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Analysis of the Pharmacokinetic Boosting Effects of Ritonavir on Oral Bioavailability of Drugs in Mice

Atsuko Tomaru, Mariko Takeda-Morishita†, Hirokazu Banba and Kozo Takayama

Department of Pharmaceutics, Hoshi University, Tokyo, Japan

Summary: Ritonavir dramatically increases the bioavailability of a variety of concurrently administered drugs by inhibition of metabolic enzymes and drug transporters. The purpose of this study was to investigate the extent to which ritonavir’s inhibition of drug transporters and/or CYP3A contributes to the increased oral bioavailability in mice. The area under the plasma concentration-time curves (AUC) for orally administered saquinavir after coadministration with 50 mg/kg ritonavir dramatically increased (325-fold). As a result, the bioavailability, F_a·F_g and F_h increased 75-, 38- and twofold, respectively. In addition, the increase in the AUC predicted from the *in vitro* Ki value was ninefold, which was derived from the inhibition of metabolic enzymes by ritonavir in the liver. The remaining 36-fold increase in the AUC was considered to be derived from the inhibition in the small intestine. The AUC_0–12 for saquinavir was affected negligibly by itraconazole. These results indicate ritonavir mainly affects the first-pass effect of saquinavir in the small intestine, increasing the bioavailability of orally administered saquinavir. Furthermore, cyp isoforms other than CYP3A, which contribute to the metabolism of saquinavir in humans, are involved in the metabolism of saquinavir in mice.

Keywords: ritonavir; drug-drug interactions; P-gp; CYP3A; oral bioavailability

Introduction

Drug-drug interactions alter the pharmacokinetic profiles of concurrently administered drugs, and may lead to a loss of efficacy or induce adverse events. Therefore, it is important to understand the mechanisms and magnitudes of drug-drug interactions. Ritonavir (RTV) boosting is a drug therapy used in the treatment of human immunodeficiency virus (HIV) that utilizes the principle of drug-drug interaction. RTV was first developed as an HIV type-1 (HIV-1) protease inhibitor (PI) and used for a single PI treatment. However, since its potent inhibitory effect on the cytochrome P450 (CYP) 3A was clarified, RTV has been used in multdrug treatment regimens for HIV. In antiretroviral therapies, adequate plasma concentrations of PIs are required for their effective anti-HIV activity. The potent inhibition of CYP3A in the small intestine and liver by RTV improves the bioavailability of drugs and prolongs their elimination half-lives. As a result, RTV boosting allows both dose and dosing frequency of the concurrently administered PI to be reduced.

Saquinavir (SQV) is also a highly selective HIV-1 PI and is administered as one of the RTV-boosted PIs. The bioavailability of orally administered SQV was only 4% in healthy volunteers after a single dose. The activity of the original hard-gel capsule formulation (Invirase®) is limited by the drug’s poor pharmacokinetics, resulting in limited long-term viral suppression. Its replacement with a soft-gel capsule formulation (Fortovase®) also failed to provide adequate drug exposure. However, the concurrent administration of SQV with RTV enhances the plasma concentration of SQV, and so SQV is used as a combination therapy with RTV. The poor bioavailability of SQV is attributed to the extensive first-pass metabolism in the small intestine and liver, because SQV is a substrate of CYP3A. Therefore, the boosting effect of RTV is considered to be mediated through its inhibitory effect on CYP3A.

RTV also inhibits many transporters, such as p-glycoprotein (P-gp), breast cancer resistance protein (BCRP), multidrug resistance-associated protein 2 (MRP2), and organic anion-transporting polypeptide (OATP). P-gp, BCRP, and MRP2 are the efflux
transporters expressed at the apical membrane in the small intestine and limit the bioavailability of their orally administered substrate drugs.\(^1\) Because SQV is also a substrate of P-gp,\(^3\) the inhibition of P-gp by RTV in the small intestine might enhance the bioavailability of SQV. In a clinical study, the AUC for fexofenadine (FEX), a substrate of P-gp, increased twofold after its oral administration with 100 mg of RTV.\(^13\) Huisman et al. reported that the AUC for 50 mg/kg SQV increased 20-fold after its oral administration to \(mdr1a^{-/-}/mdr1b^{-/-}\) mice.\(^14\) Furthermore, Washington et al. also reported a 6.5-fold increase in the AUC for 500 mg/kg SQV after its oral administration to \(mdr1a^{-/-}/mdr1b^{-/-}\) mice.\(^15\) These results indicate that the increase in the AUC for SQV can be attributed to the inhibition of P-gp by RTV. In contrast, Richter et al. reported that RTV caused only a 1.4-fold increase in the intestinal permeability in an \(in\ situ\) perfusion study.\(^16\) Thus, the detailed mechanism of RTV boosting is not well understood.

Recently, it was reported that SQV is a substrate of OATP1B1 and OATP1B3 because a significant increase in the accumulation of SQV was observed in *Xenopus laevis* oocytes injected with SLC01B1 or 1B3 cDNA.\(^17\) OATP1B1 and OATP1B3 are influx transporters and localized on the basolateral membrane of hepatocytes. As observed in the drug-drug interaction between cerivastatin and gemfibrozil,\(^18\) the inhibition of the influx transporters expressed on sinusoidal membrane increases the concentration of drugs in the liver, causing adverse events. In an \(in\ vitro\) study, the half maximal inhibitory concentration values (IC\(_{50}\)) of RTV for OATP1B1 and OATP1B3 were 1.6 and 3.6 \(\mu\)M,\(^10\) respectively. These values are close to that for P-gp (3.8 \(\mu\)M).\(^5\) Therefore, it is also important to investigate the RTV-boosting effect on influx transporters, such as OATP1B1 and OATP1B3.

In this study, we assessed the contribution of the RTV effect on the first-pass effect of SQV in the small intestines and livers of mice. The RTV boosting effects on several probe substrates were also investigated. In addition, an \(in\ vitro\) study using mouse intestinal and liver microsomes was performed to confirm the results obtained from the \(in\ vivo\) study.

**Materials and Methods**

**Materials:** RTV purchased as Norvir\(^\circledR\) (80 mg/mL) from Abbots Laboratories (Abbott Park, IL, USA) and midazolam (MDZ) as Dormicum Injection\(^\circledR\) (5 mg/mL) purchased from Astellas Pharma Inc. (Tokyo, Japan) were used in the \(in\ vivo\) study. In the \(in\ vitro\) study, RTV purchased from Biotrend Chemikalien GmbH (Cologne, Germany) and MDZ purchased from Wako Pure Chemical Industries, Inc. (Tokyo, Japan) were used. SQV was purchased from Sequoia Research Products Ltd. (Pangbourne, UK) and pravastatin (PRV) sodium was purchased from Wako Pure Chemical Industries, Inc. (Tokyo, Japan). Fexofenadine hydrochloride was purchased from Toronto Research Chemicals Inc. (Ontario, Canada). All other chemicals were of analytical grade and are commercially available.

**Animals:** Female ddY mice (11–13 weeks old) were purchased from Sankyo Labo Service Corp (Tokyo, Japan). The mice were housed in rooms maintained at 23°C and 55 ± 5% relative humidity, and allowed free access to food and water during the acclimatization period. In the \(in\ vivo\) study, the mice were fasted overnight for at least 12 h, with free access to water before dosing. The animal study was performed at Hoshi University and complied with the regulations of the Committee on Ethics in the Care and use of Laboratory Animals.

**In vivo studies:**

**Preparation of dosing solutions**

The SQV dosing solution was prepared as described previously.\(^14\) Briefly, SQV was dissolved in an 8% ethanol/4.2% glucose solution for intravenous administration and in a 16.4% ethanol/3% glucose/15.6% Cremophor EL solution for oral administration. Norvir\(^\circledR\) was diluted with ethanol and water to prepare an 8 mg/mL solution. As the control vehicle for Norvir\(^\circledR\), a 43%(v/v) ethanol solution containing Cremophor EL (105 mg/mL), propylene glycol (0.25 mg/mL), peppermint oil (3.5 mg/mL), and water-free citric acid (2.8 mg/mL) was used. This control vehicle was 10-fold diluted by ethanol and water to contain the same volume of vehicle constituents as RTV dosing solution. For the dose-dependency of RTV inhibition effect study, Norvir\(^\circledR\) was diluted with control vehicle to prepare 2.4, 8 and 40 mg/mL solutions. Then, these solutions were diluted with ethanol and water to make each RTV dosing solution (0.24, 0.8 and 4 mg/mL). The dosing solution for each probe substrate was prepared as follows. Fexofenadine hydrochloride and pravastatin sodium were dissolved in water to concentrations of 1.5 and 20 mg/mL, respectively. Dormicin Injection\(^\circledR\) was diluted with water to concentration of 3 mg/mL and used as the MDZ dosing solution. The ITZ dosing solution was prepared as described previously.\(^19\) Briefly, 20 mg of ITZ was dissolved with 0.1 mL of 12 N HCl and 1.75 mL of polyethylene glycol 400 and 0.15 mL of 8 N NaOH were added to prepare a 10 mg/mL ITZ solution. As the control vehicle for ITZ, a solution which contained the same contents of vehicle constituents was used.

**Effect of RTV on the pharmacokinetics of SQV**

The dose-dependent effects of RTV on the pharmacokinetics of SQV were investigated. The administered doses of RTV were set at 1.5, 5, 25 and 50 mg/kg. The dose of 1.5 mg/kg is the clinically relevant dose (RTV boosting dose: 100 or 200 mg/dose, i.e., 1.5–3 mg/kg for a 70 kg man). SQV (20 mg/kg) was administered orally 30 min after the oral administration of RTV.

In addition, to investigate the contribution of RTV on the first-pass effect in the liver and small intestine, SQV was administered orally (20 mg/kg) or intravenously (2 mg/kg) 30 min after the oral administration of 50 mg/kg RTV. For oral administration, mice were given each solution by sonde needle into the stomach. For intravenous administration, each solution was injected into the tail vein of mice. Thirty \(\mu\)L of blood was taken from the tail vein by using micro-haematocrit capillary tubes and put into the micro tube at predetermined time intervals. Plasma was separated by centrifugation at 15,400 \(\times\) \(g\) for 10 min. The plasma samples were stored at \(-20^\circ\)C until analysis.

**Effect of RTV on the pharmacokinetics of probe substrates**

FEX (10 mg/kg) was used as the probe substrate for P-gp, MDZ (10 mg/kg) for CYP3A, and PRV (100 mg/kg) for OATP1B1. Each probe substrate was administered orally 30 min after the oral administration of 1.5 or 50 mg/kg RTV. The effect of ITZ, an inhibitor of CYP3A in humans, on the pharmacokinetics of SQV was also investigated. SQV (20 mg/kg) was administered orally 30 min after the oral administration of 50 mg/kg ITZ. Thirty \(\mu\)L of blood was taken from the tail vein by using micro-haematocrit capillary tubes and put into the micro tube at predetermined time intervals. Plasma was separated by centrifugation at 15,400 \(\times\) \(g\) for 10 min. The plasma samples were stored at \(-20^\circ\)C until analysis.
Quantification of the plasma concentration of SQV, FEX, MDZ and PRV
For the determination of each substrate concentration in plasma, 100 µL of acetonitrile was added to the 10 µL of plasma sample. After the protein precipitation by vortex mixing and centrifugation, 80 µL of supernatant was evaporated at 40°C under nitrogen. The residue was reconstituted with the 100 µL of mobile phase and subjected to liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis on an API 4000 system (AB SCIEX, Foster City, CA, USA) equipped with the Prominence LC system (Shimadzu Co., Kyoto, Japan). The LC-MS/MS conditions are summarized in Table 1. The data were acquired with Analyst ver. 1.4.2 (AB SCIEX). The limit of quantification for all compounds was 1ng/mL.

In vitro studies:
Preparation of mouse microsomes
Pooled mouse liver and intestinal microsomes were prepared according to a previously reported method.29) Briefly, livers and intestines were homogenized separately in three volumes of ice-cold buffer (50 mM Tris-HCl, pH 7.4) containing 150 mM KCl, 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 1 mg/mL trypsin inhibitor, 10 µM leupeptin, 0.04 unit/mL aprotinin, 1 µM bestatin, and 20% (v/v) glycerol using a Teflon-tipped pestle. The homogenates were centrifuged at 9,000 × g for 20 min at 4°C, and the supernatants were then centrifuged at 105,000 × g for 60 min at 4°C. The microsomal pellets were resuspended in the same buffer and stored at −80°C. The protein concentrations were determined using a BCA Protein Assay Kit (Pierce Chemical, Rockford, IL, USA).

Inhibition study
In preliminary experiments, we confirmed that substrate depletion increased proportionally with time (to 20 min) and with protein concentration (in the range of 0.1 to 1 mg/mL protein) for both the liver and intestinal microsomes. MDZ, SQV, RTV and ITZ were dissolved in 50% methanol, 50% dimethyl sulfoxide (DMSO), methanol and DMSO, respectively. The volume of organic solvent in the incubation mixture was under 1% and the same volume of each solvent was added to the control sample.

The mouse liver and intestinal microsomes were incubated with SQV or MDZ with or without an inhibitor in an NADPH-generating system containing 2.5 mM NADPH, 25 mM glucose 6-phosphate, 2 units of glucose-6-phosphate dehydrogenase, and 10 mM MgCl2 in a total volume of 0.2 mL of 100 mM phosphate buffer (pH 7.4). After preincubation for 5 min at 37°C, the reaction was initiated by the addition of the NADPH-generating system. After incubation, the reaction was terminated by the addition of 0.4 mL of ice-cold acetonitrile and briefly vortex mixed. The samples were centrifuged at 15,400 × g for 15 min to precipitate the protein. The supernatant was evaporated at 40°C under nitrogen. The residue was reconstituted with the mobile phase and the depletion of SQV or MDZ was analyzed by high-performance liquid chromatography with ultraviolet detection (HPLC-UV).

Quantification of SQV and MDZ concentration in the in vitro samples
HPLC was performed with a Prominence LC system (Shimadzu Co.), equipped with a TSKgel® ODS-100V column (2.1 × 50 mm, 5 µm; Tosoh Co., Tokyo, Japan) for SQV and a CAPCELL PAK MGII C18 column (2.1 × 50 mm, 5 µm; Shiseido Co., Ltd, Tokyo, Japan) for MDZ. 10 mM Na2HPO4 in 35% acetonitrile was used as the mobile phase for SQV and 10 mM phosphate buffer (pH 6) in 40% acetonitrile was used as the mobile phase for MDZ. The flow rate was 0.2 mL/min at 40°C and the elution was monitored at wavelengths of 254 nm for SQV and 230 nm for MDZ.

Pharmacokinetic calculations:
In vivo study
The peak plasma concentration (Cmax) was obtained directly from the raw data. The area under the plasma concentration-time curve from time 0 to the last time point (AUC0–t) was calculated using the linear trapezoidal rule. The AUCinf was extrapolated to infinity was calculated by dividing the last measured concentration by the elimination rate constant, which was determined as the slope of regression for the terminal log-linear portion of the concentration versus time curve. The bioavailability (F), hepatic availability (Fh), fraction absorbed (Fa), and intestinal availability (Fg) were calculated using Eqs. (1)–(4). The blood-to-plasma ratio (Rb) and the hepatic blood flow (Qh) values used were 0.623) and 5.4 L/h/kg,22) respectively. Hepatic clearance was regarded as the total clearance because the urinary excretion was negligible.3)

\[
F = \frac{(\text{AUC}_{\text{po}} - \text{Dose}_{\text{iv}}) / (\text{AUC}_{\text{iv}} - \text{Dose}_{\text{po}})}{1}
\]

\[
\text{CL}_{\text{tot}} = \text{CL}_h = \text{Dose}_{\text{iv}} / (\text{AUC}_{\text{iv}} - \text{Rb})
\]

\[
F_h = 1 - \frac{\text{CL}_h}{Q_h}
\]

\[
F_a \cdot F_g = F / F_h
\]

In vitro study
The initial reaction rate was determined under linear conditions. The inhibition constant (K) was determined by plotting the slope of the Dixon plot versus inhibitor concentration.

The increase in the AUC (R) caused by the drug-drug interactions was calculated with Eq. (5) for the intravenous administration and with Eq. (6) for the oral administration.

\[
R = 1 + \left(\frac{I_{\text{in,iv}} \cdot F_h}{K_i}\right)
\]

\[
R = 1 + \frac{I_{\text{in,iv}}}{K_i}
\]

where \(I_{\text{max}}\) represents the maximum concentration of the inhibitor in the systemic blood. The first-order rate constant (K), the fraction absorbed from the gastrointestinal tract into the portal vein (Fg), and the unbound fraction in the blood (f) used for calculations are summarized in Table 2.

Statistical analysis
Each value is expressed as a mean ± standard deviation (SD). Statistical significance was examined by one-way analysis of variance (ANOVA) followed by Dunnett’s post hoc multiple-com-
Results

Effect of RTV on the pharmacokinetics of SQV: RTV increased the $C_{\text{max}}$ and $AUC_{\text{inf}}$ for SQV in a dose-dependent manner. The $AUC_{\text{inf}}$ for SQV was increased three-, 26-, 241-, and 325-fold by the coadministration of 1.5, 5, 25, and 50 mg/kg RTV, respectively. The plasma concentration-time profiles and pharmacokinetic parameters are shown in Figure 1 and Table 3, respectively.

To assess the effect of RTV on the first-pass effect in the liver and small intestine, $F_{h}$ and $F_{g}$ were calculated from the pharmacokinetic parameters after the intravenous and oral administration of SQV with and without 50 mg/kg RTV. A high dose of RTV was administered and a significant interaction was observed in this study, however, the animals tolerated it well. The plasma-concentration time profiles and pharmacokinetic parameters for SQV are presented in Figure 2 and Table 4, respectively. After the intravenous administration of SQV, the coadministration of RTV caused only a fivefold increase in the $AUC_{\text{inf}}$ for SQV. In contrast, after the oral administration of SQV, the $AUC_{\text{inf}}$ increased dramatically (325-fold) with the coadministration of 50 mg/kg RTV. As a result, the bioavailability of orally administered SQV increased from 0.0093 to 0.675. $F_{h}$ and $F_{g}$ for SQV were increased 1.7- and 38-fold, respectively, with the coadministration of RTV.

Effect of RTV on the pharmacokinetics of probe substrates: The effects of RTV on the pharmacokinetics of each probe substrate were investigated. The plasma concentration-time profiles and pharmacokinetic parameters for each probe substrate are shown in Figures 3–5 and Tables 5–7, respectively. At the clinically relevant dose (1.5 mg/kg), the $AUC_{\text{inf}}$ for FEX was not affected with the coadministration of RTV. However, the $AUC_{\text{inf}}$ for MDZ and PRV increased twofold when they were coadministered with 1.5 mg/kg RTV. The $AUC_{\text{inf}}$ for MDZ, FEX and PRV increased dramatically (325-fold).

Table 2. Parameters used in the prediction of the increase in the AUC from $K_{i}$ values

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>RTV</th>
<th>ITZ</th>
</tr>
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<tbody>
<tr>
<td>$I_{\text{max}}$</td>
<td>µM</td>
<td>24</td>
<td>2$^{(7)}$</td>
</tr>
<tr>
<td>$K_{i}$</td>
<td>min$^{-1}$</td>
<td>0.007</td>
<td>0.004$^{(17)}$</td>
</tr>
<tr>
<td>$F_{h}F_{g}$</td>
<td>—</td>
<td>0.8</td>
<td>1</td>
</tr>
<tr>
<td>$Q_{h}$</td>
<td>L/h/kg</td>
<td>5.4$^{(22)}$</td>
<td>5.4$^{(22)}$</td>
</tr>
<tr>
<td>$R_{b}$</td>
<td>—</td>
<td>0.7$^{(39)}$</td>
<td>0.6$^{(39)}$</td>
</tr>
<tr>
<td>$f_{i}$</td>
<td>—</td>
<td>0.026$^{(9)}$</td>
<td>0.003$^{(9)}$</td>
</tr>
</tbody>
</table>

$I_{\text{max}}$ represents the maximum concentration of the inhibitor in the systemic blood (the $C_{\text{max}}$ for RTV was obtained from our study). The $K_{i}$ and $F_{h}F_{g}$ for RTV was calculated using the $AUC_{\text{inf}}$ in this study and bioavailability$^{(40)}$. The maximum value was used as $F_{h}F_{g}$ for ITZ.
Table 4. Pharmacokinetic parameters of SQV after intravenous and oral administration of SQV with or without oral administration of RTV (50 mg/kg) in mice

<table>
<thead>
<tr>
<th></th>
<th>Oral</th>
<th>Intravenous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_{max}$</td>
<td>AUC_0–24</td>
</tr>
<tr>
<td>Control</td>
<td>65.6 ± 43.9</td>
<td>1,516.7 ± 608.0</td>
</tr>
<tr>
<td>with RTV</td>
<td>4,779.7 ± 1,151.2**</td>
<td>7,685.0 ± 3,127.7*</td>
</tr>
</tbody>
</table>

Values are mean ± SD ($n = 4–6$). *p < 0.05, **p < 0.01.

Fig. 3. Plasma MDZ concentration-time profiles after oral administration of MDZ with or without RTV

MDZ (10 mg/kg) was given orally after oral administration of RTV at a dose of 1.5 and 50 mg/kg. Blood samples were obtained at 5, 15, 30 min, 1, 2, 4, 6, 8, 10 (RTV 50 mg/kg only), 12, 18 and 24 h. Each point represents the mean ± SD ($n = 3$).

Fig. 4. Plasma FEX concentration-time profiles after oral administration of FEX with or without RTV

FEX (10 mg/kg) was given orally after oral administration of RTV at a dose of 1.5 and 50 mg/kg. Blood samples were obtained at 5, 15, 30 min, 1, 2, 4, 6, 8, 10 (RTV 50 mg/kg only), 12, 18 and 24 h. Each point represents the mean ± SD ($n = 3$).

Fig. 5. Plasma PRV concentration-time profiles after oral administration of PRV with or without RTV

PRV (100 mg/kg) was given orally after oral administration of RTV at a dose of 1.5 and 50 mg/kg. Blood samples were obtained at 5, 15, 30 min, 1, 2, 4, 6, 8, 12, 18 and 24 h. Each point represents the mean ± SD ($n = 3$).
increased five-, 13- and sevenfold, respectively, when they were coadministered with 50 mg/kg RTV.

Effect of ITZ on the pharmacokinetics of SQV: The inhibitory effect of 50 mg/kg ITZ on the pharmacokinetics of SQV after its oral administration was investigated. The plasma concentration-time profile and pharmacokinetic parameters are shown in Figure 6 and Table 8, respectively. There was no significant difference in the Cmax or AUCinf after treatment with or without 50 mg/kg ITZ and this result indicates that cyp isozymes other than cyp3a are involved in the metabolism of SQV in mice.

In vitro study: Before performing the inhibition study, the apparent Michaelis constant (Km) of SQV in the mouse liver was calculated and the concentration of substrate in the inhibition study was set below the Km value (data not shown). The same concentration was used in the inhibition study with mouse intestinal microsomes. Figures 7 and 8 show the Dixon plots for the inhibition by RTV of SQV or MDZ metabolism, respectively, in mouse liver and intestinal microsomes. The SQV and MDZ concentrations used were 0.25, 0.5, and 1 µM. The inhibition by RTV or ITZ was considered to be competitive or non-competitive. The Ki values for the inhibition of SQV metabolism by RTV were 95 and 75 nM in the liver and intestinal microsomes, respectively (*Table 9). The Ki value for the inhibition of MDZ metabolism was 65 nM in both the liver and intestinal microsomes. The inhibitory effect of ITZ on the metabolism of SQV was also investigated (Fig. 9). The Ki values were 3.7 and 2.6 µM in the liver and intestinal microsomes, respectively.

The fold increases in the AUC predicted from the in vitro study and their comparison with the values observed in the in vivo study are shown in Table 10. A fourfold increase in the AUC for SQV was predicted from the Ki value using Eq. (5), which is close to the value observed after the intravenous administration of SQV. In contrast, a ninefold increase in the AUC for SQV was predicted from the Ki value using Eq. (6). The remaining increase in the AUC for SQV derived from the inhibition of metabolic enzymes in the small intestine was estimated about 36-fold. This result indicates that the effect of RTV was greater in the small intestine than in the liver, which was consistent with the results obtained in the in vivo study.

Discussion

In this study, in vivo and in vitro experiments were performed in mice to clarify the underlying mechanisms of RTV boosting. Escalating doses of RTV had dose-dependent effects on the pharmacokinetics of SQV. There was a threefold increase in the AUCinf for SQV when administered with 1.5 mg/kg RTV, the clinically
relevant dose. In the clinical dose, it is reported that the increase in the AUC for SQV at SQV/RTV doses of 1,200/100 mg is six- to sevenfold.\(^2^{4,25}\) Therefore, our results indicate the effect of RTV boosting is similar in humans and in mice.

The contribution of 50 mg/kg RTV boosting to the first-pass effect on SQV in the small intestine and liver was accessed. As a result, \(F_a\) increased 38-fold, whereas \(F_h\) increased only twofold. These results suggest that RTV mainly influences the first-pass effect in the small intestine, resulting in an increase in the bioavailability of orally administered SQV.

Factors affecting the first-pass effect in the liver are metabolic enzymes and drug transporters. Among the metabolic enzymes in the liver, CYP3A is particularly important because it is responsible for the majority of phase I drug metabolism reaction, including that of PIs. CYP3A is also an important factor in the first-pass effect in the small intestine. In addition to the metabolic enzymes, the efflux transporters localized in the apical membrane are also important factors in the small intestine. Therefore, the inhibition of P-gp/

### Table 9. The kinetic parameters of SQV and MDZ inhibition by RTV and ITZ in mouse liver and intestinal microsomes

<table>
<thead>
<tr>
<th>Substrates</th>
<th>SQV (nM)</th>
<th>MDZ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver microsomes</td>
<td>95</td>
<td>65</td>
</tr>
<tr>
<td>Intestinal microsomes</td>
<td>75</td>
<td>65</td>
</tr>
</tbody>
</table>

Values are mean of \(n = 3\).

### Table 10. Comparison of the fold increase in the AUC between observed value and predicted value

<table>
<thead>
<tr>
<th>Substrates</th>
<th>SQV</th>
<th>MDZ</th>
<th>ITZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>325</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

Values are mean of \(n = 3\).

The dosage of each substrate and inhibitor are as follows. SQV: 2 mg/kg for intravenous administration and 20 mg/kg for oral administration, MDZ: 10 mg/kg, RTV and ITZ: 50 mg/kg.

CYP3A in the small intestine and the inhibition of CYP3A in the liver are considered to increase the oral bioavailability of SQV. However, regarding the probe substrate of CYP3A, the increase in the AUC\(_{\text{inf}}\) for MDZ after its oral administration with 50 mg/kg RTV was only fivefold. In addition, the increase in the AUC\(_{0.12}\) for SQV after its oral administration with ITZ was only twofold, and did not differ significantly from the control. Yamano et al. evaluated the extent of drug-drug interactions involving metabolic inhibition in the rat liver. In their report, the increase in the AUC for MDZ, in the presence of ITZ was about twofold, which is consistent with our result of the inhibition study for ITZ.

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MDZ is predominantly biotransformed to the 1'-hydroxy MDZ rather than to 4-hydroxy MDZ in both humans and mice.²⁶ In humans, CYP3A mediates its biotransformation to both 1'-hydroxy and 4-hydroxy MDZ. On the other hand, there is a report that cyp3a mediates only its biotransformation to 4-hydroxy MDZ in mice, and cyp isoforms other than cyp3a mediate its biotransformation to 1'-hydroxy MDZ.²⁷ Furthermore, van Waterschoot et al. even reported that MDZ metabolism was observed in liver microsomes prepared from cyp3a⁻/⁻ mice.²⁸ These data indicate a species difference in the MDZ metabolism in mice and humans and suggest that the cyp isoforms involved in the metabolism of SQV in mice differ from those involved in humans. Perloff et al. reported that the Ki values for the inhibition by ketoconazole of 1'-hydroxy and 4-hydroxy MDZ biont transformation were 1.7 and 0.066 µM, respectively, in mouse liver microsomes. In humans, these Ki values were 0.0054 and 0.039 µM, respectively.²⁹ These results show the Ki value for the inhibition by ketoconazole of 1'-hydroxy MDZ biotransformation in mice differed greatly from that in humans. Moreover, the Ki value for the inhibition by ketoconazole of 1'-hydroxy MDZ biotransformation was the same as our result for the inhibition by ITZ of SQV. In addition, Yamano et al. determined the Ki value for the inhibition of 1'-hydroxy biotransformation by ketoconazole and ITZ, and they found those were the same.³⁰ In our study, the inhibitory effect of RTV on the metabolism of SQV and MDZ was the same. Therefore, RTV and ITZ inhibit the cyp isoforms involved in the metabolism of SQV and MDZ, and these cyp isoforms may be the same or have the same affinity to RTV or ITZ. Komura and Iwaki reported that the cyp mRNA expression levels differ in the livers and small intestines of mice, and that cyp3a13 is predominantly expressed in the small intestine and is responsible for the first-pass metabolism of CYP3A substrates in the mouse small intestine.³¹ They also evaluated the relationships of the Ki values for the CYP3A4-mediated biotransformations of 13 drugs in intestinal and liver microsomes, and a good relationship was observed, although the mRNA expression levels differed. This observation supports our finding that the Ki values were the same for the liver and intestinal microsomes in the in vitro study.

When we considered a substrate of P-gp, there was no significant difference in the Cmax or AUCinf for FEX when it was coadministered with 1.5 mg/kg of RTV. In humans, a twofold increase in the AUCinf for FEX was observed when given with 100 mg of RTV,³² so there are also species differences in the effects of RTV on the pharmacokinetics of FEX. The oral bioavailability, Fe, Fh, and Fb of FEX in humans has been reported as 0.28, 0.31 and 0.09,³³ respectively. Therefore, it is estimated that the maximum increase in the AUC for FEX is about threefold when the inhibition by RTV is completed. In our study, the increase in the AUCinf for FEX in mice coadministered with 50 mg/kg RTV was 13-fold, which was higher than that estimated for humans. In addition, Fe, Fh, and Fb in rat were 0.014, 0.022 and 0.641,³⁴ respectively. The maximum increase in the AUC for FEX is estimated to be about 50-fold, with complete inhibition by RTV. Therefore, even 50 mg/kg RTV may not inhibit the first-pass effect in the small intestine and liver completely in mice. Tahara et al. reported that the increase in the AUCinf for FEX in Mdr1a/1b P-gp knockout mice was about sixfold.³⁵ Considering our result and the very small Fh values of FEX in mice, efflux transporters other than P-gp may be involved in the intestinal absorption of FEX in mice. Bcrp and Mrp2 are the efflux transporters expressed at the apical membrane in the mouse small intestine. It has been reported that FEX is not a substrate of BCRP in humans.³² Furthermore, Mrp2/3 are involved in the hepatic disposition of FEX in mice³²,³³ and MRP2 mediates the absorption of FEX in humans.³⁴,³⁵ These reports suggest that efflux transporters other than P-gp, such as Mrp2, might be involved in the absorption of FEX in mice and that the differences in the RTV-boosting effects on these transporters may result in the species differences observed in the pharmacokinetics of FEX.

An additional experiment was performed using mouse liver and intestinal microsomes to confirm the result obtained in the in vivo study. The fourfold increase in the AUC for SQV was predicted from the in vitro Ki value for intravenously administered SQV, which is consistent with the results of the in vivo study. This result indicates that the inhibitory effect of RTV in the liver is mainly attributable to the inhibition of metabolic enzymes with a minor contribution on the inhibition of influx transporters in the liver. On the other hand, a significant increase in the AUCinf for PRV was observed after the oral administration of 50 mg/kg RTV in the in vivo study. According to the package information for Karetroxa³⁶, which is a PI formed by the combination of lopinavir and RTV, there are no reports that Karetroxa³⁶ enhances the plasma concentration of PRV. However, it has been reported that the AUC for rosuvastatin, a substrate of OATP1B1, is increased twofold by its coadministration with Karetroxa³⁶,³⁷ In this report, they concluded this interaction might be mediated by the inhibition by lopinavir and/or ritonavir of rosuvastatin uptake at the level of absorption by BCRP or at the level of uptake into the hepatocytes by OATP1B1, by both, or by neither.

After the oral administration of SQV together with RTV, a ninefold increase in the AUC for SQV was predicted from the in vitro Ki value, and this is derived from the inhibition of metabolic enzymes in the liver. From this result, the remaining 36-fold increase in the AUC was considered to be derived from the inhibition of metabolic enzymes in the small intestine. This result corresponds to the result obtained in the in vivo study, and the inhibitory effect of RTV boosting is shown to be higher in the small intestine than in the liver.

In conclusion, RTV mainly affects the first-pass effect in the small intestine, increasing the bioavailability of orally administered SQV. The effects of RTV boosting on the pharmacokinetics of SQV are the same in humans and in mice. However, the cyp isoforms involved in the metabolism of SQV in mice differ from those in humans.

References


