Physiologically Based Pharmacokinetic Modeling of Intestinal First-Pass Metabolism of CYP3A Substrates with High Intestinal Extraction

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ABSTRACT:
Prediction of intestinal availability (\(F_a\)), in conjunction with hepatic metabolism, is of considerable importance in drug disposition to assess oral clearance and liability to drug-drug interactions. In the current study, \(F_a\) predictions were performed within a physiologically based pharmacokinetic (PBPK) model using in vitro permeability and clearance data. The prediction success was assessed in comparison with the \(Q_{out}\) model. In addition, apparent oral clearance values, predicted using the PBPK model, were compared with in vivo observations from meta-analyses. Finally, unbound intrinsic clearance values (\(CL_{int}\)) were determined for 12 CYP3A substrates in eight individual human jejunal microsome (HJM) samples to assess interindividual variability in intestinal intrinsic clearance and subsequent \(F_a\) predictions. Overall, the PBPK model improved \(F_a\) predictions in comparison with the \(Q_{out}\) model; this was apparent by a reduced bias and increased precision. In particular, \(F_a\) predictions of indinavir, saquinavir, and terfenadine were model-dependent. The predicted oral clearance values of the drugs investigated ranged from 8.79 to 6320 l/h for tacrolimus and simvastatin, respectively, and were overall within 3-fold of the observed data with the exception of indinavir, atorvastatin, and buspirone. The individual HJM \(CL_{int}\) values ranged from 17 to 14,000 \(\mu\)l/min \(\cdot\) mg \(^{-1}\) for atorvastatin and saquinavir, respectively, and corresponding interindividual variability in \(CL_{int}\) estimates ranged from 41 to 67%. These in vitro data resulted in predicted \(F_a\) values ranging from 0.03 to 0.94 for simvastatin and indinavir, respectively. The largest interindividual variability of \(F_a\) was predicted for terfenadine (65%) in contrast with the low variability in the case of simvastatin (3%).

Introduction
CYP3A enzymes represent the principle drug-metabolizing enzymes in the small intestine (Paine et al., 1997, 2006). The expression levels of CYP3A along the small intestine decline from the proximal to the distal regions (Paine et al., 1997; Zhang et al., 1999). A high drug concentration in the enterocytes during the absorption phase may lead to substantial metabolism despite the relatively low enzyme content (~1% in comparison to the liver) (Paine et al., 1997). On the other hand, it may lead to saturation or in some instances to inhibition of metabolism, resulting in nonlinear pharmacokinetics after oral drug administration. In addition, small intestinal metabolism is associated with substantial interindividual variability. For example, an up to 10-fold range in individual activity was demonstrated using metabolism data in the case of cyclosporine and tacrolimus (Lampen et al., 1995, 1996), which may partially reflect the high interindividual variability of CYP3A abundance (von Richter et al., 2004; Paine et al., 2006).

The importance of intestinal metabolism may be delineated from clinical studies after intravenous and oral drug administration or, alternatively, from grapefruit juice interaction studies under appropriate conditions. However, both in vivo methods are flawed by a number of inherent limitations discussed elsewhere (Galetin et al., 2008; Gertz et al., 2008a). The intestinal availability (\(F_G\)) for drugs such as cyclosporine, midazolam, felodipine, and tacrolimus is based on a considerable number of studies, in contrast to very limited and variable data for buspirone, statins, saquinavir, and terfenadine; hence, the in vivo \(F_G\) estimates for the latter drugs have to be regarded with caution. In addition, in vitro data may be used to make predictions of intestinal metabolism based on either in vitro clearance alone (Shen et al., 1997) or in combination with permeability data (Yang et al., 2007; Gertz et al., 2010). For the prediction of intestinal metabolism, the enterocytic, rather than the total intestinal blood flow, needs to be incorporated (Tam et al., 2003). The rationale for using enterocytic blood flow in the \(F_G\) predictions is that drugs pass through the enterocytes which contain the majority of metabolically active en-
zymes in the small intestine (Kolars et al., 1994). In general, no binding to enterocytic proteins is assumed, although this has not been satisfactorily investigated and is in contrast to the assumptions of the well-stirred model for the liver. Advancement in computational power has allowed incorporation of intestinal transit times and heterogeneous expression levels of CYP3A into mechanistic predictions of intestinal absorption and metabolism. Complex models to predict intestinal absorption generally represent adaptations of the compartmental absorption and transit model (Yu and Amidon, 1999) and may account for drug dissolution, solubility, permeability, transport, and metabolism (Agoram et al., 2001; Tam et al., 2003; Jamei et al., 2009). Limiting the current trend toward holistic and mechanistic data interpretation is the need for extensive in vitro studies (e.g., full kinetic characterization of drug affinity for metabolizing enzymes and transporters) and formulation-specific data (e.g., dissolution and solubility) in conjunction with the quality of physiological information (e.g., abundance data of relevant enzymes and transporters) to support these models.

To reduce the necessary in vitro input data, previous work investigated $F_G$ prediction success using the $Q_{Gut}$ model, which simplifies prediction of intestinal metabolism to the input of in vitro clearance and permeability data (Yang et al., 2007; Gertz et al., 2010). The $Q_{Gut}$ model has the basic structure of the well-stirred model; however, the blood flow term is modified to represent a hybrid parameter, $Q_{Gut}$, consisting of physiological flow (i.e., enterocytic blood flow) and drug permeability (Chalasani et al., 2002; Rostami-Hodjegan and Tucker, 2002). However, our analysis highlighted considerable bias and imprecision in the prediction of $F_G$ for drugs with in vivo $F_G$ values less than 0.5 (Gertz et al., 2010). In particular, the $Q_{Gut}$ model predictions of $F_G$ for indinavir, saquinavir, and terfenadine were underpredicted by up to 96%. Thus, a physiologically based pharmacokinetic (PBPK) model was developed, which retains the use of in vitro clearance and permeability data but overcomes some of the limitations of the $Q_{Gut}$ model. The PBPK model takes into account heterogeneity of metabolizing enzymes, drug concentration in the enterocytes, and any potential saturation of intestinal metabolism. The latter in particular was assumed to be responsible for some of the $F_G$ underprediction trends observed previously. Prediction success of the PBPK model is assessed in comparison with the $Q_{Gut}$ model using 12 CYP3A drugs with low intestinal availability in vivo. In addition, apparent oral clearance is predicted for the selected drugs using the PBPK modeling approach and compared with in vivo observations from meta-analyses. Finally, eight individual human jejunal microsome (HJM) samples are used to determine in vitro intrinsic clearance data of 12 selected drugs to assess interindividual variability in $F_G$ predictions.

### Materials and Methods

#### Determination of Intestinal Clearance In Vitro

In vitro clearance data were determined by substrate depletion in eight individual human jejunal microsomal samples prepared by an elution method from white donors. The microsomes from individual jejunal donors were purchased from BD Gentest (Woburn, MA) (Table 1). Substrate depletion experiments were performed in 0.1 M phosphate buffer (pH 7.4) containing 10 mM MgCl$_2$, 7.5 mM isocitric acid, 1.2 units/ml isocitric acid dehydrogenase, and 1 mM NADP. The microsomal protein concentrations ranged from 0.025 to 1.5 mg/ml for lovastatin/simvastatin and cyclosporine, respectively. The substrate concentrations were 10-fold below the reported $K_m$ values in the literature for the drugs investigated. The drugs were added from methanol stock solutions, resulting in a final concentration of organic solvent in the incubation of 0.1% v/v. Clearance incubations were prepared as replicates of two in an Eppendorf tube at 37°C and 900 rpm in an Eppendorf Thermomixer. The metabolic reaction was initialized by the addition of NADP solution to the incubation mixture, and samples were taken at six designated time points within 60 min. Noncytochrome P450-dependent loss of drug over the incubation time was monitored by preparing additional samples in the absence of NADP. Metabolic reactions were terminated by removal of aliquots into an equal volume of ice-cold acetonitrile containing internal standard. Samples were centrifuged at 1000g for 20 min at 4°C in a Mistral 3001 centrifuge [MSE (UK) Ltd., London, UK], and 150 µl of supernatant was removed from each Eppendorf vial and transferred to glass vials before analysis on the LC-MS/MS system. Detailed information on LC-MS/MS analysis and the list of chemical suppliers have been reported in our previous publication (Gertz et al., 2010). Clearance values were corrected for experimentally determined nonspecific binding values to microsomal protein (fu$_{inc}$) (Gertz et al., 2008b, 2010). For cyclosporine, binding was predicted using drug lipophilicity data. The unbound intrinsic clearance values, CL$_{uint}$, were calculated using eq. 1. Clearance values were corrected for the mean population CYP3A abundance in the intestine of 50 pmol of CYP3A/mg protein (Paine et al., 2006) prior to $F_G$ predictions.

$$\text{CL}_\text{u,ini} = \frac{V \cdot k \cdot \text{protein microsomal}^{-1}}{\text{fu}_{inc}}$$

where $k$ represents the depletion rate constant, $V$ represents initial incubation volume, and protein$_{microsomal}$ represents the initial amount of protein.

#### Determination of $K_m$ Values

Michaelis-Menten constants were determined for four drugs for which the literature indicated low and variable $K_m$ values. Substrate depletion clearance was determined at the following substrate concentration ranges: felodipine, 0.5 to 10 µM (6 concentrations); indinavir, 0.025 to 5.5 µM (10 concentrations); nisoldipine, 0.25 to 5.0 µM (6 concentrations), and saquinavir, 0.05 to 0.5 µM (6 concentrations). The depletion profiles at the different substrate concentrations were performed in one representative microsomal preparation, HJM 6 (chosen for its high testosterone 6β-hydroxylation activity and low between-day variability). The CL$_{u,ini}$ and $K_m$ values together with associated S.E.$\bar{s}$ were determined using eq. 2 (Obach and Reed-Hagen, 2002). The equation reported by the original investigators was modified by multiplying both sides of the equation with the volume of incubation to transform depletion rate constants to intrinsic (at [S] approaching 0) and apparent intrinsic clearance (at any [S]). The fitting was performed in Grafit 5.0.10 (Erithacus Software Limited, Horley, Surrey, UK).

#### Table 1

<table>
<thead>
<tr>
<th>Donor*</th>
<th>Gender</th>
<th>Age</th>
<th>CYP3A Activity ± S.D.$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>µmol · min$^{-1}$ · mg$^{-1}$</td>
</tr>
<tr>
<td>HJM 1</td>
<td>Male</td>
<td>24</td>
<td>2.09 ± 0.176</td>
</tr>
<tr>
<td>HJM 2</td>
<td>Male</td>
<td>59</td>
<td>3.08 ± 0.085</td>
</tr>
<tr>
<td>HJM 3</td>
<td>Male</td>
<td>26</td>
<td>2.63 ± 0.151</td>
</tr>
<tr>
<td>HJM 4</td>
<td>Male</td>
<td>35</td>
<td>3.38 ± 0.485</td>
</tr>
<tr>
<td>HJM 5</td>
<td>Male</td>
<td>68</td>
<td>5.15 ± 0.313</td>
</tr>
<tr>
<td>HJM 6</td>
<td>Male</td>
<td>60</td>
<td>5.56 ± 0.122</td>
</tr>
<tr>
<td>HJM 7</td>
<td>Female</td>
<td>39</td>
<td>5.20 ± 0.198</td>
</tr>
<tr>
<td>HJM 8</td>
<td>Female</td>
<td>65</td>
<td>5.88 ± 0.393</td>
</tr>
</tbody>
</table>

* Microsome preparation by elution method from the jejunum.

$^b$ Testosterone 6β-hydroxylation activity was determined at a testosterone concentration of 250 µM.
\[
\text{CL}_{\text{int,app}} = \text{CL}_{\text{int}} \left( 1 - \frac{[S]}{[S] + K_m} \right)
\]

where \(\text{CL}_{\text{int}}\) represents the unbound intrinsic clearance, \(\text{CL}_{\text{int,app}}\) is apparent intrinsic clearance, \([S]\) is substrate concentration, and \(K_m\) is the Michaelis-Menten constant.

**Preliminary Assessment of Enterocytic Drug Concentration and Possibility of Saturation of First-Pass Metabolism.** Indinavir, saquinavir, and terfenadine \(F_G\) values were previously considerably underpredicted by 75 to 96\% using the \(Q_{\text{int}}\) model (Gertz et al., 2010). Unlike the PBPK model, the \(Q_{\text{int}}\) model predictions did not take enterocytic concentrations (\(C_{\text{int}}\)) into account. An initial assessment of enterocytic drug concentration was performed on the basis of eq. 3.

\[
C_{\text{int}} = \frac{D \cdot k_s \cdot F_G}{Q_{\text{int}}}
\]

The doses (\(D\)), absorption rate constants (\(k_s\)), and fractions absorbed (\(F_G\)) considered for the preliminary analyses are summarized in Table 2; an enterocytic blood flow (\(Q_{\text{int}}\)) of 18 l/h was assumed. The selection of doses and formulations was based on the studies from which in vivo \(F_G\) estimates were obtained. The drugs investigated were ranked for their likelihood that saturation of first-pass metabolism. The drugs investigated were ranked for their likelihood that saturation of first-pass metabolism. The reevaluation of atorvastatin \(F_G\) from in vivo data compared favorably to data from grapefruit juice interaction studies (Gertz et al., 2008a).

**Reassessment of Intracellular Clearance of Cyclosporine and Tacrolimus.** In comparison with Gertz et al. (2010), the unbound fractions in plasma of cyclosporine and tacrolimus were reevaluated from the available literature. Different methods have been used to determine both cyclosporine and tacrolimus fraction unbound in plasma. The current study favored \(f_{\text{up}}\) measurements determined in stainless steel equilibrium dialysis chambers for cyclosporine (1.9\%) and tacrolimus (1.3\%), given their high reproducibility. The use of these \(f_{\text{up}}\) values resulted in changed estimates of in vivo \(C_{\text{int,app}}\) in comparison to our previous publication (Table 2).

**PBPK Model to Estimate \(F_G\) and \(C_{\text{int,app}}\) from In Vitro Data.** A PBPK model was constructed in which tissues were connected by an arterial blood supply and a collective venous return to the lungs (Nestorov, 2003). All tissues were considered to be well mixed compartments, i.e., that the unbound tissue concentration is at equilibrium with the unbound concentration in the emergent blood (eq. 4).

\[
V_T \cdot \frac{dC_T}{dt} = Q_{\text{int}} \left( C_{\text{v,A}} - C_T \right)
\]

where \(V_T\), \(C_T\), \(Q_{\text{int}}\), and \(K_m\) represent the volume, concentration, blood flow, and tissue/blood concentration ratio of the different tissues and \(C_{\text{v,A}}\) represents the arterial blood concentration.

The physiological values for blood flows and tissue volumes were taken from the literature (Brown et al., 1997; International Commission on Radiation Protection, 2002). The selected tissues accounted for >95\% of total body weight; an additional compartment representing the rest of the body was included. Tissue/blood concentration ratios were either collated from the literature or predicted with mechanistic equations (Rodgers et al., 2005; Rodgers and Rowland, 2006) using human tissue composition data (Poulin and Theil, 2002). For tissues where no human data were available, rat tissue

<table>
<thead>
<tr>
<th>Dose</th>
<th>(k_s)</th>
<th>(C_{\text{int}})</th>
<th>(pK_a)</th>
<th>(f_{\text{up}})</th>
<th>(V_{\text{T}})</th>
<th>(C_{\text{v,A}})</th>
<th>In Vivo CL_{\text{int,app}}</th>
<th>In Vitro CL_{\text{int,app}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atorvastatin</td>
<td>0.010</td>
<td>1.36</td>
<td>0.30</td>
<td>3.30</td>
<td>1950</td>
<td>213</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buspirone</td>
<td>2.0</td>
<td>1.14</td>
<td>3.45</td>
<td>Neutral</td>
<td>0.019</td>
<td>1340</td>
<td>279</td>
<td></td>
</tr>
<tr>
<td>Cyclosporine &amp;</td>
<td>0.1</td>
<td>1.11</td>
<td>3.45</td>
<td>Neutral</td>
<td>0.019</td>
<td>1340</td>
<td>279</td>
<td></td>
</tr>
<tr>
<td>Lovastatin</td>
<td>0.008</td>
<td>0.90</td>
<td>4.26</td>
<td>Neutral</td>
<td>0.017</td>
<td>5750</td>
<td>17,200</td>
<td></td>
</tr>
<tr>
<td>Mifepristone</td>
<td>0.005</td>
<td>0.88</td>
<td>2.07</td>
<td>0.1</td>
<td>1120</td>
<td>2090</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midazolam</td>
<td>0.004</td>
<td>1.0</td>
<td>4.05</td>
<td>2.1</td>
<td>38,900</td>
<td>25,200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nifedipine</td>
<td>2.0</td>
<td>1.10</td>
<td>4.71</td>
<td>Neutral</td>
<td>0.014</td>
<td>14,300</td>
<td>25,500</td>
<td></td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>0.005</td>
<td>0.5</td>
<td>5.95</td>
<td>2.6</td>
<td>7530</td>
<td>3750</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terfenadine</td>
<td>2.0</td>
<td>1.47</td>
<td>5.62</td>
<td>9.7</td>
<td>70,600</td>
<td>12,400</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*References for log\(P_{\text{ocw}}\), \(pK_a\), \(f_{\text{up}}\), and \(K_m\) are found in Supplemental Table 1 and at http://www.pharmacy.manchester.ac.uk/capkr/.*

\[t_{\text{int}} = \frac{\ln \left( \frac{k_{\text{int}}}{k_{\text{up}} - k_{\text{int}}} \right)}{k_{\text{up}} - k_{\text{int}}}\]

\[F_G = 1 - \frac{[S]}{[S] + K_m}\]

\[V_T \cdot \frac{dC_T}{dt} = Q_{\text{int}} \left( C_{\text{v,A}} - C_T \right)\]

| TABLE 2 |
| Summary of drug-related parameters used in the current PBPK model for 12 drugs investigated |

\[\text{CL}_{\text{int,app}} = \text{CL}_{\text{int}} \left( 1 - \frac{[S]}{[S] + K_m} \right)\]

\[\text{CL}_{\text{oral}} = \text{CL}_{\text{oral}} \left( 1 - \frac{[S]}{[S] + K_m} \right)\]

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References for log\(P_{\text{ocw}}\), \(pK_a\), \(f_{\text{up}}\), and \(K_m\) are found in Supplemental Table 1 and at http://www.pharmacy.manchester.ac.uk/capkr/.

\[\text{CL}_{\text{oral}} = \text{CL}_{\text{oral}} \left( 1 - \frac{[S]}{[S] + K_m} \right)\]

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composition data were used. Concentration-time profiles, as well as \( F_G \) and CL parameters, were taken into consideration in the model development using midazolam and alfentanil as test compounds because of availability of \( K_p \) data in rat (data not shown). However, subsequent analysis focused on the prediction of \( F_G \), CLapp, and CLoral.

The intestinal and liver compartments of this model are outlined in detail below, as they allowed the assessment of intestinal and hepatic availability and apparent intraintravenous and oral clearance. Systemic clearance was considered to occur exclusively in the liver (unless renal excretion was significant, e.g., for inulin) and presystemic metabolism was considered to occur in both the small intestine and the liver. The liver was separated into liver tissue and the blood residing in the liver. The rate equations (eqs. 5 and 6) describe the change in concentrations in liver blood and liver tissue, respectively.

\[
\frac{dC_{blue}}{dt} = C_{AH} \cdot Q_{HA} + C_{PV} \cdot Q_{PV} - C_{blue} \cdot Q_{AV} - PS_{L} \cdot (f_{un} \cdot C_{blue} - f_{un} \cdot C_{Li}) \tag{5}
\]

\[
\frac{dC_{Li}}{dt} = PS_{Li} \cdot (f_{un} \cdot C_{Li} - f_{un} \cdot C_{Li}) - \frac{V_{max} \cdot A_{CYP3A,Li} \cdot f_{un} \cdot C_{Li}}{K_{m} + f_{un} \cdot C_{Li}} \tag{6}
\]

where \( C_{HA}, C_{PV}, C_{Li}, \) and \( C_{blue} \) represent the concentrations in the arterial blood, portal vein, liver, and hepatic outlet (or liver blood), respectively; \( Q_{HA} \) (6.5% of cardiac output), \( Q_{PV} \) (18.5% of cardiac output), and \( Q_{AV} \) (\( Q_{PV} + Q_{HA} \)) represent the blood flows of the hepatic artery, portal vein, and hepatic vein, respectively; \( f_{un} \) and \( f_{un} \) represent the fractions unbound in blood and liver (\( f_{un} = f_{un}/K_{Li} \) for drugs with no active uptake or efflux); \( V_{un} \) and \( V_{Li} \) represent the volumes of the blood residing in the liver and the liver tissue, respectively; \( PS_{L} \), \( V_{max} \), and \( K_{m} \) represent the permeability-surface area product (10,000 times greater than hepatic blood flow to satisfy perfusion limited assumptions), the maximum metabolic velocity, and the Michaelis-Menten constant for metabolism, respectively; and \( A_{CYP3A,Li} \) represents the total hepatic amount of CYP3A.

In the current analysis, no active uptake or efflux was considered to occur in the liver for the drugs under investigation. However, eqs. 5 and 6 can be modified to accommodate these processes by inclusion of the appropriate Michaelis-Menten or intrinsic clearance terms and accounting for the extra-cellular water fraction. The portal vein concentration represents the differential of emergent blood concentrations from the intestine (including the entero-cytes), stomach, spleen, and pancreas.

The small intestine was divided into seven compartments (Fig. 1): 1, 2, 3, and 4 to 7 represent the intestinal segments of duodenum, jejunum, and ileum, respectively (Yu and Amidon, 1999). The rate equations below describe the change of drug amount in the stomach and intestinal lumen (eqs. 7–10) and drug concentration in the enterocytes (eq. 11) with respect to time.

\[
\frac{dA_{st}}{dt} = -A_{st} \cdot K_{st} \tag{7}
\]

\[
\frac{dA_{G1}}{dt} = A_{st} \cdot K_{st} - A_{G1} \cdot K_{G1} - a_{G1} \cdot A_{G1} \tag{8}
\]

\[
\frac{dA_{Gn}}{dt} = K_{Gn-1} \cdot A_{Gn-1} - K_{Gn} \cdot A_{Gn} - a_{Gn} \cdot A_{Gn} \quad n = 2-7 \tag{9}
\]

\[
\frac{dA_{c0}}{dt} = A_{C0} \cdot K_{C0} \cdot A_{C0} \cdot K_{C0} \tag{10}
\]

\[
\frac{dC_{enter}}{dt} = k_{un} \cdot A_{Gn} - Q_{Gout} \cdot C_{enter} - \frac{V_{max} \cdot A_{CYP3A,enter} \cdot f_{un} \cdot C_{enter}}{K_{m} + f_{un} \cdot C_{enter}} \tag{11}
\]

where \( A \) denotes the amounts of drug in either the stomach \( (A_{st}) \), the intestinal segments \( (A_{Gn,a}) \), or the colon \( (A_{Cn}) \); \( K_{st}, K_{G1,n}, \) and \( K_{C0} \) refer to the transit rate constants of stomach, intestinal lumen, and colon, respectively; \( k_{g} \) represents the absorption rate constant determined using eq. 15; \( f_{un} \) \( \text{CYP3A,enter} \), \( V_{max} \text{CYP3A,enter} \), and \( Q_{Gout} \) refer to the unbound fraction, concentration, volume, amount of CYP3A, and the hybrid parameter of blood flow and permeability in the enterocytes of the \( n \)th intestinal compartment.

Drug permeability was incorporated as a hybrid parameter of enterocytic blood flow and permeability, as in the \( Q_{Gout} \) model (Chalasani et al., 2002; Rostami-Hodjegan and Tucker, 2002). Absorption was considered to occur from the small intestinal compartments with the exception of suxamethonium, for which colonic absorption was also incorporated, as investigated previously (Agoram et al., 2001). The blood flow to the small intestine represents approximately 10% of the cardiac output (39 l/h) (International Commission on Radiological Protection, 2002), and the enterocytic blood flow represents approximately 50% (i.e., 18 l/h) of the small intestinal blood flow (Granger et al., 1980). The cardiac output in the current assessment was 6.5 l/h, based on male subjects aged 20 to 35 years (Brown et al., 1997; International Commission on Radiological Protection, 2002). Effect of age on cardiac output (Brown et al., 1997) was taken into account for tacrolimus \( F_G \) predictions.

Intestinal availability, apparent intraintravenous, and oral clearance data were calculated from eqs. 12 and 13; where \( t \)-last represents a time appropriate to completely recover the administered dose as metabolites or drug excreted unchanged and \( AM_{ent,a} \) represents the accumulative amount metabolized in the \( nth \) enterocyte compartment. The rate equations were solved in Matlab (version 7.10, 2010) using the ODE15s or 23s solvers. A mass balance equation was included in the script to allow monitoring of dose recovery over time.

\[
CL_{app} = \frac{Dose}{\int_{0}^{t_{-last}} C(t) \cdot dt} \tag{12}
\]

\[
F_G = 1 - \sum AM_{ent,a} \cdot \frac{Dose_{oral} \cdot F_x}{\text{Dose}_{oral}} \tag{13}
\]

where \( C(t) \) represents the drug concentration-time profile in blood or plasma, \( F_G \) represents fraction absorbed, and \( F_x \) represents the fraction of drug amount available to the enterocytes that enters the portal vein unchanged.

The main assumptions made in the current analysis are as follows: 1) there is no contribution of the small intestine to the systemic elimination of drugs; 2) there is no binding of drugs in the enterocytes (i.e., \( f_{un} = 1 \)); 3) drug distribution into tissues satisfies the well stirred assumptions (no active uptake or efflux); 4) CYP3A is exclusively responsible for the metabolism of selected drugs; 5) dissolution and solubility do not affect assessment of \( F_G \) (special
considerations for cyclosporine, indinavir, saquinavir, and terfenadine are outlined below); and 6) absorption occurs from the seven compartments of the small intestine only, with the exception of saquinavir for which colonic absorption was also incorporated.

Parameters for PBPK Modeling. The metabolism in the liver was scaled using the standard human microsomal recovery of 40 mg/g, average liver weight of 21.4 g/kg, and CYP3A content of 155 pmol/mg microsomal protein. The total CYP3A contents in the duodenum, jejunum, and ileum were 9.7, 38.4, and 22.4 nmol, respectively (Paine et al., 1997). The regional weights of the enterocytes and the transit rate constants in the duodenum, jejunum, and ileum were 18.2 g and 4.3 h⁻¹, 65.8 g and 1.7 h⁻¹, and 38.3 g and 2.1 h⁻¹, respectively (Yu et al., 1996; Paine et al., 1997). Differential blood supply to the duodenum, jejunum, and ileum was accounted for (Jamei et al., 2009; Darvich et al., 2011).

The oral absorption rate constants ($k_a$) of the drugs investigated here were estimated from apparent permeability data determined in MDCK-MDR1 cells. These data were first converted to effective permeability, $P_{eff}$, using the regression analysis previously performed on a set of 20 drugs (eq. 14) (Gertz et al., 2010). The $P_{app}$ data were then used to estimate absorption rate constants by eq. 15 (Yu and Amidon, 1999). The radii of the different intestinal compartments ($r_{SI}$) ranged from 0.85 and 1.58 cm for the ileum and duodenum, respectively (default values in GastroPlus version 7).

$$\log P_{eff} = 0.829 \cdot \log P_{app,A,B} - 1.30$$

$$k_a = \frac{2 \cdot P_{eff}}{r_{SI}}$$

For cyclosporine, indinavir, saquinavir, and terfenadine, saturation of intestinal metabolism was considered highly likely on the basis of preliminary analysis. Drug solubility data were therefore incorporated for these drugs, because luminal solubility may limit drug concentration in the enterocytes. In those cases, luminal rate equations (eqs. 7–10) were expanded to include the dissolved and undissolved drug amounts (Hintz and Johnson, 1989), as exemplified in eqs. 16 and 17. The current model assumed immediate drug release, dissolution from spherical particles, a constant particle radius over time, and equality of dissolution and precipitation rate constants. The occurrence of supersaturation was allowed.

$$\frac{dA_{\text{un},n}}{dt} = A_{\text{un},n-1} \cdot K_{t,n} - A_{\text{un},n} \cdot K_{t,n} - \frac{3D}{\rho \cdot r \cdot h} \cdot A_{\text{un},n} \cdot \left( C_{n,s} - \frac{A_{\text{dis},n}}{V_o} \right)$$

$$A_{\text{dis},n} = A_{\text{dis},n-1} \cdot K_{t,n} + \frac{3D}{\rho \cdot r \cdot h} \cdot A_{\text{un},n} \cdot \left( C_{n,s} - \frac{A_{\text{dis},n}}{V_o} \right) - A_{\text{dis},n} \cdot k_{un}$$

where $A_{\text{un},n}$ and $A_{\text{dis},n}$ represent, respectively, the undissolved and dissolved amount in the intestinal compartment $n$ (including stomach and colon); $K_{t,n}$ and $k_{un}$ represent the transit rate and absorption rate constants of the $n$th compartment; $D$ represents the diffusion coefficient; $\rho$ is the density; $r$ is the particle radius; $h$ is the diffusion layer thickness; $C_{n,s}$ is drug solubility in the $n$th is luminal compartment; and $V_o$ is luminal volume of the different intestinal segments (default values in GastroPlus version 7).

For these four drugs, measurements in fasted simulated small intestinal fluids were kindly provided by Pfizer (Pharmacokinetics, Dynamics and Metabolism group, Sandwich, UK); additional solubility profiles across different pH values, if available, were obtained from the literature (Supplemental Table 1). The diffusion coefficients were estimated from molecular weights, the diffusion layer thickness was considered to be equal to particle radius (Hintz and Johnson, 1989), and a density of 1.2 g/ml was used (default value in GastroPlus version 7 and SimCYP version 10). A summary of drug solubility and particle size used can be found in Table 3.

Michaelis-Menten constants for CYP3A metabolism were collated from the literature except for felodipine, indinavir, nisoldipine, and saquinavir, for which $K_m$ data were determined in the current study. A summary of $K_m$ data, physicochemical properties, and drug permeability and clearance data used in the current analysis is provided in Table 2.

In vitro intrinsic clearance data used for predictions were determined in either pooled intestinal and liver microsomes (Gertz et al., 2010) or eight individual HJM reported in the current study. The clearance data obtained in the intestinal and liver pools were used for a comparison of model performance between the $F_G$ predictions by the $Q_{int}^M$ and the PBPK model and for the prediction of apparent intravenous and oral clearance using the PBPK model. On the other hand, the individual HJM data were used to assess interindividual variability in $F_G$ predictions using the PBPK model alone.

Measurement of Prediction Success and Comparison with In Vivo Data and $Q_{int}^M$ Predictions. The predictions of $F_G$ and apparent intravenous and oral clearance data were compared with corresponding in vivo data summarized in Table 4. Bias and precision of $F_G$ and clearance predictions were assessed by geometric fold error and root mean squared error.

### Results

Small intestinal metabolism was assessed for 12 drugs in eight individual human jejunal microsomal preparations (six male and two female white donors) (Table 1). The mean CYP3A activity was 4.12 nmol · min⁻¹ · mg⁻¹, ranging from 2.09 to 5.88 nmol · min⁻¹ · mg⁻¹ as measured by formation of 6β-hydroxytestosterone at 250 μM testosterone concentration. The mean CYP3A activity was associated with a coefficient of variation of 36%; a 2.8-fold difference was observed between the donors with the lowest and highest CYP3A activity. The between-day variability in testosterone 6β-hydroxylation activity determinations was low, ranging from 2 to 14%.

The individual intrinsic clearance values determined in the current study showed a more than 800-fold difference, ranging from 17.0 to 14,000 μl · min⁻¹ · mg⁻¹ for atorvastatin and saquinavir, respectively (Fig. 2). HJM 1 showed the lowest CYP3A activity and resulted, on average, in the lowest intrinsic clearance values for the drugs investigated. In contrast, HJM 6 generally resulted in the highest clearance values while displaying the second highest CYP3A activity. The mean HJM intrinsic clearance values ranged from 33.5 to 7220 μl · min⁻¹ · mg⁻¹ for atorvastatin and saquinavir, respectively, whereas the coefficient of variation ranged from 41 to 67% for simvastatin and tacrolimus, respectively (Table 4). Overall, the testosterone 6β-hydroxylation activity was a reasonable predictor for intrinsic clearance values between different individual jejunal microsomal preparations ($R^2 = 0.72$ combined data of all 12 substrates). The intrinsic clearance values of atorvastatin and saquinavir were poorly correlated with testosterone 6β-hydroxylation activity ($R^2 < 0.30$), in contrast to the good correlation seen for buspirone, midazolam and simvastatin ($R^2 > 0.85$).

### Table 3

<table>
<thead>
<tr>
<th>Drug</th>
<th>Solubility (μM)</th>
<th>Particle Radius (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporine</td>
<td>9.1 (13.3)*</td>
<td>0.018, 1.87*</td>
</tr>
<tr>
<td>Indinavir</td>
<td>90*</td>
<td>25*</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>64*</td>
<td>25*</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>38*</td>
<td>25*</td>
</tr>
</tbody>
</table>

* Value in parentheses represents fasted human intestinal fluids.

* Cyclosporine (Neoral and Sandimmune, respectively).

* Refer to indinavir sulfate and saquinavir mesylate; solubility data of indinavir and saquinavir over pH range were collated from the literature and the maximal solubility was limited to the highest reported value.

* Assumed.

* Henderson-Hasselbalch equation for a monoprotic base was used to estimate pH dependent solubility; maximum solubility was limited to 8.48 μM.
To assess the $K_m$ values of indinavir, felodipine, nisoldipine, and saquinavir, intrinsic clearance values were measured via a substrate depletion approach across a range of substrate concentrations. The $K_m$ values were estimated using eq. 2 at 0.1 ± 0.01 (mean and SE from the fit), 0.3 ± 0.02, 2.1 ± 0.3, and 5.3 ± 0.4 μM for indinavir, saquinavir, nisoldipine, and felodipine, respectively.

**Mechanistic Predictions of $F_G$ and Comparison to the $Q_{Gut}$ Model**

The predictions of intestinal first-pass metabolism were based on the PBPK model outlined under Materials and Methods. Different CYP3A amounts along the small intestine, intestinal transit time, drug-specific absorption rates, and differential blood flows to the intestinal segments were taken into account. Furthermore, the inclusion of Michaelis-Menten kinetic parameters allowed the assessment of potential nonlinear first-pass metabolism in small intestine and liver.

**TABLE 4**

*Individual $\text{CL}_{\text{unint}}$ values of 12 drugs determined by a substrate depletion method in eight individual HIM preparations and comparison to previously published data in human intestinal and hepatic microsomal pools.*

<table>
<thead>
<tr>
<th>Drug</th>
<th>HIM $^\text{a}$</th>
<th>HLM $^\text{b}$</th>
<th>HIM Mean $^\text{a}$</th>
<th>CV $^\text{c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atorvastatin</td>
<td>13.6</td>
<td>59.3</td>
<td>33.5</td>
<td>47</td>
</tr>
<tr>
<td>Buspirone</td>
<td>108</td>
<td>268</td>
<td>142</td>
<td>60</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>27.7</td>
<td>79.5</td>
<td>53.5</td>
<td>66</td>
</tr>
<tr>
<td>Felodipine</td>
<td>170</td>
<td>1990</td>
<td>1610</td>
<td>51</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>2440</td>
<td>4790</td>
<td>2740</td>
<td>59</td>
</tr>
<tr>
<td>Midazolam</td>
<td>340</td>
<td>429</td>
<td>418</td>
<td>47</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>3840</td>
<td>7000</td>
<td>6180</td>
<td>47</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>3030</td>
<td>7460</td>
<td>7220</td>
<td>65</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>3480</td>
<td>7100</td>
<td>5840</td>
<td>41</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>658</td>
<td>1040</td>
<td>1970</td>
<td>67</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>1650</td>
<td>3440</td>
<td>3690</td>
<td>59</td>
</tr>
</tbody>
</table>

$^\text{a}$ Data from the present study; for tacrolimus, $\text{CL}_{\text{unint}}$ value represents the mean and CV of seven individual intrinsic clearance values.

$^\text{b}$ $\text{CL}_{\text{unint}}$ data from HIM pool ($n = 10$ donors) and three HLM pools ($n = 105$ donors in total) reported in Gertz et al. (2010).

The $F_G$ predictions obtained by the PBPK model were compared with the predictions obtained by the $Q_{Gut}$ model (Fig. 3). To allow a direct comparison between the models, our previously published in vitro intrinsic clearance and permeability data were used (Table 5). The $F_G$ predictions of atorvastatin, felodipine, midazolam, nisoldipine, and simvastatin were marginally affected by the choice of model. However, considerable differences were observed in the $F_G$ predictions of indinavir, saquinavir, and terfenadine for which the PBPK model predicted higher $F_G$ values, more in line with the observed data. The $F_G$ predictions for indinavir, saquinavir, and terfenadine using the PBPK model were 0.98, 0.56, and 0.32, respectively. Differences in the $F_G$ estimates were due to the ability of the PBPK model to account for saturation of intestinal metabolism by using $V_{\text{max}}$ and $K_m$ data rather than $\text{CL}_{\text{unint}}$. In contrast to indinavir, the predicted $F_G$ values of saquinavir and terfenadine were very sensitive to changes in the $K_m$ values used. A reduction in prediction accuracy was observed for buspirone and cyclosporine when the PBPK model was compared with the $Q_{Gut}$ model. For these drugs, $F_G$ overprediction observed using the $Q_{Gut}$ model increased further in the PBPK model (e.g., for cyclosporine from 93 to 110% overprediction). For tacrolimus, a reduction in enterocyte blood flow in response to a reduction in cardiac output with age resulted in lower $F_G$ estimates than those reported previously (clinical data of tacrolimus were generally reported in individuals aged 50 or older). This result was accurately accounted for by both models. An overall improvement in $F_G$ predictions was observed using the PBPK model as measured by the decrease in geometric mean fold error (from 2.4 to 1.8) and root mean squared error value (from 0.32 to 0.28) in comparison with those for the $Q_{Gut}$ model.

**Prediction of Apparent Oral Clearance.** In contrast with the $Q_{Gut}$ model, the PBPK model also allowed the prediction of apparent oral clearance data of the 12 drugs investigated. Analogous to the $F_G$ predictions, this analysis was performed using the mean in vitro intrinsic clearance data from the HIM and HLM pools summarized in Table 4. The predicted oral clearance values are summarized in Table 5 and Fig. 4. The in vivo oral clearance data ranged from 20.7 to 14,400 l/h for tacrolimus and saquinavir, respectively; for cyclosporine and tacrolimus, observed oral clearance values were obtained from blood data. Prediction success of apparent oral clearance inside 3-fold
of unity was observed for cyclosporine (Neoral), felodipine, lovastatin, midazolam, nisoldipine, tacrolimus, and terfenadine. For cy-

closporine, indinavir, saquinavir, and terfenadine, incomplete absorption was predicted using the drug solubility data (Table 5).

Due to a combination of underprediction of hepatic and intestinal clearance, a particularly low prediction success of oral clearance was observed for atorvastatin and buspirone (both <2% of the observed value). In contrast, overpredictions of oral clearance were observed for indinavir and simvastatin. These were due to an approximately 2-fold overprediction of hepatic intrinsic clearance (Table 2) for both drugs. In addition, for indinavir, the incorporation of drug solubility data predicted incomplete absorption (61%), which contributed to the overprediction of CLoral. Saturation of hepatic first-pass metabolism of indinavir, on the other hand, was predicted by the model, as illustrated by the ratio of the areas under the curve in hepatic out- and inlet (FH = 32 versus 11% under linear conditions). For felodipine, overprediction of intestinal metabolism (observed and predicted FG values were 0.45 and 0.20, respectively) and underprediction of ap-

parent hepatic clearance (Table 5) resulted in underprediction of CLoral. Saturation of hepatic first-pass metabolism of indinavir, on the other hand, was predicted by the model, as illustrated by the ratio of the areas under the curve in hepatic out- and inlet (FH = 32 versus 11% under linear conditions). For felodipine, overprediction of intestinal metabolism (observed and predicted FG values were 0.45 and 0.20, respectively) and underprediction of ap-

\[ \text{Observed apparent clearance (l/h)} \]

\[ \text{Predicted apparent clearance (l/h)} \]

\[ \text{HIM} \]

\[ \text{HJM} \]

\[ \text{PBPK} \]

\[ \text{HIM} \]

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\[ \text{PBPK} \]

\[ \text{HIM} \]

\[ \text{HJM} \]

\[ \text{PBPK} \]

\[ \text{HIM} \]

\[ \text{HJM} \]
Intrinsic clearance data from eight individual HM were used to assess interindividual variation of $F_G$. In the literature, very few clinical studies report individual data to allow an estimation of interindividual variability of this parameter. For felodipine, individual, and pooled data from the current study (2–3-fold, respectively).

Intrinsic clearance data from eight individual HJM were used to assess interindividual variation of $F_G$. In the literature, very few clinical studies report individual data to allow an estimation of interindividual variability of this parameter. For felodipine, individual, and pooled data from the current study (2–3-fold, respectively).

In the current study, a PBPK model was applied in order to make predictions of intestinal availability using intravenous clearance and permeability data for 12 selected drugs. The analysis focused on drugs with high intestinal extraction in vivo, because $F_G$ prediction accuracy for these drugs based on the $Q_{Gut}$ model was lower that for drugs with $F_G > 0.5$. The study showed that the PBPK model improved $F_G$ prediction, resulting in lower bias and increased precision. In contrast to the $Q_{Gut}$ model, the PBPK model accounted for substrate concentrations at the enzyme site, Michaelis-Menten constants, and potential saturation of metabolism. In addition, the PBPK model accommodated any potential regional differences in intestinal availability because of the heterogeneous expression of CYP3A enzymes along the small intestine (Paine et al., 1997). Solubility of cyclosporine, indinavir, saquinavir, and terfenadine in simulated small intestinal fluids under fasted conditions was taken into account; for cyclosporine, these data compared well with reports in actual human intestinal fluids (Table 3). Because of a lack of transporter-specific kinetic data ($k_m$ and $V_{max}$), regional abundance data for key transporters in the small intestine, the current model did not account for active uptake or efflux in either the small intestine or the liver, which may contribute to the absorption and disposition of a number of drugs in the current dataset. At present, even for P-glycoprotein, the most studied transporter in the small intestine, conflicting reports regarding its distribution exist in the literature. Data are generally based on either mRNA levels (Nakamura et al., 2002; Thörm et al., 2005; Berggren et al., 2007), or, if protein levels are determined, on a small sample size $(n = 4)$ (Mouly and Paine, 2003) or on comparison of regional preparations from different individuals (Berggren et al., 2007). We refrained from performing a sensitivity analysis on the interplay of CYP3A and P-glycoprotein, as a publication dedicated exclusively to that effect has been presented (Darwich et al., 2011).

In addition to the need for more informative data on intestinal transporters, refinement of regional cytochrome P450 contents based on reports on tacrolimus and cyclosporine in 14 small intestinal samples, for which the between-subject variability was 54 to 70%, a 4.6- to 11-fold difference between highest and lowest activity in contrast to 2.8-fold in the current study (Lampen et al., 1995, 1996). Considering the relatively small sample size, one can question whether the current HM selection accurately reflects the true population variability of intestinal CYP3A activity. However, to our knowledge this is the largest dataset of intrinsic clearance data obtained in individual intestinal microsomes in the current literature.

**Prediction of $F_G$ Using PBPK Modeling.** In this study, a PBPK model was applied in order to make predictions of intestinal availability using intravenous clearance and permeability data for 12 selected drugs. The analysis focused on drugs with high intestinal extraction in vivo, because $F_G$ prediction accuracy for these drugs based on the $Q_{Gut}$ model was lower that for drugs with $F_G > 0.5$ (Gertz et al., 2010). Use of the PBPK model improved $F_G$ prediction, resulting in lower bias and increased precision. In contrast to the $Q_{Gut}$ model, the PBPK model accounted for substrate concentrations at the enzyme site, Michaelis-Menten constants, and potential saturation of metabolism. In addition, the PBPK model accommodated any potential regional differences in intestinal availability because of the heterogeneous expression of CYP3A enzymes along the small intestine (Paine et al., 1997).

Solubility of cyclosporine, indinavir, saquinavir, and terfenadine in simulated small intestinal fluids under fasted conditions was taken into account; for cyclosporine, these data compared well with reports in actual human intestinal fluids (Table 3). Because of a lack of transporter-specific kinetic data ($k_m$ and $V_{max}$), regional abundance data for key transporters in the small intestine, the current model did not account for active uptake or efflux in either the small intestine or the liver, which may contribute to the absorption and disposition of a number of drugs in the current dataset. At present, even for P-glycoprotein, the most studied transporter in the small intestine, conflicting reports regarding its distribution exist in the literature. Data are generally based on either mRNA levels (Nakamura et al., 2002; Thörm et al., 2005; Berggren et al., 2007), or, if protein levels are determined, on a small sample size $(n = 4)$ (Mouly and Paine, 2003) or on comparison of regional preparations from different individuals (Berggren et al., 2007). We refrained from performing a sensitivity analysis on the interplay of CYP3A and P-glycoprotein, as a publication dedicated exclusively to that effect has been presented (Darwich et al., 2011).

In addition to the need for more informative data on intestinal transporters, refinement of regional cytochrome P450 contents based

**TABLE 6**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Range</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atorvastatin</td>
<td>0.83</td>
<td>0.73–0.90</td>
</tr>
<tr>
<td>Buspirone</td>
<td>0.65</td>
<td>0.48–0.85</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>0.84</td>
<td>0.71–0.94</td>
</tr>
<tr>
<td>Felodipine</td>
<td>0.11</td>
<td>0.06–0.17</td>
</tr>
<tr>
<td>Indinavir</td>
<td>0.94</td>
<td>0.92–0.98</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>0.08</td>
<td>0.03–0.16</td>
</tr>
<tr>
<td>Midazolam</td>
<td>0.33</td>
<td>0.19–0.52</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>0.04</td>
<td>0.02–0.07</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>0.36</td>
<td>0.19–0.55</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>0.03</td>
<td>0.02–0.06</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>0.08</td>
<td>0.03–0.15</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>0.12</td>
<td>0.03–0.23</td>
</tr>
</tbody>
</table>

*Based on apparent permeability (apical-basolateral) data in the presence of a P-glycoprotein inhibitor.*
on a larger number of individuals and preferably determined by a less destructive preparation technique will provide greater confidence in the predictions of intestinal metabolism. The current data presented by Paine et al. (1997) are based on a mucosal scraping technique and a relatively small sample size, in contrast to hepatic CYP3A content data obtained from a meta-analysis of a large number of individuals (Rostami-Hodjegan and Tucker, 2007).

**Prediction of Apparent Intravenous and Oral Clearance.** Successful predictions of apparent oral clearance (inside 3-fold) were obtained using pooled microsomal intrinsic clearance data for 6 of 12 drugs. In contrast, large underpredictions of oral clearance were observed for atorvastatin and buspirone (consistent with underprediction of CLint). The metabolism of buspirone has been suggested to be mainly mediated by CYP3A4 and a similar metabolite pattern in HLM was reported in comparison with that in vivo (Jajoo et al., 1989; Zhu et al., 2005). Previously reported metabolite formation data resulted in marginally higher CLUint values (correction for fu free applied) compared with our substrate depletion data (460 versus 268 µl · min⁻¹ · mg⁻¹) (Zhu et al., 2005; Gertz et al., 2010). Buspirone clinical data after intravenous administration in eight volunteers were highly variable (i.e., CLHLM = 28.3 ± 10.3 ml · min⁻¹ · mg⁻¹, F = 3.9 ± 4.3%) with negligible renal excretion of unchanged drug (Gammans et al., 1986). However, neither the variability in the in vivo data nor the differences in the in vitro data are sufficient to explain the extensive underprediction of intrinsic hepatic clearance, because the in vitro data would classify buspirone as a low to moderate extraction drug (Ea = 38%; Table 5), whereas the in vivo data show clearance values in excess of hepatic blood flow.

A number of factors may contribute to the extensive underprediction of both intravenous and oral clearance of atorvastatin. First, in vitro data have highlighted drug affinity for uptake transporters (Kameyama et al., 2005) and studies in polymorphic organic anion transporting polypeptide 1B1 populations confirmed the clinical relevance of at least organic anion-transporting polypeptide 1B1 to atorvastatin disposition (Pasanen et al., 2007). It has been suggested that interconversion between acid and lactone forms may represent the initial step in atorvastatin metabolism (Jacobsen et al., 2000); this may occur directly from the parent or from the acyl glucuronide metabolite (Pruksaritamont et al., 2002). In human liver microsomes, the lactone displays on average a 70-fold greater intrinsic clearance than the hydroxy acid form (Jacobsen et al., 2000). Incorporation of the higher intrinsic clearance of the lactone form into the current analysis resulted in apparent intravenous and oral clearance values of 54.4 and 786 l/h, respectively, and better agreement with the observed data (37.5 and 949 l/h, respectively). The acid-lactone conversion was assumed to occur in plasma and therefore only CLUint values for hepatic metabolism was modified in the PBPK model, whereas intestinal intrinsic clearance remained unaltered. However, given the large variability in the ratio of CLUint of the lactone and the acid form (14- to 160-fold reported from four individual HLM) (Jacobsen et al., 2000), more data are required to investigate the rate-limiting step in atorvastatin metabolism.

In the case of cyclosporine, the reason for an ~5-fold underprediction of intrinsic clearance is unclear. Although UDP-glucuronosyltransferase-mediated metabolism in HLM has been reported (Strassburg et al., 2001), we were unable to confirm this by a substrate depletion assay performed in alamethicin-activated microsomes in the absence and presence of bovine serum albumin (data not shown). The underestimated of CLUint of cyclosporine was masked in the reasonably well predicted oral clearance by the fact that cyclosporine absorption was also underestimated (F a of 24 and 16% for Neoral and Sandimmune, respectively).

**F G Predictions Using Individual Jejunal Microsomal Samples.** Intrinsic clearance data from eight individual HJM were used to predict F G and associated interindividual variability. Individual in vivo F G values were reported for terfenadine, tacrolimus, midazolam, and felodipine in 6, 12, 20, and 45 individuals, respectively. Use of individual HJM clearance data in the PBPK model resulted in an underprediction of felodipine F G (consistent with the pooled data); however, the predicted variability in F G reflected the variability seen in vivo. Considering extensive binding of felodipine to plasma proteins (>99%) (Table 2), binding to enterocyte proteins during absorption cannot be ruled out. Any binding to the enterocyte proteins would lead to reduced apparent intestinal clearance and therefore increased F G predictions. Midazolam F G was well predicted using the pooled microsomal CLUint data but underpredicted using the individual HJM data. Although the coefficient of variation was predicted reasonably well, the F G range was underpredicted. In particular, the ability to predict the upper limit of in vivo F G values was poor, suggesting that selected individual jejunal donors did not cover the full spectrum of CYP3A activity present in the small intestine in vivo.

Other examples for which individual estimates of F G were available in the literature include tacrolimus (Flore et al., 1997; Hebert et al., 1999) and terfenadine (Clifford et al., 1997). The interindividual variabilities of in vivo F G estimates for tacrolimus and terfenadine were 36 and 54%, respectively. Both the CV and the fold difference between the highest and lowest F G values were overpredicted for terfenadine. In addition, the mean predicted F G of terfenadine was underestimated using the individual HJM data. Extensive binding to microsomal proteins has been observed for terfenadine using equilibrium dialysis (Gertz et al., 2008b). Any inaccuracies in the fu free estimate will bias the estimate of intrinsic clearance and also the estimate of unbound K m used in the PBPK model. In contrast, prediction of the mean tacrolimus F G was more successful (0.08 versus 0.14 for the predicted and observed F G, respectively); however, both the CV and the fold difference were overpredicted using the intrinsic clearance data from eight individual HJM (CV = 58% and 5.5-fold). As an alternative, a propagation of the variability in CYP3A in the small intestine (von Richter et al., 2004) using Monte Carlo simulations may be used to predict interindividual variability of intestinal metabolism and F G. However, it has to be emphasized that CYP3A abundance of a larger population needs to be assessed to capture the interindividual variability correctly.

In conclusion, the use of a PBPK model to study intestinal metabolism represents an improvement over the previously reported F G predictions of high-extraction drugs using the Qint model. Accounting for drug concentration and the region of absorption plays an important role in the assessment of intestinal metabolism and can be propagated into assessment of potential drug-drug interactions. In addition to F G predictions, the PBPK model allowed the assessment of apparent intravenous and oral clearance, with the majority of the drugs predicted within 3-fold of the observed data. Finally, the contribution of intestinal transporters or metabolizing enzymes other than CYP3A needs to be integrated in the models; currently, this is limited by the general lack of unambiguous abundance data in tandem with drug-specific in vitro kinetic data.

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Authorship Contributions

Participated in research design: Galezit, Gertz, and Houston.

Conducted experiments: Gertz.

Performed data analysis: Gertz.

Wrote or contributed to the writing of the manuscript: Galezit, Gertz, and Houston.

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References


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