Effects of ketoconazole and quinidine on pharmacokinetics of pactimibe and its plasma metabolite, R-125528, in human

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Running title: CYP2D6-mediated metabolism of pactimibe and R-125528 in human

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Abbreviations: P450, cytochrome P450; AUC, area under the plasma concentration time curve; DDI, drug-drug interaction; CLint, intrinsic clearance; fm, fraction of the metabolic process subject to inhibition; LC/MS/MS, liquid chromatography/tandem mass spectrometry
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 solely cleared by CYP2D6 in spite of its acidity. To evaluate contribution of the P450 enzymes on pharmacokinetics of pactimibe and R-125528 in humans, DDI studies using ketoconazole and quinidine were conducted. Eighteen healthy male subjects were received a single dose of pactimibe sulfate without and with 400 mg of ketoconazole (q.d.). With the concomitant treatment, AUC₀-inf of pactimibe modestly increased 1.7-fold and AUC₀-tz of R-125528 decreased by 55%. Besides, seventeen healthy male subjects were received a single dose of pactimibe sulfate without and with 600 mg of quinidine (b.i.d.). With the concomitant treatment, AUC₀-inf for pactimibe modestly increased 1.7-fold. On the other hand, AUC₀-tz of R-125528 was markedly elevated 5.0-fold, although AUC₀-inf could not be adequately defined because the terminal elimination phase of R-125528 was not obtained in the study period up to 72 hr. As fmCYP3A4 and fmCYP2D6 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, fmCYP2D6 of R-125528 estimated to be almost 1 would well explain the accumulation of R-125528 observed in the quinidine treatment.
Introduction

Pactimibe sulfate [7-(2, 2-dimethylpropanamido)-4, 6-dimethyl-1-octylindolin-5-yl] acetic acid hemisulfate (formerly named CS-505, Figure 1A) is a novel lipophilic Acyl coenzyme A: cholesterol acyltransferase inhibitor developed for the treatment of hypercholesterolemia and atherosclerotic diseases (Kitayama et al., 2006a; Kitayama et al., 2006b; Kitayama et al., 2006c; Nissen et al., 2006). A number of Acyl coenzyme A: cholesterol acyltransferase inhibitors have been synthesized and their pharmacological profiles evaluated in animals and humans. However, several adverse effects such as adrenal toxicity (Vernetti et al., 1993; Reindel et al., 1994; Matsuo et al., 1996), diarrhea (Kashiwa et al., 1997), hepatotoxicity (Ishi et al., 1994; Nakaya et al., 1994), and various elusive 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), and 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 indoline 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 (Figure 1B.). None of the metabolites were estimated to be pharmacologically active *in vitro*. Kinetic studies using human liver microsomes (Kotsuma et al., 2008) revealed that CLint values for indoline ring oxidation (formation of R-125528), ω-1 oxidation (formation of M-1), and glucuronidation were 0.63, 0.76, and 0.16 µL/min/mg-protein, respectively. Moreover, according to a P450-isoenzyme identification study, the indoline oxidation and the 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-protein. To our surprise, although R-125528 is an atypical substrate for CYP2D6 because of its acidity, a P450-isoenzyme identification study using P450 expression microsomes revealed that CYP2D6 was the only isoform that could catalyze the reaction. In addition, the reaction
A 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 \textit{in vitro} (data not shown). Considering that R-125528 itself could not be excreted into the bile nor 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 systemic circulation.

The degree of drug-drug interaction depends largely on the fraction of the metabolic process subject to inhibition \( (f_m) \) that is inhibited (Ito et al., 2005; Gibbs et al., 2006). AUC increase ratio is simply expressed as the following equation, \( \frac{AUC_{po(+\text{inhibitor})}}{AUC_{po(\text{control})}} = \frac{1}{1-f_m} \), 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 of CYP2D6 activity is expected to have greater impact on pharmacokinetics of R-125528 than that of pactimibe. Even though R-125528 is pharmacologically inactive, to monitor the plasma concentration level of this metabolite in humans will be of great importance from 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, since ketoconazole is one of the most potent...
CYP3A4 inhibitors used in the clinical medicine (Jones, 1997; Niwa et al., 2005) and also is recommended as a prototype inhibitor to use in human drug-drug interaction studies (CDER, 2006) by the 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 quinidine study than that was used (100 mg) for ketoconazole study, because we had a safety concern of metabolite accumulation in quinidine treatment group considering high $f_m$CYP2D6 for R-125528 metabolism. Even though it was known that the pharmacokinetics of pactimibe showed non-linear trend slightly between these two doses, we believe that the contribution of CYP2D6 and CYP3A4 mediated pathway should not be different between two doses, because both of the Km values for M-1 and R-125528 formation are as high as 100 µM (Kotsuma et al., 2008).
METHODS

Subjects: The 18 healthy male subjects between 22 and 40 years of age were recruited for the ketoconazole DDI study. According to the result of genotyping, normal metabolizers via CYP2D6 were screened. The 19 healthy male subjects, aged between 20 and 41 years and genotyped as normal metabolizers via CYP2D6, were recruited for the quinidine DDI study. Among them, there was one withdrawal due to an adverse event that occurred in the wash-out period (thermal burn of the left hand). Since the blinded analytical results showed zero pactimibe plasma concentrations for all post-dose timepoints 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 trial medication, no clinically relevant abnormalities in the electro-cardiographic examinations, negative results in 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 independent ethics committee (Landesärzte-kammer, Hessen, Germany) and written informed consent was received from all subjects prior to admission into the trial. This study was conducted in one center (IMFORM GmbH, Germany).

Study design -ketoconazole DDI study-: This was a randomised, 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, II), separated by a wash-out period of seven days. After screening, eligible subjects proceeded into the first treatment period and were administered ketoconazole (400 mg, Jansen Cilag Neuss GmbH, Germany) or matching placebo once daily on Day 1 to Day 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 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 seven day wash-out period (starting on Day 8/period I), subjects began treatment period II and received the second treatment (once daily on Day 1 to Day 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 DDI study-**: This study was designed as randomised, double-blind, placebo-controlled, two-way crossover single center trial with two treatment periods separated by a wash-out period of seven to 14 days. No more than 14 days after screening, all 19 subjects that were enrolled in this study received orally two
daily doses (600 mg, Astra Pharmaceuticals Ltd., Herts, UK) of quinidine or placebo for six days. On day 4, 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 wash-out period, subjects again received two daily dose of quinidine or placebo for six days in that way that every subject received each of the two treatment regimens only once. Subjects were not received any other medication including over-the-counter preparations during the entire trial period including the safety follow-up visit.

**Safety:** Safety and tolerability was addressed in terms of occurrences of treatment-emergent adverse events (AEs), changes in vital signs (blood pressure/pulse rate), electrocardiographic (ECG) examinations, physical examinations and clinical laboratory parameters.

**Blood sampling:** Blood samples (5 mL) for the analysis of pactimibe and R-125528, were drawn pre-dose as well as 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 60 and 72 hours after administration of pactimibe of each treatment period in both study. For the determination of 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 pre-dose as well as 2, 4, 6, 8, 10 and 12 hours after administration of pactimibe on Day 4. Each sample was collected into vacuum tubes containing sodium
The samples were centrifuged immediately (1600 × g, 15 minutes 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 (LC/MS/MS) 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, Harbor City, Calif). The extracts were evaporated under nitrogen, and the residue was reconstituted with acetonitrile/1N HCl/200 mM dithiothreitol (98:1:1, v/v/v) prior to injection onto an LC/MS/MS. Pactimibe and R-125528 were separated by Metachem Metasil AQ C₁₈ (50 × 2 mm, 5 µm, Varian, Harbor City, Calif) attached to the precolumn, Phenomenex ODS C₁₈ (4 × 2 mm, Torrance, CA, USA). 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 (min)% (B): 0 → 0.5/20 → 40, 0.5 → 1.0/40 → 90, 1.0 → 2.8/90, 2.8 → 3.6/90 → 20, 3.6 → 4.0/20] was used. For detection, a PE Sciex API 3000 (Concord, ON, Canada) with atmospheric pressure ionization (API) 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 \textit{d}_6\textit{-R-125528}, respectively. The linearity of the standard curve was obtained from 1 to 1000 ng/mL and the lower limit of quantification (LLOQ) was set at 1 ng/mL for each substance. In 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 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. 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 \textmu g/mL and the LLOQ was set at 0.05 \textmu g/mL. The interday precision and accuracy were 1.35\%, 0.90\%, and 1.63\% and -1.53\%, -3.35\%, and -4.65\% for 0.15, 1.5, and 7.5 \textmu g/mL of quality control samples.

**Pharmacokinetic analysis:** The following pharmacokinetic parameters were calculated from pactimibe and R-125528 concentrations in plasma using a non-compartmental approach. Values below LLOQ were set to 0. \(C_{\text{max}}\), the maximum plasma concentration, and \(t_{\text{max}}\), the time to reach \(C_{\text{max}}\) were defined directly from measured plasma concentrations. The terminal elimination half-life, \(t_{1/2}\), given as \(t_{1/2} = \ln(2)/\lambda_z\), where \(\lambda_z\) 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, USA) using at least the three quantifiable concentration, and $\lambda_z$ corresponds to the negative slope of the fitted log-linear regression line. AUC$_{0-t_z}$, area under the plasma concentration time curve from 0 to last quantifiable time, was obtained according to the linear trapezoidal rule. AUC$_{0-\text{inf}}$, the area under the plasma concentration time curve extrapolated up to infinity, was calculated by $\text{AUC}_{0-\text{inf}} = \text{AUC}_{0-t_z} + \frac{C_z}{\lambda_z}$ where $C_z$ is the concentration at the last quantifiable time point. If %Extrapol ($\%$), the percentage proportion of AUC$_{0-\text{inf}}$ not explained by AUC$_{0-t_z}$, exceeds 20%, AUC$_{0-\text{inf}}$ is not calculated. With regard to the quinidine pharmacokinetic analysis, AUC$_{ss,\tau}$, the area under the concentration-time curve from pre-dose to $\tau=12$ h of quinidine at steady state on Day 4 after the first dose, $C_{\text{max}}$, and $t_{\text{max}}$ 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, USA) and WinNonlin Professional, Version 3.1 or 3.3 (Pharsight Corp., Mountain View, CA, USA).
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 (○) and absence (●) of 400 mg ketoconazole (q.d.) administered to healthy male volunteers are shown in Figure 2A and B, respectively. Coadministration of ketoconazole with pactimibe resulted in an increased plasma concentration of pactimibe. The AUC₀₋ₜₓ, AUC₀₋ₐₙₐₐ₄₉ and Cₘₐₓ of pactimibe were moderately increased 1.6, 1.7, and 1.2-fold, respectively, by cotreatment (Table I). In contrast, coadministration of ketoconazole with pactimibe decreased the plasma concentration of R-125528. The AUC₀₋ₜₓ and Cₘₐₓ of R-125528 were decreased by 55% and 54%, respectively. AUC₀₋ₐₙₐₐ₄₉ could only be determined reliably for 1 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 Figure 3A and B. Coadministration of quinidine with pactimibe sulfate resulted in an increased plasma
concentration compared to placebo treatment group. As shown in Table II, the \( \text{AUC}_{0-tz} \), \( \text{AUC}_{0-inf} \) and \( C_{\text{max}} \) of pactimibe were moderately increased 1.7, 1.7, and 1.3-fold, respectively by cotreatment. On the other hand, quinidine cotreatment with pactimibe sulfate drastically increased the plasma concentration of lipophilic metabolite, R-125528, when compared to placebo treatment group. The \( \text{AUC}_{0-tz} \) and \( C_{\text{max}} \) of R-125528 were 5.0 and 3.6 times higher in the quinidine treatment group, respectively. With respect to this metabolite, \( \text{AUC}_{0-inf} \) and 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 III. Plasma quinidine concentration observed in this study is consistent with previously published results in which the clinical dosage of 600-800 mg/day was used (Brinn et al., 1986; Brosen et al., 1987).

**Safety:** In quinidine DDI study, no deaths or other serious AEs occurred during the course of this 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 due to an unexpected AE during the wash-out period (thermal burn of left hand). No remarkable changes in vital signs, ECG
or physical findings were observed throughout the trial. No subject except the one who was withdrawn from the trial was treated concomitantly.

In ketoconazole DDI study, no deaths and serious AEs occurred during the course of this trial. Two significant treatment-emergent AEs occurred in one subject. This subject showed an increased level of alanine aminotransferase-serum glutamate pyruvate transaminase on Day 8 of Period I and Period 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 AUC\(_{0\text{-}\infty}\) of pactimibe (1.7-fold) relative to pactimibe when given alone. Similarly, concomitant administration of CYP2D6 inhibitor quinidine and pactimibe resulted in a modest increase in the AUC\(_{0\text{-}\infty}\) of pactimibe (1.7-fold) relative to pactimibe when given alone. On the other hand, there was a severe increase in the plasma concentrations of R-125528 (>5.0-fold) in the presence of quinidine.

Dosing regimen used in this study almost completely abolished CYP3A4 and CYP2D6 mediated pathway. The regimen of 4 daily treatment of 400 mg 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, 16-fold AUC increase of well-known CYP3A4 substrate, midazolam, was observed in the same regimen (Olkkola et al., 1994). Similarly, there are several reports that demonstrate 600-800 mg/day quinidine can change the phenotype of CYP2D6 extensive metabolizers to that of poor metabolizers by monitoring urinary metabolic ratio (Brinn et al., 1986; Brosen et al., 1987).

The AUC increase ratio can be simply expressed as AUC\(_{po(+\text{inhibitor})}/\text{AUC}_{po(\text{control})}\) = \(1/(1-f_m)\) under the condition used in this study as following reasons. In general, AUC increase ratio is expressed as the following equation, AUC\(_{po(+\text{inhibitor})}/\text{AUC}_{po(\text{control})}\) = \(1/[f_h\cdot f_m/(1+I_u/K_i)+(1-f_h\cdot f_m)]\), where \(f_h\), \(f_m\), \(I_u\), and \(K_i\) 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, $f_h$ can be approximated to 1, because of the lack of renal clearance as an intact form. The maximum plasma ketoconazole concentration in the same dosing regimen is reported as about 13 µM (Olkkola et al., 1994). The maximum quinidine plasma concentration obtained in this study was 6.5 µM (Table III). Because the plasma free fractions of ketoconazole and quinidine are reported to be 0.01 and 0.15 (Ito et al., 1998), $I_u$ is estimated to be 0.13 and 0.98 µM, respectively. If we also consider the concentration in the portal vein, the $I_u$ values could be higher. Since the $K_i$ 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 $f_h f_m/(1+I_u/K_i)$ could be negligible.

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, indoline oxidation, $\omega$-1 oxidation, and glucuronidation, of which intrinsic clearance values are 0.63, 0.76, and 0.16 µL/min/mg-protein in human liver microsomes, respectively. Since the contributions of CYP3A4 and CYP2D6 to the indoline and the $\omega$-1 oxidation were 99 and 67.8% (Kotsuma et al., 2008), $f_{mCYP3A4}$ and $f_{mCYP2D6}$ of pactimibe were calculated as 0.40 ($=0.63 \times 0.99/(0.63+0.76+0.16)$) and 0.33 ($=0.76 \times 0.678/(0.63+0.76+0.16)$), respectively. According to the equation described above, AUC increase ratios are estimated to be 1.7 ($1/(1-0.40)$) with ketoconazole and 1.5 ($1/(1-0.33)$) with quinidine. These values are well in accordance with the 1.7-fold
AUC increase observed in this study concomitantly administered with ketoconazole and quinidine. Even though we did not measure the glucuronide of pactimibe in the urine and feces in this study, these results suggested that contribution of glucuronidation should be minor in the pactimibe clearance.

In contrast to pactimibe, the plasma metabolite, R-125528, highly accumulated with quinidine treatment. As CYP2D6 mediated reaction is the crucial pathway for the elimination of R-125528 from systemic circulation, it was easily expected that the increase of plasma concentration could be very severe. Assuming that the $f_{m\text{CYP2D6}}$ of R-125528 was 0.9 based on the fact that R-125528 oxidation in human liver microsomes was inhibited as much as 90% in the presence of quinidine (data not shown), AUC increase ratio were estimated to be 10 ($1/(1-0.9)$) with quinidine according to the equation shown above. In fact, the increase ratio of AUC$_{0-tz}$ of R-125528 was 5.0 with quinidine treatment, however, the increase ratio of AUC$_{0-inf}$ could not be adequately defined because the plasma concentration of R-125528 did not decline during the study period up to 72 hr in the quinidine treatment group.

Even though R-125528 is pharmacologically inactive, to monitor the plasma concentration level of this metabolite in humans will be of great importance from toxicological point of view. To address a question whether we should apply genetic screening into the clinical development program of this compound, we need to carefully conduct pharmacokinetic studies to figure out the R-125528 profile in CYP2D6 poor metabolizers. Also we may need to conduct additional toxicological studies to guarantee
safety of R-125528, because the formation of R-125528 in toxicological animals was known to be lower than that in human.

One of the interesting points of this study is both pactimibe and R-125528 are good CYP2D6 substrates in vivo in humans, although they are quite atypical CYP2D6 substrates devoid of basic nitrogen. Especially, the Km value for CYP2D6 mediated ω-1 oxidation of R-125528 is 1.8 µM (Kotsuma et al., 2008). It has been generally recognized that the presence of basic nitrogen is essential for CYP2D6 substrates to interact with the carboxylate anion of Asp^{301} or Glu^{316} (Ellis et al., 1995; Paine et al., 2003). Although R-125528 has a nitrogen atom in the indole ring, it is not protonated in the whole pH range. Recently, Guengerich et al. (Guengerich et al., 2002) also identified an atypical CYP2D6 ligand, spirosulfonamide, devoid of basic nitrogen but having a high affinity for CYP2D6 from in vitro observation.

To conclude, in accordance with the in vitro observation, the pharmacokinetics of pactimibe is only modestly affected by co-treatment with ketoconazole and quinidine to similar extent. However, plasma concentration of its metabolite, R-125528, was markedly elevated in presence of quinidine, because its single metabolic pathway via CYP2D6 was abolished. Although the clinical significance of this drug-drug interaction remains to be established, these results suggest that safety assessments of R-125528 should be carefully conducted considering a genetic background of the patients such as CYP2D6 polymorphisms. In addition, the fact that acidic pactimibe and R-125528 are good substrates for CYP2D6 in humans will require us to make further investigation into
their CYP2D6 binding mode.
References


Metab Dispos 34:1516-1522.


Drug Metab Dispos 36:529-534.


Legends for figures

Figure 1. Chemical structure of pactimibe sulfate (A) and proposed metabolic pathway of pactimibe (B). M-1, oxidized form at the ω-1 position of the octyl chain of pactimibe; R-125528, oxidized form at the indoline ring of pactimibe; M-2, oxidized form at the ω-1 position of the octyl chain of R-125528.

Figure 2. (A) Plasma concentration-versus-time profiles for pactimibe after a single oral administration of pactimibe sulfate (100 mg) in the presence (○) and absence (●) of 400 mg ketoconazole (q.d.) administered to healthy male volunteers. Each point represents the Mean-SD for placebo treatment group and Mean+SD for ketoconazole treatment group (N=18 in each group). (B) Plasma concentration-versus-time profiles for R-125528 after a single oral administration of pactimibe sulfate (100 mg) in the presence (○) and absence (●) of 400 mg ketoconazole (q.d.) administered to healthy male volunteers. Each point represents the Mean+SD for both treatment group (N=18 in each group).

Figure 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 quinidine (b.i.d.) administered to healthy male volunteers. Each point represents the Mean+SD. (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 quinidine (b.i.d.) administered to healthy male volunteers. Each point represents the Mean+SD. (N=17 in each group).
Table I. Pharmacokinetic parameters of pactimibe and its plasma metabolite, R-125528, after oral administration of pactimibe sulfate (100 mg) in the presence and absence of 400 mg ketoconazole administered to healthy male volunteers.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Treatment</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;0-12h&lt;/sub&gt; (ng·h/mL)</th>
<th>AUC&lt;sub&gt;0-inf&lt;/sub&gt; (ng·h/mL)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pactimibe</td>
<td>Placebo + pactimibe</td>
<td>2043 ± 1152</td>
<td>1.75 ± 0.62</td>
<td>23812 ± 10208</td>
<td>24577 ± 10711</td>
<td>14.9 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole + pactimibe</td>
<td>2366 ± 1169</td>
<td>2.58 ± 1.49</td>
<td>38766 ± 22938</td>
<td>42626 ± 25003</td>
<td>21.3 ± 6.4</td>
</tr>
<tr>
<td>R-125528</td>
<td>Placebo + R-125528</td>
<td>174 ± 81.8</td>
<td>11.9 ± 7.68</td>
<td>6820 ± 2748</td>
<td>*6992 ± 2269</td>
<td>c 31.0 ± 10.8</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole + R-125528</td>
<td>79.2 ± 61.3</td>
<td>30.9 ± 22.2</td>
<td>3092 ± 1590</td>
<td>4898 ± NA</td>
<td>d 44.2 ± 17.6</td>
</tr>
</tbody>
</table>

Each data represents Mean ± SD (N = 18, unless specified)

NA = Not applicable

* N = 11 (due to unreliable determination of AUC<sub>0-inf</sub>, i.e. %Extrapol. Exceeds 20%)

b N = 1 (due to unreliable determination of AUC<sub>0-inf</sub>, i.e. %Extrapol. Exceeds 20%)

c N = 17 (due to unreliable determination of λ<sub>Z</sub>)

d N = 5 (due to unreliable determination of λ<sub>Z</sub>)
Table II. 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>Analytes</th>
<th>Treatment</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;0-tz&lt;/sub&gt; (ng·h/mL)</th>
<th>AUC&lt;sub&gt;0-inf&lt;/sub&gt; (ng·h/mL)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (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>
<td>17.3 ± 7.6</td>
</tr>
<tr>
<td></td>
<td>Quinidine + pactimibe</td>
<td>176.3 ± 72.6</td>
<td>4.9 ± 2.9</td>
<td>4087 ± 1282</td>
<td>4538 ± 1307</td>
<td>23.4 ± 4.2</td>
</tr>
<tr>
<td>R-125528</td>
<td>Placebo + R-125528</td>
<td>11.0 ± 4.8</td>
<td>15.4 ± 10.7</td>
<td>401.2 ± 242.9</td>
<td>423.2 ± 187.7</td>
<td>18.8 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>Quinidine + R-125528</td>
<td>39.4 ± 14.1</td>
<td>44.2 ± 19.8</td>
<td>2003 ± 716.2</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Each data represents Mean ± SD (N = 17, unless specified)

NA = Not applicable (due to unreliable determination of λ<sub>z</sub>)

<sup>a</sup>N = 13 (due to unreliable determination of AUC<sub>0-inf</sub>, i.e. %Extrapol. Exceeds 20%)

<sup>b</sup>N = 11 (due to unreliable determination of AUC<sub>0-inf</sub>, i.e. %Extrapol. Exceeds 20%)
Table III. Steady state pharmacokinetic parameters of quinidine (600 mg) on Day 4 (N=17).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{AUC}_{\text{ss,}\tau} , (\mu\text{g}\cdot\text{h}/\text{mL})$</td>
<td>$19.6 ± 4.4$</td>
</tr>
<tr>
<td>$C_{\text{max}} , (\mu\text{g}/\text{mL})$</td>
<td>$2.1 ± 0.5$</td>
</tr>
<tr>
<td>$T_{\text{max}} , (h)$</td>
<td>$4.1 ± 0.5$</td>
</tr>
</tbody>
</table>
Figure 1

(A)

(B)

Glucuronidation

N-dealkylation

CYP3A4

CYP2D6

Pactimibe

M-1

R-125528

M-2
Figure 2

(A) Plasma concentration (ng/mL) over time (h) for 100 mg pactimibe + placebo and 100 mg pactimibe + 400 mg ketoconazole.

(B) Plasma concentration (ng/mL) over time (h) for 100 mg pactimibe + placebo and 100 mg pactimibe + 400 mg ketoconazole.
Figure 3

(A) Plasma concentration (ng/mL) over time (h) for Actimibe 25 mg + placebo and Actimibe 25 mg + Quinidine 600 mg, b.i.d.

(B) Plasma concentration (ng/mL) over time (h) for Actimibe 25 mg + placebo and Actimibe 25 mg + Quinidine 600 mg, b.i.d.