Effects of Ketoconazole and Quinidine on Pharmacokinetics of Pactimibe and Its Plasma Metabolite, R-125528, in Humans

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ABSTRACT:

Pactimibe sulfate is a novel acyl coenzyme A:cholesterol acyltransferase inhibitor developed for the treatment of hypercholesterolemia and atherosclerotic diseases. Pactimibe has two equally dominant clearance pathways forming R-125528 by CYP3A4 and M-1 by CYP2D6 in vitro. R-125528 is a plasma metabolite and is cleared solely by CYP2D6 despite its acidity. To evaluate contributions of the cytochrome P450 enzymes on the pharmacokinetics of pactimibe and R-125528 in humans, drug-drug interaction studies using ketoconazole and quinidine were conducted. Eighteen healthy male subjects were given a single dose of pactimibe sulfate without and with 400 mg of ketoconazole (q.d.). With the concomitant treatment, the area under the plasma concentration-time curve (AUC0–τ) of pactimibe modestly increased 1.7-fold and AUC0–τ of R-125528 decreased by 55%. In addition, 17 healthy male subjects were given a single dose of pactimibe sulfate without and with 600 mg of quinidine (b.i.d.). With the concomitant treatment, the AUC0–τ of pactimibe modestly increased 1.7-fold. On the other hand, the AUC0–τ of R-125528 was markedly elevated 5.0-fold, although the AUC0–τ could not be adequately defined because the terminal elimination phase of R-125528 was not obtained in the study period up to 72 h. As the fm,CYP3A4 and fm,CYP2D6 values of pactimibe estimated from in vitro studies were 0.40 and 0.33, respectively, AUC increase ratios of pactimibe were estimated to be 1.7 with ketoconazole and 1.5 with quinidine. These values were well in accordance with the values observed in this study. Moreover, the fm,CYP2D6 of R-125528 estimated to be almost 1 would well explain the accumulation of R-125528 observed with the quinidine treatment.

Pactimibe sulfate [7-(2, 2-dimethylpropanamido)-4,6-dimethyl-1-octylindolin-5-yl] acetic acid hemisulfate (formerly named CS-305) (Fig. 1A) is a novel lipophilic acyl coenzyme A:cholesterol acyltransferase inhibitor developed for the treatment of hypercholesterolemia and atherosclerotic diseases (Kitayama et al., 2006a,b,c; Nissen et al., 2006). A number of acyl coenzyme A:cholesterol acyltransferase inhibitors have been synthesized, and their pharmacological profiles were evaluated in animals and humans. However, several adverse effects such as adrenal toxicity (Vernetti et al., 1993; Reineld et al., 1994; Matsuo et al., 1996), diarrhea (Kashiwa et al., 1997), and hepatotoxicity (Ishii et al., 1994; Nakaya et al., 1994) and various efficacies in humans (Harris et al., 1990; Hainer et al., 1994; Tardif et al., 2004) have been revealed, and none of these compounds has so far succeeded in clinical development. Pactimibe sulfate was selected as a clinical development candidate showing good oral absorbability and potent pharmacological effects in apolipoprotein E-deficient mice (Terasaka et al., 2007) and Watanabe heritable hyperlipidemic rabbits (Kitayama et al., 2006b) without showing significant adrenal toxicity even in dogs, the most sensitive animal species.

In vivo biotransformation studies in animals and healthy volunteers demonstrated that only pactimibe and its lipophilic metabolite R-125528, the oxidized form of the indolin ring in pactimibe, appeared in the plasma and that none of the other metabolites were observed. On the other hand, pactimibe and R-125528 were not detected in the urine or bile but were excreted into the bile as further metabolized forms. Thus, the clearances of pactimibe and R-125528 from systemic circulation are totally dependent on the metabolic clearance.

In vitro metabolic studies showed that pactimibe has several metabolic pathways including oxidation at the indolin ring (formation of R-125528), ω-1 oxidation at the octyl chain, N-dealkylation, and glucuronidation on the carboxylic acid (Fig. 1B). None of the metabolites was estimated to be pharmacologically active in vitro. Kinetic studies using human liver microsomes (Kotsuma et al., 2008) revealed that intrinsic clearance (CLint) values for indolin ring oxidation (formation of R-125528), ω-1 oxidation (formation of M-1), and glucuronidation were 0.63, 0.76, and 0.16 l/min/mg of protein, respectively. Moreover, according to a P450-isozyme identification study, the indolin oxidation and ω-1 oxidation were found to be catalyzed mainly by CYP3A4 and CYP2D6, respectively.

On the other hand, the metabolic reaction for R-125528 was restricted. The ω-1 oxidized form of R-125528 (M-2) was the only metabolite derived from R-125528, and no glucuronide was detected in vitro and in vivo in animals and humans. In human hepatic microsomes, the CLint value for the ω-1 oxidation was 75.0 μl/min/mg of protein. To our surprise, although R-125528 is an atypical substrate for CYP2D6 because of its acidity, a P450 isozyme identifi-
fication study using P450 expression microsomes revealed that CYP2D6 was the only isoform that could catalyze the reaction. In addition, the reaction phenotyping study indicated CYP2D6 activity was strongly ($r^2 = 0.90$) correlated with the formation of M-2 (Kotsuma et al., 2008). Furthermore, R-125528 oxidation is inhibited as much as 90% in the presence of quinidine in vitro (data not shown). Considering that R-125528 itself could not be excreted into the bile or urine as an intact form, the $\omega$-1 oxidation mediated by CYP2D6 was considered to be a crucial pathway for the elimination of R-125528 from the systemic circulation.

The degree of drug-drug interaction depends largely on the fraction of the metabolic process subject to inhibition ($f_{inhib}$) that is inhibited (Ito et al., 2005; Gibbs et al., 2006). The AUC increase ratio is simply expressed as the following equation: $\frac{\text{AUC}_{\text{po}(\text{inhibitor})}}{\text{AUC}_{\text{po}(\text{control})}} = 1/(1 - f_{inhib})$, when the related pathway is completely abolished by the inhibitor. As pactimibe has multiple metabolic pathways, it will minimize the extent of drug-drug interaction and/or genetic polymorphisms. On the other hand, R-125528 was suggested to have a single metabolic pathway mediated by CYP2D6. Therefore, the variation in CYP2D6 activity is expected to have greater impact on the pharmacokinetics of R-125528 than that of pactimibe. Even though R-125528 is pharmacologically inactive, monitoring the plasma concentration level of this metabolite in humans will be of great importance from a toxicological point of view.

In this study, we performed clinical drug-drug interaction studies to clarify the contributions of CYP3A4 and CYP2D6 on the pharmacokinetics of not only pactimibe but also R-125528. To evaluate the involvement of CYP3A4, a synthetic broad-spectrum antifungal agent, ketoconazole, was used, because ketoconazole is one of the most potent CYP3A4 inhibitors used in clinical medicine (Jones, 1997; Niwa et al., 2005) and also is recommended as a prototype inhibitor to use in human drug-drug interaction studies (Center for Drug Evaluation and Research, 2006) by regulatory guidance. To evaluate the involvement of CYP2D6, a well-known potent CYP2D6 inhibitor (Zhou et al., 1990), quinidine, was used.

We selected a lower dose (25 mg) in the quinidine study than was used (100 mg) for the ketoconazole study, because we had a safety concern about metabolite accumulation in the quinidine treatment group considering the high $f_{inhib}$ for R-125528 metabolism. Even though it was known that the pharmacokinetics of pactimibe showed a nonlinear trend slightly between these two doses, we believe that the contribution of the CYP2D6- and CYP3A4-mediated pathway should not be different between two doses, because the $K_m$ values for both M-1 and R-125528 formation are as high as 100 $\mu$M (Kotsuma et al., 2008).

**Materials and Methods**

**Subjects.** Eighteen healthy male subjects between 22 and 40 years of age were recruited for the ketoconazole DDI study. Genotyping was used to screen for normal metabolizers via CYP2D6. Nineteen healthy male subjects, aged
between 20 and 41 years and genotyped as normal metabolizers via CYP2D6, were recruited for the quindine DDI study. Among them, one subject withdrew because of an adverse effect that occurred in the washout period (a thermal burn of the left hand). Because the blinded analytical results showed zero pactimibe plasma concentrations for all postdose time points in one subject, this subject was excluded from the pharmacokinetic analysis. Subjects recruited for both studies were normotensive, with no abnormal physical findings of clinical relevance, no clinically relevant laboratory values at screening and before intake of the trial medication, no clinically relevant abnormalities in electrocardiographic examinations, and negative results for human immunodeficiency virus antibody, hepatitis B surface antigen, and hepatitis C virus tests. These studies were conducted in accordance with the Declaration of Helsinki. The protocol and informed consent form were approved by an independent ethics committee (Landesärztekammer, Hessen, Germany), and written informed consent was received from all subjects before admission to the trial. This study was conducted in one center (IMFORM GmbH, Görlitz, Germany).

Study Design—Ketoconazole DDI study. This was a randomized, placebo-controlled, double-blind, two-way crossover study. Eighteen subjects were assigned randomly in a 1:1 ratio to two treatment sequences. Subjects received both treatment combinations (pactimibe/ketoconazole and pactimibe/placebo) alternately in two treatment periods (I and II), separated by a washout period of 7 days. After screening, eligible subjects proceeded into the first treatment period and were given ketoconazole (400 mg; Jansen-Cilag GmbH, Neuss, Germany) or matching placebo once daily on days 1 to 7 (period I). A single dose of pactimibe (100 mg) was administered on day 5 in an open-label manner. Ketoconazole/placebo were taken at 8:00 AM in the morning (±60 min). On day 5, pactimibe tablets were taken immediately after administration of ketoconazole/placebo capsules after an overnight fast. No breakfast was served on day 5. Medication was administered via the oral route with a total of 200 ml of water. After a 7-day washout period (starting on day 8/period I), subjects began treatment period II and received the second treatment (once daily on days 1–7): ketoconazole for subjects who received placebo in period I and placebo for subjects who received ketoconazole in period I. On day 5 of treatment period II, subjects received another single dose of pactimibe (100 mg).

Study Design—Quinidine Study. This study was designed as a randomized, double-blind, placebo-controlled, two-way crossover single-center trial with two treatment periods separated by a washout period of 7 to 14 days. No more than 14 days after screening, all 19 subjects who were enrolled in this study received two daily doses orally (600 mg; Astra Pharmaceuticals Ltd., Herts, UK) of quinidine or placebo for 6 days. On day 6, subjects received a single dose of pactimibe (25 mg; Sankyo Co., Ltd, Tokyo, Japan) immediately after administration of the morning dose of quinidine followed by pharmacokinetic assessments of pactimibe and quinidine. After a washout period, subjects again received two daily doses of quinidine or placebo for 6 days. On day 5, subjects received each of the two treatment regimens only once. Subjects did not receive any other medication including over-the-counter preparations during the entire trial period including the safety follow-up visit. Safety. Safety and tolerability were addressed in terms of occurrences of treatment-emergent adverse events (AEs), changes in vital signs (blood pressure/pulse rate), electrocardiographic examinations, physical examinations, and clinical laboratory parameters.

Blood Sampling. Blood samples (5 mL) for the analysis of pactimibe and R-125528 were drawn predose as well as 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 60, and 72 h after administration of pactimibe of each treatment period in both study. For the determination of the plasma concentration of quinidine, blood samples (4 mL) were drawn before administration of the morning dose of quinidine on day 3. Blood samples were also collected predose as well as 2, 4, 6, 8, 10, and 12 h after administration of pactimibe on day 4. Each sample was collected into vacuum tubes containing sodium heparin. The samples were centrifuged immediately (1600g, 15 min at 4°C), and the resulting plasma was transferred into storage tubes and stored frozen at −80°C until the analysis.

Analytical Method. A validated liquid chromatography/tandem mass spectrometry method was applied for the determination of pactimibe and R-125528 in human heparinized plasma. Plasma samples were spiked with the corresponding deuterated internal standards of the analytes (d6-pactimibe and d6-R-125528). The samples were extracted by 96-well format solid-phase extraction (Versaplate Certify; Varian, Inc., Harbor City, CA). The extracts were evaporated under nitrogen, and the residue was reconstituted with acetonitrile-1 N HCl-200 mM dithiothreitol (98:1.1, v/v/v) before injection onto an liquid chromatography/tandem mass spectrometry. Pactimibe and R-125528 were separated by MetaChem MetaSil AQ C18 column (50 × 2 mm, 5 μm; Varian, Inc.) attached to the precolumn, ODS C18 (4 × 2 mm; Phenomenex, Torrance, CA). The mobile phases were (A) 2.0 mM ammonium acetate in H2O (pH 3.3) and (B) 2.0 mM ammonium acetate in acetonitrile (pH 3.3), the solvent flow rate was set at 0.3 mL/min, and a gradient of [time (minute)/percent (B): 0 → 0.5/20 → 30, 6.5 → 1.0/40 → 90, 10.0 → 8.0/90, 2.8 → 3.6/90 → 20, 3.6 → 4.0/20] was used. For detection, a PE Sciex API 3000 system (Concord, ON, Canada) with an atmospheric pressure ionization mass spectrometry turbo ion spray inlet in the positive ion-multiple reaction monitoring mode was used. The following parent and daughter ions (m/z) were monitored: 417.3 and 399.3 for pactimibe, 423.3 and 405.3 for d6-pactimibe, 415.4 and 369.3 for R-125528, and 421.4 and 375.3 for d6-R-125528, respectively. The linearity of the standard curve was obtained from 1 to 1000 ng/mL and the lower limit of quantitation (LLOQ) was set at 1 ng/mL for each substances. In the quinidine study, interday precision (coefficient of variation) and accuracy for pactimibe and R-125528 were 1.49 to 4.55% and −1.30 to 2.04% and 1.98 to 6.06% and −1.20 to 1.00%, respectively. In the ketoconazole study, interday precision and accuracy for pactimibe and R-125528 were 2.14 to 9.41% and −1.86 to 2.25% and 2.94 to 6.28% and −4.50 to 2.60%, respectively. The plasma concentration of quinidine was determined using high-performance liquid chromatography analytical methods at MDS Pharma Services (Lincoln, NE). The linearity of the standard curve was obtained from 0.05 to 10.0 μg/mL and the LLOQ was set at 0.05 μg/mL. The interday precision and accuracy was 1.35, 0.90, and 1.63% and −1.53, −3.35, and −4.65% for 0.15, 1.5, and 7.5 μg/mL of quality control samples.

Pharmacokinetic Analysis. The following pharmacokinetic parameters were calculated from pactimibe and R-125528 concentrations in plasma using a noncompartmental approach. Values below LLOQ were set to 0. Cmax, the maximum plasma concentration, and tmax, the time to reach Cmax, were defined directly from measured plasma concentrations. The terminal elimination half-life, t1/2, given as t1/2 = ln2/Cz, where Cz is the terminal rate constant, was calculated by log-linear regression of the terminal segment of the plasma concentration versus time curve. The optimal regression fit was determined by WinNonlin Professional (version 3.3; Pharsight Corp., Mountain View, CA) using at least the three quantifiable concentrations, and λz corresponds to the negative slope of the fitted log-linear regression line. AUC0–inf, area under the plasma concentration time curve from 0 to last quantifiable time, was obtained according to the linear trapezoidal rule. AUC0–tmax area under the concentration time curve extrapolated up to infinity, was calculated by AUC0–tmax = AUC0–t + Cz/t, where Cz is the concentration at the last quantifiable time point. If %Extrapol, the percent proportion of AUC0–tmax not explained by AUC0–t, exceeds 20%, AUC0–tmax is not calculated. With regard to the quinidine pharmacokinetic analysis, AUC0–t, the area under the concentration-time curve from predose to t = 12 h of quinidine at steady state on day 4 after the first dose, Cmax, and tmax were calculated.

Data Management. Data management was carried out using the SAS System for Windows (version 6.12 or 8.2; SAS Institute Inc., Cary, NC) and WinNonlin Professional (version 3.1 or 3.3).

Results

Drug-Drug Interaction between Ketoconazole and Pactimibe Sulfate. Plasma concentration versus time profiles for pactimibe and R-125528 after a single oral administration of pactimibe sulfate (100 mg) in the presence (C) and absence (D) of 400 mg of ketoconazole (q.d.) administered to healthy male volunteers are shown in Fig. 2, A and B, respectively. Coadministration of ketoconazole with pactimibe resulted in an increased plasma concentration of pactimibe. The AUC0–tmax, AUC0–tmax, and Cmax of pactimibe were moderately increased 1.6-, 1.7-, and 1.2-fold, respectively, by cotreatment (Table 1). In contrast, coadministration of ketoconazole with pactimibe decreased the plasma concentration of R-125528. The AUC0–t and Cmax of R-125528 were decreased by 55 and 54%, respectively.
AUC$_{0-\inf}$ could only be determined reliably for one subject and the extrapolated portion of the AUC exceeded the total area by more than 20% in the remaining subjects of the ketoconazole treatment group. The mean terminal elimination half-life of R-125528 after administration of ketoconazole could only be determined reliably in five subjects.

**Drug-Drug Interaction between Quinidine and Pactimibe Sulfate.** Plasma concentration versus time profiles for pactimibe and R-125528 after a single oral administration of pactimibe sulfate (25 mg) in the presence (○) and absence (●) of 600 mg quinidine (b.i.d.) administered to healthy male volunteers are shown in Fig. 3, A and B. Co-administration of quinidine with pactimibe resulted in an increased plasma concentration of pactimibe, AUC$_{0-\inf}$ and the mean terminal elimination half-life could not be determined reliably because the terminal elimination phase could not be accurately determined.

**Pharmacokinetics of Quinidine.** The pharmacokinetic parameters of quinidine after administration of quinidine (600 mg)/pactimibe (25 mg) on day 4 to healthy male volunteers is shown in Table 3. The plasma quinidine concentration observed in this study is consistent with previously published results in which the clinical dosage of 600 to 800 mg/day was used (Brinn et al., 1986; Brøsen et al., 1987).

**Safety.** In the quinidine DDI study, no deaths or other serious AEs occurred during the course of the trial. Four treatment-emergent AEs were recorded in four subjects during the course of the trial. Three of the AEs were mild in severity; one AE was moderate in severity. All subjects recovered from the AEs, and there was no action taken in three cases. One subject was withdrawn from trial participation because of an unexpected AE during the washout period (a thermal burn of the left hand). No remarkable changes in vital signs, electrocardiogram, or physical findings were observed throughout the trial. No subject except the one who was withdrawn from the trial was treated concomitantly.

In the ketoconazole DDI study, no deaths or serious AEs occurred during the course of this trial. Two significant treatment-emergent
resulted in a modest increase in the AUC0–inf of pactimibe (1.7-fold) relative to pactimibe when given alone. Similarly, concomitant administration of CYP2D6 inhibitors quinidine and pactimibe (1.7-fold) relative to pactimibe when given alone. On the other hand, there was a significant increase in the plasma concentrations of R-125528 relative to pactimibe when given alone. The regimen of four daily treatments of 400 mg of ketoconazole has been recommended to optimally assess the effect of the most potent clinical inhibition of CYP3A on the pharmacokinetics of a CYP3A substrate (Bjornsson et al., 2003). In fact, a 16-fold AUC increase of a well-known CYP3A4 substrate, midazolam, was observed in the same regimen (Olkkola et al., 1994). Similarly, there are several reports demonstrating that 600 to 800 mg/day quinidine can change the phenotype of CYP2D6 extensive metabolizers to that of poor metabolizers by monitoring the urinary metabolic ratio (Brinn et al., 1986; Brøsen et al., 1987). The AUC increase ratio can be simply expressed as AUCpo(+inhibitor)/AUCpo(control) = 1/(1 - \(f_{i,u}^{\text{h}}\)) - 1/(1 - \(f_{i,m}^{\text{h}}\)) (1 - \(f_{i,m}^{\text{h}}\)) where \(f_{i,o}^{\text{h}}, f_{i,m}^{\text{h}}, I_{i}, K_{i}\) and represent fraction of hepatic clearance in total clearance, fraction of the metabolic process subject to inhibition, unbound concentration of the inhibitor, and inhibition constant, respectively (Ito et al., 1998). In the case of pactimibe, \(f_{i}\) can be approximated to 1, because of the lack of renal clearance as an intact form. The maximum plasma ketoconazole concentration in the same regimen (Olkkola et al., 1994). The maximum quinidine plasma concentration obtained in this study was 6.5 \(\mu\)M (Table 3). Because the plasma free fractions of ketoconazole and quinidine are reported to be 0.01 and 0.15 (Ito et al., 1998), \(I_{i}\) is estimated to be 0.13 and 0.98 \(\mu\)M, respectively. If we also

![A](image1)

**FIG. 3.** A, plasma concentration versus time profiles for pactimibe after a single oral administration of pactimibe sulfate (25 mg) in the presence (○) and absence (●) of 600 mg of quinidine (b.i.d.) administered to healthy male volunteers. Each point represents the mean ± S.D. (n = 17 in each group). B, plasma concentration versus time profiles for R-125528 after a single oral administration of pactimibe sulfate (25 mg) in the presence (○) and absence (●) of 600 mg of quinidine (b.i.d.) administered to healthy male volunteers. Each point represents the mean ± S.D. (n = 17 in each group).

### TABLE 2

**Pharmacokinetic parameters of pactimibe and its plasma metabolite, R-125528, after oral administration of pactimibe sulfate (25 mg) in the presence and absence of 600 mg quinidine (b.i.d.) administered to healthy male volunteers**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
<th>(C_{\text{max}}) (μg · h/ml)</th>
<th>(T_{\text{max}}) (h)</th>
<th>(AUC_{\text{po,inf}}) (ng · h/ml)</th>
<th>(t_{1/2}) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pactimibe</td>
<td>Placebo + pactimibe</td>
<td>137.0 ± 49.5</td>
<td>3.8 ± 2.9</td>
<td>2446 ± 802</td>
<td>2599 ± 867</td>
</tr>
<tr>
<td>R-125528</td>
<td>Placebo + pactimibe</td>
<td>11.0 ± 4.8</td>
<td>15.4 ± 10.7</td>
<td>401.2 ± 182.9</td>
<td>423.2 ± 187.7</td>
</tr>
<tr>
<td>Quinidine + pactimibe</td>
<td>39.4 ± 14.1</td>
<td>44.2 ± 19.8</td>
<td>2003 ± 716.2</td>
<td>N.A.</td>
<td></td>
</tr>
</tbody>
</table>

N.A., not applicable (attributable to unreliable determination of AUCpo,inf); \(n = 13\) (attributable to unreliable determination of \(AUC_{\text{po,inf}}\), i.e., %Extrapol exceeds 20%); \(n = 11\) (attributable to unreliable determination of \(AUC_{\text{po,inf}}\), i.e., %Extrapol exceeds 20%).

### TABLE 3

**Steady-state pharmacokinetic parameters of quinidine (600 mg) on day 4 (n = 17)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(AUC_{\text{po,inf}}) (μg · h/ml)</td>
<td>19.6 ± 4.4</td>
</tr>
<tr>
<td>(C_{\text{max}}) (μg/ml)</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>(T_{\text{max}}) (h)</td>
<td>4.1 ± 0.5</td>
</tr>
</tbody>
</table>

AEs occurred in one subject. This subject showed an increased level of alanine aminotransferase-serum glutamate pyruvate transaminase on day 8 of periods I and II. The level of the laboratory parameter decreased to normal values without any action taken.

**Discussion**

Concomitant administration of CYP3A4 inhibitor ketoconazole and pactimibe resulted in a modest increase in the AUC0–inf of pactimibe (1.7-fold) relative to pactimibe when given alone. Similarly, concomitant administration of CYP2D6 inhibitors quinidine and pactimibe resulted in a modest increase in the AUC0–inf of pactimibe (1.7-fold) relative to pactimibe when given alone. On the other hand, there was a significant increase in the plasma concentrations of R-125528 (>5.0-fold) in the presence of quinidine.

The dosing regimen used in this study almost completely abolished the CYP3A4- and CYP2D6-mediated pathway. The regimen of four
consider the concentration in the portal vein, the $I_{fa}$ values could be higher. Because the $K_i$ values of ketocazoline and quinidine are reported to be much lower than those values (von Moltke et al., 1996; Palkama et al., 1999; Ito et al., 2004), the contribution of $f_{m,CYP2D6}(1 + 1/K_i)$ could be negligible.

An increase of the plasma concentration of pactimibe observed in this study was in good agreement with prediction based on the in vitro metabolic studies. Pactimibe has multiple metabolic pathways, indole oxidation, $\omega$-1 oxidation, and glucuronidation, of which intrinsic clearance values are $0.63, 0.76$, and $0.16 \mu$mol/min/g of protein in human liver microsomes, respectively. Because the contributions of CYP3A4 and CYP2D6 to the indole and the $\omega$-1 oxidation were 99 and 67.8% (Kotsuma et al., 2008), $f_{m,CYP3A4}$ and $f_{ia,CYP2D6}$ of pactimibe were calculated as $0.40 \pm 0.03$ and $0.33 \pm 0.06$, respectively. According to the equation described above, AUC increase ratios are estimated to be $0.9 \pm 0.10$ with ketocazoline and $1.1 \pm 0.33$ with quinidine. These values are well in accordance with the 1.7-fold AUC increase observed in this study concomitantly administered with ketocazoline and quinidine. Even though we did not measure the glucuronide of pactimibe in the urine and feces in this study, these results suggested that the contribution of glucuronidation should be minor in the pactimibe clearance.

In contrast to pactimibe, the plasma metabolite, R-125528, accumulated highly with quinidine treatment. As the CYP2D6-mediated reaction is the crucial pathway for the elimination of R-125528 from the systemic circulation, it was certainly expected that the increase in the plasma concentration should be very significant. Assuming that the $f_{m,CYP2D6}$ of R-125528 was 0.9 on the basis of the fact that R-125528 oxidation in human liver microsomes was inhibited as much as 90% ($K_i$ values of ketoconazole and quinidine are reported to be much lower than those values (von Moltke et al., 1996; Palkama et al., 1999; Ito et al., 2004), the contribution of $f_{m,CYP2D6}(1 + 1/K_i)$ could be negligible.

The metabolic pathways of pactimibe and R-125528 are good substrates for CYP2D6 in humans will require further investigation of their CYP2D6 binding mode.

### References


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