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Hepatic and intestinal contributions to pharmacokinetic interaction of indinavir with amprenavir, nelfinavir and saquinavir in rats

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To elucidate the aspects of pharmacokinetic interactions among HIV protease inhibitors (PIs), we investigated the effects of indinavir (IDV) on the hepatic and intestinal first-pass metabolism of other HIV PIs, amprenavir (APV), saquinavir (SQV) and nelfinavir (NFV), in rats. After oral co-administration with IDV, the area under the concentration versus time curves (AUC) of APV, SQV and NFV increased significantly by 1.6-, 9.5- and 2.3-fold, respectively, compared with mono-administration. After intravenous administration, the AUC of APV, SQV and NFV also increased in the presence of IDV by 1.4-, 1.2- and 1.5-fold, respectively. Mean concentrations of APV, SQV and NFV in the liver extracellular fluid, measured using a liver microdialysis method, were very low compared with their Michaelis constants regardless of co-administration of IDV, suggesting that APV, SQV and NFV metabolism follows linear kinetics in the liver. This finding also indicates that metabolism of PIs depended on the metabolic clearance rate in the liver microsomes. The oral bioavailability of SQV in the presence of IDV increased markedly by 8.5-fold, and that of APV and NFV also increased by 1.2- and 1.5-fold, respectively. On the basis of the well-stirred model, the hepatic availabilities of APV, SQV and NFV in the presence of IDV increased by 1.1-, 1.4- and 1.5-fold, and the intestinal availabilities increased by 1.1-, 6.2- and 1.1-fold, respectively. These results suggest that both hepatic and intestinal metabolism were essentially involved in the interactions between IDV and other HIV PIs, and the degree of those contributions varied with each combination of HIV PIs.

Keywords: HIV protease inhibitor, indinavir, amprenavir, saquinavir, nelfinavir, pharmacokinetic interaction, first-pass effect,

Introduction

A combination therapy with two kinds of reverse transcriptase inhibitors and an HIV protease inhibitor (PI), namely highly active antiretroviral therapy (HAART), has significantly delayed the progression of HIV-related disease and prolonged the survival of AIDS patients (Finzi *et al.*, 1999). More recently, treatment with two kinds of HIV PIs (double-protease therapy) has been advocated because of the poor adherence, side-effects and virus resistance with HAART. However, when selecting a combination of HIV PIs, preventing drug interactions remains one of the major problems because all the PIs are metabolized via CYP3A and are substrates and/or inhibitors of the membrane efflux transporter, P-glycoprotein (P-gp) (Caroline *et al.*, 1998; Barry *et al.*, 1999; Coo *et al.*, 2000).

Currently, five HIV PIs, indinavir (IDV), amprenavir (APV), saquinavir (SQV), nelfinavir (NFV) and ritonavir (RIV) have been used in clinical practice for the treatment of AIDS patients (Williams & Sinko, 1999). IDV is a potent and highly selective inhibitor of HIV protease. It is a substrate of the CYP3A system and P-gp in rats, humans and various *in vitro* systems (Lin *et al.*, 1982; Chiba *et al.*, 1997; Kim *et al.*, 1998). According to previous studies (Yamaji *et al.*, 1999; Shibata *et al.*, 2000), the rank order of *in vitro* liver metabolic clearance rate of these drugs is SQV>NFV>IDV>APV>RIV, and the degree of *in vivo* pharmacokinetic interaction between any two-drug combination, selected out of these five, is highly variable. Although, IDV had the strongest inhibitory effect on APV metabolism in rat liver microsomes, the *in vivo* effects of an HIV PI, after co-administration with IDV, cannot always be predicted from *in vitro* results, suggesting that other interaction process occur besides metabolism in the liver (Shibata *et al.*, 2002). In the case of some drugs, the role of intestinal metabolism may be greater than that of hepatic

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metabolism in the overall first-pass effect (Thummel *et al.*, 1996; Kim *et al.*, 1999; Kim *et al.*, 2000). Therefore, in the case of interaction among HIV PIs, the drug–drug interaction is thought to occur in both liver and intestine. However, the degree of hepatic or intestinal contribution for drug–drug interaction between HIV PIs is still not clear.

The purpose of the present investigation was to determine the hepatic and intestinal availabilities of APV, SQV and NFV in combination with IDV, and to find out whether there is any change in the degree of contribution of hepatic or intestinal metabolism to pharmacokinetic interactions following co-administration of HIV PIs in rats.

Materials and methods

Chemicals

IDV, APV and SQV were kindly supplied by Vertex Pharmaceuticals (Cambridge, Mass., USA), Merck Sharp & Dohme Research Laboratories (Rahway, NJ, USA) and Hoffman-LaRoche (Nutley, NJ, USA), respectively. NFV was extracted from commercial tablets, and the crude extract was purified by a preparative high performance liquid chromatography (HPLC) method. The chemical structures of the four PIs are shown in Figure 1. N-methyl-2-pyrrolidone (Pharmasolve®) was kindly supplied by International Specialty Products (Tokyo, Japan). Acetonitrile (HPLC grade) and diethyl ether were obtained from Kanto Chemical Co. (Tokyo, Japan). All other reagents were of analytical grade and were used without further purification.

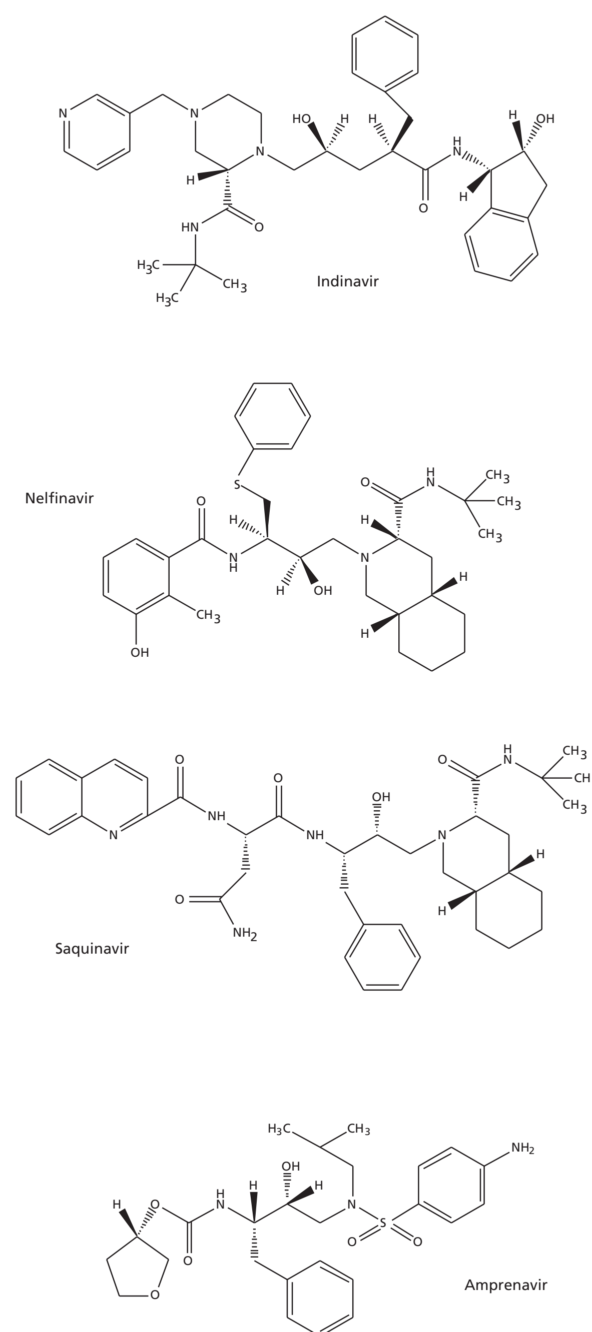
Preparation of standard and test solutions

The standard stock solutions of the four HIV PIs were prepared by dissolving them in ethanol at various concentrations and were stored at 4°C in the dark. Calibration curve samples were prepared by adding known amounts of these standard stock solutions to plasma or dialysate in a volume ratio of 1:100. The test solutions of HIV PIs for intravenous administration were prepared by dissolving 50 mg of each HIV PI in 10 ml of a vehicle composed of 5% ethanol, 5% polyoxyethylene(40) hydrogenated castor oil (HCO-40) and 5% Pharmasolve® in distilled water, and for oral administration by suspending 200 mg of each HIV PI in 2% aqueous sodium carmellose dispersion.

Animals

Male Wistar rats, weighing approximately from 280 to 320 g were obtained from Nippon SLC Co. (SLC, Hamamatsu, Japan). Rats had free access to food and water and were maintained in a temperature-controlled facility with a 12 h light/dark cycle for at least 1 week before use.

Figure 1. Chemical structures of HIV protease inhibitors



All animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of Kyoto Pharmaceutical University.

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Oral administration

Rats were fasted for at least 12 h with free access to water. They were given orally each HIV PI alone or in combination with IDV by stomach intubation at a dose of 20 mg/kg. To avoid a possible effect of physiochemical interactions between IDV and other PIs in the gastrointestinal tract, IDV was given to rats 30 min before the administration of APV, SQV and NFV. Then, 0.25 ml aliquots of blood samples were collected into heparinized centrifuging tubes at 5 min before administration and at 0.5, 1, 1.5, 2, 3, 4 and 6 h after administration. The plasma samples were obtained by centrifuging blood samples at 9000 *g* for 10 min and were immediately frozen in a deep freezer at −20°C until analysis.

Liver microdialysis after intravenous administration

For the hepatic microdialysis, under pentobarbital anaesthesia (32 mg/kg), the liver was exposed by making a midline incision beginning at the xiphoid cartilage and extending it by about 2.5 cm. A needle supplied with the microdialysis probe (PC-20, BAS Tokyo, Japan) was used to gently pierce the outside surface of the liver tissue to make a small hole in the center of the median lobe of the liver and parallel to the midline. A microdialysis probe was then inserted into the liver through the hole made by the needle and fixed by a surgical glue (Aron Alpha®, Sankyo Co., Tokyo, Japan). The rat plasma, diluted to 50% with 0.9% saline, was passed through the probe as a dialysate at a constant flow rate (2.5 µl/min) using an automatic infusion pump (MF-9090, BAS Inc., USA). After 1 h of stabilization, APV, SQV and NFV were intravenously administered at a bolus dose of 5 mg/kg either alone or in combination with IDV (5 mg/kg). Then, 0.25 ml aliquots of blood samples were collected into heparinized centrifuge tubes 5 min before administration and at 2, 15, 30 min and at 1.5, 2.5, 3.5 and 4.5 h after administration. The plasma samples were obtained by centrifuging blood samples at 9000 *g* for 10 min. The dialysates were collected at 1, 2, 3, 4 and 5 h post intravenous bolus administration. All biological samples were stored at −80°C until analysis.

Assay of HIV PIs in plasma and dialysate

The extraction procedure and liquid chromatography-mass spectrometry (LC/MS) analysis used for biological samples were similar to those described previously (Gao *et al.*, 2002). Briefly, an equal volume of 2 M K_3PO_4 was added to 100 µl aliquots of plasma or 150 µl aliquots of dialysate and mixed. Then, 1 ml of diethyl ether was added, the mixture was vortexed for 30 s and centrifuged at 12 000 *g* for 5 min. The aqueous phase was frozen in an ethanol-cooled bath at −10°C, and the ether phase was transferred to a clean HPLC vial. The ether phase was evaporated to dryness at 40°C in a water bath with air draught. The residues were reconstituted with 50 µl of the mobile phase, and a 30 µl sample was injected to the LC/MS system. All elutions of the four HIV PIs were completed within 5 min.

Data analysis

To estimate the *in vivo* recoveries (R_{vivo}) of HIV PIs, the microdialysis probe was perfused with 0.5, 1.0 and 2.0 µg/ml of the four HIV PIs in a perfusate of 50% rat plasma in saline at a flow rate of 2.5 µl/min. The inlet (C_{in}) and outlet (C_{out}) concentrations of the four HIV PIs were determined by the LC/MS method. The recoveries of the four HIV PIs were calculated by the following equation:

$$(C_{in}-C_{out}) = R_{vivo}\times C_{in} \quad \text{(equation 1)}$$

The above equation was used with the assumption that the amount of drug across the probe from tissue to the perfusate is equal to the amount of drug from the perfusate to the tissue. The value of R_{vivo} was calculated by plotting ($C_{in}-C_{out}$) against C_{in}, and was given as the slope of linear regression line. The values of R_{vivo} for IDV, APV, NFV and SQV were 0.62, 0.17, 0.19 and 0.36, respectively. The concentrations of the four HIV PIs in the liver extracellular fluid (ECF), C_{ECF}, after intravenous administration were calculated using the following equation (Sato *et al.*, 1996):

$$C_{ECF} = C_{out}/R_{vivo} \quad \text{(equation 2)}$$

The hepatic intrinsic clearance ($Cl_{int,H}$) for single and co-administration was calculated using the following equations:

in the case of single administration

$$Cl_{int,H} = \frac{V_{max}\times MY_H\times W_H}{(K_m+C_{ECF})\times f_{m,H}} \quad \text{(equation 3)}$$

in the case of co-administration

$$Cl_{int,H} = \frac{V_{max}\times MY_H\times W_H}{[K_m\times(1+C_{ECF,i}/K_i)+C_{ECF}]\times f_{m,H}} \quad \text{(equation 4)}$$

where V_{max}, K_m, MY_H, W_H, $f_{m,H}$, $C_{ECF,i}$, and K_i represent the maximum reaction velocity in liver microsomes, the Michaelis constant, the protein yield (mg/g) in liver microsomes, the liver weight (g for 300 g body weight of rats),the unbound fraction of individual HIV protease inhibitor in the microsomal reaction mixture, the C_{ECF} of the inhibitor (IDV) and the inhibition constant of IDV for APV, SQV and NFV, respectively. The values of MY_H in rats were taken from the literature, 50 mg/g liver (Houston, 1994), and the value of W_H in rats represents estimate based on

the allometric relationship (Boxenbaum, 1980): liver weight (g/kg)=0.037×(body weight)$^{0.85}$. The values of K_m and V_{max} for APV, SQV and NFV, and the K_i values of IDV for other HIV PIs were taken from our previous report (Shibata *et al.*, 2000). The hepatic clearance (Cl_H) was determined according to the 'well-stirred' model as follows (Pang & Rowland, 1977):

$$Cl_H = \frac{Q_H \times f_B \times Cl_{int,H}}{Q_H + f_B \times Cl_{int,H}} \quad \text{(equation 5)}$$

where f_B is the unbound fraction of each protease inhibitor in the blood, given by $f_B=f_p/R_B$, and f_p and R_B represent unbound fraction of each PI in plasma and blood-to-plasma concentration ratio, respectively. These values were taken from our previous report (Shibata *et al.*, 2002). Q_H represents the liver blood flow, which was equal to 17.6 ml/min. Then, assuming the metabolism of HIV PIs occurs at the liver and intestine, the oral bioavailability, F, can be defined as a product of three availabilities as follows:

$$F = F_A \times F_G \times F_H \quad \text{(equation 6)}$$

where F_A is the fraction of drug absorbed from the intestinal tract, and F_H and F_G are the availabilities in the liver and intestine, respectively. The value of F was calculated by the equation:

$$F = \frac{AUC_{po} \times Dose_{iv}}{AUC_{iv} \times Dose_{po}} \quad \text{(equation 7)}$$

where AUC_{po}, AUC_{iv}, $Dose_{po}$ and $Dose_{iv}$ represent the area under the concentration versus time curve (AUC) following oral (po) or intravenous (iv) administration and the oral and intravenous doses, respectively. The value of F_H was obtained by the following equation:

$$F_H = 1-(Cl_{tot}/Q_H) \quad \text{(equation 8)}$$

The intestinal clearance (Cl_G) was calculated as follows:

$$Cl_G = \frac{Cl_{tot}-Cl_H}{F_H} \quad \text{(equation 9)}$$

where Cl_{tot} represents the total body clearance after intravenous administration. Consequently, the hybrid intestinal availability ($F_A \times F_G$) was calculated from the equation:

$$F_A \times F_G = F/F_H \quad \text{(equation 10)}$$

The values of AUC_{po}, AUC_{iv}, Cl_{tot} and other pharmacokinetic parameters were obtained by applying a non-compartmental pharmacokinetic analysis to the plasma concentration-time data using the computer program, WinHARMONY (Yoshikawa *et al.*, 1998). The terminal elimination rate constant, λz, was determined by a linear regression of at least three data points from the terminal portion of the plasma concentration-time plots. The AUC was calculated using the linear trapezoidal rule, up to the last measured plasma concentration, Cp(last), and extrapolated to infinity using a correction term, namely Cp(last)/ λz. The area under the first-moment curve to the last measured plasma concentration (AUMC) was also calculated using the linear trapezoidal rule and the addition of the concentration term after the last measured point [t(last)] to infinity, namely, t(last)×Cp(last)/λz+Cp(last)/λz^2. The terminal elimination half-life ($t_{1/2}$) was determined by dividing ln2 by λz. The mean residence time, MRT, was calculated by dividing AUMC by AUC. The apparent clearance after oral administration (Cl_{app}) was calculated by dividing $Dose_{po}$ by AUC_{po}.

Statistical analysis

All values are expressed as the mean ±SD. Statistical differences of the means were assumed to be significant when $P<0.05$ (two-sided *t*-test).

Results

To prepare the intravenous test solutions of APV, SQV and NFV, a mixed vehicle composed of 5% ethanol, 5% HCO-40 and 5% Pharmasolve® in distilled water was used. IDV was used to investigate the effect of the vehicle on its pharmacokinetic profile, because IDV is the only water-soluble drug among the HIV PIs tested. IDV plasma concentration versus time curves, after rats were given intravenous IDV dissolved in the mixed vehicle or saline, are shown in Figure 2. The curves for the two vehicles were very similar, indicating that the mixed vehicle had no marked effect on IDV pharmacokinetic profile in rats. Therefore, this vehicle was used as solvent for the intravenous administrations of IDV, APV, SQV and NFV in the hepatic microdialysis studies.

The plasma concentration versus time profiles of APV, SQV and NFV given alone or by oral co-administration with IDV are shown in Figure 3. The pharmacokinetic parameters based on the non-compartmental pharmacokinetic analysis are summarized in Table 1. There were no episodes of diarrhoea in rats when 2% of sodium carmellose was used in oral study. Though the dissolution rate of a drug is dependent on the concentration of sodium carmellose, which may reflect the time to peak plasma

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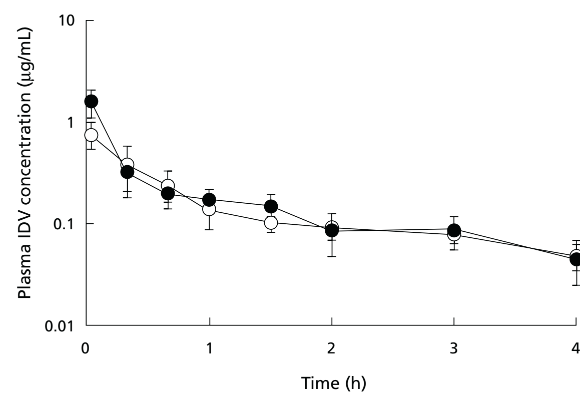
Figure 2. Plasma concentration-time profiles following intravenous administration of indinavir (5 μg/kg) in saline or mixed vehicle

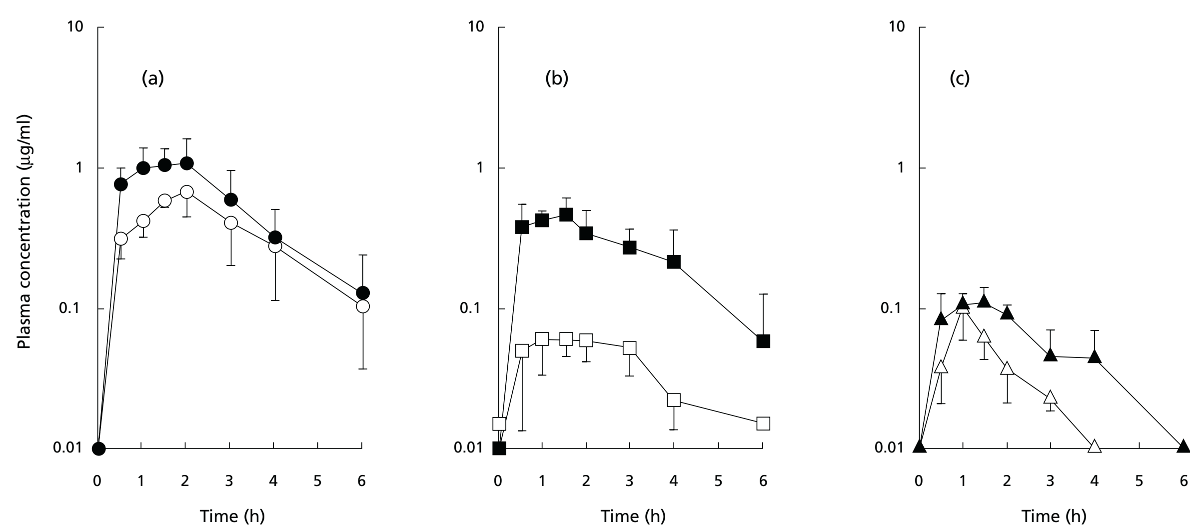


Indinavir (IDV) was dissolved in saline (closed circles) or mixed vehicle (open circles) containing 5% EtOH, 5%HCO-40 and 5% Pharmasolve® in distilled water. Bars represent the mean ±SD of three rats.

concentration, it is considered not to have any effect in comparing the contribution of the metabolism. The plasma concentrations of all the three HIV PIs were higher in the presence of IDV than those for single administration. When APV, SQV and NFV were co-administered with IDV to rats, the C_{max} values increased significantly for APV from 0.71 ±0.18 μg/ml to 1.20 ±0.45 μg/ml (P<0.05), and for SQV from 0.07±0.02 to 0.58±0.05 μg/ml (P<0.01). In the presence of IDV, the AUC values of all three HIV PIs after oral administration increased significantly compared with those in the absence of IDV, by 1.6-, 9.5- and 2.3-fold for APV, SQV and NFV, respectively. The presence of IDV significantly decreased the Cl_{app} from 42.22 ±12.55 to 4.18 ±1.63 l/h for SQV (P<0.01) and from 33.10 ±7.96 to 16.10 ±6.63 l/h for NFV (P<0.05).

The pharmacokinetic profiles after intravenous administration of HIV PIs either alone or in combination with IDV and the concentrations of HIV PIs in the liver ECF using the microdialysis method are shown in Figures 4 and 5, respectively. The pharmacokinetic parameters based on the non-compartmental pharmacokinetic analysis and the mean C_{ECF} ($C_{ECF,ave}$) of the HIV PIs are summarized in Table 2. The value of $C_{ECF,ave}$ for HIV PIs in the liver was estimated by dividing AUC of the C_{ECF} versus time curve by the entire dialysate sampling time interval. After intravenous co-administration with IDV, the $C_{ECF,ave}$ values increased by 1.6-, 1.7 and 5.4-fold for APV, SQV and NFV, while their AUC values increased by 1.2-, 1.4- and 1.5-fold, respectively. However, there were no significant changes in the initial concentration after intravenous administration (C_0), AUC, $t_{1/2}$, MRT and Cl_{tot} of APV and SQV, but the C_0 and AUC of NFV increased significantly (P<0.01). During this experiment, the mean

Figure 3. Plasma concentration-time profiles following oral administration of APV, NFV and SQV either alone or in combination with IDV



The dose of each drug was 20 mg/kg. (a) Open circle, amprenavir (APV) alone; closed circle, APV with indinavir (IDV). (b) Open square, saquinavir (SQV) alone; closed square, SQV with IDV. (c) Open triangle, nelfinavir (NFV) alone; closed triangle, NFV with IDV. Bars represent the mean ±SD of four to six rats.

Table 1. Pharmacokinetic parameters of APV, SQV and NFV after oral administrations alone and with IDV

Case	C_{max} (µg/ml)	AUC (µg×h/ml)	$t_{1/2}$ (h)	MRT (h)	Cl_{app} (l/h)
APV alone	0.71 ±0.18	2.33 ±0.81	1.25 ±0.57	2.63 ±0.57	3.47 ±1.64
+ IDV	1.20 ±0.45*	3.78 ±1.38*	1.45 ±0.51	2.81 ±0.57	2.62 ±0.97
SQV alone	0.07 ±0.02	0.21 ±0.09	1.85 ±0.68	3.06 ±0.58	42.22 ±12.55
+ IDV	0.58 ±0.05**	1.99 ±0.96**	2.33 ±2.02	3.67 ±2.32	4.18 ±1.63**
NFV alone	0.11 ±0.01	0.21 ±0.08	0.98 ±0.35	1.98 ±0.39	33.10 ±7.96
+ IDV	0.15 ±0.05	0.49 ±0.18*	1.63 ±1.32	3.09 ±1.89	16.10 ±6.63*

The pharmacokinetic parameter values were calculated by a non-compartmental analysis. Each value represents the mean±SD of three to six rats.APV, amprenavir; SQV, saquinavir; NFV, nelfinavir; IDV, indinavir; $t_{1/2,}$ half-life; AUC, area under curve; MRT, mean residence time; Cl_{app}, apparent clearance after oral administration.
*P<0.05; **P<0.01, compared with corresponding controls, respectively.

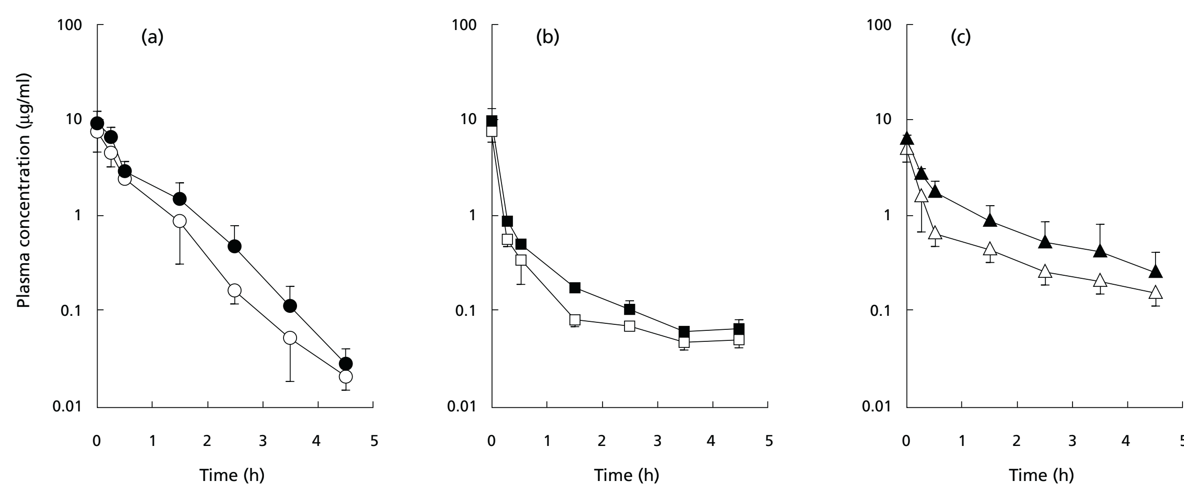
$C_{ECF,ave}$ value of IDV without other HIV PIs was 73 ng/ml, and with APV, SQV and NFV were 204, 21 and 825 ng/ml, respectively (ranged from approximately 0.03–1.35 µM).

Using the mean data of oral and intravenous administrations, the values of F, organ clearances (Cl_H, Cl_G) and availabilities (F_H, F_A×F_G) were calculated (Table 3). The F value of SQV with IDV was substantially different to the F value without IDV. In the presence of IDV, Cl_H of APV and NFV showed about a 10% decrease, whereas that of SQV showed no notable change. On the other hand, Cl_G of APV, SQV and NFV in the presence of IDV showed about 32%, 53% and 85% decrease, respectively. The values of F_H in the presence of IDV increased from 0.695 to 0.769 for APV, from 0.360 to 0.497 for SQV and from 0.481 to 0.711 for NFV. The F_A×F_G value of SQV with IDV showed a noteworthy increase (0.07 versus 0.41).

Discussion

Since the mid 1990s, the clinical management of patients infected with HIV has become more efficient by the introduction of potent and specific HIV PIs. These drugs have provided a dramatic decrease in morbidity and mortality in HIV-infected patients because of a HAART composed of a combination of two kinds of reverse transcriptase inhibitors and an HIV PI. HAART has been found to be a better therapy than each drug alone in reducing HIV RNA levels and increasing CD4 cell counts (Hoetelmans *et al.*, 1998). However, treatment failure

Figure 4. Plasma concentration-time profiles following intravenous administration of APV, NFV and SQV either alone or in combination with IDV



The dose of each drug was 5 mg/kg. (a) Open circle, amprenavir (APV) alone; closed circle, APV with indinavir (IDV). (b) Open square, saquinavir (SQV) alone; closed square, SQV with IDV. (c) Open triangle, nelfinavir (NFV) alone; closed circle, NFV with IDV. Bars represent the mean ±SD of four to six rats.

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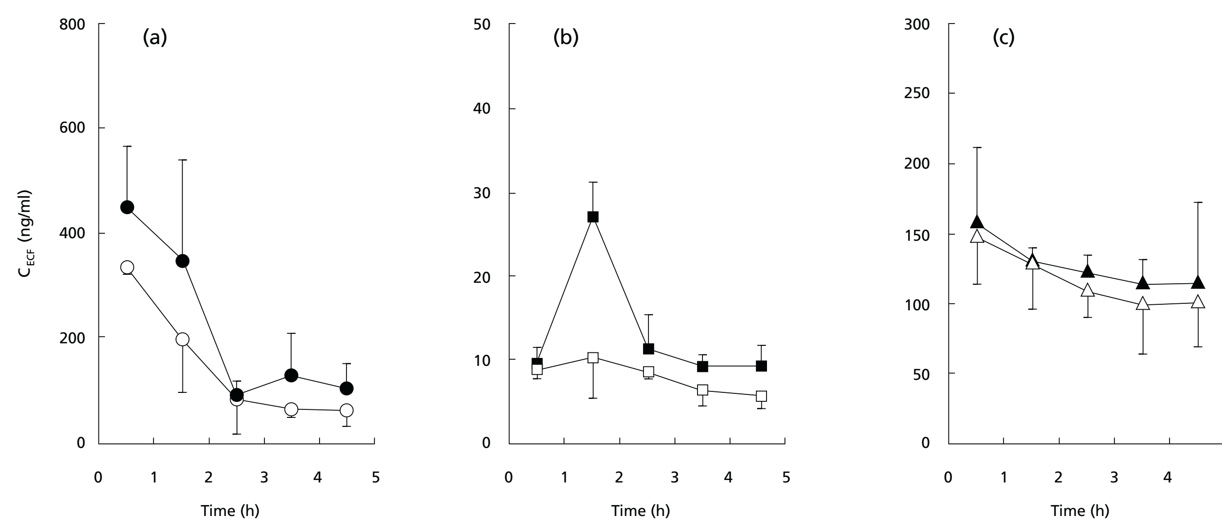
Figure 5. Liver extracellular fluid concentration-time profiles following intravenous administration of APV, SQV and NFV either alone or in combination with IDV



The intravenous dose of each drug was 5 mg/kg. (a) Open circle, amprenavir (APV) alone; closed circle, APV with indinavir (IDV). (b) Open square, saquinavir (SQV) alone; closed square, SQV with IDV. (c) Open triangle, nelfinavir (NFV) alone; closed circle, NFV with IDV. Bars represent the mean ±SD of four to six rats.

(a result of poor adherence to therapy in many cases), development of viral resistance and pharmacokinetic problems have come to light with HAART (Vanhove *et al.*, 1997). Recently, a combination therapy with two kinds of HIV PIs showed clinical effectiveness in preventing development of tolerance by HIV, and this double protease therapy was attempted in clinical practice (Barry *et al.*, 1997). However, it has been difficult to decide which combination of HIV PIs should be used because these drugs have considerable inter- and intra-patient variability in plasma concentrations and marked potential for drug interactions via CYP3A4 (Hsu *et al.*, 1998; Von Moltke *et al.*, 1998).

In our earlier studies (Yamaji *et al.*, 1999; Shibata *et al.*, 2000; Shibata *et al.*, 2002;) on pharmacokinetic interactions among HIV PIs currently used in clinical practice, we have observed the rank order of metabolic clearance rate in rat liver microsome fraction as RIV<APV<IDV<NFV<SQV, and RIV inhibited the metabolism of APV, IDV, NFV and SQV with inhibition constants (K_i) of 2.29, 0.95, 1.01 and 1.64 µM, respectively. However, IDV inhibited the metabolism of APV, NFV and SQV with K_i values of 0.67, 2.76 and 3.55 µM, respectively. Thus, RIV was found to be the strongest metabolic inhibitor for other four HIV PIs in the liver CYP3A system, and IDV showed the smallest value of K_i for APV.

Table 2. Pharmacokinetic parameters after intravenous administrations of APV, SQV and NFV alone and with IDV under liver microdialysis

Case	C_O (µg/ml)	AUC (µg×h/ml)	$t_{1/2}$ (h)	MRT (h)	Cl_{tot} (l/h)	$Cl_{ECF, ave}$ (ng/ml)
APV alone	6.44 ±0.56	5.01 ±0.23	0.65 ±0.05	0.65 ±0.03	0.32 ±0.02	668 ±470
+ IDV	9.08 ±3.07	6.83 ±2.25	0.53 ±0.15	0.75 ±0.13	0.24 ±0.01	1043 ±297
SQV alone	7.88 ±3.29	2.02 ±0.57	2.38 ±0.11	0.98 ±0.18	0.68 ±0.19	38 ±3
+ IDV	9.78 ±2.15	2.50 ±0.59	1.79 ±0.66	0.88 ±0.28	0.53 ±0.11	63 ±11
NFV alone	5.03 ±1.3	3.13 ±1.19	2.03 ±0.52	2.19 ±0.79	0.55 ±0.29	164 ±36
+ IDV	6.31 ±0.29**	4.64 ±0.80**	2.04 ±0.15	2.08 ±0.33	0.31 ±0.05	890 ±501

The pharmacokinetic parameter values were calculated by a non-compartmental analysis. Each value represents the mean ±SD of three to six rats. APV, amprenavir; SQV, saquinavir; NFV, nelfinavir; IDV, indinavir; $t_{1/2,}$ half-life; AUC, area under curve; MRT, mean residence time; Cl_{tot}, total body clearance.
***P*<0.01, compared with corresponding controls, respectively.

Table 3. Hepatic and intestinal clearances and availabilities of APV, SQV and NFV with and without IDV

Case	$Cl_{int,H}$ (ml/min)	Cl_H (ml/min)	Cl_G (ml/min)	F	F_H	$F_A \times F_G$*
APV alone	20.579	0.402	7.160	0.116	0.695	0.167
+ IDV	18.034	0.353	4.841	0.138	0.769	0.180
SQV alone	65.965	4.439	18.988	0.024	0.360	0.066
+ IDV	65.711	4.427	8.911	0.204	0.497	0.412
NFV alone	95.560	4.256	10.167	0.017	0.481	0.035
+ IDV	86.784	4.018	1.502	0.026	0.711	0.037

Theses pharmacokinetic parameter values were calculated using the mean data obtained in *in vitro* and *in vivo* studies according to the equations defined in the text. APV, amprenavir; SQV, saquinavir; NFV, nelfinavir; IDV, indinavir; $Cl_{int,H}$, hepatic intrinsic clearance; Cl_H, hepatic clearance; F, oral bioavailablility; F_H, availability in liver.
*This parameter shows an intestinal availability presented as a hybrid form of product composed of a real fraction absorbed from intestine (F_A) and a fraction unmetabolized in gut cells (F_G).

After oral administration RIV increased the AUC values of APV, SQV, IDV and NFV by 9.1-, 84.0-, 19.3- and 11.2-fold (Shibata *et al.*, 2000), whereas IDV increased the AUC values of APV, SQV and NFV by 1.6-, 9.5- and 2.3-fold, respectively. Thus, the *in vivo* pharmacokinetic interactions between two HIV PIs after oral administration cannot always be predicted by the *in vitro* results with rat liver microsome. In the intravenous administration study, the AUC values of APV, SQV and NFV in the presence of IDV did not show any significant increase compared with oral administration. In addition, the $C_{ECF,\ ave}$ values had no clear correlation with the AUC values after oral administration. The increase in the C_{ECF} levels of APV, SQV and NFV in the presence of IDV indicate metabolic inhibitions by IDV within liver cells, and the increasing effect on the mean of $C_{ECF,ave}$ was in inverse proportion to corresponding K_i values of IDV. Moreover, $C_{ECF,ave}$ values for APV, SQV and NFV were very low compared with their corresponding Michaelis constants, K_m, suggesting that these drugs had linear kinetics in liver metabolism regardless of the presence of IDV at dose used clinically. These observations indicate clearly that there is another site of pharmacokinetic interaction between IDV and other PIs. The small intestine is a possible site for these interactions because the calculated values of Cl_G decreased with sizable differences in the presence of IDV. In the present study, therefore, we have focused on determining whether the contribution of hepatic or intestinal metabolism is altered by combining other HIV PIs, with IDV. Judging from the values of F_H and $F_A \times F_G$ obtained, both liver and intestines contribute to increase the SQV availability when SQV is co-administered orally with IDV. The intestinal contribution was much larger than the hepatic contribution. However, hepatic contributed more to the increase in APV and NFV availabilities when they were orally co-administered with IDV. These observations suggest that both hepatic and intestinal metabolisms were involved in the pharmacokinetic interactions between IDV and other HIV PIs, and also indicate that the degree of hepatic or intestinal contributions vary in combination with IDV.

For drugs that are metabolized via CYP3A, the liver and intestines are the main sites of metabolism. Based on the assumption that all HIV PIs are transformed via CYP3A in rat liver and intestines, we have constructed a physiologically based pharmacokinetic (PB-PK) model to predict pharmacokinetic interaction between HIV PIs (Shibata *et al.*, 2002). We calculated the values of intestinal intrinsic clearance, $Cl_{int,G,}$ using the *in vitro* metabolic clearance rate of each HIV PI in the intestine. Hence, relatively underestimated values of $Cl_{int,G}$ for each PI were obtained. A scaling-up process based on the *in vitro* metabolic clearance rate in the gut is required because it is involved with metabolism via CYP3A, which might cause an under estimation in our study using the PB-PK model (Shibata *et al.*, 2002). However, IDV, SQV, APV and NFV were substrates of both of the membrane efflux transporter, P-gp, which is an ATP-driven efflux pump capable of transporting a wide variety of structurally diverse compounds from the cell interior into the extracellular space and the CYP3A system (Caroline *et al.*, 1998; Kim *et al.*, 1998; Williams & Sinko, 1999; Jerome *et al.*, 2001). Therefore, elimination of the drugs from body through the intestine depends on the synergetic effect of CYP3A and P-gp function. Summarized equations, such as equations 8 and 9 in this study, provided a quantitative increase in the values of Cl_G for APV, SQV and NFV with IDV, compared with our previous study using the PB-PK model, because the value of Cl_{tot} calculated using iv data indicated the total sum of drug removal via CYP3A or P-gp from the blood circulation. Although the degrees of inhibitory effects of IDV against CYP3A or P-gp *in vivo* are unknown at present, these observations correlated well with the view that the intestine is an important site of HIV PIs metabolism. Hence, the overlapping substrate specificities of P-gp and CYP3A for IDV, APV, SQV and NFV result in modulation of the oral bioavailability and subsequent pharmacokinetic changes when they

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are administered in combination. We could assume that two mechanisms were involved in dramatically increasing the oral bioavailability of SQV. However, further detailed studies about the synergetic action of CYP3A and P-gp are required to explain the pharmacokinetic interactions among HIV PIs. In this study, we have focused the interactions between the peptidomemitic PIs. Drug interactions between the new and non-peptidomemitic PIs could be possible and complex, and further studies are going on to investigate the interactions among different types of PIs.

In summary, our results showed the potential drug interactions between IDV and the other three HIV PIs: APV, SQV and NFV. Both hepatic and intestinal metabolisms were involved in the pharmacokinetic interactions between IDV and other HIV PIs, and the degree of contribution varies with every PI and IDV combination. The results of this study may provide useful information for the treatment of AIDS patients when they receive a combination therapy with two kinds of HIV PIs. We are now studying the degree of *in vivo* inhibitory effect of IDV on CYP3A or P-gp activity when double protease inhibitors are administered.

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