Differentiation of Gut and Hepatic First-Pass Effect of Drugs: 1. Studies of Verapamil in Ported Dogs

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Purpose. To investigate the relative contributions of the gut and liver to the first-pass loss of verapamil (VL) using an *in vivo* intestinal-vascular access port (IVAP) dog model.

Methods. Basic pharmacokinetics of VL were determined after intravenous (IV: 0.5 mg/kg), portal venous (PV: 2 mg/kg), and duodenal (ID: 2 mg/kg) administration in IVAP dogs. Serial blood samples were collected for 8 h after dosing, and plasma was analyzed for unchanged drug by a high-performance liquid chromatography-fluorescence method. Extraction ratios in the liver and intestinal tract were determined from the area under the concentration-time curves for ID, PV, and IV administration. The functional role of CYP450 or secretory transporters such as P-gp on the gut and liver first-pass loss of VL was further studied using ritonavir, a known substrate or inhibitor of these processes.

Results. The liver had a high intrinsic capacity for clearing VL because the absolute bioavailability (BA) of VL was 21.7% after PV administration. The BA of VL after ID administration was 23.5%; therefore, intestinal absorption was complete and intestinal extraction was negligible ($ER_{GI} \sim 0$). The BA of VL increased from 23.5% to 66.2% in the presence of ritonavir primarily due to a reduction in hepatic extraction.

Conclusions. Although the liver had a high intrinsic capacity for extracting VL, the contribution of gut to the first-pass loss of VL was negligible. Because of the additive effects of intestinal CYP3A-mediated metabolism and secretory transport, a significant gut first-pass effect was expected, but not observed in dogs. These studies demonstrate the utility of the *in vivo* IVAP dog model for evaluating the relative contribution of the gut and liver to the first-pass loss of drugs and for characterizing the functional role that CYP450 metabolism and/or secretory transporters play in drug–drug interactions and reduced oral bioavailability.

KEY WORDS: pharmacokinetics; oral absorption; first-pass effect; CYP3A; P-glycoprotein; IVAP dogs.

INTRODUCTION

Numerous factors, such as gastrointestinal (GI) motility (i.e., residence time and gastric emptying), regional differences in pH and membrane permeability, digestive proteolytic and colonic microflora enzymatic activity, GI metabolism, and hepatobiliary secretion significantly influence the oral bioavailability (BA) of many drugs. Although our understanding of these factors has increased dramatically in recent years, identifying the specific *in vivo* roles of the gut and liver has been difficult to assess using conventional experimental models.

Cytochrome P450 3A4 (CYP3A4) is an enzyme that plays an important role in the first-pass metabolism of drugs such as cyclosporine and VL after oral administration (1,2). CYP3A4 is highly expressed in the duodenum and jejunum of the small intestine, and its expression level in the small intestine is second only to the liver in humans (3). Recent oral pharmacokinetic studies have suggested that the contribution of gut wall metabolism to the total first-pass loss of cyclosporine, midazolam, and verapamil (VL) was significant (4–7). It has also been suggested that P-glycoprotein (P-gp) and other secretory transporters present in gut epithelial cells may play a role in the intestinal secretion of drugs (6,8). Recent studies have revealed that many substrates of CYP3A4 are also substrates or inhibitors of P-gp, suggesting overlapping substrate specificity between CYP3A4 and P-gp (9). Other researchers have proposed that the synergistic effects of CYP3A4-mediated metabolism and P-gp-mediated secretion in the gut epithelium may result in an unexpectedly high first-pass effect after oral administration (9,10). Thus, the gut may play a significant role in drug–drug interactions as a result of the inhibition or induction of P450s and/or secretory transporters.

Studies in conscious *in vivo* animal models provide a realistic evaluation of the functional role of gut absorption and metabolism compared to *in vitro* and/or *in situ* models. In our group (11,12), beagle dogs were implanted long term with intestinal and vascular access ports (IVAPs) to investigate drug absorption and to assess the potential roles of the intestine and liver. In these studies, a portal vein (PV) port was used to administer VL to assess the role of the liver or take PV blood samples to assess absorption across the intestinal membrane. The roles of the liver and intestine were determined using these tools. VL was chosen as a model drug for these studies because it is completely absorbed into the gut tissue (i.e., absorption fraction, $F_{ABS} = 1$) (13), but a high gut first-pass effect was anticipated because it is a substrate of CYP3A4 and P-gp (6,14).

MATERIALS AND METHODS

Materials

^{3}H-Verapamil HCl was obtained from ARC (St. Louis, MO). Verapamil HCl and propranolol HCl were obtained from Sigma Chemical Co. (St. Louis, MO). Ritonavir (Norvir, 80 mg/mL) and 20G IV catheter, Abbocath® were obtained from Abbott Labs (North Chicago, IL). Intestinal and vascular access ports and 22-G Huber needles were obtained from Access Technologies (Skokie, IL). Heparinized syringe, Monovette® was obtained from Sarstedt (Newton, NC). Dog slings were obtained from Alice King Chatham Medical Arts (Hawthorne, CA). All other materials were obtained from Fisher Scientific (Fair Lawn, NJ) or Sigma Chemical Co. and were used as received.

Preparation of IVAP Dogs

All animal studies were performed under approved protocols (IRB–UCA, Rutgers University and IACUC, Univer-

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sity of Medicine and Dentistry of New Jersey). Male beagle dogs (11–16 kg) were surgically fitted with portal vein (PV) and duodenum (ID) access ports. A detailed description of the surgical procedure is given elsewhere (15). The animals were allowed to recover at least 2 weeks before the initiation of the studies. Six dogs were used and fasted overnight prior to the study. Water was allowed *ad libitum*. During the study, the animals were restrained in a dog sling, and the ports for IVAP infusions were accessed transcutaneously with a 22-G Huber needle. The IVAP dogs were reused once every 1 or 2 weeks.

IV, PV, and ID Bolus Administration

Doses of 0.5, 2, and 2 mg/kg were administered IV, PV, and ID, respectively. The dosing amounts were calculated based on its therapeutic concentration (16) and linear pharmacokinetics (13). The IV dose was administered into the other brachial vein of the dog. The PV or ID doses were injected into the PV or ID port over 1 min with a final flush of 3 mL of saline. The dosing solution was prepared using saline, and the dosing volume was 0.2 mL/kg for IV, PV, and ID, respectively. To insure sterility, all dosing solutions were filtered through Millipore filter (Millex-GV_{13}) before administration. The mean recovery of VL after a single pass through the filter was complete in all cases. Systemic blood samples were drawn at 0, 1, 5, 10, 20, 40, 70, 120, 180, 270, 360, and 480 min through a 20-G IV catheter with a heparin lock, which inserted in the brachial vein, and the catheter was flushed with heparinized saline (50 IU/mL). Two to three milliliters of samples were drawn directly into 4.5-mL Monovette heparinized syringes and placed on ice. For IV and ID administration, PV blood samples were also drawn at the same times from PV ports. Drug interaction studies were also performed using ritonavir, the substrate and inhibitor of P-gp and CYP3A (17–19). Ritonavir (5 mg/kg) was administered orally using hard gelatin capsules (#00) at 30 min before VL dosing. Plasma was prepared within 30 min of sampling by centrifuging the blood sample for 10 min at 3000 × g at 4°C. Analytical quality control samples, 100, 500, and 2500 ng/mL, were prepared by serial dilution of the IV dosing solution, 2.5 mg/mL in saline, by blank dog plasma. The study and quality control samples were stored at −80°C pending analysis by high-performance liquid chromatography (HPLC).

Blood-to-Plasma Concentration Ratio

Three concentrations (10, 50, and 250 μg/mL) of verapamil working solution were prepared in saline containing ^{3}H-verapamil (20 μCi/mL). Two 1-mL aliquots of heparinized dog blood (heparin, 50 IU/mL) and 10-μL aliquots of working solutions were added to make final concentrations of 100, 500, and 2500 ng/mL containing ^{3}H-verapamil (0.2 μCi/mL) (n = 3 for each concentration). After incubation for 30 min at 37°C, a 100-μL aliquot was taken from each blood sample to measure the blood concentration, and the remaining sample was centrifuged for 10 min at 3000 × *g*. After centrifugation, a 100-μL aliquot of plasma was taken to measure the plasma concentration. This 100-μL aliquot of plasma (or blood) was diluted to 100 μL of blood (or plasma for blood sample) and 4 mL of liquid scintillation cocktail, and samples were counted using a liquid scintillation counter. The data were expressed as the ratio of radioactivity, determined by LSC, in equal volumes of blood and plasma, referred to as the blood-to-plasma ratio.

HPLC Analysis

VL in the plasma was extracted using liquid–liquid extraction, and analyzed by HPLC using a C_{18} column (3.9 × 150 mm, particle size 5 μm). The mobile phase was a mixture of acetonitrile and 0.1% trifluoroacetic acid (35:65). VL and its related metabolites were monitored fluorometrically at Excitation 208 nm and Emission 321 nm. Five-hundred microliter of plasma sample was mixed with a 50 μL of internal standard solution (IS: propranolol, 1 μg/mL in water), 100 μL of 1 M Na_2CO_3, and 5 mL of diethyl ether in a 15 mL screw-capped conical polypropylene tube, vortexed for 5 min, and centrifuged at 1500 × g for 10 min. The supernatant was transferred to a 15-mL screw-capped conical polypropylene tube containing 200 μL of 0.1 N HCl, vortexed for 5 min, and centrifuged at 1500 × g for 10 min. The supernatant was discarded while 100 μL of the aqueous phase containing drug, its related metabolites, and IS was injected into the HPLC. The extraction recovery was greater than 95% for VL and IS. The calibration was linear through 5–5000 ng/mL in the plasma, and the detection limit of drug was less than 3 ng/mL based on the signal to noise ratio of 5. Under these conditions, the intra- and inter-run variations were less than 7 and 11%, respectively.

Pharmacokinetic Analysis

Plasma concentration-time data were analyzed by noncompartmental pharmacokinetic methods (20). The highest observed concentration and the corresponding sampling time were defined as C_{max} and t_{max}, respectively. The elimination half-life ($t_{1/2}$) was estimated from $t_{1/2} = \ln 2/\lambda$ where λ is the slope of the regression line that best fit the terminal portion of the log-linear concentration time curve. The area under the concentration time curve (AUC) was calculated by a combination of the trapezoidal and log-trapezoidal methods (21) and then extrapolated to infinity. The total plasma clearance (CL) and the volume of distribution at steady state (V_{dss}) after IV dose were estimated by standard methods, CL = Dose/AUC, V_{dss} = CL · MRT where MRT is the mean residence time (AUMC/AUC). BA, hepatic extraction ratio (ER_H), and gut extraction ratio (ER_{GI}) were calculated by Equations 1–3 on the basis of the AUC and dose (D) values at multiple administration routes.

$$\begin{aligned} BA &= (AUC_{ID}/AUC_{IV}) \cdot (D_{IV}/D_{ID}) \\ &= F_{ABS} \cdot (1 - ER_{GI}) \cdot (1 - ER_H) \end{aligned} \quad (1)$$

$$1 - ER_H = (AUC_{PV}/AUC_{IV}) \cdot (D_{IV}/D_{PV}) \quad (2)$$

$$1 - ER_{GI} = (1/F_{ABS}) \cdot (AUC_{ID}/AUC_{PV}) \cdot (D_{PV}/D_{ID}) \quad (3)$$

where AUC_{ID}, AUC_{PV}, and AUC_{IV} are the ID, PV, and IV AUC values, where D_{ID}, D_{PV}, and D_{IV} are the ID, PV, and IV dose values. F_{ABS} is defined as the fraction of the dose absorbed into the intestinal tissue. In the past F_{ABS} was synonymous with BA; however, as the role of the liver was increasingly recognized F_{ABS} was redefined as the fraction absorbed into the PV (22). However, to account for intestinal cell metabolism and secretory transport F_{ABS} is now defined

as the fraction absorbed into intestinal tissue and F_{GI} ($= 1 - ER_{GI}$) is the fraction entering the PV.

On the other hand, the amount absorbed into portal vein can also be described by

$$= D_{ID} \cdot F_{ABS} \cdot (1 - ER_{GI})$$
$$= \int Absorption\ fluxdt$$
$$= \int Qpv \cdot (CB_{pv} - CBsys)dt$$

Therefore, the ER_{GI} can be calculated by an indirect method using only ID data.

$$1 - ER_{GI} = (1/D_{ID}) \cdot (1/F_{ABS}) \cdot \int Q_{pv} \cdot (C_{Bpv} - C_{Bsys})dt \quad (4)$$

where Q_{pv}, C_{Bpv}, and C_{Bsys} are the portal blood flow rate, the blood concentration in the PV, and the blood concentration in the systemic circulation (brachial vein). C_B can be calculated from the plasma concentration (C_P) using measured blood-to-plasma concentration ratio (C_B/C_P). Portal vein blood flow rate (Q_{pv}) of 30 mL/min/kg was used (23).

Statistical Analysis

All statistical tests were performed using Jandel Sigma Stat (Version 2.0, San Raphael, CA). One-way ANOVA was performed, and a minimum P value of 0.05 was used as the significance. All data are reported as the mean ± standard deviation (SD) unless otherwise noted.

RESULTS

Analytical quality control samples were included in each run to assess the accuracy and precision of the assay procedure. The intra- and inter-run recoveries (mean ± SD, n = 6) were 106.5 ± 7.3% and 94.9 ± 11.2% for 100 ng/mL, 99.4 ± 2.0% and 97.4 ± 3.8% for 500 ng/mL, and 100.9 ± 1.6% and 105.0 ± 4.7% for 2500 ng/mL, respectively. The plot of VL-to-IS peak area ratios versus nominal concentration was linear (0.002856 ± 0.000165, mean ± SD, $r^2 > 0.9999$, n = 6). These results indicated that the analytical procedure is accurate, precise, and reproducible.

The mean plasma concentration time profiles of VL after IV, PV, and ID administration are shown in Figure 1; and the estimated pharmacokinetic parameters are listed in Table I.

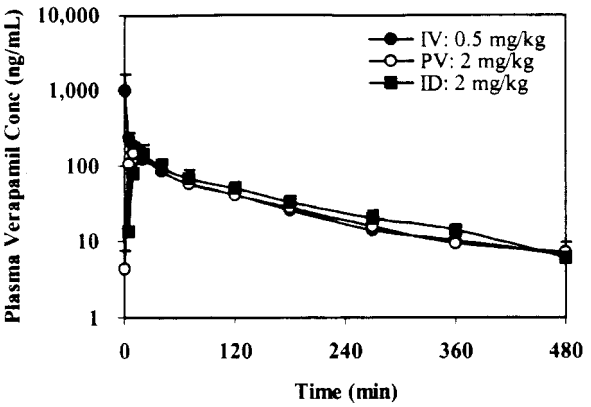

Fig. 1. Plasma concentration (mean ± standard deviation) of verapamil versus time curves after bolus IV, PV, and ID administration in IVAP dogs (n = 4).

The mean systemic concentrations of VL declined in a two-exponential behavior. The $t_{1/2}$ was not different after IV and PV administration. The mean systemic concentrations of VL reached its peak at 10–20 min (PV) or 10–30 min (ID). The calculated BA, 23.5%, compared favorably to that previously reported in dogs and humans (13,16,24). VL absorption was complete ($F_{ABS} \sim 1$) and intestinal extraction was negligible ($ER_{GI} \sim 0$), because the AUC after PV and ID administration were not different. The normalized AUCs of VL at 1 mg/kg dose after IV, PV, and ID administration were 37907, 8232, and 8894 ng.min/mL, respectively (Fig. 2).

The mean plasma concentration time profiles of VL after IV, PV, and ID administration in the presence of ritonavir are shown in Figure 3; and the estimated pharmacokinetic parameters are listed in Table I. After IV administration of VL, the mean CL decreased 57%, but the AUC and $t_{1/2}$ increased 149 and 158%, respectively. After PV administration of VL, the mean AUC increased 873%, and the $t_{1/2}$ increased 165%. After ID administration of VL, the mean AUC increased 602%. The BA of VL increased from 23.5% to 66.2% in the presence of ritonavir.

After ID administration of VL, the mean portal venous concentrations reached its peak at 1 to 5 min (i.e., the first and second sampling times) indicating that VL absorption is rapid (Fig. 4). The mean portal venous AUC value of VL after ID administration, 56562 ± 36153 ng.min/mL, was significantly greater than its systemic AUC value (17788 ± 5832 ng.min/mL) indicating a significant hepatic first-pass effect. In contrast, the mean portal venous AUC after IV administration was not significantly different from its systemic plasma AUC, indicating negligible intestinal extraction during systemic circulation (data not shown). The portal venous AUC values of VL (56562 ± 36153 and 153143 ± 92577 ng.min/mL) were significantly different in the absence and presence of ritonavir, respectively.

The range of the *in vitro* dog blood-to-plasma concentration ratio (C_B/C_P) of VL was similar (0.65 ± 0.04, 0.61 to 0.69) at three different concentrations (100, 500, and 2500 ng/mL). The intestinal extraction ratio (ER_{GI}) of VL was 0.62, when it is calculated by an indirect method using eq 4 and $F_{ABS} = 1$ (13). Therefore, there was a clear inconsistency when the ER_{GI} was estimated using the direct (Eq. 3) and indirect methods (Eq. 4).

DISCUSSION

Recent studies have indicated that the small intestine contributes substantially to the overall first-pass extraction of cyclosporine, nifedipine, midazolam, verapamil, and other drugs (4,5,7,25,26). Some of these studies have even suggested that the role of the intestine is quantitatively greater than that of the liver. Many authors have suggested that gut-wall CYP3A4 and P-gp act in a concerted manner to control the absorption of drugs (10,25,27,28). This is based on the large overlap of substrates between the two and the proximity of their expression within the gut wall. These findings raise a question as to whether intestinal extraction by either CYP3A or P-gp or other enzymes and transporters truly play a role in the first-pass effect. Therefore, we used a well-established ported dog model, and two drugs, verapamil and ritonavir, which are known substrates of CYP3A-mediated metabolism, and P-gp-mediated secretory transport to dissect the role of the intestine and liver in the extraction of verapamil. Al-

TABLE I. Pharmacokinetic Parameters of Verapamil (Mean ± Standard Deviation) in the Systemic Plasma after IV (0.5 mg/kg), PV (2 mg/kg), and ID (2 mg/kg) Administration with and without Ritonavir (5 mg/kg) in IVAP Dogs

	Dose (mg/kg)	AUC (ng.min/mL)	CL (mL/min/kg)	$t_{1/2}$ (min)	V_{dss} (mL/kg)	C_{max} (ng/mL)	t_{max} (min)	ER_H	BA (%)	F_{ABS}, ER_{GI}
Drug only										
IV (n = 4)	0.5	18953 ± 2674	26.8 ± 3.5	119 ± 14	3796 ± 390					
PV (n = 4)	2	16464 ± 4789		116 ± 19		177 ± 64	16.3 ± 7.5	0.78 ± 0.06	21.7 ± 6.3	
ID (n = 4)	2	17788 ± 5832				145 ± 40	22.5 ± 12.6		23.5 ± 7.7	$F_{ABS} \approx 1$ $ER_{GI} \approx 0$
With Riton avir (5 mg/kg)										
IV (n = 3)	0.5	47207 ± 14794	11.4 ± 3.8	307 ± 37	4541 ± 1566					
PV (n = 3)	2	160236 ± 35673		307 ± 42		481 ± 270	20 ± 0.0	0.17 ± 0.15	82.7 ± 15.2	
ID (n = 3)	2	124944 ± 28155				349 ± 38	26.7 ± 11.5		66.2 ± 14.9	$F_{ABS} < 1$ $ER_{GI} \approx 0$
P										
IV		<0.05	<0.05	<0.05						
PV		<0.05		<0.05		<0.05		<0.05		
ID		<0.05				<0.05			<0.05	

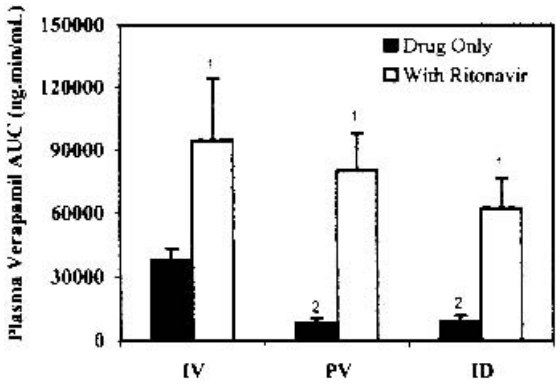

Fig. 2. Dose-normalized AUC (mean ± standard deviation) of verapamil after bolus IV, PV, and ID administration with/or without 5 mg/kg ritonavir (n = 3–4). [1]Indicates the significant difference from drug only by $P < 0.05$ at one-way ANOVA and [2]indicates the significant difference from IV drug only by $P < 0.05$ at one-way ANOVA.

though the oral BA of VL is reported to be 10–30% in dogs and humans, it has been reported to be absorbed completely from the gastrointestinal tract (13,16,24). Recent studies have found that VL is a substrate of P-gp in various cell culture models and intact intestines (6,14), and that it inhibits the efflux of various P-gp substrates (19,29,30). VL has also been shown to be a CYP3A substrate in the intestine (7,31). In this study, the drugs were administered directly into the duodenum through an ID port to avoid any potentially confounding effects caused by the stomach.

The absolute BA of VL was 21.7% after PV administration; therefore, the liver had a high intrinsic capacity for clearing VL. The absolute bioavailability of verapamil (23.5%) after ID administration observed in this study was in good agreement with the values reported previously in dogs and humans (13,16,24). Intestinal absorption was considered complete ($F_{ABS} \sim 1$), and the intestinal extraction was considered negligible ($ER_{GI} \sim 0\%$) because the AUC was not different after PV and ID administration. Because the PV input rate and the ID absorption rate of VL were similar, the observation is not likely due to the differential saturation of hepatic enzymes. Actually, the mean portal venous concentrations of VL reached its peak at 1 to 5 min after ID administration. On the one hand, a considerable first-pass effect was expected in

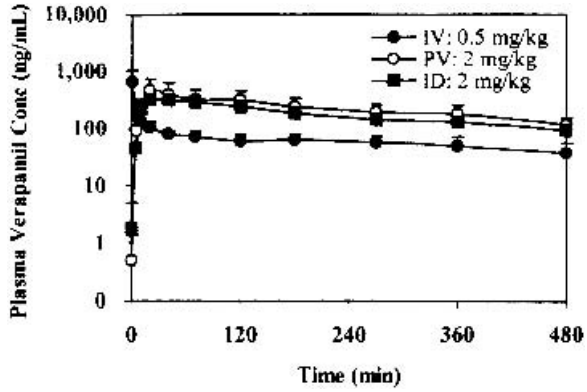

Fig. 3. Plasma concentration (mean ± standard) deviation of verapamil versus time curves after bolus IV, PV, and ID administration in IVAP dogs with the pre-PO administration of 5 mg/kg ritonavir (n = 3).

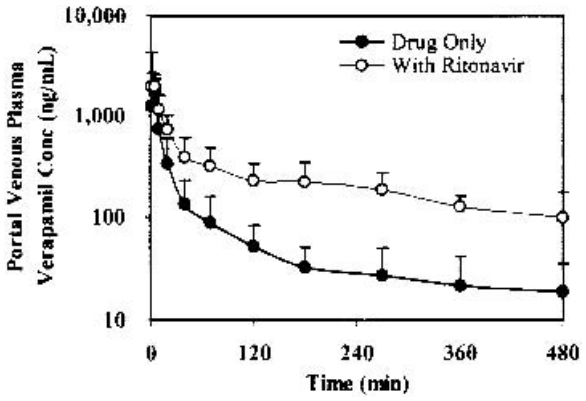

Fig. 4. Portal venous plasma concentration (mean ± standard deviation) of verapamil versus time curves after bolus ID administration of 2 mg/kg in IVAP dogs with/or without 5 mg/kg ritonavir (n = 3–4).

the gut because VL is a substrate of CYP3A and P-gp (6,14), gut-wall metabolism was reported in humans (7,31) and was inducible when given with rifampin (7). In fact, Fromm *et al.* reported that the gut wall metabolism of VL increased to about 90% in induced subjects (7). Although VL has been reported to have a rather high affinity to P-gp, VL, and the R/S-enantiomers are classified as high permeability drugs according to the recently proposed biopharmaceutical drug classification. Supporting this was a regional single-pass perfusion study of the proximal jejunum in humans that reported the VL P_{eff} was high enough ($>2 \times 10^{-4}$ cm/s) to expect complete intestinal absorption after oral dosing (32). The fast rate of permeation of VL across the gut and the short residence time in the enterocyte would suggest that there is less opportunity for intestinal metabolism. Therefore, the high rate of VL permeation across the gut wall probably saturated apical recycling mechanisms and CYP3A metabolism in enterocytes. Consequently, even though VL is a substrate for P-gp located in the apical enterocyte membrane, it does not appear to affect its transport. In the present study, the intestinal extraction of VL appears to be negligible. Chiou *et al.* (33) also demonstrated an apparent lack of P-glycoprotein efflux effect on the gastrointestinal absorption of tacrolimus in normal mice using intravenous and oral data obtained from the literature. In contrast, more direct evidence for a role of intestinal P-gp in limiting drug absorption was derived from *in vivo* studies with *mdr1a* (–/–) mice. The plasma AUC of paclitaxel was 6-fold higher in with *mdr1a* (–/–) mice than in *mdr1a* (+/+) mice after oral administration, whereas there were no significant differences in biliary excretion between *mdr1a* (–/–) mice and *mdr1a* (+/+) mice after IV dosing (34). When intestinal excretion was measured after the interruption of bile flow, the intestinal excretion of digoxin was approximately 2% of the dose in the *mdr1a* (–/–) mice, but 16% of the dose in the *mdr1a* (+/+) mice over 90 min after IV dosing (35). These *in vivo* results indicated that P-gp limited the oral absorption of some drugs by excreting the drug from the epithelial cells into the intestinal lumen. In addition, more direct evidence for a role of intestinal CYP3A in limiting drug absorption has been demonstrated *in vivo* in clinical studies. The oral BA of midazolam in humans was approximately 36% due to extensive first-pass metabolism (36). It was shown that the intestinal extraction ratio of midazolam was 0.43 when midazolam was instilled into the duodenum of anhepatic patients, suggesting an effect of intestinal CYP3A4 on systemic drug clearance in addition to oral absorption (5).

The intestinal extraction ratio was evaluated using numerous approaches, including an indirect measurement. As shown in Equation 1, oral bioavailability (BA) is the product of the fraction of the drug absorbed into the gastrointestinal membranes (F_{ABS}), the fraction of the absorbed dose that passes through the gut into the hepatic portal blood unmetabolized ($F_{GI} = 1 - ER_{GI}$) and the hepatic first-pass availability ($F_H = 1 - ER_H$). The parameters, ER_H and ER_{GI}, can be assessed only by comparing AUCs after drug administration and blood sampling at a number of sites relative to the intestine and liver using specialized surgical procedures (Eqs. 2 and 3). Various conscious animal models using multiple routes of administration (or sampling) have been used to evaluate the extent of extraction by different organs (15,23,37,38). This technique, however, has not been attempted in humans. In contrast, some clinical studies have used pharmacokinetic results after IV and PO administration to calculate the intestinal and hepatic first-pass clearance of drugs (4,7,26). The intestinal and hepatic first-pass effects were calculated based on comparisons of AUCs or other pharmacokinetic parameters after IV and PO administration, i.e., $ER_H = CL/Q_H$ and $ER_{GI} = 1 - BA/[F_{ABS}(1 - ER_H)]$, in the absence and presence of a CYP3A modulator. In fact, estimates of intestinal extraction calculated by this indirect method often contradicted those determined from direct measurements. For example, nifedipine, a well-absorbed drug, is subject to substantial first-pass metabolism, which results in an oral BA of 30–50%. Using an indirect method, Holtbecker *et al.* concluded that the contribution of intestinal metabolism was quantitatively as important as that of hepatic metabolism to the overall first-pass effect of nifedipine in humans (26). However, Kleinboesem *et al.* demonstrated that the intestinal metabolism of nifedipine in patients with a portalcaval shunt was absent because the BA of nifedipine in these patients was complete (100%) (39). Similarly, inconsistencies were noted between the direct and indirect estimation of intestinal metabolism for verapamil in humans (7,40), although the direct estimation of intestinal extraction was based on the bioavailability data from a single hepatic patient (40). The validity of human ER_{GI} calculated by the indirect method using IV and PO AUC data was reviewed by Lin *et al.* (41). Their research clearly showed that the overestimation of ER_{GI} was due to the several assumptions, such as the systemic clearance after the IV dose reflects only hepatic elimination; metabolism of the systemically available drug by intestinal enzymes is negligible; use of hepatic blood flow rate (Q_h); and $F_{ABS} = 1$. ER_{GI} can also be calculated by directly measuring the drug concentrations of the portal vein and the systemic circulation after ID administration. As shown in this study, the intestinal extraction ratio (ER_{GI}) of VL was 0.62 when it was calculated by the indirect method (Eq. 4) using the measured C_B/C_P ratio (0.65) and literature information, $Q_{PV} = 30$ mL/min/kg (23) and $F_{ABS} = 1$ (13). Therefore, there were clear inconsistencies between ER_{GI} estimations using the direct ($ER_{GI} \sim 0$, eq 3) and indirect methods ($ER_{GI} = 0.62$, Eq. 4). These findings suggest that intestinal and hepatic first-pass clearance of drugs should be carefully examined using appropriate experimental and pharmacokinetics models.

The identification of compounds as being substrates or

inhibitors of intestinal P-gp and/or CYP3A has involved *in vitro* experiments. This suggests that, for some compounds at least, *in vitro* experiments may overemphasize the functional role of P-gp and CYP3A on *in vivo* intestinal extraction. VL has been reported to have a rather high affinity to P-gp (42) with the ratios of efflux to influx permeability equal to 2.5, 3.6, 3.1, 2.8, and 1.4 in the rat jejunum, ileum, and colon, human ileum, and Caco-2, respectively (14). We also observed that the VL (100 μM) efflux ratios in rabbit duodenum, jejunum, ileum, and colon, and Caco-2 were 10.2, 5.5, 6.9, 6.3, and 23.8, respectively when tested in Ussing side-by-side diffusion chambers (pH 6.5 apical and pH 7.4 basolateral, unpublished data). These results suggest the possibility of high secretory flux *in vivo*. However, the effect of P-gp on the intestinal metabolism and absorption of drugs may be quantitatively less important than for *in vitro* studies. For example, after a 2 mg/kg ID dose of verapamil into IVAP dogs (10 kg), the estimated initial intestinal VL concentrations were 4080, 815, and 163 μM at 10, 50, and 250 mL dilutions, respectively. However, K_m values of verapamil efflux *in vitro* in jejunum, ileum, and colon were 30.8, 28.5, and 4.5 μM, respectively, in rat intestine (14). Furthermore, in *in vivo* studies, the mucosal side typically has higher drug concentrations than the basolateral side because of the presence of serum protein and continuous removal of the drug by systemic blood flow. As a result, for drugs with a high absorptive permeability such as VL, active secretory transport may have little overall effect on net absorption into the portal vein.

The pharmacokinetics of VL were differentially modulated by ritonavir, a substrate of P-gp and a strong metabolic inhibitor of CYP3A (17–19), depending upon the route of administration. Interestingly, the effect of ritonavir on VL concentration was dramatically different between PV and IV dosings. In the presence of ritonavir, the AUC after PV dosing increased 873% whereas it increased only 149% after IV dosing. The lower IV effect occurred because the hepatic clearance (CL_H) of VL is high and approaches liver blood flow (i.e., $CL_H \sim Q_H$) (13,24); therefore, its elimination after IV administration seems to be not influenced by the change of intrinsic hepatic metabolism. In contrast, after PV administration, the effect is greater because the portal venous clearance (CL_{PV}) of VL approaches hepatic intrinsic clearance (CL_I) (i.e., $CL_{PV} \sim CL_I$) (43), so its elimination after PV administration seems to be influenced by the change of hepatic intrinsic metabolism in the presence of ritonavir. Because VL is a CYP3A substrate, ritonavir is considered to inhibit the oxidative metabolism of verapamil. CYP3A is involved in the formation of two major metabolites, norverapamil by N-demethylation (by CYP3A4) and D-617 by N-dealkylation (by CYP3A4 and 1A2) in humans (44). There were two major metabolites in this study. After PV administration, two metabolites were comparable to that of VL, whereas they were negligible after IV administration, assuming their extraction recovery and fluorescence extinction are identical to that of VL. Ritonavir reduced the formation of VL metabolites significantly when VL was dosed PV, thereby increasing the C_{max} and AUC of VL in the plasma. Using ritonavir, the observed increase of AUC by 873% after PV dosing was primarily due to a reduction in the hepatic extraction ratio caused by the inhibition of CYP3A metabolism. There is also a report that coadministration of some drugs, saquinavir, indinavir, nelfinavir, and VX-478 (amprenavir), with ritonavir in rats and dogs produced elevated plasma drug levels by 8- to 46-fold by inhibition of CYP3A mediated metabolism (17). A >50-fold enhancement of the concentration of saquinavir in plasma was observed in humans after a single co-dose of ritonavir (600 mg) and saquinavir (200 mg). Because VL is a P-gp substrate, and ritonavir is a strong P-gp modulator (45), ritonavir may possibly inhibit the biliary excretion of VL, thereby increasing the C_{max} and AUC of VL in the plasma, but the modulation is unclear because there are no reports on the biliary excretion of VL.

After ID administration of VL, $F_{ABS} \cdot (1\text{-}ER_{GI}) \sim 1$ (i.e., $F_{ABS} \sim 1$ and $ER_{GI} \sim 0$), $ER_H = 0.78$, and BA = 23.5%. In the presence of ritonavir, $F_{ABS} \cdot (1 - ER_{GI}) < 1$ (i.e., $F_{ABS} < 1$ and $ER_{GI} \sim 0$), $ER_H = 0.17$, and BA = 66.2%. Because ritonavir is a strong inhibitor of CYP3A or P-gp (17–19), ER_{GI} is considered ~ 0 and, therefore, F_{ABS} is considered <1, although it is unclear why F_{ABS} of VL is decreased in the presence of ritonavir. In the presence of ritonavir, the BA of VL increased 182% (from 23.5% to 66.2%), but F_{ABS} decreased. Because VL is a substrate of P-gp and CYP3A, and ritonavir is a strong P-gp and CYP3A modulator, we expected strong modulation effect after ID administration caused by inhibition of intestinal and hepatic extraction mediated by either CYP3A or P-gp, or both in the presence of ritonavir. However, these data indicated that it is unlikely that ritonavir coadministration will substantially affect the contribution of intestinal extraction. The effect of ritonavir on VL concentration was less when administered ID than PV because the contribution of the gut to the first-pass loss of VL was negligible.

In conclusion, it is now recognized that the intestinal extraction of drugs by intestinal P-gp, CYP3A, and possibly other transporters and enzymes may contribute significantly to the poor oral bioavailability and high variability observed for many drugs *in vivo*. This appears to be the case for cyclosporine and midazolam and may be the case for other P-gp and/or CYP3A substrates that exhibit poor and variable oral bioavailability. However, as shown in this study, the intestinal extraction of VL is negligible in the dog. Another study (33) has suggested the same for tacrolimus in the mouse even though *in vitro* studies have implicated secretory transport and P450 metabolism mechanisms in its clearance. Therefore, the functional role of intestinal metabolism and secretion on *in vivo* oral absorption should be carefully interpreted using appropriate *in vivo* as well as *in vitro* models. Species differences must also be accounted for, however, little work to quantitate these differences has been published. These studies demonstrate the utility of *in vivo* IVAP dog models for evaluating the relative contribution of the gut and the liver to the first-pass clearance of drugs and for characterizing functional role of CYP450 or secretory transporters such as P-gp on oral drug absorption.

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