUC: 3.01-fold, C_{max}: 2.38-fold) after coadministration of BFE1224, compared with that after the i.v. infusion of midazolam (AUC: 1.4-fold, C_{max}: 1.2-fold). The 90% CIs for the ratios of the AUC and C_{max} in Period II/I were outside the range of bioequivalence (0.8–1.25) after the oral administration of midazolam, whereas these ratios were mostly within the range of bioequivalence after the i.v. infusion of midazolam.

Therefore, the evidence that BFE1224 decreases the metabolism of orally administered midazolam to a greater extent than that of intravenously administered midazolam suggest that BFE1224 affects the first-pass metabolism of midazolam, mainly through the inhibition of intestinal CYP3A by the active component, ravuconazole.

Although the oral antifungal agents, itraconazole (triazole) and terbinafine (allylamine), are currently the most effective treatments for onychomycosis,[44] they both are associated with a risk of DDIs caused by the potent inhibition of CYP3A (primarily in the liver) and 2D6, respectively.[3,12] Administration of itraconazole resulted in 27-, 10.8-, 19-, and 6.3-fold increases in the AUC of triazolam,[45] midazolam,[46] simvastatin,[14] and felodipine,[47]

respectively. In addition, itraconazole is an inhibitor of P-gp and clinical DDI with digoxin has been observed in healthy volunteers.[17] Itraconazole caused a 6.5-fold increase in the AUC of aliskiren, which was mainly explained by the inhibition of the P-gp-mediated efflux of aliskiren in the small intestine.[48]

In conclusion, the results of these two clinical DDI studies demonstrated that coadministration of BFE1224 is unlikely to affect the clearance of drugs metabolized by CYP1A2, 2C8, 2C9, 2C19, and 2D6, or the transport activities of P-gp, BCRP, OATP1B1, and OATP1B3, but is a moderate inhibitor of CYP3A that largely inhibits intestinal CYP3A. These results indicated that BFE1224 is more likely to have a lower risk of clinical DDI than the current onychomycosis agents on the market, itraconazole and terbinafine.

The CYP and transporter cocktails and clinical study design presented in this report provided a valuable and efficient tool for the investigation of the DDI risks of a new chemical entity in clinical settings and can be used to accelerate drug development.

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Conflict of interest. Y.I., Y.I., and K.S. are employees of Sato Pharmaceutical Co. E.L.S. is an employee of Eisai Inc. O.O. and K.T. are employees of Seren Pharmaceuticals Inc. S.M. is an employee of Soseikai and a paid principal investigator of this project. N.U. is fully employed by Oita University and partially employed by Osaka University and RIKEN, and is a board member of Clinical Research Support Center Kyushu (CREST), a member of Technological Review Board for Advance Medicine, Ministry of Health, Labor and Welfare of Japan. N.U. is a paid medical monitor for this project. As an Associate Editor for *Clinical and Translational Science*, Naoto Uemura was not involved in the review or decision process for this article.

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