CYP2D6-Mediated Metabolism of a Novel Acyl Coenzyme A:Cholesterol Acyltransferase Inhibitor, Pactimibe, and Its Unique Plasma Metabolite, R-125528

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ABSTRACT:

Pactimibe sulfate is a novel acyl coenzyme A:cholesterol acyltransferase inhibitor. We conducted metabolic studies of pactimibe and its plasma metabolite, R-125528. Pactimibe had multiple metabolic pathways including indolin oxidation to form R-125528, \( \omega-1 \) oxidation, N-dealkylation, and glucuronidation. Among them, the indolin oxidation and the \( \omega-1 \) oxidation were dominant and were mainly catalyzed by CYP3A4 and CYP2D6, respectively. The intrinsic clearance (\( \text{CL}_{\text{int}} \)) values for these pathways in human hepatic microsomes were 0.63 and 0.76 \( \mu\text{l/min/mg-protein} \), respectively. On the other hand, the metabolic reaction for R-125528 was restricted. It was demonstrated that \( \omega-1 \) oxidation was the only pathway that could eliminate R-125528 from the systemic circulation. To our surprise, only CYP2D6-expressing microsomes could catalyze the reaction, and \( \omega-1 \) oxidation was strongly correlated with the CYP2D6 marker reaction, dextromethorphan O-demethylation (\( r^2 = 0.90 \)), in human hepatic microsomes. Although R-125528 is an atypical substrate for CYP2D6 because of its acidity, the \( K_m \) value was 1.8 \( \mu\text{M} \) for the reaction in human hepatic microsomes and the \( \text{CL}_{\text{int}} \) value was as high as 75.0 \( \mu\text{l/min/mg-protein} \). These results suggested that the systemic clearance of R-125528 was highly dependent on CYP2D6 activity and that several studies with CYP2D6 including drug-drug interaction and polymorphism sensitivity should be performed during development from the viewpoint of metabolite safety assessment. The finding that R-125528, an acidic compound devoid of basic nitrogen, was a good substrate for CYP2D6 raised a question about previously reported CYP2D6 models based on a critical electrostatic interaction with Asp\(^{301}\) and/or Glu\(^{216}\).

Pactimibe sulfate (formerly named CS-505) (Fig. 1) is a novel acyl coenzyme A:cholesterol acyltransferase inhibitor used to treat hypercholesterolemia and atherosclerotic diseases (Nissen et al., 2006; Kitayama et al., 2006a,b,c). A number of acyl coenzyme A:cholesterol acyltransferase inhibitors have been evaluated by several investigators. However, because of poor pharmacokinetics (Peck et al., 1995), adverse effects such as adrenal toxicity (Vernetti et al., 1993; Reindel et al., 1994; Matsuo et al., 1996), diarrhea (Kashiwa et al., 1997), and hepatotoxicity (Ishi et al., 1994; Nakaya et al., 1994), and elusive efficacies in humans (Harris et al., 1990; Hainer et al., 1994; Tardif et al., 2004), none of these compounds have 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. Pactimibe is a weak acidic compound and has several metabolic pathways including oxidation at the indolin ring, \( \omega-1 \) oxidation at the octyl chain, N-dealkylation, and glucuronidation on the carboxylic acid. Kinetic studies using human liver microsomes revealed that indolin ring oxidation (formation of R-125528) and \( \omega-1 \) oxidation (formation of M-1) were equally dominant and that glucuronidation and N-dealkylation were minor. In addition, none of the metabolites were estimated to be pharmacologically active in vitro.

In vivo biotransformation studies in animals demonstrated that only pactimibe and R-125528, the oxidized form of the indolin ring in pactimibe, appeared in the plasma and that none of the other metabolites were observed. After oral administration of pactimibe sulfate at a dose of 1 mg/kg to rats, dogs, and monkeys, AUC\(_{R-125528}/\text{AUC}_{\text{pactimibe}}\) ratios were calculated to be 3, 14, and 55%, respectively. 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.

R-125528 is quite a unique metabolite that has higher lipophilicity than the parent compound does. In general, biotransformation converts nonpolar, lipophilic pharmacologically active drug molecules into polar, inactive, or nontoxic metabolites that are readily eliminated from the body (Venkatakrishnan et al., 2001). Interestingly, however,
oxidation from the indolin ring to the indole ring leads pactimibe (logP 4.68) into more lipophilic R-125528 (logP 5.83). Then, R-125528 has to be further metabolized to be eliminated from systemic circulation, forcing us to conduct extensive biotransformation studies of R-125528.

To our surprise, metabolic studies using human liver microsomes and human P450 expressing systems revealed that R-125528 was significantly metabolized by CYP2D6, even though R-125528 is an acidic compound with a carboxyl group and without a basic nitrogen in the structure, suggesting it is an atypical CYP2D6 substrate. When the drug has only a single elimination pathway mediated by a polymorphic enzyme such as CYP2D6, its AUC could drastically increase in patients who lack the enzyme genetically and/or who take concomitant medications, which inhibit the enzyme activity, leading to enhanced side effects. Thus, we performed metabolic studies of pactimibe and its plasma metabolite, R-125528, and in this article we discuss the clinical outcomes of drug-drug interactions and genetic polymorphism on the metabolic enzymes responsible for clearance.

Materials and Methods

Test Substances and Reagents. Pactimibe sulfate [7-(2,2-dimethylpropanamido)-4,6-dimethyl-1-octylindolin-5-yl] acetic acid hemisulfate and R-125528 were synthesized at the Process Development Laboratories of Sankyo Co., Ltd. (Tokyo, Japan). Pactimibe was synthesized at Amersham Pharmacia Biotech Limited (Tokyo, Japan). M-1, δ6-M-1, and M-2 were synthesized at Sankyo Co., Ltd. (Tokyo, Japan). [14C]Pactimibe was synthesized at Amer- sham Pharmacia Biotech Limited (Tokyo, Japan). MgCl2 and glucose-6-phosphate dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO). The chemical structure of pactimibe sulfate is shown in Fig. 1. NADP, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Chemtec Laboratories, Inc. (Tokyo, Japan). The chemical structure of pactimibe sulfate is shown in Fig. 1. NADP, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO). MgCl2 and glucose 6-phosphate were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Acetonitrile of HPLC grade and 1 N HCl of volumetric analysis grade were purchased from Wako Pure Chemical Industries, Ltd. Other reagents and solvents were of analytical grade and were used without further purification.

In Vitro Metabolism Study. Microsomes expressing human P450 isoforms. Human lymphblast microsomes expressing human P450 isoforms, CYPIA1 (lot 36), CYPIA2 (lot 69), CYPIB1 (lot 9), CYPIA26 (lot 41), CYPIB6 (lot 48), CYPIB8 (lot 31), CYPIB9 (lot 44), CYPIB19 (lot 38), CYPIB26 (lot 43), CYPIB2E1 (lot 41), CYPIA3A (lot 73), CYPIA11 (lot 8), and control microsomes (lot 28) were purchased from BD Gentest (Woburn, MA). Microsomes were stored at −80°C until use.

Human liver microsomes. Human liver microsomes (mixed pool microsomes from 15 donors) were purchased from In Vitro Technologies (Baltimore, MD) and used to determine enzyme kinetic parameters. A Reaction Phenotyping Kit (version 5) including 16 individual human liver microsomes was purchased from Xenotech, LLC (Kansas City, KS). The microsomes had been characterized with respect to the following enzyme activities: 7-ethoxysorufin O-dealkylation (CYPIA2), coumarin 7-hydroxylation (CYPIA2A), S-mephenytoin N-demethylation (CYPIB6), paclitaxel 6-O-hydroxylation (CYPIB8), Diclofenac 4′-hydroxylation (CYPIB9), S-mephenytoin 4-hydroxylation (CYPIB19), dextromethorphan O-demethylation (CYPIB26), chloroxazone 6-hydroxylation (CYPIB2E1), testosterone 6β-hydroxylation (CYPIA4A45), and lauric acid 12-hydroxylation (CYPIA4A). Microsomes were stored at −80°C until use.

Microsomal incubations. P450-mediated metabolic reactions were performed at 37°C using a 100 mM potassium phosphate buffer (pH 7.4) and a NADPH-generating system that contained final concentrations of 1.25 to 2.5 mM NADP, 1 to 10 mM MgCl2, 6 H2O, 12.5 to 25 mM glucose 6-phosphate, and 0.5 to 1 U/ml glucose-6-phosphate dehydrogenase. The rate of formation of the metabolite (V: picomoles per minute per milligram of protein or picomoles per hour per milligram of protein or picomoles per minute per piconole of P450) was determined in each experiment.

In the experiments measuring the enzyme kinetic parameters in human hepatic microsomes, final concentrations of 7.8 to 250 μM pactimibe and 0.78 to 100 μM R-125528 were incubated for 30 and 15 min, respectively. To determine the enzyme kinetic parameters for glucoronidation, final concentrations of 5 to 2000 μM [14C]pactimibe were incubated for 120 min. Because of the absence of an authentic sample of the glucuronide of pactimibe, structure analysis of the metabolite separated by HPLC was performed by mass spectrometry. Under the conditions used, the formation of the metabolites was linear with respect to incubation time and protein concentrations.

In the experiment with human P450-expressing microsomes, protein concentrations were set at 0.5 to 1 mg/ml; 100 mM Tris buffer (pH 7.4) was used instead of a 100 mM potassium phosphate buffer for the CYP2A6, CYP2C9, and CYP4A11 reactions. Final substrate concentrations and reaction times were set at 7.8 to 250 μM and 30 min to determine the enzyme kinetic parameters for the formation of M-1 and R-125528 from pactimibe. For the formation of M-2 (the α1 oxidized form of R-125528) from R-125528, a metabolic reaction was performed at a final concentration of 5 μM R-125528 for 15 min.

In correlation experiments, final protein concentrations, substrate concentrations, and reaction times were set at 1 mg/ml, 100 μM, and 30 min and 0.5 mg/ml, 10 μM, and 15 min when pactimibe and R-125528 were used as substrates, respectively. The reaction was stopped by the addition of acetoni- trile-1 N HCl-200 mM dithiothreitol (98:1.1:1, v/v/v) containing internal standards. The mixture was centrifuged at 15,000 rpm for 3 min at 4°C (Himac CF15R; Hitachi Koki, Ltd., Tokyo, Japan). The supernatant fraction was injected into a HPLC or LC-MS/MS system for analysis.

Free fraction of test substance in microsomes. In the experiments measuring the enzyme kinetic parameters, final concentrations of pactimibe and R-125528 were corrected by multiplying the free fraction percentage of each test substance in the microsomal incubation mixture. Free fraction was experimentally determined by a modification of a method described previously (Nakai et al., 2004). In brief, a microsomal incubation mixture was made without the use of a NADPH-generating system. An aliquot of the mixture was collected and combined with acetoni- trile-1 N HCl-200 mM dithiothreitol (98:1.1:1, v/v/v). The rest of the mixture was ultracentrifuged at 105,000g (Optima Max, Beckman Coulter, Fullerton, CA) for 30 min at 4°C, and an aliquot of the supernatant was collected and combined with acetoni- trile-1 N HCl-200 mM dithiothreitol (98:1.1:1, v/v/v). The concentrations of the test substances and postultracentrifugation samples were determined and the ratio of the free form in the sample was calculated by dividing the concentra- tion in the postultracentrifugation sample by the concentration in the preultra- centrifugation sample.

Analysis. LC-MS/MS was used to measure pactimibe, R-125528, and M-1 in an incubation mixture with δ6-M-1 as a deuterated internal standard. Separation by HPLC was performed with a Waters Alliance 2795 Separation Module (Waters, Milford, MA) with a Hypersil C18 column (150 × 2 mm, 5 μm; YMC Co., Ltd., Kyoto, Japan). The mobile phases were (A) 95% H2O, 5% acetonitrile, 0.1% formic acid, and 5 mM ammonium acetate and (B) 5% H2O, 95% acetonitrile, 0.1% formic acid, and 5 mM ammonium acetate. The mobile phase was set at 33% A/67% B in an isocratic mode, and the solvent flow rate was 0.2 ml/min. Mass spectra were determined with a Micromass Quattro LC-MS/MS system (Micromass UK Ltd., Manch, UK). The following parent and daughter ions (m/z) were monitored: 417.5 and 399.5 for pactimibe, 415.5 and 369.5 for R-125528, 433.5 and 415.4 for M-1, and 439.5 and 421.5 for δ6-M-1.

The analysis of M-2 was performed via HPLC using an LC-10AT (Shi- madzu, Kyoto, Japan) pump and an SPD-10AV (Shimadzu) detector. A Sym- metry C18 column (5 μm, 4.6 mm i.d. × 150 mm; Waters) was used for the column separation. The UV wavelength and column temperature were set at 230 nm and 40°C, respectively. The mobile phases were (A) 50 mM phosphate

![Fig. 1. Chemical structure of pactimibe sulfate.](image-url)
buffer (pH 2.5) and (B) acetonitrile, and a gradient of [time (minutes)/percent B: 0 → 8/35 → 70, 8 → 12/70, 12 → 17/35] was used. The solvent flow rate was set at 1 ml/min.

For the analysis of pactimibe glucuronide, a Symmetry C18 (5 μm, 4.6 mm i.d. × 150 mm; Waters) was used for column separation. The mobile phases were (A) H₂O containing 0.1% trifluoroacetic acid and (B) acetonitrile containing 0.1% trifluoroacetic acid. The time programming was as follows: time (minutes)/percent B: 0 → 7.5/40 (constant), 7.5 → 8.5/100 (constant), 8.5 → 15/40 (constant). The solvent flow rate was set at 1 ml/min. The HPLC eluents were collected in scintillation vials with a fraction collector (L-5200; Hitachi Koki, Ltd.) at 30-s intervals, and the scintillator (Pico-Fluor40; Packard BioScience, Groningen, The Netherlands) was added to each vial. The radioactivity of the fraction corresponding to pactimibe glucuronide was measured using a liquid scintillation analyzer (Tri-Carb 2250CA, Tri-Carb 2300TR; Packard BioScience).

Data Presentations. Kᵣ (micromolar concentration), Vₘₐₓ (picomoles per minute per milligram of protein or picomoles of P450), and intrinsic clearance (CLᵣₛₜ), defined as Vₘₐₓ/Kᵣ (microliters per minute per milligram of protein or picomoles of P450), were calculated using WinNonlin (version 1.5 or 4.0.1; Scientific Consulting Inc., Apex, NC) and Microsoft Excel (Microsoft, Redwood, WA) by fitting the data to the following equation; V = Vₘₐₓ × [S]/(Kᵣ + [S]), where V and [S] represent the initial rate of metabolite formation and free concentration of the test substance in microsomal incubation mixture, respectively. Correlation coefficients (r²) obtained from linear regression analysis were calculated with Microsoft Excel.

Results

Metabolism of Pactimibe. The enzyme kinetic parameters for the formation of M-1, R-125528, and glucuronide form from pactimibe were determined using pooled human hepatic microsomes. The formation of the N-dealkylated form was not examined because of the low metabolic activity for the reaction in human hepatic microsomes (data not shown). CLᵣₛₜ values for the formation of M-1 and R-125528 were almost the same (0.76 and 0.63 μl/min/mg-protein, respectively) (Table 1). The CLᵣₛₜ for glucuronide formation from pactimibe was quite low (0.16 μl/min/mg-protein) compared with P450-mediated oxidation.

To elucidate the P450 isoform that is involved in the formation of M-1, microsomes expressing human P450 isoforms were incubated with pactimibe. As shown in Table 2, CYP2C9, CYP2C19, and CYP2D6 showed metabolic activity for M-1 formation. CLᵣₛₜ values of CYP2C9, CYP2C19, and CYP2D6 for the formation of M-1 were 0.001, 0.026, and 0.124 μl/min/pmol of P450, respectively. Considering the P450 content (picomoles per milligram of protein) in human liver microsomes (Rodrigues, 1999), contributions of each P450 isoform involved in the formation of M-1 were calculated to be 5.2, 27, and 67.8%, respectively, suggesting that mainly CYP2D6 contributes to the ω-1 oxidation of pactimibe. As shown in Table 2, P450 expression microsomes revealed that CYP2C19 and CYP2D6, and CYP3A4 showed metabolic activity in R-125528 formation. However, CLᵣₛₜ values for CYP2C19 and CYP2D6 were quite low (<0.002 μl/min/pmol P450). Considering the high amount of CYP3A4 protein content compared with CYP2C19 and CYP2D6 in human hepatic microsomes, CYP3A4 was estimated to be the major contributor of indolin oxidation in pactimibe.

To obtain further information on P450 isoforms involved in the metabolism of pactimibe, human liver microsomes from 16 different donors, for which P450 activities had been known, were incubated. Correlations between the rate of formation of pactimibe metabolites (M-1 and R-125528) and different P450 marker activities are listed in Table 3. The relationship between dextromethorphan O-demethylation activity, a marker for CYP2D6 activity (Schmid et al., 1985), and the rate of M-1 formation was moderately correlated (r² = 0.52). With regard to the rate of R-125528 formation, testosterone 6β-hydroxylation (r² = 0.86), a marker for CYP3A activity (Waxman et al., 1988), was strongly correlated, whereas no remarkable correlation was observed for other P450 activities. These results support the idea that mainly CYP2D6 and CYP3A4 are involved in the formation of M-1 and R-125528 from pactimibe, respectively.

Metabolism of R-125528. To clarify the elimination pathway of R-125528, the main and only metabolite in systemic circulation, we performed a metabolic study using pooled human hepatic microsomes (Fig. 2), microsomes expressing human P450 isoforms (Fig. 3A), and a reaction phenotyping kit (Fig. 3B). As depicted in Fig. 2, enzyme kinetic parameters, Kᵣₛₜ, Vₘₐₓ, and CLᵣₛₜ, for the ω-1 oxidation of R-125528 (formation of M-2) in pooled human liver microsomes were calculated to be 1.8 μl, 135.0 pmol/min/mg-protein, and 75.0 μl/min/mg-protein, respectively.

To elucidate the P450 isoform that is involved in the formation of M-2, microsomes expressing human P450 isoforms were incubated with R-125528. The ω-1 oxidation of R-125528 was observed only in CYP2D6-expressing microsomes (Fig. 3A). In addition, the relationship between the rate of ω-1 oxidation of R-125528 and CYP2D6 activity was strongly correlated (r² = 0.90), suggesting that CYP2D6 is highly involved in the metabolism of R-125528 (Fig. 3B).

TABLE 1

<table>
<thead>
<tr>
<th>Metabolism</th>
<th>Kᵣₛₜ (μM)</th>
<th>Vₘₐₓ (pmol/min/pmol P450)</th>
<th>CLᵣₛₜ (μl/min/pmol P450)</th>
<th>Contents (pmol/mg)</th>
<th>Contribution (%)</th>
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<td>M-1 formation</td>
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<td>0.5</td>
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<tr>
<td>CYP3A4</td>
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<td>0.037</td>
<td>108</td>
<td>99</td>
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</table>

*a Data from Rodrigues (1999).

*b Calculated from CLᵣₛₜ for each P450 isoform and P450 content.

TABLE 2

<table>
<thead>
<tr>
<th>Metabolism</th>
<th>P450 isoform</th>
<th>Kᵣₛₜ (μM)</th>
<th>Vₘₐₓ (pmol/min/pmol P450)</th>
<th>CLᵣₛₜ (μl/min/pmol P450)</th>
<th>Contents (pmol/mg)</th>
<th>Contribution (%)</th>
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</table>

*a Data from Rodrigues (1999).

*b Calculated from CLᵣₛₜ for each P450 isoform and P450 content.

CYP2D6-MEDIATED METABOLISM OF PACTIMIBE AND R-125528
A proposed main metabolic pathway of pactimibe in humans is illustrated in Fig. 4. Pactimibe has multiple metabolic pathways, that is, oxidation at the indolin ring, \( \text{H} \text{9275} -1 \) oxidation at the octyl chain, \( \text{N} \)-dealkylation, and glucuronidation on the carboxylic acid. Metabolic studies using human liver microsomes revealed that indolin ring oxidation (formation of R-125528) and \( \text{H} \text{9275} -1 \) oxidation (formation of M-1) were dominant and the CLint values were 0.63 and 0.76 l/min/mg-protein, respectively (Table 1). Moreover, according to a P450 isozyme identification study using P450 expression microsomes and a correlation analysis using human liver microsomes, the indolin oxidation and the \( \text{H} \text{9275} -1 \) oxidation were found to be catalyzed mainly by CYP3A4 and CYP2D6, respectively (Tables 2 and 3).

On the other hand, the metabolic reaction for R-125528 was restricted. Using human hepatic microsomes, R-125528 was only metabolized in the P450 reaction system but was not conjugated in the UGT reaction system. In rat bile, the only metabolite derived from R-125528 was the \( \text{H} \text{9275} -1 \) oxidized form. No glucuronide form was observed (data not shown). In human hepatic microsomes, the CLint value for the formation of M-2, the \( \text{H} \text{9275} -1 \) oxidized form of R-125528, was 75.0 \( \mu \)/min/mg-protein (Fig. 2). The P450 isozyme identification study using P450 expression microsomes revealed that CYP2D6 was the only isozyme that could catalyze the reaction (Fig. 3A). In addition, the reaction phenotyping study indicated that CYP2D6 activity was strongly correlated \( (r^2 = 0.90) \) with the formation of M-2 (Fig. 3B).

### Table 3

<table>
<thead>
<tr>
<th>P450 Isoforms</th>
<th>Marker Reaction</th>
<th>M-1 formation</th>
<th>R-125528 formation</th>
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<td>CYP 1A2</td>
<td>7-Ethoxyresorufin O-dealkylation</td>
<td>0.019</td>
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<td>CYP 2A6</td>
<td>Coumarin 7-hydroxylation</td>
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<td>S-Mephenytoin N-demethylation</td>
<td>0.25</td>
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<td>CYP 2C8</td>
<td>Paclitaxel 6α-hydroxylation</td>
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<td>CYP 2C9</td>
<td>Diclofenac 4'-hydroxylation</td>
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<td>CYP 2C19</td>
<td>S-Mephenytoin 4'-hydroxylation</td>
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<td>CYP2D6</td>
<td>Dextromethorphan O-demethylation</td>
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<td>CYP2E1</td>
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<td>CYP3A4/5</td>
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<td>Lauric acid 12-hydroxylation</td>
<td>0.04</td>
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**Discussion**

A proposed main metabolic pathway of pactimibe in humans is illustrated in Fig. 4. Pactimibe has multiple metabolic pathways, that is, oxidation at the indolin ring, \( \text{H} \text{9275} -1 \) oxidation at the octyl chain, \( \text{N} \)-dealkylation, and glucuronidation on the carboxylic acid. Metabolic studies using human liver microsomes revealed that indolin ring oxidation (formation of R-125528) and \( \text{H} \text{9275} -1 \) oxidation (formation of M-1) were dominant and the CLint values were 0.63 and 0.76 l/min/mg-protein, respectively (Table 1). Moreover, according to a P450 isozyme identification study using P450 expression microsomes and a correlation analysis using human liver microsomes, the indolin oxidation and the \( \text{H} \text{9275} -1 \) oxidation were found to be catalyzed mainly by CYP3A4 and CYP2D6, respectively (Tables 2 and 3).

On the other hand, the metabolic reaction for R-125528 was restricted. Using human hepatic microsomes, R-125528 was only metabolized in the P450 reaction system but was not conjugated in the UGT reaction system. In rat bile, the only metabolite derived from R-125528 was the \( \text{H} \text{9275} -1 \) oxidized form. No glucuronide form was observed (data not shown). In human hepatic microsomes, the CLint value for the formation of M-2, the \( \text{H} \text{9275} -1 \) oxidized form of R-125528, was 75.0 \( \mu \)/min/mg-protein (Fig. 2). The P450 isozyme identification study using P450 expression microsomes revealed that CYP2D6 was the only isozyme that could catalyze the reaction (Fig. 3A). In addition, the reaction phenotyping study indicated that CYP2D6 activity was strongly correlated \( (r^2 = 0.90) \) with the formation of M-2 (Fig. 3B).
3B). Considering that R-125528 itself could not be excreted into the bile or urine as an intact form, the \(-3\) oxidation mediated by CYP2D6 was considered to be a crucial pathway for the elimination of R-125528 from the systemic circulation.

CYP2D6 is one of the best known P450 isoforms that leads to large interindividual variations in drug concentration, drug response, therapeutic outcome, and/or toxicity because of its polymorphic nature (Brynne et al., 1998; Molden et al., 2002; Fux et al., 2005). The variability in CYP2D6 activity is mainly determined genetically, and 5 to 10% of Caucasians are reported to express a poor CYP2D6-metabolizing phenotype, resulting from the inheritance of two mutant null alleles (van der Weide and Steijns, 1999). Therefore, it was necessary to examine whether the CYP2D6 polymorphism is clinically significant for pharmacokinetic behavior of pactimibe and R-125528 in humans.

It was reported elsewhere (Ito et al., 2005; Gibbs et al., 2006) that the AUC increase in CYP2D6 poor metabolizers can be calculated from the following equation: \( \frac{\text{AUC}_{\text{PM}}}{\text{AUC}_{\text{EM}}} = \frac{1}{1 - f_m \text{CYP2D6}} \), where \( \text{AUC}_{\text{EM}} \) is the area under the plasma concentration time curve in CYP2D6 extensive metabolizers, \( \text{AUC}_{\text{PM}} \) is the area under the plasma concentration time curve in CYP2D6 poor metabolizers, and \( f_m \text{CYP2D6} \) is the fraction of metabolism via CYP2D6. Pactimibe was suggested to have multiple metabolic pathways (i.e., similar contributions of indolin oxidation and \(-3\) oxidation beside glucuronidation to some extent) (Table 1). If we assume that the \( f_m \text{CYP2D6} \) of pactimibe is estimated to be 0.5 at most, the AUC increase of pactimibe in CYP2D6 poor metabolizers is expected to be within 2-fold. Similarly, even if the CYP3A4-mediated pathway is completely abolished by concomitant use of strong CYP3A4 inhibitors such as ketoconazole, the AUC increase of pactimibe would be within 2-fold. It can be said that the multiple metabolic pathways of pactimibe will minimize the extent of drug-drug interactions and/or the effect of genetic polymorphisms.

On the other hand, on the basis of the equation above, small changes in percentage contribution can result in large pharmacokinetic differences when the fraction metabolized exceeds 60%, because of the nonlinear shape of the relationship between \( f_m \text{CYP2D6} \) and % of R-125528. Therefore, the effect of CYP2D6 polymorphism on the pharmacokinetics of R-125528 is expected to be more significant 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. Metabolite safety testing has also been recommended in the U.S. Food and Drug Administration guide for industries (CDER, 2005). This guide stated that major human metabolites should be monitored in clinical trials and that exposure to subjects should be guaranteed by nonclinical safety studies within an adequate margin.

In the case of pactimibe, when CYP2D6 activity is decreased, the exposure of R-125528 could be drastically increased because of its considerably high \( f_m \text{CYP2D6} \). This idea reminded us of the concern that exposure of R-125528 after multiple doses of pactimibe sulfate in CYP2D6 poor metabolizers might exceed the animal no observed adverse effect level. If changes in the pharmacokinetics have a significant impact on both the safety and pharmacological activity of this compound, dose adjustment in CYP2D6 poor metabolizers and/or exclusion of CYP2D6 poor metabolizers may be necessary in clinical settings. To assess the safety of this compound in humans, it has been suggested that the following studies be performed during development: 1) drug-drug interaction studies with CYP2D6 inhibitors; 2) studies to confirm the exposure and safety levels of pactimibe and R-125528 in CYP2D6 poor metabolizers after single-dose administration and simulation of exposure levels after multiple dose in CYP2D6 poor metabolizers; and 3) studies to confirm the exposure and safety of pactimibe and R-125528 in CYP2D6 poor metabolizers after multiple dose administration.

Several groups have tried to predict binding affinity against CYP2D6 using in silico methods (Strobl et al., 1993; de Groot et al., 1999a,b; Ekins et al., 2003). Ellis et al. (1995) demonstrated that Asp301 plays an important role in determining the substrate specificity and activity of CYP2D6 and provided experimental evidence for the electrostatic interaction between the basic nitrogen in CYP2D6 substrates and the carboxylate group of Asp301. Paine et al. (2003) also suggested the importance of Glu216 in addition to Asp301 as a key
determinant factor for substrate specificity and product regioselectivity in CYP2D6. Therefore, it has been recognized that the presence of basic nitrogen is essential for CYP2D6 substrates to interact with the carboxylate anion of Asp<sup>301</sup> or Glu<sup>216</sup>.

In this study, we found that both pactimibe and R-125528 are CYP2D6 substrates and, to our surprise, that R-125528 is an especially good specific substrate for CYP2D6. The <i>K<sub>m</sub></i> value for CYP2D6-mediated <i>ω</i>-1 oxidation of R-125528 was 1.8 µM in human hepatic microsomes, indicating that this acidic compound has a very high affinity for CYP2D6. Although R-125528 has a nitrogen atom in the indole ring, it is not protonated in the whole pH range. Thus, R-125528 is thought to be a very unique CYP2D6 substrate devoid of basic nitrogen. Recently, Guengerich et al. (2002) also identified a ligand, spiroisulfonamide, devoid of basic nitrogen but having a high affinity for CYP2D6.

In conclusion, we performed metabolic studies of pactimibe and its plasma metabolite, R-125528. We found that R-125528 needs to be metabolized solely by CYP2D6 to be eliminated, suggesting that several studies with CYP2D6, including drug-drug interaction and polymorphism sensitivity, should be performed during clinical development from the viewpoint of metabolite safety assessment. The finding that R-125528, an acidic compound devoid of basic nitrogen, was a good substrate for CYP2D6 raises questions about the previously reported CYP2D6 models based on a critical electrostatic interaction with Asp<sup>301</sup> and/or Glu<sup>216</sup>.

References


Gibbs JP, Hyland R, and Youdim K (2006) Minimizing polymorphic metabolism in drug CYP2D6 substrates to interact with the carboxylate anion of Asp<sup>301</sup> or Glu<sup>216</sup>. In this study, we found that both pactimibe and R-125528 are CYP2D6 substrates and, to our surprise, that R-125528 is an especially good specific substrate for CYP2D6. The <i>K<sub>m</sub></i> value for CYP2D6-mediated <i>ω</i>-1 oxidation of R-125528 was 1.8 µM in human hepatic microsomes, indicating that this acidic compound has a very high affinity for CYP2D6. Although R-125528 has a nitrogen atom in the indole ring, it is not protonated in the whole pH range. Thus, R-125528 is thought to be a very unique CYP2D6 substrate devoid of basic nitrogen. Recently, Guengerich et al. (2002) also identified a ligand, spiroisulfonamide, devoid of basic nitrogen but having a high affinity for CYP2D6.

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