Differentiation of Gut and Hepatic First Pass Metabolism and Secretion of Saquinavir in Ported Rabbits

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ABSTRACT
The current study was performed in intestinal and vascular access ported rabbits to quantify and differentiate the components of intestinal and hepatic first pass extraction (i.e., metabolism and secretion) of saquinavir (SQV) mediated by P-glycoprotein (P-gp) and CYP3A. SQV was administered i.v. (1–5 mg/kg) or into the upper small intestine (USI) (5 mg/kg). The roles of intestinal and hepatic secretion by means of P-gp and/or metabolism by CYP3A on the first pass gastrointestinal extraction of SQV were differentiated by using N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl]-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide (GF120918) (a P-gp inhibitor), midazolam (an inhibitor of CYP3A), or cyclosporine A (an inhibitor of P-gp and CYP3A). The bioavailability (BA) of SQV after USI dosing was 4%. In the presence of CYP3A and P-gp inhibitors, the BA of SQV increased 2- to 11-fold. Based on a relatively unchanged $C_{\text{max}}$ but prolonged $t_{\text{max}}$ and $t_{1/2}$, P-gp and CYP3A inhibition appeared to alter SQV disposition (i.e., enhanced oral bioavailability by diminishing SQV elimination and by increasing its net intestinal absorption). In conclusion, the current results substantiate the role of the liver and, for the first time, experimentally establish an important role for the intestine in the net absorption and disposition of SQV. The results also demonstrate that changes in SQV disposition due to the modulation of metabolism and secretion were important and may potentially have considerable implications on multiple drug therapeutic regimens used in the treatment of AIDS.

It has been widely hypothesized that intestinal apical secretion and/or CYP3A-mediated metabolism may profoundly affect a drug’s bioavailability (BA); however, the ability to directly assess the potential importance of these two intestinal processes in vivo has represented a major technological challenge with little success being demonstrated to date. P-glycoprotein is thought not only to affect the secretion/excretion of a variety of drugs in the small intestine, colon, brain, liver, kidney, and other parts of the body (Georges et al., 1990; Tsuji et al., 1992), but it can also affect the metabolism and disposition of drugs (Kim et al., 1999; Schuetz et al., 2000) since it is an important factor in controlling cellular drug concentrations and the residence time of drugs inside cells. Although the contribution of P-gp toward pharmacokinetics has not yet been clearly elucidated, the potential influence on the rate and extent of drug absorption may be profound. The impact of P-gp on drug disposition has been better characterized. For example, several drugs including ivermectin, digoxin, cyclosporine A, and vinblastine were shown to cross the blood brain barrier more significantly in P-gp knockout mice than in normal mice (Schinkel et al., 1995). Pharmacokinetic studies in P-gp knockout mice have demonstrated that mdr1a gene deficiency enhanced the oral BA of tacrolimus by 3-fold compared with normal mice (Yokogawa et al., 1999), although a clear functional effect of P-gp could not be established given the involvement of CYP3A-mediated intestinal and hepatic metabolism. CYP3A is a major phase I drug metabolizing enzyme that potentially

**ABBREVIATIONS:** BA, bioavailability; IVAP, intestinal and vascular access ported; SQV, saquinavir; HIV, human immunodeficiency virus; P-gp, P-glycoprotein; PV, portal vein; USI, upper small intestine; LC, liquid chromatography; MS/MS, tandem mass spectrometry; GF120918, N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl]-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide; SPE, solid phase extraction; $F_{\text{USI}}$, bioavailability after USI administration; AUC, area under plasma concentration time curve; $AUC_{0\rightarrow t}$, AUC to the last sampling time point; $F_{\text{abs}}$, the fraction that enters the intestinal cell; $F_{\text{gen}}$, the fraction that survives from gut wall elimination; $F_{\text{Fg}}$, fraction escaped from hepatic extraction; $E_{\text{Fg}}$, hepatic extraction ratio; $E_{\text{gw}}$, gut wall extraction ratio; PK, pharmacokinetic; CL, hepatic clearance; Fr, relative bioavailability; Fr(l), relative intestinal availability; SA, systemic artery; MDZ, midazolam; CsA, cyclosporine A; NZW, New Zealand White.
mediates the biotransformation of more than 50% of known drugs with its gene expression levels being 30% and 70% of total cytochromes P450 in human liver and intestine, respectively (Kolars et al., 1994; Shimada et al., 1994). In addition to the relatively high CYP3A gene expression levels in the intestine, mdr1a/1b knockout mice were shown to display a further increased expression of CYP enzymes to compensate for the loss of P-gp (Schuetz et al., 2000). In addition, increased expression of mdr1b was observed in mdr1a knockout mice, presumably to compensate for the loss of mdr1a (Schuetz et al., 2000). Therefore, the potential for underestimating the influence of intestinal P-gp in knockout models cannot be ruled out. In light of the above limitations with knockout models, the use of normal animal species may prove valuable for elucidating the effect of intestinal secretion and/or metabolism, in particular on the oral BA of drugs.

In the current study, an IVAP rabbit model was used to differentiate and quantify the contribution of intestinal and hepatic metabolism and secretion of drugs by measuring the amount of intact SQV appearing in the portal circulation (i.e., immediately after absorption through the intestine but before entering the liver) and comparing it with systemic (i.e., postliver) concentrations.

Saquinavir, among other HIV protease inhibitors, has been approved for use in the treatment of patients with AIDS. However, treatment of HIV with SQV is plagued by its low and highly variable oral BA (Williams and Sinko, 1999). The varied oral BA of SQV has been attributed in part to extensive CYP3A-mediated metabolism (Fitzsimmons and Collins, 1997) and P-gp-mediated secretion as demonstrated in Caco-2, MDR1-transfected LLC-PK1, and MDCKII/P-gp cells (Polli et al., 1999; Kim et al., 1998b; Williams et al., 2003). The rate and extent of intestinal P-gp- and/or CYP3A-mediated SQV loss, however, can only be assessed by in vivo studies since in vitro studies lack many of the physiological factors critical to intestinal or entero-hepatic recycling.

The present studies utilized an intestinal and vascular access rabbit model developed and validated by us previously (Kunta et al., 2001) that allowed for the direct instillation of SQV into the intestinal segment, avoiding the confounding influence of gastric emptying. Blood samples were taken from the portal vein (i.e., representing net intestinal absorption (i.e., immediately after absorption through the intestine but before entering the liver) and comparing it with systemic (i.e., postliver) concentrations.

Materials. Saquinavir [N-tert-etyl-decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarnbonyl)-1-asparaginy]l]aminobutyl]-[4aS,8aS]-quinoine-3(S)-carboxamide] (Ro 31–8959) and GF120918 were given as gifts by Roche Pharmaceuticals (Nutley, NJ) and GlaxoSmithKline (Uxbridge, Middlesex, UK), respectively. Cyclosporine A (cyclosporine inj., USP; 250 mg/5 ml dose; Bedford Laboratories, Bedford, OH), midazolam (MDZ) (versed; 5 mg/ml dose; Roche Pharmaceuticals) were purchased through the Rutgers University Health Center Pharmacy. In vivo study supplies, such as sterile saline, 50% dextrose, suture material, isoflurane, and heparin, were obtained through commercial sources. Cannulation supplies such as 22-gauge Huber needles and 24-gauge i.v. catheters were purchased from Instech Solomon (San Antonio, TX), and BD BioScience (San Jose, CA), respectively. Millex-GV filters (0.22 μm) that were used for sterilizing dosing solutions were purchased from Millipore Corporation (Bedford, MA). C2 Bond Elut SPE cartridges were obtained from Varian, Inc. (Palo Alto, CA). Other reagents and solvents used are the highest grade commercially available.

Animals. Twenty New Zealand White (NZW) female rabbits weighing 4 to 5 kg were used in accordance with the protocol (99-054) approved by the Institutional Review Board Use and Care of Animal Committee and housed in Association for Assessment and Accreditation of Laboratory Animal Care-accredited facilities at Rutgers University. All animals were individually housed in an environmentally controlled facility and allowed to acclimatize for several days before study. Food and water were provided ad libitum.

IVAP Port Cannulation and Maintenance. The surgical procedure for placement of portal vein (PV) and upper small intestine (USI) cannulas was described in detail elsewhere (Kunta et al., 2001). In brief, through a survival surgery, cannulas were placed into portal vein and upper small intestine (10 cm distal to the ligament of Treitz). The catheters were tunneled out and affixed to a titanium reservoir on the subcutaneous layer of the dorsal side of the animals. After surgery, the catheters in the PV and USI ports were flushed once a week with 1 to 2 ml of glucose D50 with and without heparin (2000 IU/ml, respectively). The ports were accessed through a transdermal injection into the titanium reservoir using a 22-gauge Huber needle. The procedure was conducted in a sterile manner to prevent infection.

Dosing and Sampling. On the day of the study, rabbits were restrained in a stainless steel restrainer. The skin surrounding the ports was shaved and sterilized with scrubbing povidone-iodine solution and alcohol. A 22-gauge Huber needle was placed in the PV port and capped with a standard heparin lock cap to facilitate blood sampling. Meanwhile, a 24-gauge i.v. infusion catheter was placed into an auricular artery (left ear in general) for systemic blood sampling. Both catheters were flushed with −1 ml sterile heparinized saline (50 IU/ml) at the beginning of the study and after each blood sampling to prevent the catheters from clotting.

Saquinavir dosing solution (5 mg/ml) in a mixture of ethanol/propylene glycol/water (20:30:50, v/v/v) (Kempf et al., 1997), was sterilized through a Millipore filter before use. Saquinavir dosing solution was injected into either marginal ear vein (10 μg/kg) or USI (5 mg/kg). For CYP3A and P-gp inhibition studies, GF120918 (15 mg/kg in the same dosing vehicle as for SQV), midazolam (1 mg/kg), and cyclosporine A (15 mg/kg) were administered into the USI port 10 min prior to saquinavir USI dosing (5 mg/kg). Each dose regimen was tested in three to five animals except for GF120918 (n = 2) due to the limited supply of the compound. Periodic blood samples (500 μl) were drawn at 0, 3, 5, 10, 20, 40, 60, 90, 120, 180, 270, and 360 min from systemic and/or PV and emptied into 2-ml Vacutainers. The blood samples were then centrifuged at 4°C for 10 min at 3000g. The plasma was decanted and transferred to clean centrifuge tubes and frozen at −80°C until analyzed by LC-MS/MS. Between studies, rabbits were allowed to recover for 1 to 2 weeks before being assigned to the next set of studies.

Plasma Sample Purification. Frozen plasma samples were thawed at room temperature. To 250 ml of plasma, 50 μl of internal standard (quinidine bisulfate; 50 μg/ml in methanol) and 650 μl of 0.1 M ammonium acetate, pH 6.5, were added. Calibration standards and quality control samples were prepared in the same manner. The diluted plasma samples were vortexed and centrifuged at 3000 rpm for 5 min to remove any precipitate. The supernatants were extracted by a solid phase extraction (SPE) technique. C2 Bond Elut SPE cartridges were conditioned with 1 ml of acetonitrile and 1 ml of ammonium acetate buffer (pH 6.5; 0.1 M). Plasma samples were loaded onto the SPE cartridges and washed with 1 ml of acetonitrile.
ammonium acetate buffer (pH 6.5, 0.1 M) mixture (3:7 v/v). Saquinavir was then eluted with 0.5 ml of acetonitrile-ammonium acetate (pH 6.5, 2.5 mM) mixture (8:2 v/v). The average recovery for quality control samples at 200 ng/ml was 91.5 ± 6.1%.

LC-MS/MS Analysis. SQV plasma concentrations were determined using LC-MS/MS analysis. The LC-MS/MS system consisted of a PerkinElmer LC-200 HPLC (PerkinElmer Life and Analytical Sciences, Boston, MA) and a PerkinElmer Sciex MS/MS model API III* mass analyzer (PerkinElmer Life and Analytical Sciences). On an Eclipse XDB-C8 column (2.1 × 50 mm, particle size 3.5 μm, Agilent Technologies, Palo Alto, CA), analytes were eluted by a mobile phase of acetonitrile and 2.5 mM ammonium acetate buffer (pH 6.5) at 8:2 (v/v) at a flow rate of 0.2 ml/min. MS/MS spectra were acquired by the electrospray ionization method with turbo ion spray at 350°C. The orifice potential and electron multiplier settings were +40 V and −2500 V, respectively. The dwell time was 200 ms. Collision energy for SQV and quinidine was 127 and 40 eV, respectively. Parent to fragment ions for SQV and quinidine were monitored at 671.4/128.17 and 325.2/183.8, respectively. The detection limit of the assay was 1 ng/ml.

Calculation of Bioavailability. The bioavailability of SQV after USI was defined as $F_{oral}$ and was calculated as the following:

$$ F_{oral} = \frac{AUC_{USI}}{AUC_{IV}} \times \frac{D_{IV}}{D_{USI}} \quad (1) $$

where area under plasma concentration time curve (AUC) and $D_{IV}$ and $D_{USI}$ are the AUCs of SQV after i.v. (IV) and USI administration and the doses through each dosing route. $F_{oral}$ is the product of the fraction of SQV absorbed from the gastrointestinal tract, $F_{abs}$, which is the fraction that enters the intestinal cell, $F_{gw}$, is the fraction that escaped degradation in the intestinal cell or secretion out of the cell back into the intestinal lumen, and $F_{H}$ is the fraction that escaped liver extraction:

$$ F_{oral} = F_{abs} \times F_{gw} \times F_{H} \quad (2) $$

Since $F_{abs}$ cannot be directly measured but the amount of SQV entering the PV can be measured, equation 2 can be redefined as:

$$ F_{oral} = F_{I} \times F_{H} \quad (3) $$

where $F_{I}$ (availability of SQV in the PV after intestinal extraction) is the product of $F_{abs} \times F_{gw}$ or:

$$ F_{oral} = (1 - E_{I}) \times (1 - E_{H}) \quad (4) $$

where $E_{I}$ and $E_{H}$ represent the intestinal and hepatic extraction ratios, respectively.

In the current study, plasma concentrations of SQV in the PV and systemic circulation were both measured. If the drug concentrations in PV are considered to be the same as the inlet drug concentrations to the liver ($C_{L}$) and systemic drug concentrations as outlet concentrations ($C_{V}$), $E_{H}$ can be calculated using the equation $E_{H} = (C_{L} - C_{V})/C_{A}$, where $C_{A}$ and $C_{V}$ are the steady-state drug concentrations (Rowland and Tozer, 1995). After integrating $C_{A}$ and $C_{V}$ over time, $E_{H}$ can be calculated from AUCs in PV (AUC$_{USI}$) in and systemic circulations (AUC$_{USI}$):  

$$ E_{H} = \frac{AUC_{USI} - AUC_{IV}}{AUC_{USI}} \quad (5) $$

By similar approach, $E_{gw}$ can be calculated through systemic and portal AUCs following an i.v. administration:

$$ E_{gw} = \frac{AUC_{gw} - AUC_{IV}}{AUC_{gw}} \quad (6) $$

Through $E_{H}$ and $E_{gw}$, $F_{abs}$ can be estimated:

$$ F_{abs} = \frac{F_{oral} - F_{gw}}{(1 - E_{gw})} \quad (7) $$

### Pharmacokinetic Analysis

Pharmacokinetic (PK) parameters were calculated using a noncompartmental analysis (WinNonlin Enterprise version 3.1; Pharsight, Mountain View, CA). $F_{oral}$ after USI administration of SQV alone was calculated using equation 1, where AUC$_{USI}$ was the AUC$_{oral}$ after i.v. dosing at 5 mg/kg. $F_{oral}$ was calculated based on the average AUC$_{oral}$ (AUC to the last sampling time point) for all USI dosing treatments using equation 5. Hepatic clearance (CL$_{H}$) was estimated as the product of $E_{H} \times Q_{H}$ (Rowland and Tozer, 1995), where $Q_{H}$ (the hepatic blood flow) in rabbits was reported as 2.66 l/h/kg (Boxenbaum, 1980). $E_{gw}$ was estimated using equation 6 and based on the AUC$_{oral}$ in the systemic and portal circulations after i.v. dosing at 1 mg/kg. From $F_{oral}$ ($F_{H} = 1 - E_{H}$), $F_{gw}$ ($F_{gw} = 1 - E_{gw}$), and $F_{oral}$, $F_{abs}$ was determined using equation 7. The relative bioavailability ($Fr$) of SQV after USI dosing in the presence of CYP3A and P-gp inhibitors was the ratio of AUCs in absence (SQV alone) and presence of the inhibitors ($Fr = AUC_{oral:inhibitor}/AUC_{oral:SQV}$). Relative intestinal availability ($Fr(I)$) under P-gp or CYP3A modulation was calculated as the portal AUC ratios of SQV in the presence or absence of inhibitors.

### Statistical Analysis

Results in this paper are reported as mean and standard error (S.E.M.) with three significant figures. To evaluate changes in the PK parameters for SQV under P-gp and CYP3A inhibition, the PK data obtained from USI administration of SQV alone were used as control. The statistical differences in PK parameters between the control and P-gp or CYP3A inhibitor coadministered groups were tested using either paired or unpaired Student’s t tests, depending on the animal involvement in the control and test groups. The statistical differences among test groups were also analyzed using analysis of variance coupled with the Student-Newman-Keuls Range test (Hicks and Turner, 1999). The significance level was defined as $p < 0.05$.

### Results

### Pharmacokinetics of Saquinavir

The PK parameters and plasma concentration time profiles of SQV after i.v. and USI dosing with systemic artery (SA) and PV sampling are summarized in Tables 1 to 3 and Figs. 1 and 2. Following i.v. administration at 1, 3, and 5 mg/kg, SQV plasma concentrations were dose-dependent, and the AUCs increased proportionally with dose, indicating that SQV PKs were linear in this dose range. SQV was eliminated from plasma with a terminal half-life of approximately 2 to 3 h and a mean residence time ranging from 0.7 to 2.3 h. Its large volumes of distribution during the terminal phase and at steady state indicate that SQV was extensively distributed in the peripheral tissues. Its total clearance, similar to the rabbit’s hepatic blood flow rate (2.66 l/h/kg), demonstrates that SQV is a high extraction ratio drug. Based on the AUC data at 1 mg/kg (i.v.), $E_{gw}$ was found to be $0.391 \pm 0.089$ (mean ± S.E.M.), revealing that intestinal metabolism plays a significant role in SQV disposition.

Following USI administration, SQV plasma concentrations were much lower than those after i.v. administration, demonstrating a significant loss during the first pass through the intestine and liver. $F_{oral}$ after USI dosing was found to be 4% of the dose, confirming that SQV has very limited oral bioavailability in rabbits. $F_{oral}$ values after USI dosing were found to be 56% (using equation 5) and $F_{oral} = 1 - E_{H}$, revealing that the liver is a major degradation site for SQV. From the values of $F_{oral}$ (4%), $F_{oral} = 56\%$, and $F_{gw}$ (61%), $F_{abs}$ (1 - $E_{gw}$), the fraction absorbed ($F_{abs}$) from the gastrointestinal tract fol-
phases paralleled each other. Those in the systemic circulation, and their elimination of CYP3A or P-gp inhibitors

TABLE 2
Pharmacokinetic parameters of saquinavir in New Zealand White female rabbits after USI administration at 5 mg/kg in the absence or presence of CYP3A or P-gp inhibitors

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>SQV + GF</th>
<th>SQV + MDZ</th>
<th>SQV + CsA</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_{\text{max}} ) (min)</td>
<td>9.00 ± 3.92</td>
<td>17.5 ± 2.5</td>
<td>5.33 ± 2.33</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng/ml)</td>
<td>218 ± 67</td>
<td>220 ± 25</td>
<td>141 ± 57</td>
</tr>
<tr>
<td>( k_r ) (min)</td>
<td>0.184 ± 0.040</td>
<td>0.07030 ± 0.0028</td>
<td>2.59 ± 1.30</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{Cmax}} ) (ng·h/ml)</td>
<td>134 ± 57</td>
<td>126 ± 24</td>
<td>227 ± 160</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{Cmax}} ) (ng·h/ml)</td>
<td>143 ± 62</td>
<td>150 ± 46</td>
<td>360 ± 282</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>1.83 ± 0.69</td>
<td>3.42 ± 3.01</td>
<td>4.25 ± 1.72</td>
</tr>
<tr>
<td>( F_r )</td>
<td>0.0809 ± 0.0202</td>
<td>2.52 ± 1.97</td>
<td>5.36 ± 3.37</td>
</tr>
</tbody>
</table>

\( F_r = \frac{\text{Relative intestinal availability}}{\text{PV AUC}_{\text{SQV}} + \text{inhibitor}} \frac{\text{PV AUC}_{\text{SQV}}}{\text{AUC}_{\text{SQV}}}. \)

\( p < 0.05 \) between groups.

TABLE 3
Pharmacokinetic parameters of saquinavir in New Zealand White female rabbits after USI administration at 5 mg/kg in the absence or presence of CYP3A or P-gp inhibitors

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>SQV + GF</th>
<th>SQV + MDZ</th>
<th>SQV + CsA</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_{\text{max}} ) (min)</td>
<td>7.00 ± 1.22</td>
<td>17.5 ± 2.5</td>
<td>8.33 ± 1.67</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng/ml)</td>
<td>91.8 ± 30.2</td>
<td>93.8 ± 28.7</td>
<td>58.6 ± 23.4</td>
</tr>
<tr>
<td>( k_r ) (min)</td>
<td>0.164 ± 0.028</td>
<td>0.0876 ± 0.0192</td>
<td>0.365 ± 0.100</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{Cmax}} ) (ng·h/ml)</td>
<td>64.1 ± 16.9</td>
<td>76.6 ± 20.2</td>
<td>123 ± 84</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{Cmax}} ) (ng·h/ml)</td>
<td>72.7 ± 18.4</td>
<td>132 ± 15</td>
<td>406 ± 359</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>2.22 ± 0.34</td>
<td>16.7 ± 1.7</td>
<td>5.68 ± 3.4</td>
</tr>
<tr>
<td>( CL/F ) (l/h/kg)</td>
<td>88.9 ± 20.6</td>
<td>38.4 ± 4.4</td>
<td>103 ± 0</td>
</tr>
<tr>
<td>( V/F ) (l/kg)</td>
<td>269 ± 70</td>
<td>606 ± 164</td>
<td>307 ± 134</td>
</tr>
<tr>
<td>( E_{H0} )</td>
<td>0.445 ± 0.130</td>
<td>0.404 ± 0.048</td>
<td>0.326 ± 0.165</td>
</tr>
<tr>
<td>( CL_{\text{NH}} ) (l/h/kg)</td>
<td>0.948 ± 0.382</td>
<td>1.08 ± 0.13</td>
<td>0.869 ± 0.438</td>
</tr>
<tr>
<td>( F_{\text{oral}} )</td>
<td>0.0408</td>
<td>1.05 ± 0.32</td>
<td>2.52 ± 1.97</td>
</tr>
<tr>
<td>( F_r )</td>
<td>1.00</td>
<td>1.82 ± 0.21</td>
<td>5.58 ± 4.95</td>
</tr>
</tbody>
</table>

\( \text{AUC}_{\text{Cmax}} \) at a 5-mg/kg intravenous dose. \( F_r = \frac{\text{PV AUC}_{\text{SQV}}}{\text{AUC}_{\text{SQV}}}. \)

\( \text{inhibitor} = \text{PV AUC}_{\text{SQV}}. \)

\( p < 0.05 \) in comparison with the control.

\( ** p < 0.05 \) between groups.

lowing USI administration was approximately 12% of the dose. Since, as demonstrated in many in vitro studies, SQV is a slow permeability drug (Kim et al., 1998b; Polli et al., 1999), and it was dosed as a solution, the limited oral absorption of SQV in rabbits appears to be controlled mainly by its intestinal permeability rather than its solubility.

**SQV Pharmacokinetics in the Presence of CYP3A and P-gp Inhibitors.** Plasma concentration time profiles of SQV coadministered with GF120918, MDZ, and cyclosporine A (CsA) are shown in Figs. 2 to 4. All drugs and modulators were dosed as solutions. The PK parameters are listed in Tables 2 and 3. In general, SQV concentrations in the portal circulation following USI administration were higher than those in the systemic circulation, and their elimination phases paralleled each other.

In the presence of GF120918, SQV plasma concentrations in both the portal and systemic circulation increased slightly; however, the elimination \( t_{1/2} \) was significantly prolonged. Coadministration of GF120918 resulted in an increase of SQV oral bioavailability by nearly 2-fold. This was due primarily to the prolonged plasma concentrations, particularly 3 h after the study began. The relatively unchanged E\(_{\text{H0}}\) suggests that P-gp inhibition had little effect on SQV hepatic metabolism, whereas the relatively unchanged Fr(I) indicates that P-gp inhibition also had a limited effect in increasing the extent of SQV absorption from the gastrointestinal tract. This could be due to many factors that cannot be teased out from the currently used animal model. From the prolonged \( T_{\text{max}} \) and elimination half-life and relatively unchanged AUC, P-gp inhibition appears to have a significant
effect on SQV disposition. It is important to keep in mind that intestinal secretion and reabsorption, even at later time points when absorption is generally thought to be complete, would be reflected in the "elimination" phase of the plasma level versus time curve and would have the effect of prolonging the "elimination" half-life by decreasing the slope of the elimination phase. This is discussed further under Discussion.

Coadministration of MDZ resulted in lowered SQV C_{max} values (to nearly one-half of those in controls) and a prolonged elimination t_{1/2} in the portal and systemic circulation. In the presence of MDZ, the oral bioavailability of SQV increased significantly. Decreased E_{H} and CL_{H} data demonstrate that SQV metabolism in the liver was inhibited by MDZ. Increased Fr(I) indicates that the fraction absorbed through intestine was increased via inhibition of intestinal CYP3A as well. These results reveal that CYP3A activity was compromised in both the liver and intestine in rabbits when SQV was coadministered with MDZ.

From plasma concentration profiles, CsA coadministration appears to have little impact on the initial absorption of SQV and led to a slow elimination of SQV from plasma. This resulted in a plateau concentration at ~100 ng/ml to the last sampling time point and to a 10-fold increase in SQV oral bioavailability in rabbits. Significantly increased Fr(I), E_{H}, and CL_{H} demonstrate that the functions of responsible enzymes and transporters in both the intestine and liver were significantly inhibited.

Based on these study results, it appears that the intestinal metabolism and secretion of SQV is significant but less than that in the liver. The rank order for Fr and Fr(I) in the presence of the various inhibitors is CsA > MDZ > GF120918, confirming the numerous published hypotheses suggesting a synergistic effect between the actions of CYP3A and/or P-gp on the oral bioavailability of drugs (Wu et al., 1995). However, there are a number of possible reasons why P-gp inhibition alone did not increase net intestinal absorption. These are discussed in the next section.

Discussion

Numerous factors contribute to the low and erratic oral bioavailability of drugs such as low aqueous solubility, poor dissolution properties, and poor apparent permeability due to intrinsically low absorptive membrane permeability. Extensive CYP3A-mediated metabolism in the liver, and, to some extent, in intestinal tissues is also considered a significant factor (Wu et al., 1995). More recently, secretory membrane transporters (e.g., P-gp and MRP2) also have been implicated in controlling the oral bioavailability and variability of drug absorption. The roles of CYP3A and P-gp on the metabolism and transport of SQV and other HIV protease inhibitors have been demonstrated in vitro and in vivo (Fitzsimmons and Collins, 1997; Alsenz et al., 1998; Kim et al., 1998a). However, a common experimental limitation is that the contributions of the liver and intestine cannot be directly assessed. Therefore, previous studies did not address the importance of
intestinal P-gp or CYP3A on the oral absorption and elimination of SQV. The current study was designed to directly assess and differentiate the roles of the intestine and liver with respect to metabolism and secretion by coadministering SQV with specific P-gp and CYP3A inhibitors in IVAP rabbits.

The in vivo delineation and interpretation of intestinal and entero-hepatic drug secretion is a challenging task given that secretion simultaneously involves an input process (i.e., absorption) and an output process (i.e., elimination elements of disposition). Although the IVAP rabbit model allows for the macroscopic separation of intestinal and hepatic processes, it is somewhat limited since it cannot separate out processes at the cellular membrane level. However, as more specific inhibitors and better knockout models become available, in vivo investigations at the membrane domain level will be feasible. Another confounding aspect to in vivo studies is the commonality of metabolism and secretion processes in the various organs. In other words, the same enzymes and membrane transporters are present in the intestine, liver, and many other organ systems. Another aspect that the results of the current studies have highlighted is the calculation and interpretation of bioavailability and how it relates not only to just drug absorption but also to disposition. Every time concentration (x, y) point on a plasma level versus time curve represents the net drug concentration resulting from the competing processes of absorption and elimination. The difference in these rates determines the slope and the process that is dominating at that point in time. Therefore, changes in the terminal phase slope maybe due a prolonged secretion-reabsorption process resulting in an apparent prolonged half-life of elimination. It is typically assumed that “absorption” is nearly complete at the time point T_{\text{max}}, C_{\text{max}} (i.e., where the net rate of absorption = net rate of elimination). However, the current studies clearly suggest that changes in metabolism, which relate solely to disposition, and secretion, which relate to absorption and disposition, affect overall disposition and confound the interpretation of in vivo results especially during the terminal disposition phase where absorption is considered minimal. This underappreciated concept and its confounding effect on data interpretation must be strongly considered when planning and analyzing results from any in vivo study that modulates membrane transporter and/or metabolizing enzyme activity.

Three putative transporter/enzyme drug modulators of SQV absorption and disposition were used in this study. The first was GF120918, an acridine carboxamide derivative, which is a potent inhibitor of P-gp activity and has been shown to fully reverse multidrug resistance at concentrations as low as 0.05 to 0.1 \mu M in the CHRC5 cell line (Hyafil et al., 1993). A definitive mechanism of action of GF120918, however, is still not clear, although it has been previously demonstrated to reverse multidrug resistance by modulating P-gp transport rather than acting as a competitive substrate (Martin et al., 1999). GF120918 probably not only interacts with P-gp at a site distinct from the substrate-binding site but also reduces the number of binding sites on P-gp, possibly through a negative allosteric interaction (Martin et al., 2000). Coadministration of GF120918 led to a significant improvement in SQV bioavailability (by nearly 2-fold) in IVAP rabbits. This result is consistent with an earlier report where the in vitro absorptive transport of SQV across Caco-2 cell monolayers was enhanced 4-fold in the presence of GF120918 (Pulli et al., 1999). A significantly prolonged elimination t_{1/2} of SQV can be attributed to P-gp inhibition by GF120918 since it was observed that P-gp inhibition can cause a significant decrease in fecal drug excretion. The increase in fecal drug excretion is likely due to the inhibition of intestinal and/or hepatic secretory transporters responsible for intestinal and entero-hepatic recycling, respectively. For example, it was observed that the fecal excretion of paclitaxel decreased approximately 40-fold in mdr1a (-/-) knockout mice as compared with wild-type mice (Sparreboom et al., 1997). It was not possible to differentiate intestinal versus entero-hepatic recycling effects due to the animal model used in those studies.

The minimal inhibitory effect of GF120918 on hepatic extraction of SQV may be due to many factors, including the limited dose that was used in the current studies. In other words, it may have not been sufficient to completely inhibit P-gp in the canalicular membranes of hepatocytes compared with higher doses used in other preclinical and clinical studies (Letrent et al., 1998; Malingre et al., 2001). However, a broad-range P-gp inhibition in other parts of the body including the brain cannot be ruled out, resulting in sequestration of SQV in the brain, cerebrospinal fluid, and other organs or tissues. Apart from P-gp, it is important to realize that GF120918 may also modulate cMOAT/MRP2 and breast cancer resistance protein (Maliepaard et al., 2001). The affinity and the extent of MRP2 and breast cancer resistance protein modulation by GF120918 are unclear, however, Given the high inhibitory potency of GF120918, a more likely rationale for the limited observed effect was that, due to recycling, the hepatic and/or intestinal extraction of SQV might be difficult to assess. In other words, “extraction” is classically used to indicate removal of parent drug from a biological system. If parent drug can be recycled a number of times, it is not truly “extracted.” In this case, the classic interpretation of extraction ratios must be reexamined. Increasing the number of absorption-secretion cycles may result in increased exposure of the drug to metabolizing enzymes such as CYP3A as suggested by Benet (Benet et al., 2003). On the other hand, it is also possible that by inhibiting P-gp activity, the residence time of a drug inside a cell may increase, resulting in a higher degree of metabolism by CYP3A. Therefore, the effects of P-gp inhibition in the presence of CYP3A may be difficult to discern due to the complexity of the effect at the molecular/cellular level and the inability of the IVAP model to examine events at that level. Despite the complexity of interpreting the current in vivo data, it is clear that fundamental changes in drug disposition (secretion and elimination) were observed in the presence of the various modulators. The effect of CYP3A-mediated metabolism on the oral bioavailability of SQV was determined in the presence of MDZ, a classical CYP3A inhibitor. The current studies reveal a significant interaction between SQV and MDZ. In the presence of MDZ, SQV bioavailability increased more than 5-fold, whereas the hepatic clearance decreased only slightly. Although these results suggest that MDZ increases SQV bioavailability by inhibiting both hepatic and intestinal CYP3A, the current results directly show, for the first time, that intestinal CYP-mediated metabolism is significant. Previous attempts to assess the extent of CYP-mediated intestinal metabolism relied on measuring systemic concentrations and...
interpretation of the data using pharmacokinetic models to ascertain the hepatic and intestinal components. The mean plasma concentration of SQV was significantly higher ($p < 0.05$) than control studies and remained so for the duration of study (6 h). The pharmacokinetic interaction between SQV and MDZ can be hypothesized to be competitive in nature with MDZ exhibiting a higher affinity to CYP3A. This is based on the argument that SQV, being a substrate for P-gp, may undergo repeated secretion and, as a consequence, has a higher probability of being exposed to CYP3A. The competitive inhibition of CYP3A may also depend on the duration and the dose of “inhibitor” administered. For instance, in a clinical study, SQV (1200 mg; fortovase administered three times a day for 5 days, followed by oral administration of 7.5 mg of MDZ on day 3) increased oral bioavailability of MDZ from 41% to 90% in healthy volunteers (Palkama et al., 1999). In yet another clinical report, SQV dosed at 600 mg three times a day when added to the treatment regimen prolonged the sedative effect of MDZ (Merry et al., 1997). In the current study, the oral absorption of SQV was shown to be rapid with peak plasma concentrations achieved within 10 min. The peak systemic concentration of MDZ when administered orally in 20 healthy volunteers was reported to be between 15 and 60 min (Thummel et al., 1996). In our studies, MDZ was dosed 10 min before SQV administration to allow maximal interaction with CYP3A. Moreover, the slower absorption rate of MDZ relative to SQV and the appreciable absorption of MDZ at the time of SQV administration may have resulted in a sustained and prolonged inhibition of the metabolism of SQV in the gut and in the liver.

CsA has been shown to alter P-gp (Kwei et al., 1999) and CYP3A (Whalen et al., 1999) activity. Other than CYP3A4, SQV was shown to exhibit minimal or no interaction with human CYP enzymes including CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP2E1 (Fitzsimmons and Collins, 1997) when studied for its oxidative metabolism in the presence of inhibitors of the various CYP isoforms in vitro. A few reports suggest that cyclosporine A modulates intestinal P-gp more than CYP3A (Lown et al., 1997; Edwards et al., 1999), whereas other reports maintain that CYP3A plays a major role in the intestinal extraction of CsA (Wu et al., 1995). However, the current belief is that CsA inhibits both P-gp- and CYP3A-mediated extraction of drugs in the intestine and liver, although to varied extents. In a clinical setting, 75 mg of CsA administered twice daily with SQV (600 mg twice daily) enhanced SQV systemic AUC by 4.3-fold (Brinkman et al., 1998). In the current study, coadministration of CsA remarkably increased SQV BA in rabbits more than 10 fold. The disposition profiles of SQV were characterized as slightly increased $C_{\text{max}}$ but significantly delayed $T_{\text{max}}$ and dramatically prolonged elimination $t_{1/2}$. Since up to 20 min postdose SQV concentrations in portal circulation were similar to those without coadministration of CsA (Fig. 4), these findings indicate that CsA increases SQV BA mainly by decreasing SQV clearance rather than by increasing its intestinal absorption. Because CsA is a potent inhibitor for both P-gp and CYP3A, its modulation effect on SQV BA was much more profound than that of GF120918 or MDZ alone, resulting in the lowest clearance parameters ($E_{\text{IC50}}, E_{\text{CL/F}},$ and $E_{\text{CL/IT}}$) and the highest BA parameters [Fr, Fr(I), $C_{\text{max}}$, and AUCs]. Our results demonstrate that the functions of P-gp and CYP3A are synergistic in regulating SQV BA in vivo.

In conclusion, the current results demonstrate that the intestine and liver both play an important role in the net absorption and disposition of SQV. The IVAP model was successful in delineating the roles of the liver and intestine in a direct and quantitative manner. The results also suggest that P-gp and CYP3A alter the disposition properties of SQV. However, due to the complexity of the in vivo model, the role of P-gp and other putative secretory efflux transporters is not yet clear and needs to be further elucidated. Comparatively, the effect of CYP3A-mediated metabolism of SQV was more prominent than P-gp-mediated secretion on altering the apparent pharmacokinetics of SQV. These results point out the complexity of investigating secretion processes in vivo and the difficulty in interpreting in vivo results. However, repeated recycling of certain drugs by P-gp and other secretory transporters may affect their oral bioavailability in a positive or negative way depending on the importance of metabolism for any given drug. In addition, it is important to realize that the modulation of P-gp will affect transporter activity in other tissues and may result in unanticipated changes in drug disposition such as enhanced uptake and sequestration of SQV into the brain that ultimately can be misinterpreted as a reduction in bioavailability as measured in the systemic circulation.

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