Evaluation of Factors to Decrease Plasma Concentration of an HIV Protease Inhibitor, Saquinavir in Ethanol-Treated Rats

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Although alcohol consumption is a factor in which the bioavailability of saquinavir (SQV) are retarded, the cause for this phenomenon remains to be uncertain. In the presence study, we examined factors to decrease plasma concentration of SQV in ethanol-treated rats. The ethanol-treated rats were prepared by making them freely access to 15% ethanol solution for 14 d (Day 14 rats). The exsorption clearance of SQV from the blood circulation to the jejunal lumen in the Day 14 rats increased by 6-fold as compared to ethanol non-treated (NT) rats. In the presence of 25 μM ketoconazole (KCZ) or 10 μM cyclosporin A (CsA) in the jejunal lumen, the plasma concentration of SQV in the portal vein increased significantly, and this effect of 10 μM CsA was superior to that of 25 μM KCZ. The biliary excretion clearance of SQV in Day 14 rats also increased by 1.8-fold as compared to that in the NT rats. The metabolic clearance rate (Vmax/Km) of SQV in the intestinal microsomes from the Day 14 rats increased significantly, while in the liver microsomes the Vmax/Km did not change. The phase II metabolism processes in the Day 14 rats based on UDP-glucuronosyltransferases and gultathion-S-transferase activities were activated, however, they were not likely to be one of factors to decrease the bioavailability of oral SQV, because CYP3A activity in the liver and intestine was not activated to such an extent and SQV itself was not conjugated. These observations suggest that a main possible factor to explain the reducing effect on the SQV oral bioavailability during ethanol consumption is an enhanced efflux of SQV at the intestine and liver, where it is suggested that functional enhancement or excessive expression of P-glycoprotein is caused by ethanol consumption.

Key words protease inhibitor; saquinavir; ethanol consumption; pharmacokinetics; P-glycoprotein; CYP3A

The mid-1990s, HIV protease inhibitors (PIs) have been largely responsible for recent successful results in the treatment of HIV infected patients. A combination use of two kinds of reverse transcriptase inhibitors and an HIV protease inhibitor, highly active anti-retroviral therapy (HAART), has been found to be a better therapy than either drug alone in reducing HIV RNA levels and increasing CD4 cell counts. On the other hand, a systematic review about alcohol use and HIV pharmacotherapy by Kresina et al. reported that alcohol consumption by persons infected with HIV is one of important medical management issues, because alcohol consumption is a risk factor for poor medication adherence and can modify liver drug metabolism. They also concluded that research areas that are of particular importance for HIV therapy is clarifying the relationships and interactions among alcohol metabolism, HIV drug metabolism and pharmacogenetics. However, there had been no study on pharmacokinetic interaction between alcohol and HIV drugs.

In our previous reports, we recognized that bioavailability of saquinavir (SQV), a potent protease inhibitor, after oral administration was retarded during alcohol consumption both in HIV-infected patients and ethanol-treated rats. The bioavailability of SQV after oral administration alone to rats treated with 15% ethanol solution for 14 d (Day 14 rats) decreased significantly by 51% as compared to non treated (NT) rats. Moreover, total body clearance of SQV after intravenous administration alone to the Day 14 rats increased slightly by 30% as compared to the NT rats. Although most alcoholic beverages contain 0.13 to 0.5% isopentanols that inhibit CYP3A, it has been well known that the greater part of constituent of alcohol beverages, ethanol, induces mainly CYP2E1. Since SQV is one of PIs to be used for HAART therapy and is mainly metabolized via CYP3A4, a room to clarify the reason that ethanol decreases the bioavailability of SQV after oral administration has been remained.

In this study, to solve this contradiction that we observed in both clinical practice and ethanol-treated rats, the factors to decrease the bioavailability of SQV during ethanol consumption were evaluated. Where, we focused on the metabolism of SQV via CYP enzymes, the efflux pump; P-glycoprotein (P-gp) and the phase II metabolism in ethanol-treated rats.

MATERIALS AND METHODS

Chemicals SQV was kindly supplied by Hoffman-LaRoche Inc. (Nutley, NJ, U.S.A.). Rhodamine 123 (Rho123), ketoconazole (KCZ), cyclosporin A (CsA), Glucose-6-phosphate (G6P), G6P dehydrogenase (G6PDH) and nicotinamide adenine dinucleotide phosphate (NADP) were obtained from Sigma Chemicals (St. Louis, MO, U.S.A.). Testosterone, 1-naphthol, 4-methylumbelliferone (4-MU) and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Nacalai tesque (Kyoto, Japan). Ethanol and all other reagents used were of analytical grade and were used without further purification.

Preparation of Ethanol-Treated Rats Male Wistar rats of about 8–9 weeks old (300±10 g) were obtained from Nippon SLC Co. Ltd. (Hamamatsu, Japan). Rats were housed independently at least 2 d to be acclimatized under controlled environmental conditions with free access to general food and water. Then, they were divided into two groups, namely ethanol-treated rats and non-treated (NT) rats. Thereafter, the NT rats were housed for 14 d with free access to general food and water, and then they were provided to experiments at 15-th day from the treatment. The ethanol-treated (Day 14) rats were housed for 14 d with free access to...
general food and 15% ethanol solution, and then they were provided to the experiments at 15-th day after 24 h of ethanol removal interval. During the breeding, volume of water or 15% ethanol solution consumed and body weight were monitored. All animal experiments were performed in accordance with the Guideline for Animal Experimentation in Kyoto Pharmaceutical University.

Preparation of Solutions The standard stock solutions of SQV were prepared by dissolving in ethanol at various concentrations, and were stored at 4 °C in the dark. Rho123 for intravenous administration was prepared by diluting with saline containing 5% (v/v) dimethylsulfoxide at a final concentration of 0.17 mg/ml. Calibration curve samples for the liquid chromatography–mass spectrometry (LC/MS) analysis were prepared by adding known amounts of these standard stock solutions to drug-free plasma in a volume ratio of 1: 100. The test solution of SQV for intravenous administration was prepared by dissolving 50 mg SQV with 10 ml of a mixture containing 5% ethanol, 5% HCO-40 and 5% Pharmasolve+, and for intrajejunal administration by suspending 200 mg SQV in 10 ml of 1% sodium carmelllose solution (CMC-Na).

In Situ Intestinal Esxorption Studies After fasted overnight, rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (32 mg/kg) and were fixed on a heating pad under a surgical lamp to maintain body temperature at 37 °C. After placing a bile-duct cannula, each group of rats received an intravenous SQV solution at 5 mg/kg. Then, bile sample was collected every 10 min into 1.5 ml microcentrifuging tubes. Samples were immediately frozen in a deep freezer at −80 °C until the day of assay.

In Vitro Metabolism Using Rat Liver and Intestinal Microsomes Preparation of rat liver microsomes was performed according to the method of Hayes et al.9) For intestinal microsomes, the intestinal cells were isolated according to the methods of Fasco et al. with a minor modification.10) Microsomal protein concentration was determined by the method of Lowley et al. using bovine serum albumin as a standard.11) The metabolism of SQV in the liver or intestinal microsomes was measured in an NADPH-generating system according to the following method. To a clean 1.5 ml microcentrifuging tube, 50 μl of 5 mM NADP in 0.1 M phosphate buffer (pH 7.4), 50 μl of 50 mM G6P in 0.1 M phosphate buffer, 2 μl of 500 units of G6PDH in 0.1 M phosphate buffer, 50 μl of 50 mM MgCl2, in 0.1 M phosphate buffer and 293 μl of 0.1 M phosphate buffer were added. When inhibition experiment was performed, each 5 μl of SQV ethanol solution was added. After a 15-min preincubation at 37 °C in a water bath, the metabolic reaction was initiated by adding 50 μl of rat liver or intestinal microsomal suspension (final concentration: 0.8 and 0.4 mg protein/ml of microsomal suspension, respectively) and incubated for 10 min at 37 °C. The reaction was stopped by the addition of 200 μl of ice-cold 2 M K2PO4.

UDP-Glucuronosyltransferases (UGTs) and Gultathione-S-Transferase (GST) Activities in Liver and Intestine According to the method of Narayan et al.,12) the activities of UGT isoforms, 1A6, 2B1 and 2B12 in rat liver or intestinal microsomes were measured using 4-MU, testosterone and 1-napthol as respective substrates. The GST activity in the soluble fraction of liver and intestine were measured in accordance with the method of Habig et al.,13) using CDNB as a substrate.

Extraction and Assay Procedure for Liquid Chromatography–Mass Spectrometry (LC-MS) Method The assay for SQV in all biological samples was performed by an LC-MS method we developed.14) The extraction procedure of SQV in plasma and bile samples had been already described in our previous reports.15) For the in vitro microsomal samples, to the resultant aqueous layer after adding 100 μl of ice-cold 2 M K2PO4, 0.8 ml of diethyl ether was added, and then the mixture was volaxed vigorously for 15 s, and was centrifuged at 12000×g for 5 min. For the extraction of CsA and KCZ in plasma, to 100 μl aliquots of plasma after adding 500 ng cyclosporin D (CsD) as an internal standard, 100 μl of 2% ZnSO4 in 50% methanol solution was added to voltexed vigorously for 15 s, and then the mixture was centrifuged at 12000×g for 5 min. After the supernatant being decanted to a clean microcentrifuging tube, 1.0 ml of diethyl ether was added. Then the mixture was voltexed vigorously for 15 s and was centrifuged at 12000×g for 5 min. The aqueous phase in test tube was frozen in a cold bath at −10 °C and the ether phase was transferred to a clean glass test tube. The organic phase was evaporated to dryness at 50 °C under a stream of nitrogen gas. The residue was reconstituted with 50 μl of mobile phase and then 30 μl was injected into the LC-MS system. Mass spectrometry for SQV and indinavir (IDV) used as
an internal standard was performed utilizing atmospheric pressure chemical ionization (APCI) at a negative mode. The voltages of APCI probe and curved desolvation line (CDL) were set at $-5 \text{kV}$ and $0 \text{V}$, respectively. The voltages of deflectors were set at $-50 \text{V}$, and the peaks of SQV and IDV were detected at 669 and $612 m/\text{z}$, respectively. For CsA, KCZ and CsD, mass spectrometry was performed utilizing the APCI at a positive mode. The voltages of APCI probe and CDL were set at 5 kV and 0 V, respectively. The voltage of deflectors was set at 75 V, and the peaks of CsA, KCZ and CsD were detected at 1203, 531 and 1216 m/\text{z}, respectively. Elution was carried out isocratically at a flow-rate of 0.2 ml/min with 90% acetonitrile containing 1% acetic acid. The flow rate of nebulizing gas (N$_2$) was set at 2.5 l/min. The temperatures of APCI probe, prove and CDL were set at 400 °C and 230 °C, respectively.

**Pharmacokinetic Analysis**

A noncompartmental pharmacokinetic analysis was applied to the plasma concentration-time data using a computer program, WinHARMONY.$^{15}$ The terminal elimination rate constant, $\lambda_z$, was determined by a linear regression of at least three data points from the terminal portion of the plasma concentration–time plots. The area under the plasma concentration–time curve, $AUC$, was calculated using the linear trapezoidal rule up to the last measured plasma concentration, $C_{p(last)}$, and extrapolated to the infinity using a correction term, namely $C_{p(last)}/\lambda_z$. The area under the first-moment curve to the last measured point ($t_{(last)}$) to infinity, namely, $t_{(last)}C_{p(last)}/\lambda_z + C_{p(last)}/\lambda_z$. The terminal elimination half-life, $t_{1/2}$, was determined by dividing $\ln 2$ by $\lambda_z$. The mean residence time (MRT) was calculated by dividing $AUMC$ by $AUC$. The total body clearance ($CL_{tot}$) was calculated by dividing $D/AUC$, where $D$ represents the dose administered. The intestinal exsorption clearance ($CL_{int}$) was calculated by dividing the cumulative amount of SQV in the perfusate by the $AUC$. The portal $AUC$ was also calculated by a trapezoidal rule from time zero to 120 min after administration of SQV. The in vitro kinetic parameters, Michaelis constant ($K_m$) and maximum reaction rate ($V_{max}$), were estimated by a non-linear least square method utilizing MULTI program,$^{16}$ and then the in vitro clearance rate was given as $V_{max}/K_m$.

The values from in vivo and in vitro studies are expressed as the mean±S.E. Statistical comparisons of in vivo data and in vitro pharmacokinetic parameters were performed using one-way analysis of variance (ANOVA) followed by Tukey’s multiple range test with a significance level of below 0.05.

### RESULTS

#### In Situ Intestinal Exsorption of Rho123 and SQV in Ethanol-Treated Rats

Figures 1 and 2 show the intestinal exsorption of Rho123 or SQV from the blood circulation to the intestinal lumen after intravenous administration to the NT rats and Day 14 rats. The pharmacokinetic parameters of Rho123 and SQV are listed in Table 1. The exsorbed amounts of Rho123 and SQV at every interval increased significantly (Figs. 1a, 2a), while plasma concentrations of Rho123 and SQV in the Day 14 rats were reduced (Figs. 1b, 2b). The total amount of Rho123 excreted over 120 min in the NT and Day 14 rats were 0.511±0.151 and 1.133±

<table>
<thead>
<tr>
<th></th>
<th>$AUC$ (μg h/ml)</th>
<th>$t_{1/2}$ (h)</th>
<th>$CL_{tot}$ (ml/h)</th>
<th>$CL_{int}$ (ml/h)</th>
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<tr>
<td>Rho123</td>
<td>NT 0.151±0.018</td>
<td>2.1±0.4</td>
<td>372.4±35.3</td>
<td>3.64±0.82</td>
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<td></td>
<td>Day 14 0.104±0.008*</td>
<td>1.8±0.3</td>
<td>442.1±47.6*</td>
<td>11.63±1.86**</td>
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<tr>
<td>Saquinavir</td>
<td>NT 3.783±0.132</td>
<td>1.0±0.1</td>
<td>399.6±12.7</td>
<td>0.19±0.03</td>
</tr>
<tr>
<td></td>
<td>Day 14 2.235±0.761**</td>
<td>0.9±0.2</td>
<td>741.1±84.6**</td>
<td>1.12±0.37*</td>
</tr>
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**$p<0.01$ compared to the control; *$p<0.05$ compared to the NT rats.**

Fig. 1. In Situ Exsorption of Rho123 from the Bloodstream to the Intestinal Perfusate (a) and Plasma Concentration (b) after Intravenous Administration of Rho123 (0.17 mg/kg) to NT (○, ○) and Day 14 (■, ●) Rats

After intravenous administration, the perfusate was passed through the intestinal loop placed at the jejunal portion at a flow rate of 1.0 ml/min. Each column with bar or each symbol with bar represents the mean±S.E. of 6 rats. *$p<0.05$, **$p<0.01$. 

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Table 1. Pharmacokinetic Parameters of Rhodamine 123 and Saquinavir after Intravenous Administration to Rats in the in Situ Perfusion Method

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Table 2. Effect of Inhibitors for CYP3A and P-gp on the AUC Measured in Portal Vein

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<th>Rat</th>
<th>Portal AUC&lt;sub&gt;0—120&lt;/sub&gt; (µg min/ml)</th>
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<td>NT</td>
<td>Control 25 µM KCZ 10 µM CsA</td>
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<tr>
<td>Day 14</td>
<td>45.5±10.9 50.9±9.1 105.5±17.3**</td>
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<td>7.3±2.1 34.5±7.3** 54.5±3.6**</td>
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Each point with bar represents the mean±S.E. of 5 to 6 rats. **p<0.01 compared to respective control, a) p<0.001 compared to the CsA treated group in the NT rats.

notable changes as compared to the rats without inhibitor (control), whereas in the presence of 10 µM CsA the portal plasma concentration of SQV was increased significantly (Fig. 4a). In the Day 14 rats, the portal plasma concentration of SQV in the presence of 25 µM KCZ or 10 µM CsA increased significantly (Fig. 4b). The portal AUC<sub>0—120</sub> of SQV in the Day 14 rats decreased in all categorical rats, and in the presence of 10 µM CsA the portal AUC<sub>0—120</sub> in the in the Day 14 rats was one-half as compared to that in the NT rats (Table 2).

Biliary Excretion of SQV after Intravenous Administration in Ethanol-Treated Rats The biliary excretion of SQV after intravenous administration in the NT and Day 14 rats was shown in Fig. 5. There was no significant difference in the flow rate of bile between the NT and Day 14 rats (Fig. 5b). The cumulative amounts of SQV excreted into the bile over 120 min in the NT and Day 14 rats were 36.9±5.2 and 65.0±6.6 µg, respectively, and it was found to be that the biliary excretion of SQV in the Day 14 rats was 1.8-fold as compared to that in the NT rats.

Effect of Ethanol Consumption on SQV Metabolism in Liver or Intestinal Microsomes In vitro metabolism of SQV in the liver and intestinal microsomes from the NT and Day 14 rats were represented using a S–V plots in Fig. 6. The cumulative amounts of SQV excreted into the bile over 120 min in the NT and Day 14 rats were 36.9±5.2 and 65.0±6.6 µg, respectively, and it was found to be that the biliary excretion of SQV in the Day 14 rats was 1.8-fold as compared to that in the NT rats.

In Situ Absorption of SQV in Ethanol-Treated Rats The systemic plasma concentrations of CsA and KCZ after intralooop administration of SQV in normal rats during in situ loop method were shown in Fig. 3. The mean plasma concentrations of CsA or KCZ comes from 10 µM CsA or 25 µM KCZ were 0.08 and 0.29 µg/ml, respectively. Figure 4 shows effect of 10 µM CsA or 25 µM KCZ on the portal plasma concentration of SQV in the portal vein in the NT and Day 14 rats. The values of AUC in the portal vein over 120 min (AUC<sub>0—120</sub>) were listed in Table 2. In the NT rats, the portal plasma concentration of SQV in the presence of 25 µM KCZ showed no

Fig. 3. Mean Concentration of CsA (▲) and KCZ (●) in the Systemic Plasma as Inhibitors after Intraloop Administration of 10 µM CsA or 25 µM KCZ to Normal Rats

The looped-jejunum was preincubated by 10 µM CsA or 25 µM KCZ for 30 min, then the absorption experiment was started. Each symbol with bar represents the mean±S.E. of 3 rats.

Fig. 2. In Situ Exsorption of SQV from the Bloodstream to the Intestinal Perfusate (a) and Plasma Concentration (b) after Intravenous Administration of SQV (5.0 mg/kg) to NT (▲, ○) and Day 14 (■, ●) Rats

In the NT rats, the portal plasma concentration of SQV was increased significantly (Fig. 4a). In the Day 14 rats, the portal plasma concentration of SQV in the presence of 25 µM KCZ or 10 µM CsA increased significantly (Fig. 4b). The portal AUC<sub>0—120</sub> of SQV in the Day 14 rats decreased in all categorical rats, and in the presence of 10 µM CsA the portal AUC<sub>0—120</sub> in the in the Day 14 rats was one-half as compared to that in the NT rats (Table 2).

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the NT rats (Fig. 6b). The values of \( K_m \) for the NT and Day 14 rats in the intestinal microsomes were 66.3±15.2 and 93.5±24.6 \( \mu \text{M} \), respectively, and in the liver microsomes were 37.2±14.9 and 42.3±10.2 \( \mu \text{M} \), respectively. The values of \( V_{\text{max}} \) for the NT and Day 14 rats in the intestinal microsomes were 5.3±1.6 and 12.4±2.8 \( \mu \text{mol/min/mg protein} \), and in the liver microsomes 4.7±0.8 and 4.3±0.4 \( \mu \text{mol/min/mg protein} \), respectively. The values of metabolic clearance rate (\( V_{\text{max}}/K_m \)) of SQV in the intestinal microsomes for the NT and Day 14 rats were 80.1±6.2 and 132.6±13.2 \( \mu \text{l/min/mg protein} \), respectively, and in the liver microsomes 126.3±12.0 and 101.7±4.5 \( \mu \text{l/min/mg protein} \), respectively.

**Effects of Ethanol Consumption on Phase II Metabolism of SQV** To check if the phase II metabolism processes affect the pharmacokinetics of SQV during ethanol consumption, UGTs and GST activities were measured using known substrates and SQV in the NT and Day 14 rats. As shown in Fig. 7, the UGT2B12 activity in the liver (Fig. 7a) and intestine (Fig. 7b) using 1-naphthol as a known substrate, increased significantly in the Day 14 rats. On the other hand, the activities of UGT1A6 (4-MU as a known substrate) and UGT2B1 (testosterone as a known substrate) in the liver and
intestine showed no changes between the control and Day 14 rats. However, SQV was not metabolized directly by the UGTs in the NT and Day 14 rats. Moreover, as shown in Fig. 8, the GST activities in the liver (Fig. 8a) and intestine (Fig. 8b) using CDNB as a known substrate increased significantly in the Day 14 rats as compared to the NT rats, but SQV was not metabolized directly by the GST in both rat groups.

DISCUSSION

The efflux pump, P-gp has been shown to be present and function as a transporter in the plasma membranes of many normal tissues, and P-gp in the enterocyte brush border limits the bioavailability of many drugs that are metabolized by CYP3A. Recently, in the mdr1a(−/−) knockout mouse, lacking P-gp expression, the brain levels of SQV, indinavir and nelfinavir were 7- to 35-fold higher than in syngeneic wild-type mice. More recently, it was confirmed that SQV is transported via a multidrug resistance-associated protein isoform 2 (MRP2), but not MRP1, MRP3, MRP5 and breast cancer resistance protein 1 (BCRP) in human-related culture cells. In addition, the expression of a multidrug resistance-associated protein isoform 2 (mrp2) which locates in the apical brush-border membrane of the enterocyte or in the bile-duct lateral membrane of hepatocytes has been proved. Therefore, SQV is a potent substrate for CYP3A, P-gp and mrp2 in rats. Essentially, a significant increase in the intestinal exsorption and biliral excretion of SQV suggests that SQV excretion from the systemic circulation was facilitated by enhanced efflux due to ethanol consumption (Figs. 2, 5), where P-gp function might be greatly facilitated. Although it is considered that mrp2, which locates in the apical brush-border membrane of the enterocyte and in the bile-duct lateral membrane of hepatocyte, are also related to the SQV excretion, the magnitude of contribution of this transporter under ethanol consumption is unknown.

Generally, it is accepted that the total body clearance of a drug after intravenous administration reflects mainly the hepatic clearance that the drug is metabolized via CYP enzymes in the liver. However, as shown in Fig. 6a, enzymatic activity in the liver microsomes from the Day 14 rats had no change as compared to the NT rats, suggesting that an increase in the CLint of SQV in the Day 14 rats is not responsible for the CYP enzymes in the liver. On the other hand, a significant increase in the biliary excretion of SQV was observed in the Day 14 rats after intravenous administration (Fig. 5), suggesting an enhancement of P-gp efflux system that is adjacent to the inner side of bile duct. This speculation is further supported by the fact that a significant increase in the CLtot of SQV with unchanged values of t1/2 was found in the Day 14 rats after intravenous administration (Table 1). Contrary, the enzymatic activity for SQV in the intestinal microsomes from the Day 14 rats increased (Fig. 6b), suggesting that an increase in the CYP activities except for CYP2E1 contributes partly to the low bioavailability of SQV after oral administration in cooperating with an enhanced intestinal P-gp efflux by ethanol consumption.

In order to examine whether the lowering of SQV bioavailability after oral administration in the Day 14 rats originates in which of CYP3A or P-gp, SQV concentrations in the portal vein were measured after intraloop administration of SQV in the presence of KCZ or CsA (Fig. 4). Since the plasma free fraction of CsA and KCZ are 5.0 and 4.0%, respectively, their substantial plasma concentrations, which show pharmacological effect, are far below (Fig. 3) than those that reported in vitro studies using hepatic microsomal fraction or Caco-2 cell monolayers. Hence, the results in Fig. 4 show the effect of CsA or KCZ on the intestinal efflux system or the intestinal metabolism. Essentially, in the Day 14 rats, the portal AUC0−120 of SQV in every treated group showed significant decreases as compared to the NT rats, suggesting that the intestinal exsorption was facilitated in the Day 14 rats. The portal AUC0−120 of SQV in the presence of 10 µM CsA in the NT rats increased significantly by 2.3-fold as compared to the control, but not in the presence of 25 µM KCZ (Table 2). In the Day 14 rats, the portal AUC0−120 of SQV in the presence of 25 µM KCZ or 10 µM CsA increased significantly by 4.7- or 7.5-fold as compared to their controls (Table 2). However, in the Day 14 rats the portal AUC0−120 in the presence of 10 µM CsA was one-half lower than that in the NT rats. Since KCZ and CsA are potent inhibitors for CYP3A and P-gp, respectively, these observations clearly indicate that the intestinal disposition of SQV mainly depends on the enhanced efflux system caused by ethanol consumption. As one of underlying mechanisms, it is speculated that an activation of P-gp function or an enhancement of P-gp expression in the intestinal cells due to ethanol consumption is responsible for the enhanced efflux of SQV in the intestine. On the other hand, it is reported that CsA inhibits not only P-
gp but also other transporters such as MRPI, MRP2 and BCRP. In addition, it is evidenced that SQV is a substrate for MRPI but not for MRP1 and BCRP. Therefore, further detailed experiments are required to clarify the contribution of MRPI that locates in the liver and intestine to the excretion of SQV during ethanol consumption.

Our data regarding the pharmacokinetic interaction between indinavir (IDV) and SQV after oral administration showed that the value of SQV bioavailability with IDV increased markedly from 0.024 to 0.204. Assuming the metabolism of SQV occurs at the liver and intestine, the hepatic and intestinal availability of SQV increased from 0.360 to 0.497 and from 0.066 to 0.412, respectively. Therefore, co-administered with IDV, the intestinal availability contributes strongly to a noteworthy increase in the oral bioavailability of SQV. These observations strongly indicate that the intestinal interaction between SQV and other drugs such as indinavir, which inhibits the P-gp function, contributes greatly to increasing in the oral bioavailability of SQV. In contrast, it is suggested that the intestinal interaction between SQV and a P-gp inducer such as ethanol leads to decreasing in the oral bioavailability of SQV.

For the phase II metabolism as shown in Figs. 7 and 8, the UGT2B12 activity by 1-naphthol and GST activity by CDN in the liver and intestine increased in the Day 14 rats. Munoz et al. reported that glutathion levels and GST activity by CDN in the liver cells increased significantly by at least 2-week consumption of 36% ethanol diet in rats. The phase II biotransformation pathways are an important detoxification route for electrophilic metabolites of xenobiotics and reactive oxygen intermediates, which are derived from the process of phase I biotransformation via CYP enzymes. However, from the facts that CYP3A activity for SQV in the liver did not change in the Day 14 rats, and that SQV itself was not metabolized directly by UGTs or GST, these enhanced phase II metabolism induced by ethanol is not considered to be possible factor to decrease the oral bioavailability of SQV.

In conclusion, main possible mechanism to explain the reducing effect of SQV bioavailability after oral administration during ethanol consumption is an enhanced efflux of SQV from the systemic circulation to the intestinal lumen or bile duct. Where, a functional enhancement or excessive expression of P-gp, which was induced by ethanol consumption, was suspected as one of factors to decrease the SQV oral bioavailability. Although CYP2E1 or other CYP enzymes were induced by ethanol, they did not seem to be responsible for the reducing effect on the SQV oral bioavailability during ethanol consumption. In addition, a phase II metabolism induced by ethanol was not likely to have contribution to the lowering SQV oral bioavailability.

REFERENCES