ABSTRACT:

Low and varied oral bioavailability (BA) of some drugs has been attributed to extraction by the intestine and liver. However, the role of the intestine is difficult to directly assess. We recently developed an in vivo intestinal and vascular access-ported (IVAP) rabbit model that allows for a direct assessment of the contributions of the gut and the liver to the first-pass loss of drugs. The current studies validate the utility of the IVAP rabbit model using verapamil (VL). VL pharmacokinetics (PK) were determined after intravenous administration. The IVAP method that allows for a direct assessment of the contributions of the gut and the liver to the first-pass loss of drugs. The current results demonstrate the utility of the rabbit IVAP model in studying the first- and second-pass intestinal and hepatic loss of drugs and other xenobiotics.

Oral PK studies have demonstrated the significance of the contribution of gut wall metabolism to the total first-pass effect for cyclosporine, midazolam, and verapamil (Wu et al., 1995; Fromm et al., 1996; Paine et al., 1996). However, in these studies the gut component was not assessed directly by measuring portal venous (PV) drug concentrations. Rather, PV concentrations were estimated using systemic drug concentrations and a specific PK model. Although apically located intestinal transporters such as P-glycoprotein (P-gp) and MRP2/cMOAT have been implicated in the secretory absorption of drugs back into the gut lumen (Saitoh and Aungst, 1995), the net effect on intestinal-hepatic extraction is not clear since secreted drugs can be reabsorbed and recycled numerous times before their ultimate fate is determined (i.e., reaching the systemic circulation, becoming metabolically transformed, or not absorbed and excreted in the feces). If drugs are exposed to metabolizing enzymes during repeated recycling events, increased extraction is possible (Wu et al., 1995). Another possibility is that systemic absorption may be delayed due to intestinal- or enterohepatic recycling as manifested by a prolonged time to peak blood drug concentration (t_{max}). The effect of intestinal recycling on drug disposition has not been appreciated and is the subject of a recent report from our group (Sinko et al., 2004).

In the recent past, attempts have been made to assess the intestinal extraction of drugs by inserting catheters into the portal vein of rats (Hoffman et al., 1995), dogs (Tam-Zaman et al., 2004), and monkeys (Ward et al., 2004). Placement of a catheter into the portal vein provides a way to accurately quantitate drug absorption from the gut lumen into portal venous circulation and distinguish the intestinal extraction of drugs from that of the liver. These animal models, however, are either short-lived (e.g., rats) or expensive to maintain (e.g., dogs or primates). The rabbit model has been commonly used for the PK evaluation of numerous drugs including cyclosporin A (Guo et al., 2001), methotrexate (Ilo et al., 2001), and cisplatin (Najjar and Saad, 2001), among others, and rabbit intestine has been widely used to assess the membrane permeability characteristic of drugs (Grass and Sweetana, 1989). Rabbits are known to express P450 enzymes (Donato and Castell, 2003). A partial list of secretory transporters such as P-gp and cMOAT/Mrp2 in intestinal epithelial cells (Freeman, 1995; van Aubel et al., 1998) and various P450 enzymes (Donato and Castell, 2003). A partial list of secretory transporters and P450 enzymes expressed in rabbits, dogs, and humans is provided for comparison (Table 1). Of the expressed phase 1 P450 enzymes in rabbits, CYP3A6 has drawn particular attention due
to its predominant expression in both intestine and liver as human CYP3A4 (McKinnon et al., 1993) and its strong similarities to CYP3A4 (Franklin, 1995). As a result, rabbits were used to study the CYP3A-inductive potential of rifampicins (Weber et al., 2001) and the comparative microsomal CYP3A6- and CYP3A4-inhibitory potential of α-naphthoflavone (Boek-Dohalsa et al., 2001), among other studies.

Based on common features in P450 and secretory transporter expression across species, we have proposed the rabbit as a viable and less expensive alternative and complementary model to characterize the specific in vivo roles of intestinal and hepatic extraction of drugs. Previously, we characterized the IVAP rabbit model with respect to placement of the USI and PV catheters and their effects on normal intestinal motility patterns and PV blood flow, respectively (Kunta et al., 2001). More recently, we investigated the effect of P-gp and CYP3A modulation on saquinavir absorption and disposition using the rabbit IVAP model (Sinko et al., 2004). The current study further validates the IVAP rabbit model with respect to pharmacokinetic (intestinal and hepatic extraction) evaluation of verapamil (VL). Since VL is a substrate for CYP3A4 and, potentially, its isoform CYP3A6 in rabbit and P-gp (Saitoh and Aungst, 1995; Fromm et al., 1996), high intestinal secretion and metabolism were anticipated in vivo. Previously, VL was shown to be completely absorbed from the gastrointestinal tract (Echizen and Eichelbaum, 1986), and it yet exhibits low and varied oral bioavailability (BA), ranging from 7 to 30% in rats, dogs, and humans (Echizen and Eichelbaum, 1986; Bhatti and Foster, 1997; Lee et al., 2001). In this report, the relative contribution of the gut and liver toward the first-pass extraction of VL was assessed in IVAP rabbits.

### Materials and Methods

Verapamil and propranolol were obtained from Sigma-Aldrich (St. Louis, MO). Bacteriostatic 0.9% sodium chloride injection, USP, and the 20 gauge i.v. catheter, Abbocath, were obtained from Abbott Diagnostics (Abbott Park, MO). A Millipore filter, Millex-GV13, was obtained from Millipore Corporation (Bedford, MA). Intestinal and vascular access ports and 22 gauge Huber needles were obtained from Intech Solomon (San Antonio, TX). Suture material (Ethicon Inc., Somerville, NJ), ketamine and xylazine (Abbott Diagnostics), isoflurane (Schein Pharmaceutical Inc., Cherry Hill, NJ), buprenorphine (Reckitt & Colman, Hull, U.K.), Hypaque-76 (diatrizoate meglumine and diatrizoate sodium solution; Nycomed Inc., Princeton, NJ), 50% dextrose (Abbott Diagnostics), heparin (Eikins-Simm Inc., Cherry Hill, NJ), and 24 gauge i.v. catheters (BD Biosciences, Franklin Lakes, NJ) were purchased. All other materials were obtained from Fisher Chemicals (Fairlawn, NJ) or Sigma-Aldrich.

### Preparation of IVAP Rabbits

All rabbit experiments were carried out in accordance with the “Guide for the Care and Use of Laboratory Animals” (National Institutes of Health Publication 1996) and the protocol (99-054) approved by the Institutional Animal Care and Review Board of Rutgers University. New Zealand White female rabbits weighing 3 to 4 kg were surgically fitted with PV and USI access ports. Detailed surgical and postoperative procedures are described elsewhere by us (Kunta et al., 2001). After the surgery, the animals were allowed to recover for 2 weeks before being assigned to pharmacokinetic studies. Before the studies, rabbits were restrained in rabbit slings, and their back was shaved near the subcutaneous ports to facilitate port access. The left ear was shaved and wiped with alcohol swabs to ease the cannulation of the auricular artery for systemic sampling. After inserting and securing the catheter, it was flushed with heparinized saline at the beginning of and during the study to maintain its viability between samples. The skin lining the intestinal and PV ports was scrubbed with Povidone-Iodine solution and wiped clean with alcohol swabs to ease the cannulation of the arterial vascular for systemic sampling. Before the studies, rabbits were restrained in rabbit slings, and their back was shaved near the subcutaneous ports to facilitate port access. The left ear was shaved and wiped with alcohol swabs to ease the cannulation of the auricular artery for systemic sampling. After inserting and securing the catheter, it was flushed with heparinized saline at the beginning of and during the study to maintain its viability between samples. At the end of the study, intestinal and PV ports were flushed with 50% dextrose solution and heparinized saline, respectively. The rabbits were used once every 2 weeks.

### Dosing the Rabbits

Doses of 0.2, 0.6, and 0.8 mg/kg i.v. and 6 mg/kg USI or PV, respectively, were administered to rabbits. The i.v. bolus dose was administered into the marginal ear vein. Intestinal or PV bolus doses were administered into the USI or PV ports, respectively, followed by a 1- to 2-ml saline flush. For PV infusion, a rate of 0.82 ml/min was maintained for 5 min using an infusion pump (Harvard Infusion Pump, Harvard Apparatus Inc., Holliston, MA). Fifteen minutes before intestinal dosing, the IVAP rabbit was flushed with 5 ml of saline. The dosing solutions were prepared in bacteriostatic water or saline for injection. To ensure sterility, the dosing solutions were filtered (Millex-GV 13 filters) before administration. The dosing volumes were 0.3 ml/kg for i.v. and 1 ml/kg for the USI and PV dosages, respectively. Blood samples were drawn at 0, 1, 3, 6, 10, 20, 40, 60, 90, 120, 180, 240, 300, and 360 min. At each sample point, approximately 1 ml of blood was drawn into 2-ml Vacutainers with EDTA (7.5%) and placed on ice. For i.v. and USI administration, portal and systemic blood samples were drawn simultaneously from the ports. For PV administration, systemic blood samples were drawn. The blood samples were centrifuged within 10 min of sampling at 3000 g for 10 min at 4°C. The plasma was decanted and stored at −80°C until analyzed by HPLC.

### Treatment of Plasma Sample

A total of 0.5 ml of in vivo plasma sample containing VL was mixed with 0.05 ml of the internal standard solution (propranolol, 1 μg/ml in water), 0.1 ml of 1 M sodium carbonate, and 5 ml of diethyl ether in 15-ml polypropylene tubes. For the calibration plot, serial dilutions of VL were made from the same stock solution used for the study and were spiked into the blank plasma. The in vivo plasma and standard samples were then vortexed for 10 min and centrifuged at 1500 g for 10 min at 4°C. The centrifuged samples were placed on dry ice for 1 min and the supernatant was

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### TABLE 1

<table>
<thead>
<tr>
<th>Name</th>
<th>Rabbit</th>
<th>Dog</th>
<th>Human</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-gp</td>
<td>√</td>
<td></td>
<td></td>
<td>Conrad et al., 2001; Freeman, 1995</td>
</tr>
<tr>
<td>BCRP</td>
<td>NA</td>
<td></td>
<td></td>
<td>Conrad et al, 2001</td>
</tr>
<tr>
<td>MRP1</td>
<td>NA</td>
<td></td>
<td></td>
<td>van Kuijk et al., 1997; van Aubel et al., 1998; Conrad et al, 2001</td>
</tr>
<tr>
<td>MRP2</td>
<td>√</td>
<td></td>
<td></td>
<td>van Kuijk et al., 1997; van Aubel et al., 1998; Conrad et al, 2001</td>
</tr>
<tr>
<td>MRP3–6</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td>van Kuijk et al., 1997; van Aubel et al., 1998; Conrad et al, 2001</td>
</tr>
<tr>
<td>CYP1A</td>
<td>1, 2</td>
<td>1, 2</td>
<td>1, 2</td>
<td>Donato and Castell, 2003</td>
</tr>
<tr>
<td>CYP2A</td>
<td>10, 11</td>
<td></td>
<td>6, 7, 13</td>
<td>Donato and Castell, 2003</td>
</tr>
<tr>
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<td>4, 5</td>
<td>NA</td>
<td>6, 7</td>
<td>Donato and Castell, 2003</td>
</tr>
<tr>
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<td>21, 41, 42</td>
<td>8, 9, 18, 19</td>
<td>Donato and Castell, 2003</td>
</tr>
<tr>
<td>CYP2D</td>
<td>24</td>
<td>15</td>
<td>6</td>
<td>Roussel et al., 1998; Donato and Castell, 2003</td>
</tr>
<tr>
<td>CYP2E</td>
<td>1, 2</td>
<td></td>
<td>1</td>
<td>Lankford et al., 2000; Donato and Castell, 2003</td>
</tr>
<tr>
<td>CYP3A</td>
<td>6</td>
<td>12, 26</td>
<td>3, 4, 5, 7</td>
<td>Frasier et al., 1997; Nishibe et al., 1998; Donato and Castell, 2003</td>
</tr>
</tbody>
</table>

NA, not available; BCRP, breast cancer resistance protein.
√, presence was reported.
transferred to another polypropylene tube containing 0.2 ml of 0.1 N hydrochloric acid, vortexed for 10 min, and centrifuged at 1500g for another 10 min at 4°C. The supernatant was discarded and 0.1 ml of the aqueous phase containing drug and internal standard was analyzed by HPLC fluorometrically (Lee et al., 2001). The extraction recovery was greater than 95% for VL and propranolol.

Quantitation of Verapamil by HPLC Method. VL in plasma was analyzed by HPLC under isocratic conditions on a reverse phase NovaPak symmetry C18 column (3.9 × 150 mm, particle size 5 μm) (Waters, Milford, MA). The HPLC system consisted of Millennium® system control software (Waters), two LC dual pumps (model 510, Waters), an autosampler (model 717+, Waters), and a spectrofluorometric detector (model RF-551; Shimadzu, Tokyo, Japan). The mobile phase was a mixture of 35 parts of acetonitrile and 65 parts of 0.1% trifluoroacetic acid solution. VL was monitored fluorometrically at an excitation wavelength of 208 nm and emission wavelength of 321 nm. The calibration was linear through 5 to 5000 ng/ml in plasma and the lower limit of quantitation was less than 3 ng/ml.

Pharmacokinetic Analysis. Time-dependent plasma concentration data were analyzed by noncompartmental pharmacokinetic methods (Gibaldi and Perrier, 1982). The maximum drug concentration and the corresponding sampling time were defined as Cmax and tmax, respectively. The area under the concentration curve (AUC) was calculated by a combination of the trapezoidal and log-trapezoidal methods (Chiou, 1978).

Direct and Indirect Calculation of the Gut and Liver Extraction Ratio. The BA, hepatic extraction ratio (Eh), and gut extraction ratio (Eg) were calculated by a direct method as follows (Lee et al., 2001):

\[ BA = \frac{AUC_{\text{USI}}}{AUC_{\text{IV}}} \]  

\[ 1 - E_{\text{H}} = \frac{AUC_{\text{PV}}}{AUC_{\text{IV}}} \]  

\[ 1 - E_{\text{I}} = \frac{BA}{F_{\text{abs}}(1 - E_{\text{H}})} \]  

where AUCUSI, AUCIV, and AUCPV are the systemic AUC values after USI, i.v., and PV administration, and DUSI, DIV, and DPV are the doses after USI, i.v., and PV administration. Fabs is defined as the fraction of the dose absorbed into the portal vein through the intestinal epithelial membrane and Fab was assumed to be unity from the fast absorption of VL in rabbit (tmax < 6 min) and the complete absorption of VL in other species such as dog (Lee et al., 2001) and human (von Richter et al., 2001). Ei was introduced to emphasize gut extraction by intestinal metabolism and/or secretory transport.

The indirect approach estimates the roles of the intestine and liver by calculating rather than by measuring portal drug concentrations (von Richter et al., 2001) as below:

\[ E_{\text{I}} = \frac{CL_{\text{abs}}}{Q_{\text{I}}} \]  

\[ CL_{\text{abs}} = CL_{\text{a}} + CL_{\text{i}} \]  

The Ei was calculated from eq. 3, whereas the hepatic extraction ratio was calculated using the blood clearance as shown in eq. 4. Blood clearance equals plasma clearance, since Cblood/Cplasma is unity (Lee et al., 2001). QI in eq. 4 stands for the hepatic blood flow rate \( Q_{\text{H}} \sim 56 \text{ ml/min/kg} \) in rabbit (Davies and Morris, 1993) and the total body clearance (CLblood) includes hepatic clearance (CLa) and intestinal clearance (CLi).

As an alternative model, the Ei can also be calculated using the measured arterial and PV drug concentrations (Carterg and CPV) after i.v. administration.

\[ E_{\text{I}} = \frac{\text{Rate of extraction}}{\text{Rate of presentation}} = \frac{C_{\text{artery}} - C_{\text{PV}}}{C_{\text{artery}}} \]  

Statistical Analysis. Data are reported as mean ± standard error of means (S.E.M.). A minimum of three to four animals per study was used. All statistical tests were performed using Sigma Stat (Version 2.03; SPSS Inc., Chicago, IL). One-way analysis of variance was performed, and a minimum P value of 0.05 was used as the significance level for all tests. All pair-wise comparison procedures were performed by the Student-Newman-Keuls method.

Results

Intravenous Administration. The plasma concentration-time profiles of VL after i.v. administration to rabbits (0.2, 0.6, or 0.8 mg/kg) are illustrated in Fig. 1. The decline in VL plasma concentrations followed a two-exponential process. The PK parameters are listed in Table 2. The AUC of VL was proportional to the dose, indicating linear PK behavior in the studied concentration range. A broader dose range study was not conducted due to the reported toxicity of VL at higher doses and the lower limit of detection of the assay that was used to analyze the samples. The mean systemic and portal venous VL plasma concentration profiles are compared at the highest dose studied (0.8 mg/kg) and are shown in Fig. 2. The portal venous concentrations of VL were significantly lower than the corresponding systemic concentrations.

Portal Venous and Intestinal Administration. The mean systemic concentration versus time profiles of VL after PV and USI administration are shown in Fig. 3. The systemic plasma concentrations of VL were significantly higher after PV dosing compared with USI dosing. The systemic clearance after PV bolus administration was comparable to the value after PV infusion (Table 2), suggesting that the rate of VL administration did not cause a temporary saturation of the enzymes in the liver resulting in the observed linear pharmacokinetics after the PV dose. The mean systemic and PV concentration versus time profiles of VL after USI administration are shown in Fig. 4. The maximal PV concentration of VL (Cmax) occurred at about 5 min, confirming fast VL absorption across rabbit gut epithelium.

Differentiation of Intestinal and Hepatic Extraction. A comparison of the calculation of the intestinal and hepatic extraction by the indirect and direct methods is shown in Table 3. The indirect and direct calculation methods produced a large difference in the resulting extraction ratios, especially for Ei. In the case of the previously published dog data, the resulting Ei values are quantitatively different, but both methods predicted that Ei was negligible in the dog. Eh calculated using the indirect method was based on the current rabbit data using the 0.8 mg/kg i.v. dose. Since the reported rabbit blood flow rate was 130% of CLblood, the contribution of intestinal extraction to BA was calculated to be zero. However, the Eh calculated...
Drugs that undergo extensive first-pass elimination often have low bioavailability. Although numerous in vitro studies have suggested that intestinal P-gp and/or CYP3A may contribute to the in vivo first-pass extraction of drugs, the precise role of these factors on bioavailability has not been clearly defined because of the lack of reliable models that can translate in vitro to in vivo results in a generalized manner. As an alternative to the direct measurement of drug concentrations in the portal vein, knockout/knock-in and surgical models have been developed that eliminate the influence of specific factors such as secretion or metabolism that may affect bioavailability. However, these newer models still have many limitations including the fact that the roles of the liver and intestine cannot be distinguished. For instance, in a clinical study involving anhepatic patients, Paine et al. (1996) found that 43% of midazolam was extracted in intestinal tissues when administered into the small bowel, suggesting a role for intestinal extraction of drugs, the precise role of these factors on bioavailability remains to be elucidated.

**Discussion**

VL has been classified as a high permeability drug according to the biopharmaceutical drug classification system (Amidon et al., 1995). High effective permeability of VL (2 × 10^{-4} cm/s) was reported in a regional single-pass perfusion study of the proximal human jejunum (Sandstrom et al., 1998). Consistent with this observation, we previously demonstrated that the absorption of VL was fast (t_{max} < 30 min) and almost complete (F_{abs} = 1) using the dog IVAP model (Lee et al., 2001). Although the results of the current study also demonstrate that the intestinal absorption of VL is rapid, unlike in dogs, the BA of VL is reduced in rabbits due to significant first-pass intestinal extraction. This result is significant since this behavior is similar to what has been hypothesized for VL in humans. However, for a variety of ethical and technical reasons, the direct measurement of portal VL concentrations in humans is not possible, and so this cannot be confirmed.

Drugs that undergo extensive first-pass elimination often have low and variable bioavailability. Since quantitative and mechanistic studies using PV catheterization techniques in humans are not possible, the role of the intestine in the extraction of drugs is difficult to assess, and commonly used animal model results are often confounded by other factors. Although numerous in vitro studies have suggested that intestinal P-gp and/or CYP3A may contribute to the in vivo first-pass extraction of drugs, the precise role of these factors on bioavailability has not been clearly defined because of the lack of reliable models that can translate in vitro to in vivo results in a generalized manner. As an alternative to the direct measurement of drug concentrations in the portal vein, knockout/knock-in and surgical models have been developed that eliminate the influence of specific factors such as secretion or metabolism that may affect bioavailability. However, these newer models still have many limitations including the fact that the roles of the liver and intestine cannot be distinguished. For instance, in a clinical study involving anhepatic patients, Paine et al. (1996) found that 43% of midazolam was extracted in intestinal tissues when administered into the small bowel, suggesting a role for intestinal CYP3A on systemic drug clearance. The absence of the liver, however, may have resulted in an overexpression of P-gp and CYP3A in other parts of the body to compensate for the lack of a liver. Recently, Schuetz et al. (2000) demonstrated that mdr1a/1b knockout mice displayed an increased expression of P450 enzymes to compensate for the lack of P-gp. In addition, increased expression of mdr1b was observed in mdr1a knockout mice, presumably to compensate for the loss of mdr1a expression. These results suggest that models with a disease condition or altered gene expression may under- or overestimate the significance of intestinal extraction on drug BA. Therefore, in the current studies, we have proposed and used a normal but

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**Table 2**

Pharmacokinetic parameters of verapamil (mean ± S.E.M.) in the systemic and portal plasma (only in USI dosing) after i.v. (0.2, 0.6, or 0.8 mg/kg), PV (6 mg/kg bolus and infusion), and USI (6 mg/kg) administration in IVAP rabbits.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>i.v. Dose (n = 3)</th>
<th>PV Dose (n = 3–4)</th>
<th>USI Dose (n = 3–4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2 mg/kg</td>
<td>0.6 mg/kg</td>
<td>0.8 mg/kg</td>
</tr>
<tr>
<td>C_{max} (ng/ml)</td>
<td>397 ± 75.7</td>
<td>284 ± 49.9</td>
<td>38.73 ± 7.75</td>
</tr>
<tr>
<td>t_{max} (min)</td>
<td>9701 ± 499</td>
<td>11598 ± 1851</td>
<td>1948 ± 447</td>
</tr>
<tr>
<td>AUC_{0–t} (ng · min/ml)</td>
<td>10698 ± 1053</td>
<td>13224 ± 2191</td>
<td>2286 ± 518</td>
</tr>
<tr>
<td>CL (ml/min/kg)</td>
<td>117 ± 113</td>
<td>161 ± 161</td>
<td></td>
</tr>
<tr>
<td>V_{ss} (ml/kg)</td>
<td>560 ± 453</td>
<td>2624 ± 2624</td>
<td></td>
</tr>
<tr>
<td>BA (%)bj</td>
<td>8.0 ± 1.0</td>
<td>10.0 ± 2.0</td>
<td>1.65 ± 0.37</td>
</tr>
</tbody>
</table>

a Infusion rate, 0.82 ml/min.
b BA calculation was made using 0.8 mg/kg i.v. data.
FIG. 4. Systemic (○) and (□) portal plasma concentration (mean ± S.E.M.) of verapamil after USI administration at 6 mg/kg dose in IVAP rabbits (n = 3).

TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Rabbits</th>
<th>Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct</td>
<td>Direct</td>
</tr>
<tr>
<td>Gut extraction ratio ($E_I$)</td>
<td>&gt;0</td>
<td>0.79</td>
</tr>
<tr>
<td>Hepatic extraction ratio ($E_H$)</td>
<td>&gt;1</td>
<td>0.92</td>
</tr>
<tr>
<td>Bioavailability (BA) (%)</td>
<td>1.65</td>
<td>41.9</td>
</tr>
</tbody>
</table>

“instrumented” animal model to overcome the possible compensatory issues that are known to occur with the previously described genetically or surgically altered models.

PK models have been used as an indirect approach to estimate the roles of the intestine and liver by estimating portal drug concentrations. von Richter et al. (2001) showed that the intestinal extraction of VL in humans was the same as the hepatic extraction (0.49 versus 0.48), when calculated using the indirect method. But the blood clearance of VL ($CL_{blood}$) includes not only hepatic clearance ($CL_H$) but also intestinal clearance ($CL_I$) as shown in eq. 5. Moreover, they found that intestinal secretion contributed to drug elimination to an extent similar to biliary excretion using a multilumen perfusion catheter. This resulted in an overestimation of blood clearance that, in turn, led to an overestimation of $E_H$ and an underestimation of $E_I$. In other words, the actual value of $E_I$ might be much higher than 0.49, but it could not be precisely calculated without the portal venous concentration data. Therefore, an overestimation of $E_H$ is likely reflected in the Table 3 calculation using the indirect method. Other problems with using the indirect calculation method are apparent as well, as evidenced by intestinal extraction ratios equal to zero and the hepatic extraction ratios higher than unity in both rabbits and dogs. Clearly, the calculations using the indirect method are erroneous since the difference between the systemic AUC after PV and USI dosing (10,698 versus 2286 ng · min/ml) indicates that substantial intestinal extraction of VL occurs.

In the case of low permeability drugs, the intestinal extraction ratio after USI administration is not the same as that after i.v. dosing, since the rate of blood flow is likely to be too rapid for the drug molecules to permeate and pass through the basolateral membrane. However, for the passage of highly permeable drugs such as VL, the residence time in intestinal cells is short. Application of eq. 6 to the plasma concentration data after i.v. administration in rabbit and our previous dog study results in $E_I$ values of 0.49 and 0, respectively. Although these values are smaller than those calculated from the direct method (0.49 versus 0.79 in rabbit, 0 versus 0 in dogs), trends in species differences of intestinal extraction ratios could be detected. The calculated $E_I$ using the indirect method ($E_I = 0$) failed to explain the differences between arterial and PV concentration after i.v. administration in rabbits.

To truly estimate the first-pass effect of the intestine and liver, the role of the sampling site cannot be underestimated. For example, when a systemic blood sample is taken, the resulting drug concentration can be either from a venous or an arterial source. Although the role of the lung in first-pass elimination (i.e., both secretion and metabolism) has been largely ignored, the potential bias introduced by this omission could be significant. The lung first-pass effect segregates the systemic circulation into at least two compartments, that is, arterial and venous. The systemic blood-sampling catheter in the current study was implanted in the ear artery, blocking direct contact with the venous blood-sampling site. This suggests that the so-called systemic concentrations are closer to arterial than to venous concentrations. Furthermore, blood sampled at this site passed through the lung, which expresses not only metabolizing enzymes like CYP3A but also drug transporters such as P-gp, MRPs, and lung resistance protein (Sugawara et al., 1997).

In humans, VL metabolites such as D-617, D-620, and norverapamil accumulated in an isolated intestinal segment, implicating active secretion into the gut lumen (von Richter et al., 2001). We previously showed that the mean portal venous AUC after i.v. administration was, contrary to human results, not significantly different from its systemic AUC in the dog model (Lee et al., 2001). This discrepancy suggests that intestinal extraction is negligible in dogs compared with humans and rabbits. The high contribution of intestinal extraction in humans and rabbits ($E_I > 0.49$ and 0.79) suggests that the rabbit behaves more like humans rather than dogs ($E_I = 0$), despite the relatively low bioavailability of VL in rabbit compared with human or dog.

Results for other drugs also suggest that the dog intestine may not be functionally similar to that of humans in certain critical aspects (Tam-Zaman et al., 2004). Dog intestine exhibited the lower metabolic activity against CYP2D6 and CYP3A4 marker substrates, and the immunoblot failed to detect CYP1A2 and CYP2D6 in dog intestine (Prueksaritanont et al., 1996). The formation of D-617 and norverapamil, major metabolites of VL, was mainly mediated by CYP3A and CYP1A2 in humans (Kroemer et al., 1993). CYP2D6 had a minor contribution to the metabolism of norverapamil in a P450 cDNA-expressed cell system (Tracy et al., 1999). The lower enzyme expression level and/or lower metabolic activity of CYP1A2, 2D6, and 3A in dog intestine compared with human intestine are likely causes of the relatively low $E_I$ in the dog. Little information is available about the expression level of drug transporters in the dog intestine.

The current studies shed some light on species differences as they relate to intestinal and hepatic extraction. Also, the current results suggest that using the indirect methods to estimate intestinal first-pass extraction should be cautiously used, given the problems discussed in this paper. The liver and intestine demonstrated a high extraction of VL in the rabbit model in a pattern similar to that of humans but substantially different from the negligible $E_I$ of VL in dogs (Lee et al., 2001). Although these results suggest the suitability of IVAP rabbit model for explaining VL clinical pharmacokinetics and drug interactions based on P-gp or CYP3A interactions, it also more broadly
suggests that the rabbit model may be more generally suitable for studying intestinal/liver biopharmaceutics than the dog model. Since typical human dosage forms can be administered to rabbits and they have a considerably larger blood volume than mice or rats, the rabbit IVAP model offers many advantages for directly assessing the roles of CYP3A-mediated metabolism and drug transporter interactions by means of P-gp and possibly MRP2.

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References


Address correspondence to: Dr. Patrick J. Sinko, Rutgers, The State Uni-
versity of New Jersey, Ernest Mario School of Pharmacy, 160 Frelinghuysen
Road, Piscataway, NJ 08854. E-mail: sinko@rci.rutgers.edu