A Pharmacokinetic Model for Evaluating the Impact of Hepatic and Intestinal First-Pass Loss of Saquinavir in the Rat

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ABSTRACT:
The aim of this study was to quantify the intestinal and hepatic first-pass loss of saquinavir and to assess the effect of coadministration of ritonavir on this first-pass loss. Single doses of 12, 24, and 48 mg of saquinavir and a dose of 24 mg of saquinavir/6 mg of ritonavir were orally, intravenously, or intraperitoneally administered to 94 rats. Ten groups of animals were studied. A semiphysiological pharmacokinetic model incorporating a population pharmacokinetic analysis [nonlinear mixed-effects model (NONMEM)] was developed to analyze plasma concentration-time profiles after administration via each of the three above-mentioned routes. This model confirmed that saturable metabolism in hepatocytes and enterocytes and dose-dependent precipitation in the peritoneal cavity after intraperitoneal administration characterize the pharmacokinetics of SQV. It also demonstrated that low oral bioavailability of saquinavir is due mainly to intestinal rather than hepatic first-pass metabolism. In addition, it was shown that ritonavir diminished saquinavir clearance through competitive inhibition. The present report presents a new pharmacokinetic model applied in rats to evaluate the impact of hepatic and intestinal first-pass loss on oral bioavailability.

Introduction
Saquinavir (SQV) and ritonavir (RTV) are two protease inhibitors widely used in the management of AIDS and are characterized by variable oral bioavailability, which results in an inconsistent therapeutic response (Guiard-Schmid et al., 2003; Plosker and Scott, 2003). Both drugs have been criticized in recent years because of their high pill burden and side effects, especially in patients receiving long-term therapy, although they continue to be an essential component of highly active antiretroviral therapy. SQV exhibits low oral bioavailability that increases when it is coadministered with RTV, as both are substrates of the metabolic enzymes cytochrome P450 isoform 3A4 (CYP3A4), glycoprotein P, and multidrug-resistant protein (Hsu et al., 2004). Other isoenzymes known to be involved to a lesser extent are CYP2A6, CYP2C9, and CYP4A1 (Williams and Sinko, 1999). SQV has also been described as a weak inhibitor of CYP3A4 and a substrate and inhibitor of P-glycoprotein (Eagling et al., 1997).

Until now, there has been lack of consensus regarding the roles of the liver and intestine in SQV first-pass metabolism. It has been postulated that, in humans, a first-pass gut metabolism was the principal cause of the low bioavailability of SQV (Devine et al., 1997; Lown et al., 1997; Kupferschmidt et al., 1998). However, Tam-Zaman et al. (2004) have demonstrated that the gut wall does not play an important role in the low bioavailability of SQV in dogs. Therefore, the primary aim of this study was to assess the nonlinear processes of SQV pharmacokinetics in rats, because this animal model is commonly used in pharmacokinetic studies. We assessed the nonlinear processes of SQV pharmacokinetics by administering different doses of SQV via intravenous, intraperitoneal, or oral routes. Gut absorption and hepatic and gut first-pass metabolism of SQV were evaluated to throw light on the mechanism responsible for low SQV bioavailability. The secondary aim was to study the interaction of SQV and RTV when coadministered by intravenous and oral routes. For this purpose, a semiphysiological pharmacokinetic model was developed for the administration of SQV alone or in combination with RTV.

Materials and Methods

Animals and Surgery Preparation. All the assays described in the present study adhere to the Principles of Animal Care and were approved by the Institutional ethics committee of the University of Valencia (Spain) according to RD 1201/2005. Male Wistar rats weighing between 260 and

ABBREVIATIONS: SQV, saquinavir; RTV, ritonavir; NONMEM, nonlinear mixed-effects model; IIV, interindividual variability; MOFV, minimum value of objective function; QMA, maximum amount of drug that binds to proteins; F_E, the fraction of dose escaping from the intestine; F_C, the fraction escaping from the liver; Cl_int, clearance of intestinal secretion.
300 g were subjected to jugular vein cannulation using a previously described technique (Lledo´-García et al., 2007).

**Drug Administration and Blood Sample Collection.** SQV was obtained from F. Hoffman-La Roche Ltd. (Welwyn Garden City, Hertfordshire, UK), and RTV was obtained from Abbott Laboratories (Chicago, IL). A total of 94 animals and 915 plasma samples were used in the study. Solutions were prepared by dissolving the corresponding amount of drug in 1 ml of a mixture of cosolvents containing saline solution, propylene glycol, and ethanol in proportions of 25:25:50. The study design is shown in Table 1.

**SQV Administration.** Rats were randomly allocated into eight groups according to the administration route to be used (intravenous, oral, and intraperitoneal).

- **Intravenous infusion.** The 48-mg dose was administered via the jugular cannula with the aid of a volumetric pump IVAC (30-min infusion). The cannula was immediately injected with 0.4 ml of heparinized saline solution (20 IU/ml) to draw out any remaining traces of the drug. The exteriorized cannula was then replaced with a new one to avoid possible contaminations when sampling.
- **Intravenous bolus.** Doses of 24 and 12 mg were administered via the jugular cannula. After administration, the above-mentioned procedure was repeated.
- **Intraperitoneal administration.** Doses of 24 and 12 mg were injected 2 cm above the genital area while animals were held in a supine position.

**Oral administration.** Doses of 48, 24, and 12 mg were administered by gastric intubation.

**SQV and RTV Administration.** Rats were randomly allocated into two groups that received a 24- and 6-mg dose of SQV and RTV, respectively, by either intravenous infusion (30-min infusion) or orally.

After intravenous administration, samples were collected at 8 to 10 h, which was considered to represent the terminal phase, because previous studies have estimated the half-life ($t_{1/2}$) of SQV to be 1.6 h in rats (Shibata et al., 2003). However, after intraperitoneal and oral administration, profiles showed that the initial protocol sampling times (up to 8–10 h) had not adequately captured the terminal phase and were therefore modified. Because longer sampling periods were necessary for accurately determining the terminal phase of the curves after intraperitoneal and oral administration, each group was divided into two subgroups from which samples were drawn for the first 12 h after administration from one and during the second 12-h period from the other.

Blood samples (0.2 ml) were drawn from the jugular vein cannula with heparinized syringes at a scheduled time point. After each sample was drawn, the blood volume was replaced with the same volume of a saline solution. The number of samples processed within a 24-h period to obtain the curve of the plasma level of SQV was never higher than 11. The animals' weight and hematocrit were recorded; losses in hematocrit during the assay period were lower than 19%, and thus pharmacokinetic parameters were not considered to be affected. After collection, each blood sample was centrifuged at 5000 rpm for 5 min, and the plasma was transferred to an unused polypropylene tube and stored at $-30^\circ$C until it was assayed for SQV content.

**Analytical Procedures.** SQV plasma levels were measured by reverse-phase high-performance liquid chromatography with UV detection (235 nm), which provided adequate separation and quantification of the drug. SQV content was extracted from the plasma with organic solvents, as described previously for RTV (Lledo´-García et al., 2007). The mobile phase consisted of a combination of acetonitrile and an aqueous solution of phosphate buffer (99% bidistilled water and 1% 1/15 M phosphate buffer, pH 6.9, 57:43, v/v). A flow rate of 1 ml/min was used.

Calibration curves covering the whole range of SQV concentrations in the plasma samples were prepared and assayed in triplicate on each day of analysis. The peak area of SQV was measured in each sample and correlated with SQV concentration. Excellent linear plots of the peak areas and SQV concentrations were obtained ($r >$)
0.999). Accuracy and precision were evaluated by calculating the relative error and coefficient of variation, which were always less than 14.46 and 8.4%, respectively. The limit of quantification was 0.051 μg/ml. The results obtained were considered to be completely reliable (Karnes and March, 1993).

**Pharmacokinetic Calculations and Statistical Analysis.** A stepwise population pharmacokinetic approach was followed by using a nonlinear mixed-effects model and the first-order estimation method, implemented with a nonlinear mixed-effects model (NONMEM, version VI) (Beal and Sheiner, 1989) in conjunction with a G77 FORTRAN compiler and Wings for NONMEM (http://wfn.sourceforge.net). Different subroutines were used, namely, ADVAN 3, 11, and 9. The experimental data used to build the model was total plasma concentration of SQV.

The model was developed sequentially, so that whenever modifications had to be made to the base structure of the model, a backward procedure was performed. Data were also incorporated in a sequential fashion. The analysis consisted of the following steps:

- **Step 1:** SQV intravenous data from groups 1, 2, 3, and 9.
- **Step 2:** SQV intravenous data from groups 1, 2, 3, and 9 and intraperitoneal data from groups 7 and 8.
- **Step 3:** SQV intravenous data from groups 1, 2, 3, and 9 and intraperitoneal data from groups 7 and 8 and oral data from groups 4, 5, and 6.
- **Step 4:** SQV intravenous data from groups 1, 2, 3, and 9 and intraperitoneal data from groups 7 and 8 and oral data from groups 4, 5, 6, and 10.

As a first approximation to the data, a more empirical analysis was performed at each step using a linear pharmacokinetic model, which allowed different parameter values to be estimated for each of the groups assayed. Based on the information obtained, a more mechanistic analysis was subsequently carried out. The processes involved in the pharmacokinetic profiles were described in physiological terms; namely, expressions of flow, clearances, and volumes. Physiological parameters were fixed according to data in the literature. In the last stages of the model’s development (steps 3 and 4), the values for disposition and intraperitoneal absorption were fixed at those obtained in step 2, because the addition of oral data did not affect them.

The differences between individual parameters were regarded as random and were modeled in terms of η variables. η variables were assumed to be normally distributed with a mean of zero and to have an estimated variance of η^2. The need to include interindividual variability (IIV) terms was evaluated for all parameters. The differences between the observed plasma concentrations and the predicted plasma concentrations were regarded as random and were modeled in terms of ε variables. Each ε variable was assumed to have a mean of zero and an estimated variance σ^2. A slope-intercept error model was used, in which the residual variability (σ) of the exponential term was interpreted as a coefficient of variation and the added component as a S.D.

In the selection of the best model, the minimum value of objective function (MOVF) provided by NONMEM was adopted. For hierarchical models, the difference between objective function values is distributed as χ^2, which allows the best model to be selected. A p level of 0.005 was chosen as the criterion for accepting a more complex model over a reduced one. The difference between the objective function values of hierarchical models varying by one or two parameters was 7.879 and 10.596, respectively. The precision of the estimation of the parameter values, quantified as the relative S.E. (percentage), was also evaluated, and a graphical goodness-of-fit analysis was performed using S-Plus for Windows (version 7.0; Insightful Corporation, Seattle, WA).

**Results**

The SQV total plasma concentration-time profiles obtained after intravenous, intraperitoneal, and oral administration are shown in Fig. 1. The experimental data used in the development of the model are described in Table 2.

**Table 2**

| Parameters considered in the model (number of parameters estimated in the step), random variables estimated (IIV), and MOVF are shown. Models selected appear in boldface. |
|---|---|---|---|
| Step 1: intravenous data | No. Parameters | No. IIV* | MOVF |
| 1 | Two compartments; MM elimination | 5 | 3 | 604.9 |
| 2 | Two compartments; MM elimination + K_m different G9 | 6 | 3 | 543.5 |
| 3 | Three compartments; MM elimination + K_m different G9 | 8 | 4 | 514.9 |
| 4 | Two compartments; plasma protein dynamic binding + MM elimination (K_m different G9) | 9 | 6 | 483.5 |
| Step 2: intraperitoneal data added | | | |
| 5 | K_m and F different for each intraperitoneal group (F for G9 fixed to 1 rest as in model 4) | 12 | 6 | 357.9 |
| 6 | Dissolution as limiting factor for absorption (rest as in model 4) | 14 | Restriction | 334.8 |
| Step 3: oral data added | | | |
| 7 | Same absorption (k_a and F) parameter values for all oral groups (rest as in model 6) | 2 | Restriction | 192.5 |
| 8 | Considers a portal space compartment with a MM elimination process and two intestinal transit compartments + efflux process to one of lumen compartments + two different k_a from each intestinal transit compartment (rest as in model 6) | 7 | Restriction | 163.2 |
| Step 4: oral data from G10 added | | | |
| 9 | Considers hepatocyte and enterocyte compartments with MM elimination processes and two intestinal transit compartments + efflux process to one of lumen compartments + two different K_a from each intestinal transit compartment (rest as in model 6); also takes into account the interaction between SQV and RTV at the enzymatic level | 13 | Restriction (estimated in this step: 8) | 195.3 |
| 10 | Considers hepatocyte and enterocyte compartments with MM elimination processes and one intestinal transit compartments + one K_a from intestinal compartment; dissolution as limiting factor for intraperitoneal absorption (rest as model 2); takes also into account the interaction between SQV and RTV at the enzymatic level | 8 | Restriction (estimated in this step: 6) | 342.8 |
| 11 | Hepatocyte compartment is not considered (rest as in model 10) | 6 | Restriction (estimated in this step: 5) | 342.8 |

MM, Michaelis-Menten kinetics; G, group; K_{aIP}, intraperitoneal absorption rate constant; k_a, intestinal absorption rate constant; F, bioavailability.

*Restriction indicates restriction of the random variables (were fixed to the values obtained in prior steps).
were total plasma concentrations. Table 2 shows the key models of a series of models tested at different stages of the data analysis. The parameters considered by the model, random estimated variables (IV) and MOFV, are also shown.

A Michaelis-Menten elimination process was performed to elucidate the distribution, metabolism, and excretion processes (disposition phase, step 1) for which the empirical model had revealed nonlinear phenomena but failed to provide any relevant information (models 1 and 2, Table 2). Taking into account the fact that 97% of SQV binds to plasma proteins (Holladay et al., 2001), dynamic and saturable plasma protein binding could explain the variability in clearance ($Cl_{(G1)} < Cl_{(G2)} < Cl_{(G3)} < Cl_{(G4)}$) highlighted by the empirical model, which showed that clearance (CI) and volume of the central compartment ($V_c$) differed in each group (G) (Campos Moreno et al., 2007). Thus, with the 24-mg dose, saturation of binding to plasma proteins would deliver more drug, which would in turn be eliminated, so that clearance would be higher than that observed with the 12-mg dose. Likewise, with the 48-mg dose, there could be saturation of both the plasma protein binding and elimination processes.

After incorporation of the intraperitoneal data (step 2) and taking into account the low solubility of the drug, precipitation of the drug in the peritoneal cavity was considered. Experimental observation confirmed this theory (data not shown), which suggests that dissolution limited the absorption of the drug (model 6, Table 2). In this model, the amount of precipitation depends on the dose administered ($Q_{pp(G1)}$), because the amount of drug dissolved is the same as that administered ($Q_{dis(G1)}$). Subsequently, the precipitated fraction ($FR_{(G1)}$) and dissolution rate constant ($k_D$) are estimated for each group.

Then, after incorporation of the oral data (step 3), a more semi-physiological approach was considered to define the pharmacokinetic profile of SQV (models 8–11, Table 2). Physiological expressions were incorporated into the model; $Q_{lh}$ represents hepatic flow, $Q_{ah}$ represents arteriohepatic flow, and $Q_{mes}$ represents mesenteric flow. On the basis of the data in the literature, $Q_{lh}$ and $Q_{mes}$ were considered to constitute 13 and 87% of the total hepatic flow, respectively, and the hepatic flow for a 300-g rat was considered to be 1.15 l/h (Birnbaum et al., 1994). Physiological parameters related to flow and volumes in liver and intestine were fixed to values in the literature ($Q_{lh}$, $V_{lh}$, and $V_{mes}$) (Birnbaum et al., 1994). Figure 2 illustrates the schematic structure of the model that best defined the pharmacokinetic profile of SQV and the corresponding differential equations. It is important to emphasize that unbound and bound SQV plasma concentrations were described in different compartments (compartments 1 and 3, respectively). The absorption rate constant was different in each intestinal compartment (0.24 and 0.17 h$^{-1}$ in the first and second compartments, respectively), whereas the transit compartment rate constant ($K_{t1}$) was 0.58 h$^{-1}$, indicating that the drug remained in each intestinal transit compartment for 1.71 h. There was a weak process of secretion from the enterocyte to the second intestinal transit compartment, characterized by a Michaelis-Menten secretion constant ($K_{s2}$) of 0.02 mg/l and an intrinsic blood clearance of intestinal secretion ($Cl_{int}$) of $2.83 \times 10^{-6}$ l/h. In addition, a metabolic process was detected in the liver and intestine, with a Michaelis-Menten constant ($K_{m}$) of 72.0 mg/l in both. Because RTV was a potent inhibitor of intestinal and hepatic metabolism and intestinal secretion processes, a factor was introduced to quantify the modification of $K_{m}$ when RTV was coadministered. A value of 5.16 was estimated for group 9 ($FR_{m(G9)}$) and a value of 10.2 was estimated for group 10 ($FR_{m(G10)}$). Precipitation of the drug in the intraperitoneal cavity and dissolution constants $k_D$ for the 24 and 12 mg were also estimated. The dissolution constant $k_D$.
versus individual predictions are also shown.

residuals versus time, and absolute individual weighted residuals observed SQV plasma concentrations are plotted in Fig. 3. Weighted 9) are shown in Fig. 1. Individual and population predicted versus final model (model 9, covariance step aborted). Individual SQV reports the estimated values for the pharmacokinetic parameters of the namic equilibrium characterized by the maximum amount of drug that varies from one dose to another, indicating that different sizes of the

It is well documented that CYP3A4 is the major enzyme responsible for approximately 90% of SQV metabolism. Because of the relative abundance of CYP3A4 enzyme in the human gut, first-pass gut metabolism was thought to be a significant factor in the low bioavailability of SQV in humans (Fitzsimmons and Collins, 1997; Steimer et al., 1998). In line with this thought, it has been observed that SQV and grapefruit juice coadministration produces an increase in the absolute bioavailability of SQV without affecting its clearance (Lown et al., 1997; Kupferschmidt et al., 1998), which suggests a decrease in the intestinal metabolism. A similar tendency is observed in rabbits, in which the oral bioavailability of SQV is approximately 4% because of the intestinal metabolism and secretion processes in these animals (Sinko et al., 2004). In contrast, CYP3A4 in the dog gut wall plays no significant role in clearance of SQV, and its bioavailability is greater than in any other species tested to date (Tam-Zaman et al., 2004).

In the present study, the impact of liver and intestine function on the pharmacokinetics of SQV in the rat, an animal model of the projection of human intestinal permeability (Amidon et al., 1995), was evaluated using a modelistic approach. The influence of RTV on the metabolism SQV was also assessed, because the combination of SQV and RTV has been shown to be clinically effective owing to the way in which the former augments exposure to the latter (Hoffman et al., 2007).

To imitate clinical practice, we selected a 24-mg dose of SQV, because, when scaled in rats (U.S. Food and Drug Administration,

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Definition</th>
<th>Model 9</th>
<th>Value</th>
<th>%</th>
<th>IIIV</th>
<th>%</th>
<th>Model 11</th>
<th>Value</th>
<th>%</th>
<th>IIIV</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cli (l/h)</td>
<td>Intrinsic blood clearance in hepatocytes</td>
<td>0.31</td>
<td>91</td>
<td>N.E.</td>
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<tr>
<td>$K_m$ = $K_m^e$ (mg/l)</td>
<td>Michaelis-Menten constant of metabolic process in liver and enterocyte</td>
<td>72.0</td>
<td>N.E.</td>
<td>148</td>
<td>N.E.</td>
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<tr>
<td>$k_{12}$ (h$^{-1}$)</td>
<td>Intercompartmental rate constant</td>
<td>2.80</td>
<td>N.E.</td>
<td>2.31</td>
<td>N.E.</td>
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<tr>
<td>$k_{13}$ (h$^{-1}$)</td>
<td>Intercompartmental rate constant</td>
<td>1.14</td>
<td>73</td>
<td>0.63</td>
<td>71</td>
<td>60</td>
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<tr>
<td>$V_e$ (l)</td>
<td>Volume of distribution in central compartment</td>
<td>0.24</td>
<td>40</td>
<td>0.38</td>
<td>60</td>
<td></td>
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<tr>
<td>$k_a$ (l/mg·h)</td>
<td>Protein-binding rate constant</td>
<td>1.12</td>
<td>60</td>
<td>0.38</td>
<td>N.E.</td>
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<tr>
<td>$k_{l1}$ (h$^{-1}$)</td>
<td>Protein-unbinding rate constant</td>
<td>0.20</td>
<td>78</td>
<td>1</td>
<td>N.E.</td>
<td>1</td>
<td>N.E.</td>
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<tr>
<td>QMA (mg)</td>
<td>Maximum binding capacity of the proteins for SQV</td>
<td>21,700</td>
<td>51</td>
<td>0.38</td>
<td>N.E.</td>
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<tr>
<td>$k_{12}$ (l/h)</td>
<td>Absorption rate constant for intraperitoneal administration</td>
<td>1.09</td>
<td>166</td>
<td>0.87</td>
<td>N.E.</td>
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<tr>
<td>BIDMA</td>
<td>Fraction of amount absorbed by intraperitoneal route</td>
<td>1</td>
<td>N.E.</td>
<td>1</td>
<td>N.E.</td>
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<tr>
<td>FR(G8)</td>
<td>Fraction of dose that precipitate after intraperitoneal administration in group 8</td>
<td>0.97</td>
<td>0.93</td>
<td>0.93</td>
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<tr>
<td>FR(GT7) = (1 + FR(G8))/2</td>
<td>Fraction of dose that precipitated after intraperitoneal administration in group 7</td>
<td>0.98</td>
<td>0.93</td>
<td>0.93</td>
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<tr>
<td>$k_0$ (h$^{-1}$)</td>
<td>Dissolution rate constant for group 7</td>
<td>0.04</td>
<td>0.04</td>
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<td>$k_{02}$ (h$^{-1}$)</td>
<td>Dissolution rate constant for group 8</td>
<td>0.48</td>
<td>N.E.</td>
<td>4.46</td>
<td>N.E.</td>
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<tr>
<td>$K_p$ (h$^{-1}$)</td>
<td>Absorption rate constant for oral administration from intestinal compartment 1</td>
<td>0.24</td>
<td>56</td>
<td>0.24</td>
<td>1.5</td>
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<tr>
<td>$k_e$ (h$^{-1}$)</td>
<td>Intestinal transit rate constant</td>
<td>0.58</td>
<td>61</td>
<td>0.12</td>
<td>126</td>
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<tr>
<td>$Q_h$ (l/h)</td>
<td>Hepatic flow</td>
<td>1.15</td>
<td>6.5</td>
<td>1.15</td>
<td>6.5</td>
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<tr>
<td>$V_h$ (l)</td>
<td>Volume of distribution in hepatocyte compartment</td>
<td>0.01</td>
<td>0.01</td>
<td>6.5</td>
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<tr>
<td>$V_e$ (l)</td>
<td>Volume of distribution in enterocyte compartment</td>
<td>0.006</td>
<td>0.006</td>
<td>6.5</td>
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<tr>
<td>Cie (l/h)</td>
<td>Intrinsic blood clearance due to metabolism in enterocytes</td>
<td>4.35</td>
<td>2.5</td>
<td>2.5</td>
<td>113</td>
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<tr>
<td>$K_m^e$ (mg/l)</td>
<td>Michaelis-Menten constant of enterocyte secretion process</td>
<td>2.83</td>
<td>10</td>
<td>2.83</td>
<td>6.9</td>
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<tr>
<td>$K_m$ (h$^{-1}$)</td>
<td>Absorption rate constant after oral administration from intestinal compartment 2</td>
<td>0.17</td>
<td>59</td>
<td>0.17</td>
<td>59</td>
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<tr>
<td>$F_K_{m(G10)}$</td>
<td>Factor that modified the Michaelis-Menten constant for group 9</td>
<td>5.16</td>
<td>5.05</td>
<td>5.05</td>
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<tr>
<td>$F_K_{m(G11)}$</td>
<td>Factor that modified the Michaelis-Menten constant for group 10</td>
<td>10.2</td>
<td>38</td>
<td>38</td>
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<tr>
<td>$\sigma_1$ (CV %)</td>
<td>Residual variability; exponential component</td>
<td>12.2</td>
<td>24</td>
<td>24</td>
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<tr>
<td>$\sigma_2$ (mg/l)</td>
<td>Residual variability; additive error</td>
<td>0.29</td>
<td>0.23</td>
<td>0.23</td>
<td></td>
<td></td>
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N.E., not estimated; CV, coefficient of variation.

*Parameters fixed to the estimated values obtained in previous steps.

**Parameters fixed to physiological values from the literature.

varies from one dose to another, indicating that different sizes of the solid particle precipitated from each of the doses administered.

In addition, plasma protein binding, which occurs through a dy- namic equilibrium characterized by the maximum amount of drug that binds to proteins (QMA), was also taken into consideration. Table 3 reports the estimated values for the pharmacokinetic parameters of the final model (model 9, covariance step aborted). Individual SQV plasma concentration values predicted with the selected model (model 9) are shown in Fig. 1. Individual and population predicted versus observed SQV plasma concentrations are plotted in Fig. 3. Weighted residuals versus time, and absolute individual weighted residuals versus individual predictions are also shown.

Finally, Fig. 4 shows the predicted fractions of the drug dose that escaped the effects of the intestinal and hepatic first-pass loss (first-stage), which depended on the hepatocyte and enterocyte concentrations of the drug.

### Discussion

Evidence in the literature indicates that SQV is a substrate of CYP3A4, P-glycoprotein, and CYP3A5 (Fröhlich et al., 2004; Muñoz et al., 2005) and that the low and variable bioavailability of SQV is not just a result of hepatic metabolism. Moreover, contradictory data exist with respect to the effect of intestinal first-pass loss on the oral bioavailability of SQV. Such discrepancies may be explained, at least in part, by the extent to which this process varies, depending on the animal model used.

It is well documented that CYP3A4 is the major enzyme responsible for approximately 90% of SQV metabolism. Because of the relative abundance of CYP3A4 enzyme in the human gut, first-pass gut metabolism was thought to be a significant factor in the low bioavailability of SQV in humans (Fitzsimmons and Collins, 1997; Steimer et al., 1998). In line with this thought, it has been observed that SQV and grapefruit juice coadministration produces an increase in the absolute bioavailability of SQV without affecting its clearance (Lown et al., 1997; Kupferschmidt et al., 1998), which suggests a decrease in the intestinal metabolism. A similar tendency is observed in rabbits, in which the oral bioavailability of SQV is approximately 4% because of the intestinal metabolism and secretion processes in these animals (Sinko et al., 2004). In contrast, CYP3A4 in the dog gut wall plays no significant role in clearance of SQV, and its bioavailability is greater than in any other species tested to date (Tam-Zaman et al., 2004).

In the present study, the impact of liver and intestine function on the pharmacokinetics of SQV in the rat, an animal model of the projection of human intestinal permeability (Amidon et al., 1995), was evaluated using a modelistic approach. The influence of RTV on the metabolism SQV was also assessed, because the combination of SQV and RTV has been shown to be clinically effective owing to the way in which the former augments exposure to the latter (Hoffman et al., 2007).

To imitate clinical practice, we selected a 24-mg dose of SQV, because, when scaled in rats (U.S. Food and Drug Administration,
in humans; namely, 1000 mg administered in combination with 100 mg of RTV (b.i.d.) (U.S. Food and Drug Administration, 2005, http://www.fda.gov/cder/).

The noncompartmental analysis showed nonlinearities in the absorption and elimination process (data not shown). However, results for area under the curve, clearance (Cl), and half-life ($t_{1/2}$) were contradictory, and a consistent interpretation was not possible. Moreover, it also was not possible to distinguish the roles of the liver and intestine in the elimination process. In this scenario, individual analyses of data were aborted, and a population analysis by means of nonlinear mixed-effects modeling was performed, because this has

**FIG. 3.** Goodness-of-fit plots (model 9). The top panels show observed concentrations versus predicted concentrations for the typical individual on the left and versus predictions for each individual on the right, together with the identity line. The bottom panels show the weighted residuals for the typical individual (WRES) versus time and the absolute individual weighted residuals ($|\text{WRES}|$) versus predictions for each individual.

**FIG. 4.** Graphical representation of the relationship between the $F_h$ and $F_e$ and the concentrations detected in those compartments. Population pharmacokinetic parameters and the range of concentrations of SQV in hepatocyte ($C_7$) and enterocyte ($C_9$) compartments are those predicted by model 9. The interaction was considered when $F_h$ and $F_e$ for groups 9 and 10 was calculated, so that fractions were calculated, taking into account the possibility that clearance was affected by the empirical factor, which multiplied $K_{m}$. This is a constant empirical factor and depends on the RTV concentration in these two compartments.
proved to be a very useful tool to model different kinetic processes (Ruiz-Carretero et al., 2004; Muñoz et al., 2005; Campos Moreno et al., 2007). The advantage of the nonlinear mixed-effects models is that the data may be “sparse” with a little as one observation per subject, “rich” with many observations per subject, or a combination of both with some subjects having many samples collected in a dose interval, whereas others have only a few (Bonate, 2006).

The SQV pharmacokinetic model developed and supported by our experimental data considers two absorption sites in the gastrointestinal tract, saturable metabolism in hepatocytes and enterocytes, saturable intestinal efflux, dose-dependent precipitation in the peritoneal cavity after intraperitoneal administration, and protein binding kinetics.

The C1iso indicates low affinity for the intestinal efflux carrier, and the fact that the metabolic process detected in the liver and intestine is governed by an enzymatic system characterized by the same Km value indicates that the drug has the same affinity for the metabolic enzyme regardless of where the process occurs.

The factor used to quantify the modification of Km when RTV was coadministered indicates that there was a loss of affinity for the enzyme when RTV was coadministered due to competitiveness between the two drugs for the enzyme. The fact that a higher factor was obtained for group 10 (oral) than for group 9 (intravenous) suggests that inhibition of the first-pass intestinal metabolism was greater than that of the first-pass hepatic metabolism. In line with this finding, the blood intrinsic clearance in hepatocytes (Cli) and enterocytes (C1ie) again indicated that the main metabolic process of SQV occurs in the intestine in rats.

By using the semiphysiological model, it is possible to calculate the extraction rate according to the corresponding concentration in the enterocyte or hepatocyte compartments at any time point (Fig. 4). In this sense, the fraction of dose escaping from the intestine (Fe) was shown to undergo variations of 15 to 60% as the concentration increased and the enzymatic system became saturated. On the other hand, the fraction escaping from the liver (Fh) did not undergo considerable oscillations, maintaining values of 80 to 86%, which are far from that of 56% reported by Sinko et al. (2004).

When RTV was coadministered, values of 85 to 93% and 96 to 98% were observed for Fh and Fe, respectively. These results indicate that there was an important inhibition of the SQV metabolism that was most evident at the intestinal level.

Shibata et al. (2002) used in vitro and in vivo data (rats) to develop a physiological model to describe the interaction between protease inhibitors, and among the combinations they assayed was that of SQV and RTV. These authors considered the enterocyte and hepatocyte compartments, each with its own metabolic process, but did not take into account the intestinal secretion process. They concluded that the main metabolic process took place in the liver (Fh and Fe, 51.1 and 97.4%, respectively), which is opposite to the trend observed in the present study. Oral bioavailability was 4.1%, with a fraction of absorption of 8.3. In contrast, Shen et al. (1997) reported that the considerable presystemic losses of SQV could be due to intestinal first-pass metabolism, a hypothesis that the present results support.

The SQV pharmacokinetic model developed in this study also takes into consideration plasma protein binding, which occurs through a dynamic equilibrium that does not reach saturation. The QMA suggests that this binding produces a delay in the distribution process, so that it performs the role of a peripheral compartment.

The values obtained for the parameters QMA and C1iso lacked physiological significance, and, as a consequence, the model was reevaluated without plasma protein binding and intestinal secretion processes (model 10, Table 2). This new model provided a value for C1iso, which represented 0.27% of the C1ie. This result indicates that the most important organ for elimination of SQV in rats is the gut wall and that the liver does not play a significant role. Consequently, in the final evaluation, the hepatocyte compartment was not considered (model 11). This model confirms that SQV intestinal metabolism is the process by which the oral bioavailability of SQV in rats is restricted and indicates that orally administered RTV acts a potent competitive inhibitor of this process, because the increase in Km was higher when the two protease inhibitors were coadministered orally (model 11: FPKm(G9G10) = 38 and FPKm(G10G9) = 5, by the oral and intravenous routes, respectively). However, the statistical criteria (MVOF) for model 11 (Table 2) show that eliminating plasma protein binding, intestinal secretion, and hepatocyte metabolism processes from consideration was not justified.

The pharmacokinetic model developed in this study could only be extrapolated to humans in terms of compartments and processes. It is interesting to note that bioavailability of SQV is very low in all animal species studied, and intestinal first-pass metabolism has been also identified in humans (Noble and Faulds, 1996; Fitzsimmons and Collins, 1997; Eagling et al., 2002) and rabbits (Sinko et al., 2004). On the contrary, first-pass metabolism associated with the liver seems to be the main cause in dogs (Tam-Zaman et al., 2004). However, pharmacokinetic parameter values could be only used in the animal species that have been characterized. Therefore, although the mean values estimated in this study for the maximum plasma concentration after oral administration of a single dose of SQV alone or coadministered with RTV could be considered comparable to those of human (Plosker and Scott, 2003; Dickinson et al., 2008), the impact on the interaction and saturation of protein binding in both species could be very different. Results obtained in this study indicate that saturation of this process in rats could be unlikely.

In conclusion, the pharmacokinetics of SQV in rats is complex and nonlinear, and intestinal first-pass loss seems to be the main cause of its poor oral bioavailability. Nevertheless, we believe that the application of the nonlinear mixed-effect technique allows a robust estimation of the population pharmacokinetic parameters of SQV and their variability and despite the complexity is a useful method for evaluating the impact of hepatic and intestinal first-pass loss on oral bioavailability in rats.

Authorship Contributions
Participated in research design: Merino-Sanjuan and Casabó. Conducted experiments: Lledo-García and Nácher. Contributed new reagents or analytic tools: Lledó-García. Performed data analysis: Lledó-García, Merino-Sanjuan, and Casabó. Wrote or contributed to the writing of the manuscript: Lledó-García, Merino-Sanjuan, Nácher, and Casabó.

References


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