Factors Responsible for the Variability of Saquinavir Absorption: Studies Using an Instrumented Dog Model

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Purpose. To study the effect of dose and food on the bioavailability of saquinavir in dogs.

Methods. A Youden Square block design was used for six female mongrel dogs (20–24 kg) who received six saquinavir treatments. The six randomized treatments were 1 mg/kg intravenous infusion over 30 min; 200, 400, 600, and 800 mg of saquinavir in the form of 200-mg capsules given orally with food; and 400 mg of saquinavir given orally after an overnight fast. A 200-mg ^{14}C-saquinavir capsule was used to replace one of the 200-mg unlabeled saquinavir capsules in the 200- and 800-mg oral study.

Results. Absorption of saquinavir from the gut was variable. (F_A: 49–95%). The ^{14}C-saquinavir study shows that the total radioactivity absorbed from the gut was insignificantly different from that of unlabeled saquinavir, suggesting first-pass gut metabolism was unimportant. The bioavailability of saquinavir under fasting condition was significantly lower ($8.41 \pm 4.7\%$ vs. $20.3 \pm 2.6\%$, $p < 0.05$). Saquinavir underwent significant first-pass liver metabolism because hepatic clearance values (22 to 30 ml $min^{-1}kg^{-1}$) approached that of liver blood flow.

Conclusions. Incomplete gut absorption and extensive first-pass liver metabolism are the causes for low bioavailability of saquinavir in dogs. Absorption was further reduced under fasted conditions.

KEY WORDS: first-pass liver metabolism; HIV protease inhibitor; instrumented dog study; low bioavailability; poor gut absorption; saquinavir.

INTRODUCTION

Saquinavir (SQV), Ro-31-8959, is a potent HIV protease inhibitor with an IC_{90} of 20 nM (1). It has been approved for use in the treatment of patients with acquired immunodeficiency syndrome (AIDS). SQV is effective in combination with reverse transcriptase inhibitors in reducing viral load. The doses of SQV used range from 600 to 1200 mg, and the most common regimen is 1000 mg SQV plus 100 mg ritonavir b.i.d. This drug is well-tolerated and at the doses administered exhibits little adverse effects (2).

The bioavailability of SQV is low and variable. In healthy volunteers, the bioavailability of SQV is 4% when consumed with food; this value is considerably higher than that obtained under fasting condition (3). A combination of first-pass metabolism in the gut and liver and incomplete absorption have resulted in low SQV bioavailability (1). However, the relative contributions of these eliminating organs to the first-pass effect and the significance of incomplete absorption of saquinavir have not been explored. Of the cytochrome P450 enzymes, CYP 3A4 has been found to be the major enzyme metabolizing SQV in the human gut and liver. The relative bioavailability of SQV is significantly increased when the drug is coadministered with potent CYP 3A4 inhibitors such as ritonavir (4), ketoconazole (3), or cimetidine (5). The bioavailability of SQV decreases when it is given with enzyme inducers such as rifampicin (3). Furthermore, studies have shown that other protease inhibitors, which are also substrates for CYP 3A4, when given with SQV in combination therapy can suppress first-pass metabolism of SQV (4,6). Consequently, the oral bioavailability of SQV is increased, and this change enhances HIV protease inhibition.

Oral escalating dose studies have shown that plasma levels plateaued after 1600 mg kg^{-1} day^{-1} in rats and 600 mg kg^{-1} day^{-1} in rabbits and dogs (data on file at Roche). The reduction of exposure to SQV has been attributed to saturable absorption. *In vitro* data indicate that substrate inhibition and saturable metabolism characterize SQV elimination. This nonlinear pharmacokinetic behavior further complicates accurate determination of SQV bioavailability. A clear understanding of the cause of nonlinearity and its implications on the concentration–time profiles are crucial for further evaluation of this drug.

The dog has been shown to be a useful animal model during the research and development of SQV (data on file at Roche). In this study, the objective was to use a chronically instrumented dog model (7) to measure separately gut absorption and hepatic liver first-pass metabolism of SQV, such that the mechanism responsible for the low SQV bioavailability can be elucidated.

MATERIALS AND METHODS

SQV capsules (Ro 31-8959/000) containing 200 mg SQV free base each, SQV mesylate (Ro 31-8959/008) powder, Tube Air-Fills 2A gelatin capsules, Capmul MCM90 (Ro 31-8959/A24), and ^{14}C-SQV free base (Ro 31-8959/005) were supplied by Roche Products Ltd. (Welwyn Garden City, Hertfordshire UK). The specific activity of the radiolabeled substance was 0.59 μCi/mg.

Preparation of ^{14}C-SQV Capsules

Appropriate amounts of Capmul MCM90 (Karlshamns USA, Inc., Newark, NJ), which is a mixture of mono- and di-glycerides of medium-chain fatty acids and ^{14}C-SQV free

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ABBREVIATIONS: AUC, area under the curve; C_{CA}, carotid artery concentration; C_{HA}, hepatic artery concentration; CL_H, hepatic clearance; CL_S, systemic clearance; C_{max}, maximum concentration; C_{PV}, portal vein concentration; C_{RH}, right heart concentration; D, dose; E_H, hepatic extraction ratio; F, absolute bioavailability; F_A, fraction of intact drug absorbed; F_G, fraction of total radioactivity available from gut; F_H, fraction available after hepatic elimination; MRT, mean residence time; Q_H, liver blood flow; Q_{HA}, hepatic vein flow; Q_{PV}, portal vein flow; SQV, saquinavir; $T_{1/2}$, half life; T_{max}, Time at which maximum concentration is reached; V_{SS}, volume of distribution at steady state.

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base, were previously mixed in a 100-ml beaker. The mixture was heated at 57°C on a heater and stirred until the drug was dissolved. The solution was then cooled at room temperature. A ^{14}C-SQV capsule was prepared by transferring 0.986 ± 0.009 g of solution using a 3 ml-syringe equipped with an 18-gauge needle into an empty gelatin capsule (0.44 ± 0.006 g) providing a total weight of 1.43 ± 0.006 g. The content of ^{14}C-SQV in each capsule was 0.201 ± 0.009 g. Saquinavir was labeled in the benzene ring of the quinoline group (8).

Preparation of Intravenous Solution

Intravenous solution was prepared by dissolving the mesylate salt of SQV in a 5% aqueous dextrose to give a concentration of 1.14 mg/ml, equivalent to 1 mg/ml of free base.

Animal Studies

Random source female dogs weighing between 20 to 24 kg were used for the study. The University of Alberta Animal Policy and Welfare Committee approved the study protocol. Prior to entering the University Facility, each animal was subjected to a 3-week quarantine period. Physiological parameters such as body weight, temperature, hematocrit, and blood and liver biochemistry were monitored in all dogs starting from the arrival date at the animal facility until the end of experimentation.

Prior to each experiment, a blood sample was withdrawn from each animal to perform hematological measurements. After each experiment, another blood sample was taken for the same purpose. The analysis was performed at the Veterinary Pathology Laboratory (Health Sciences Laboratory Animal Services, 140 Heritage Medical Research Center, University of Alberta). According to the report provided by this laboratory, the six dogs that participated in the study were judged to be healthy.

Surgery and Postoperative Care

A detailed description of the surgical procedure is given elsewhere (7). Briefly, after an overnight fast, each dog was sedated using a preanesthetic mixture of 2% halothane/l and oxygen. Four individual catheters were inserted into the carotid artery, right heart, and portal and hepatic veins. A blood flow probe was placed on the portal vein and another one on the hepatic artery. For blood flow measurements, a Transonic flow meter (Transonic Systems, Ithaca, NY, USA) connected to the two ultrasonic flow probes was used. The blood flow rates were recorded on a computer loaded with the WINDAQ software (Transonic Systems). After completion of the surgery, dogs were fitted with jackets equipped with side pockets (Alice King Chatam Medical Arts, Los Angeles, CA, USA) to protect the catheters. Upon waking, dogs were allowed to recover overnight in heated cages in the intensive care unit and at this time they were given 0.01–0.02 mg/kg buprenorphine (Recket and Colman, PL, Erie, IRL) subcutaneously. The following morning they were brought to their normal kennel and allowed to recover. Buprenorphine, given twice daily, was continued for 2 days, and oral cloxacillin, 500 mg, twice a day, was given prophylactically for 5 days post surgery. A recovery period of at least 10 days was provided for each animal before initiation of any experiment. Dogs were fed daily by 10:00 a.m. with PMI Certified Canine Diet (#5007). The quantity was approximately 1oz/kg body weight. The water consumed by the animal was obtained from the PETWA system in the Health Sciences Laboratory Animal Service facility. Water was given *ad libitum*. The catheters were flushed daily with sterile saline, followed by a solution containing acid citrate dextrose (ACD) to fill the catheter dead space. The ACD solution consisted of 0.4% anhydrous citric acid, 1.32% sodium citrate (dihydrate), 1.47% dextrose (monohydrate), and 1.5% of 37% formaldehyde and was left for 5 min to sterilize the catheter lumen prior to removal. Finally, the catheters were flushed with sterile saline and then filled with heparin solution 500–1000 U/ml. This procedure was performed for 5–7 days post-op. Thereafter, heparin dose was increased to 10,000 U/ml, and catheters were flushed every second day. A sling was used to provide restraint and support to the animals during the flushing procedures.

Experimental Protocol

Animals were randomly assigned according to a Youden Square block design so that each animal received the six treatments randomly. The treatments were 1 mg/kg IV; 200, 400, 600, and 800 mg orally with food; and 400 mg orally without food. One ^{14}C-labeled SQV capsule was included in the 200-mg and 800-mg oral studies. Each treatment was separated at least 7 days apart.

Intravenous Dosing. The animal was fasted overnight, and food was withdrawn by 4:00 p.m. the day before the study. On the day of the experiment, the animal was placed in a sling frame in the laboratory by approximately 9 a.m. The dog was able to stand freely or be supported along its entire ventral surface, thus taking the weight off its legs. All dogs were trained to this procedure before experimentation. The flow probes were then attached to the flow meter via a cable, and baseline flow was measured for 10–15 min. Four cups of PMI Certified Canine Diet (#5007) previously soaked with water were fed to the dog before an intravenous dosing. The left cephalic vein was cannulated using a 21-gauge cannula to which a three-way stopcock was attached. A 1 mg/kg base equivalent of saquinavir mesylate salt, dissolved in 5% dextrose solution, was infused using a single syringe infusion pump (KDS-100, Fisher Scientific, Ottawa, Ontario, Canada) at a constant rate over 30 min. Blood samples (3 ml) were withdrawn from the four catheters simultaneously at 0, 5, 10, 15, 30, and 45 min and 1, 2, 3, 4, 6, 8, and 24 h after the commencement of infusion. A heparin lock (10 U/ml) was used throughout the experiment to maintain the patency of the catheters. Blood samples were collected in sterile vacutainers containing 45 USP units of lithium heparin. Blood samples were kept on ice for 30 min and then were centrifuged at 1000 × *g* for 15 min. After centrifugation, plasma samples were harvested into labeled tubes and stored at –80°C until samples were sent for analysis.

Oral Dosing. For oral studies, dogs were fasted in the same fashion as that described in the intravenous experiments. Prior to dosing, the dog was fed two cups of certified diet. After oral dosing, the dog was given 15–30 ml of PETWA water via a syringe. Subsequent to drug administration, the animal received another two cups of food. The entire procedure took less than 10 min. Blood samples (3 ml) from the four catheters were withdrawn at 0, 15, 30, and 45 min, and 1, 2, 3, 4, 6, 8, and 24 h post dosing. For the radiolabeled

studies, (200 mg and 800 mg), 3 ml of blood samples were collected from 0 to 3 h and 6 ml of blood samples were collected at 4 h post-dose and thereafter. The higher volume at the latter part was required due to smaller concentration at these times. Blood and plasma samples were handled as that described in the intravenous dosing section except each plasma sample obtained from the labeled study was divided into two equal volumes prior to storage. One set of the sample was used for radioactivity measurements and the other for Liquid Chromatography/Mass Spectroscopy/Mass Spectroscopy (LC/MS/MS) quantification.

Analytical Procedure

SQV samples were shipped to BRI (Biopharmaceutical Research Inc., formerly known as Axelson and Kwok Research Associates Inc.) in Vancouver for analysis. An APcI LC/MS/MS method was used for SQV quantification in dog plasma (9). Calibration standard curve samples were prepared in duplicate with control blank dog plasma at concentrations ranging from 0.2 to 400 ng/ml in 250 μl plasma using d_5-labeled drug as an internal standard. Quality control (QC) samples within this range were also analyzed along with the standard samples. However, concentrations of SQV were commonly found to exceed the upper limits of the standard curve. Therefore, to permit direct quantitation of plasma samples using 250 μl plasma, the concentration range in the calibration curve was extended to 10,000 ng/ml. QC samples within this range were included and analyzed with the standard samples. Validation of this extended calibration range for linearity, precision, and accuracy was performed over three batches of calibration samples. The intra-day and inter-day relative error or accuracy of the standard samples (1–10,000 ng/ml) was within ±10%. The intra-day and inter-day precision for concentrations 1–4 ng/ml and 10–10,000 ng/ml were within ± 20% and ± 10%, respectively. The inter-day and intra-day accuracy and precision of the QC samples for concentration 1–100 ng/ml and 400–8000 ng/ml were <15% and <10%, respectively. The standard curves were linear over the range studied. A weighting factor of 1/x was used to perform linear regression of the data. The coefficient of determination was found to be >0.99.

For estimating the concentration of the radiolabeled samples, a standard curve was prepared by spiking 1 ml of blank plasma with various concentrations of C^{14} SQV ranging from 0.07 to 5 μg/ml. The standard curve was linear over the range studied ($r^2 > 0.998$). The quantifiable limit of this assay was 0.05 μg/ml when 1 ml of blank plasma was used. Wallac 1410 Liquid Scintillation Counter (Fisher Scientific, Ottawa, Ontario, Canada) was used for radioactivity measurements. The inter-day and intra-day precision and accuracy was within ±10%.

Pharmacokinetic Analysis

Concentration vs. time data collected from the right heart catheter was used to calculate noncompartmental pharmacokinetic parameters. WinNonlin version 3.1 (Pharsight Corporation, Mountain View, CA) was used for the computation. For the calculation of absorption flux, influx ($flux_{in}$) and efflux ($flux_{out}$), the LAGRAN computer program was used (10).

Measurements of Physiological Kinetic Parameters

Concentration–time data from individual blood vessels combined with electronically measured portal vein and hepatic artery blood flows were used to quantify processes. The following parameters were calculated where F_A is fraction of dose absorbed from the gut.

$$F_A = \frac{\text{Amount absorbed}}{\text{Dose}} \tag{1}$$

F_G, the fraction of radiolabeled dose absorbed from the gut was calculated using Eq. 2:

$$F_G = \frac{\text{Amount absorbed } (^{14}\text{C SQV})}{\text{Dose}} \tag{2}$$

The absolute bioavailability (F) was calculated using Eq. 3:

$$F = F_A \cdot F_H \tag{3}$$

where F_H is fraction available after hepatic elimination. The amount of drug absorbed from the gut was calculated using Eq. 4:

$$\begin{aligned}\text{Amount absorbed} &= \int_{t_1}^{t_2} \text{Absorption flux} \cdot dt \\ &= \int_{t_1}^{t_2} Q_{PV}(C_{PV} - C_{CA}) \cdot dt\end{aligned} \tag{4}$$

where Q_{PV} is the portal vein blood flow rate and C_{PV} and C_{CA} were and portal vein and carotid artery plasma concentrations, respectively, after being analyzed either by LC/MS/MS for F_A or by the scintillation counter for radioactivity for F_G measurements. A negative flux value was considered to be zero due to the precision of the LC/MS/MS assay. Hepatic extraction ratio (E_H) and F_H were calculated using Eqs. (5) and (6):

$$E_H = \frac{flux_{in} - flux_{out}}{flux_{in}} \tag{5}$$

$$F_H = 1 - E_H \tag{6}$$

where $flux_{in}$ and $flux_{out}$ are fluxes of SQV going into and out from the liver, respectively. The corresponding fluxes were calculated according to Eqs. (7) and (8). Fluxes were integrated from the first appearance of the drug in the body to the last measured plasma concentration point.

$$flux_{in} = \int_{t_1}^{t_2} (C_{PV} \cdot Q_{PV} + C_{CA} \cdot Q_{HA}) \cdot dt \tag{7}$$

$$flux_{out} = \int_{t_1}^{t_2} [C_{HV} \cdot (Q_{PV} + Q_{HA})] \cdot dt \tag{8}$$

where Q_{HA} was the blood flow rate of the hepatic artery and C_{HV} was the hepatic vein plasma SQV concentration. Hepatic blood flow rate was the sum of portal vein flow rate and hepatic artery flow rate at each time point and was calculated using Eq. (9):

$$Q_H = Q_{PV} + Q_{HA} \tag{9}$$

Hepatic clearance (Cl_H) was calculated using Eq. (10):

$$Cl_H = Q_H \cdot E_H \quad (10)$$

Systemic clearance (Cl_s) was calculated using Eq. (11):

$$Cl_s = F \cdot D/AUC \quad (11)$$

Where D is the intravenous or oral dose, AUC is the area under the plasma concentration–time curve, and F is obtained from Eq. (3).

Statistical Analysis

A repeated measures design was used where animals were assigned according to a Youden Square block design so that each animal received six treatments randomly. All parameters undergoing statistical evaluation were first tested for normal distribution using Shapiro-Wilk test and by plotting normal probability and detrended normal plots. Parameters deviating from normality were subjected to Friedman's nonparametric analysis of variance. If significant F statistics was present at $p = 0.05$, Duncan's multiple range test was used to compare treatment means. An independent *t* test was performed for all the parameters between the two 400-mg doses in fast and fed conditions, Cl_s and Cl_H at various dose levels, and F_G and F_A at 200- and 800-mg doses. All values are reported as mean ± SD. SPSS for Windows version 9.0 was used for all statistical computations (SPSS Inc., Chicago IL, USA).

RESULTS

Five out of six animals completed all six experiments. One dog died from a stroke caused by a clot that was dislodged from the carotid artery catheter. This happened within 24 h after the catheter was surgically repaired. The 400-mg and 600-mg oral experiments were not completed by this particular dog. Intravenous data from one dog were not included in the kinetic analysis because the concentration of the dosing solution was unexpectedly low.

Mean pharmacokinetic data are summarized in Tables I and II. Mean (±SD) plasma concentration vs. time profiles after a 30 min intravenous administration of SQV after a 1 mg/kg dose in five dogs is shown in Fig. 1. The systemic clearance of SQV (20.5 ± 6.0 ml min^{-1} kg^{-1}) obtained in this study is similar to that reported in an in-house study conducted by Roche Product Ltd. The half-life of SQV, however, is a lot shorter than that reported in the same study (1.5 vs. 5 h).

Representative mean (±SD) plasma concentration vs. time profiles after 400 mg saquinavir dose in fasted and fed states are shown in Figs. 2 and 3, respectively. F_A and F of SQV after fast and fed conditions are shown in Table II. The fraction of SQV absorbed, estimated based on intact SQV (F_A) and total ^{14}C activity available from the gut (F_G), is shown in Table II.

The pharmacokinetics of oral SQV is variable and appears to be linear up to 600 mg under the fed state. This is reflected by a dose-dependent increase in the mean values of C_{max} and AUC (Table I). After the 800-mg oral dose, both C_{max} and AUC values decreased; however, these values did not reach statistical significance when compared to that of the 600-mg dose (Table I). This deviation of SQV kinetics from linearity was not due to a change in the SQV disposition kinetics, as the systemic and hepatic clearance values of SQV are independent of dose (Table II). The difference is mainly due to lower gut absorption and liver availability at the 800-mg dose (Table I).

The food effect on SQV kinetics was tested under a fasted and fed condition after a 400-mg oral dose of SQV. The absorption of SQV is lower under the fasted condition as evidenced by the lower gut absorption [F_A: 0.49 ± 0.3 (fasted) vs. 0.95 ± 0.3 (fed), $p < 0.05$), which results in an approximately 50% reduction in AUC ($p < 0.05$) (Table I). Similarly, the oral bioavailability (F) was reduced to half under the fasted condition [F%: 8.41 ± 4.7 (fasted) vs. 20.3 ± 2.6 (fed),

Table I. Mean (±SD) Right-Heart-Based Pharmacokinetic Parameters of Saquinavir After an IV and Five p.o. Doses in Dogs

PK-parameters	1 mg/kg IV (n = 5)	200 mg p.o. (n = 6)	400 mg p.o. (n = 5)	400 mg fasted p.o. (n = 6)	600 mg p.o. (n = 5)	800 mg p.o. (n = 6)
C_{max} (ng/ml)	1290 ± 188	597 ± 289*	1592 ± 336‖‖	634 ± 327†	1871 ± 1156	1405 ± 1045
T_{max} (min)	19 ± 10.8‡	52.5 ± 35.2	45 ± 10.6	105 ± 64.3§	84 ± 33	72.5 ± 38.4
$AUC_{o\text{-}t}$ (μg min^{-1} ml^{-1})	45.9 ± 9.9‖	61.7 ± 49.9	160 ± 31.4‖‖	83.9 ± 41.0	309 ± 193¶	204 ± 103
V_{ss} (l/kg)	0.57 ± 0.2	n/a	n/a	n/a	n/a	n/a
$T_{1/2}$ (min)	93.3 ± 26.2	75.6 ± 31.5**	202 ± 134	171 ± 138	256 ± 189	113 ± 60.6
MRT (min)	23.9 ± 7.7††	99.8 ± 46.2‡‡	114 ± 28.8	158 ± 37.4	196 ± 105§	147 ± 32.2
AUC_{po}/AUC_{iv}¶¶		0.09 ± 0.05	0.16 ± 0.02*	0.08 ± 0.03	0.20 ± 0.09§§	0.11 ± 0.07

n/a = not applicable.
* Significantly different from 400, 600, 800 mg p.o. doses ($p < 0.05$).
† Significantly different from 400 and 600 mg p.o. doses ($p < 0.05$).
‡ Significantly different from 400 mg fasted, 600 mg, and 800 mg p.o. doses ($p < 0.05$).
§ Significantly different from 200, 400 mg p.o. and 1 mg/kg IV doses ($p < 0.05$).
‖ Significantly different from 600 and 800 mg p.o. doses ($p < 0.05$).
¶ Significantly different from 1 mg/kg IV, 200, 400 mg fasted and fed ($p < 0.05$).
** Significantly different from 600 mg p.o. dose ($p < 0.05$).
†† Significantly different from all other doses ($p < 0.05$).
‡‡ Significantly different from 1 mg/kg IV and 600 mg p.o. dose ($p < 0.05$).
§§ Significantly different from 200, 400 mg fasted and 800 mg p.o. doses ($p < 0.05$).
‖‖ $p < 0.05$ versus 400 mg fasted dose.
¶¶ Dose normalized AUC ratios.

Table II. Mean (±SD) Parameters for Saquinavir Absorption, Hepatic Elimination, and Absolute Bioavailability in Dogs

PK-parameters	1 mg/kg IV (n = 5)	200 mg p.o. (n = 6)	400 mg p.o. (n = 5)	400 mg fasted p.o. (n = 6)	600 mg p.o. (n = 5)	800 mg p.o. (n = 6)
Q_H (ml min^{-1}/kg^{-1})	35.0 ± 4.3	35.7 ± 10.7	37.2 ± 6.7*	26.9 ± 4.2†	30.7 ± 4.5	34.7 ± 4.1
E_H	0.74 ± 0.13	0.86 ± 0.09	0.77 ± 0.06	0.81 ± 0.08	0.76 ± 0.06	0.78 ± 0.11
Cl_H (ml min^{-1}/kg^{-1})	25.9 ± 5.9	30.5 ± 8.4‡	28.9 ± 6.9	22.0 ± 5.1	23.0 ± 1.7	27.1 ± 5.0
Cl_s (ml min^{-1}/kg^{-1})¶	20.5 ± 6.0	17.4 ± 13.5	24.1 ± 6.4	19.9 ± 8.6	18.4 ± 9.3	25.2 ± 13.9
F_G	n/a	0.58 ± 0.27	n/a	n/a	n/a	0.58 ± 0.32
F_A	n/a	0.56 ± 0.3	0.95 ± 0.3*	0.49 ± 0.3	0.69 ± 0.21	0.64 ± 0.39
F_H	0.26 ± 0.13	0.14 ± 0.1	0.22 ± 0.1	0.19 ± 0.1	0.24 ± 0.06	0.22 ± 0.11
F%		8.4 ± 5.9§	20.3 ± 2.68*	8.41 ± 4.7§	15.8 ± 1.4	11.8 ± 4.5‖

* $p < 0.05$ versus 400 mg fasted dose.
† Significantly different from 200 and 400 mg po doses ($P < 0.05$).
‡ Significantly different from 400 mg fasted po dose ($P < 0.05$).
§ Significantly different from 400 and 600 mg po doses ($P < 0.05$).
‖ Significantly different from 400 mg po dose ($P < 0.05$).
¶ $Cl_H > CL_S$: This apparent discrepancy can be resolved if one takes the difference in plasma and blood concentrations into account; the blood to plasma ratio (B/P) is 0.7 (data on file at Roche).

$p < 0.05$]. The elimination of SQV is not affected by fasting because the systemic and hepatic clearance values after fasted and fed condition are not significantly different (Table II). The rate of absorption is significantly slower under the fasted condition; this is evidenced by a mean T_{max} value that is more than twice than that of the fed state. As a result of lower and slower gut-wall absorption, the mean C_{max} value is less than half of that of the corresponding fed value ($p < 0.05$) (Table I).

Systemic clearance values remain unchanged with escalating doses of SQV suggesting there is no dose dependency (Table II). Similarly, hepatic clearance (Cl_H) also shows no dose dependency (Table II); however, the value observed after a 200-mg dose seemed to be significantly higher when compared to the values observed after 400-mg fasted p.o. dose (Table II) ($p < 0.05$). Lung elimination of SQV is minimal because there is a lack of difference in SQV concentration between carotid artery and right heart (Figs. 2 and 3). Similarly, the concentration profiles of SQV in the carotid artery and the portal vein are superimposable after intravenous administration of the drug, suggesting that elimination of SQV from the blood stream by the gut is not significant (Fig. 1). This is substantiated by the fact that the fraction of total radioactivity available from the gut, F_G, was found to be insignificantly different from F_A at the two dose levels studied [200 mg: 0.58 ± 0.27 (F_G) vs. 0.56 ± 0.30 (F_A); and 800 mg: 0.58 ± 0.32 (F_G) vs. 0.64 ± 0.39 (F_A)] suggesting lack of gut-wall metabolism (Table II). The elimination of SQV is predominantly carried out by the liver. This is substantiated by high Cl_H values that approach that of hepatic blood flow, Q_H. As a result, a high hepatic extraction ratio, mean values ranging from 0.74 ± 0.13 to 0.86 ± 0.09, was observed (Table II). The mean absorption value of SQV from the gut (F_A) ranges from 49 ± 14% to 95 ± 30% at the dose range studied. The overall bioavailability of SQV in this study is 20% or less. Apart from incomplete gut absorption, high first-pass liver extraction plays an important role in the low oral bioavailability of SQV.

DISCUSSION

The mechanisms of SQV absorption were evaluated using a chronic instrumented dog model that has previously been used successfully to quantify intestinal absorption, first-

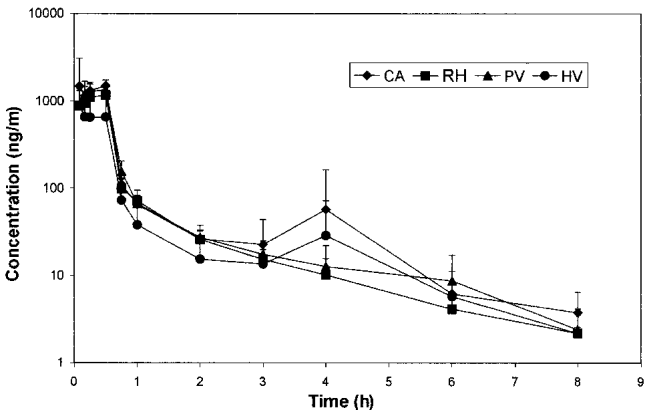

Fig. 1. Mean (±SD) plasma concentration vs. time profiles after a 30-min intravenous administration of saquinavir (1 mg/kg) in five dogs (CA, carotid artery; RH, right heart; PV, portal vein; HV, hepatic vein).

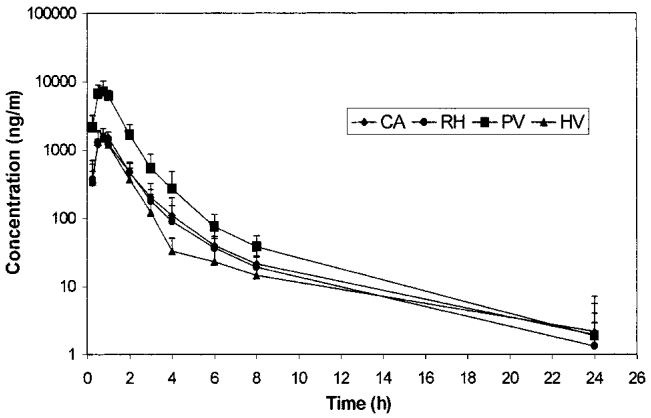

Fig. 2. A representative mean (±SD) concentration vs. time profile after an oral dose of 400 mg of saquinavir in dogs with food (n = 5) (CA, carotid artery; RH, right heart; PV, portal vein; HV, hepatic vein).

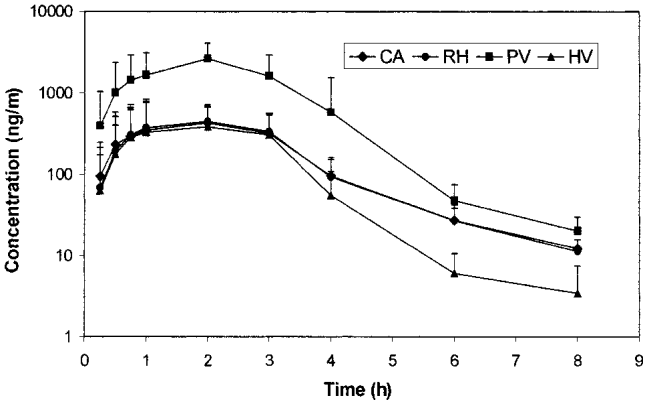

Fig. 3. Mean (±SD) concentration vs. time profile after an oral dose of 400 mg of saquinavir in dogs without food (n = 6) (CA, carotid artery; RH, right heart; PV, portal vein; HV, hepatic vein).

pass gut metabolism, and liver extraction (7,11,12). The results of the study clearly show that the absorption of saquinavir from the gut of the dog is variable and often low, the range being between 20% and 100%, depending on dose and whether the drug is taken with food. The low absorption from the gut is not due to first-pass metabolism in the gut-wall because the total radioactivity absorbed was not significantly different from that of the unlabelled species (Table II). Additional evidence for the lack of importance of the gut-wall in the low bioavailability of saquinavir in the dog was that the concentration profiles of the drug in the carotid artery and portal vein were superimposable after intravenous administration, suggesting that elimination of SQV from the blood stream by the gut is not significant (Fig. 1). The absorption of saquinavir was slow; the average portal vein concentrations being higher than those of the carotid artery for 8 to 24 h after oral drug administration suggests that absorption is not limited to the upper small intestine.

There are two barriers to a drug's entering the portal vein once it has been absorbed into the enterocyte. It can be metabolized by cytochrome P450 or it can be pumped back into the lumen of the gut by the permeability p-glycoprotein (P-gp). Saquinavir is a good substrate for the cytochrome P450 isoform, CYP3A, found in human gut-wall (1), but the current study has shown that the enzyme in the dog does not reduce the bioavailability of the compound. Saquinavir is also a good substrate for P-gp (13–15), and it is likely that this transporter limits its absorption. Because P-gp is saturable, the higher absorption of the 400 mg compared to the 200 mg dose is an indication. Reduced absorption at higher doses, as found in this study, has been observed in many species (data on file at Roche) and is probably due to relatively lower dissolution. Because P-gp is found lower down the gut (16) than is CYP3A (17), the exposure of SQV to P-gp is high because of its low solubility, and this could explain the very long period over which saquinavir is absorbed. Absorption, rather than elimination, controls the pharmacokinetics of saquinavir, and its very slowness is probably responsible for the maintenance of efficacious plasma levels in man between doses.

The inter-subject variability in bioavailability was largely caused by variability in absorption, which ranged from 49% to 95%, depending on dose (Table II); the coefficients of variation in this parameter (F_a) were between 30% and 60%. By comparison, the hepatic extraction ratio was essentially constant at about 0.8 with coefficients of variation between 8% and 18% (Table II). Restricted oral absorption plus high first-pass metabolism in the liver resulted in average bioavailability ranging between 8% and 20%.

Under fasting conditions, bioavailability was less than half of that when the dogs were fed (8.4% vs. 20.3%). Food had no effect on the hepatic clearance of saquinavir, even though hepatic blood flow was increased, (37.2 ± 6.7 ml min^{-1} kg^{-1} compared to 26.9 ± 4.2 ml min^{-1} kg^{-1}, Table II), and the reduction in bioavailability was strictly related to reduced absorption from the gut (Table II). The physiological reason for this has not been elucidated, but the higher absorption of SQV in fed dogs could be a result of increased solubility accomplished by secretion of bile acids.

The major eliminating organ for saquinavir in the dog is the liver; neither the gut-wall nor the lung plays a role in the removal of the compound. The parallel curves for saquinavir levels in the hepatic and portal veins (Figs. 2 and 3) demonstrate the constant extraction ratio and the large difference, its magnitude. Hepatic clearance was consistently about 30% higher than systemic clearance. Taking the measured whole blood:plasma concentration ratio of 0.74 ± 0.24 and converting plasma levels of saquinavir into blood levels would mean that hepatic and systemic clearance would not be significantly different. This observation again demonstrates that the liver is the main organ for eliminating saquinavir.

These data appear to be generally consistent with those observed in man. SQV bioavailability is low and hepatic extraction ratio is high (18). When administered with food, the bioavailability of the Invirase formulation of SQV in human has been reported to be 4%, considerably higher than in the fasting state (3).

It has been well documented that cytochrome P450 3A4 (CYP 3A4) is the major enzyme responsible for about 90% of SQV metabolism (1,3,19). Owing to the relative abundance of CYP 3A4 enzyme in the gut, first-pass gut metabolism was postulated to be a significant factor in causing low bioavailability of SQV in man. Using human intestinal microsomes, Fitzsimmons and Collins (1) reported that SQV was metabolized to its respective mono- and dihydroxylated metabolites with K_m values ranging from 0.3 to 0.5 μM and V_{max} values from 1.33 to 2.63 nmol min^{-1} mg^{-1} protein. Additional evidence for this hypothesis is that grapefruit juice can selectively inhibit CYP3A4 in the gut and significantly increase bioavailability of drugs such as SQV after oral administration (20,21). For example, Kupferschmidt *et al.* (20) have demonstrated that simultaneous consumption of grapefruit juice with SQV doubled the absolute bioavailability of SQV without affecting its clearance, which suggests that the increase in bioavailability of SQV is due to decreased intestinal metabolism.

A physiologically based dose–AUC model for SQV, which correlates the different pharmacokinetics of the Invirase and Fortovase formulations, the greater exposure observed in HIV-patients compared to healthy volunteers, and the increased exposure attained after repeated dosing, has been developed (22). This model incorporates metabolism by CYP3A4 in the gut-wall and liver as well as p-glycoprotein-mediated efflux of SQV from the gut-wall and quantitatively

explains the effects of food, grapefruit juice, and ritonavir in raising systemic exposure to SQV.

The pharmacokinetics of SQV in the dog appear to differ from those in man in that gut-wall CYP 3A4 plays no significant role in the clearance of the drug whereas the human enzyme is believed typically to reduce bioavailability by a factor of two (22). This could also explain the relatively high bioavailability of SQV in the dog compared to all other species (data on file at Roche).

In conclusion, the bioavailability of SQV in the dog is controlled by a combination of solubility in the gut lumen (increased markedly by the presence of food), p-glycoprotein mediated efflux in the gut-wall, and hepatic first-pass metabolism. In these respects, the instrumented dog is a good model for the human pharmacokinetics of SQV but appears to lack the gut-wall CYP3A4 metabolism that also serves to reduce human exposure to the drug.

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