Prediction of Human Intestinal First-Pass Metabolism of 25 CYP3A Substrates from In Vitro Clearance and Permeability Data

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Received February 8, 2010; accepted April 5, 2010

ABSTRACT:

Intestinal first-pass metabolism may contribute to low oral drug bioavailability and drug-drug interactions, particularly for CYP3A substrates. The current analysis predicted intestinal availability (F₂) from in vitro metabolic clearance and permeability data of 25 drugs using the Qout model. The drug selection included a wide range of physicochemical properties and in vivo F₂ values (0.07–0.94). In vitro clearance data (CLint) were determined in human intestinal (HIM) and three liver (HLM) microsomal pools (n = 105 donors) using the substrate depletion method. Apparent drug permeability (Papp) was determined in Caco-2 and Madin-Darby canine kidney cells transfected with human MDR1 gene (MDCK-MDR1 cells) under isotonic conditions (pH 7.4). In addition, effective permeability (Peff) data, estimated from regression analyses to Papp or physicochemical properties were used in the F₂ predictions. Determined CLinout values ranged from 0.022 to 76.7 μl/min/pmol of CYP3A (zolpidem and nisoldipine, respectively). Differences in CLinout values obtained in HIM and HLM were not significant after normalization for tissue-specific CYP3A abundance, supporting their interchangeable usability. The F₂ predictions were most successful when Papp data from Caco-2/MDCK-MDR1 cells were used directly; in contrast, the use of physicochemical parameters resulted in significant F₂ underpredictions. Good agreement between predicted and in vivo F₂ was noted for drugs with low to medium intestinal extraction (e.g., midazolam predicted F₂ value 0.54 and in vivo value 0.51). In contrast, low prediction accuracy was observed for drugs with in vivo F₂ <0.5, resulting in considerable underprediction in some instances, as for saquinavir (predicted F₂ is 6% of the observed value). Implications of the findings are discussed.

CYP3A enzymes represent the principle drug-metabolizing system in the small intestine, accounting for approximately 80% of total cytochrome P450 (P450) content (Lin et al., 1999; Paine et al., 2006). Although the total amount of CYP3A expressed in the human small intestine represents approximately 1% of the hepatic estimate (Paine et al., 1997), considerable drug extraction occurs during absorption of orally administered drugs (Hall et al., 1999; Galetin et al., 2008; Gertz et al., 2008a). This is due to the relatively high enterocytic drug concentration and the considerably lower blood flow to the intestine in comparison to the liver that allows prolonged exposure to the intestinal metabolizing enzymes. The contribution of intestinal first-pass metabolism has been shown indirectly for a number of CYP3A drugs administered both intravenously and orally in the absence and presence of inhibitors/inducers (Galetin et al., 2010). Such studies have allowed delineation of the relative roles of the liver and intestine and are particularly abundant for midazolam and to a lesser extent for cyclosporine, tacrolimus, alfentanil, and nifedipine.

The ability to predict the intestinal first-pass metabolism of drugs is of considerable importance for the assessment of oral clearance and the drug-drug interaction potential of CYP3A substrates. In both cases prediction models are very sensitive to the accuracy of the F₂ estimate, and the contribution of intestinal first-pass metabolism cannot be ignored. Two indirect methods have been proposed to estimate F₂ in vivo, namely use of plasma concentration-time profiles after either oral and intravenous administration or in the presence and absence of grapefruit juice. Both methods have several assumptions that may lead to potential bias in the F₂ estimates (Galetin et al., 2008; Gertz et al., 2008a).

In addition, in silico approaches have been proposed to estimate F₂. These are based on the incorporation of drug permeability and metabolism data and enterocytic blood flow together with zonal and negence of inhibitors/inducers (Galetin et al., 2010). Such studies have allowed delineation of the relative roles of the liver and intestine and are particularly abundant for midazolam and to a lesser extent for cyclosporine, tacrolimus, alfentanil, and nifedipine.

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In addition, in silico approaches have been proposed to estimate F₂. These are based on the incorporation of drug permeability and metabolism data and enterocytic blood flow together with zonal and

ABBREVIATIONS: P450, cytochrome P450; F₂, intestinal availability; Qout, hybrid parameter of blood flow and drug permeability; Papp, apparent permeability; A, apical; B, basolateral; MDCK-MDR1, Madin-Darby canine kidney cells transfected with multidrug resistance 1; Peff, effective permeability; CLinout, intrinsic clearance; CLint,h, hepatic intrinsic clearance; CLint,g, intestinal intrinsic clearance; CLinout,g, unbound intrinsic clearance; Qout, enterocytic blood flow; fu, fraction unbound; HLM, human liver microsomes; LC, liquid chromatography; MS/MS, tandem mass spectrometry; HIM, human intestinal microsome(s); Rb, blood/plasma distribution ratio; AUC, area under the curve; Qh, hepatic blood flow; CLh, hepatic blood clearance; P-gp, P-glycoprotein; CP-100356, N-(3,4-dimethoxyphenethyl)-4-(6,7-dimethoxy-3,4-dihydroisoxoquinolin-2(1H)-yl)-6,7-dimethoxyquinazolin-2-amine; MEM, minimal essential medium; HBSS, Hanks’ balanced salt solution; ER, efflux ratio; gmfe, geometric fold error; rmse, root mean square error; UGT, UDP glucuronosyltransferase.
cellular heterogeneous distribution of metabolic enzyme and efflux/uptake transporters along the length of the intestine (Ito et al., 1999; Tam et al., 2003; Badhan et al., 2009; Jamei et al., 2009). In contrast to complex physiologically based models, a “minimal” \( Q_{\text{Gut}} \) model has been proposed. This model allows prediction of \( F_G \) using in vitro drug clearance and permeability (Chalasani et al., 2002; Rostami-Hodjegan and Tucker, 2002; Yang et al., 2007) and accounts for either permeability or perfusion as the rate-limiting process in the small intestine (see Materials and Methods) (eq. 2). However, the suitability of this model has yet to be demonstrated for a broad range of drugs. Yang et al. (2007) investigated the \( F_G \) prediction success of the \( Q_{\text{Gut}} \) model using in vitro clearance and permeability data collated from a variety of sources; although results were promising, this study did not allow a comprehensive assessment of the \( Q_{\text{Gut}} \) model. In addition, a systematic assessment of the predictive utility of different in vitro systems to generate either clearance or permeability data as input parameters in this model is currently lacking.

The aim of this study was to investigate several aspects of the use of the \( Q_{\text{Gut}} \) model to predict \( F_G \) from in vitro data. First, a comparison was made between human intestinal and liver microsomes in their capacity to assess the metabolic drug clearance of a large number of structurally diverse drugs using a range of commercially available microsomal pools. The current analysis involves 25 drugs with differing physicochemical, metabolic, and permeability properties (Tables 1 and 3). These drugs also show differing extents of intestinal first-pass metabolism in vivo with \( F_G \) values ranging from 0.07 to 0.94 for lovastatin and alprazolam, respectively. The suitability of these in vitro clearance data to predict both intravenous and oral clearance (incorporating the available in vivo \( F_G \) values) was explored. Second, the use of permeability data \( [P_{\text{app}} (A-B)] \) generated under standardized conditions in both MDCK-MDR1 and Caco-2 cell lines was investigated, together with effective permeability \( (P_{\text{eff}}) \), as permeability input parameters in the \( Q_{\text{Gut}} \) model. The latter parameter was estimated either from regression analysis of the Caco-2 and MDCK-MDR1 data or from physicochemical parameters. Finally, the value of using midazolam as a calibrator within the \( Q_{\text{Gut}} \) model was investigated given the abundance of information on this drug both in vivo and in vitro.

The basis of these analyses, recommendations on the suitability of the various in vitro input parameters in the \( Q_{\text{Gut}} \) model and associated prediction accuracy are discussed.

Materials and Methods

**Prediction of Intestinal Availability.** The \( CL_{\text{intra}} \) values were scaled by the total amount of intestinal CYP3A to an intestinal intrinsic clearance, \( CL_{\text{int,g}} \) (eq. 1). The total CYP3A content used in the current analysis was 70.5 nmol (Paine et al., 1997). The \( F_G \) values were predicted using the \( Q_{\text{Gut}} \) model (Chalasani et al., 2002; Rostami-Hodjegan and Tucker, 2004) as defined in eq. 2. \( Q_{\text{Gut}} \), a model parameter, represents a hybrid parameter of enterocytic blood flow and drug permeability as defined by eq. 3 (Yang et al., 2007). \( F_G \) predictions from the \( Q_{\text{Gut}} \) model were compared with in vivo \( F_G \) estimates obtained either from intravenous/oral or grapefruit interaction data (Gertz et al., 2008b; Gertz et al., 2008a) and are summarized in Table 5. For indinavir, the \( F_G \) value was estimated from intravenous/oral data reported by Yeh et al. (1999).

\[
CL_{\text{intra}} = CL_{\text{unb}} \cdot \text{Content}_{\text{CYP3A}}
\]

\[
F_G = \frac{Q_{\text{Gut}}}{Q_{\text{Gut}} + \text{fu}_{\text{intra}} \cdot CL_{\text{intra}}}
\]

\[
Q_{\text{Gut}} = \frac{CL_{\text{perm}} \cdot CL_{\text{int,g}}}{CL_{\text{perm}} + CL_{\text{int,g}}}
\]

where \( F_G \) represents intestinal availability, \( Q_{\text{Gut}} \) represents mucusal blood flow (liters per hour), \( CL_{\text{intra}} \) represents unbound intrinsic clearance (micromoles per minute per picomole CYP3A), \( \text{fu}_{\text{intra}} \) represents fraction unbound in the enterocytes, and \( CL_{\text{perm}} \) represents permeability clearance (liters per hour), the product of intestinal surface area and either apparent (nanometers per second) or effective permeability (micrometers per second).

The fraction unbound in the enterocytes was assumed to be 1 (Yang et al., 2007) and an average enterocytic blood flow \( (Q_{\text{ent}}) \) of 18 l/h was used for the predictions (Granger et al., 1980). Use of either \( fu \) in plasma or blood as an alternative to \( \text{fu}_{\text{intra}} \) = 1 resulted in complete loss of prediction success and \( F_G \) values approaching 1 for all drugs investigated. When effective permeability was used to predict \( F_G \), an intestinal surface area of 0.66 m² (intestine treated as a tube as \( P_{\text{eff}} \) accounts for the surface area magnifications of the fold of Kerckering, the villi, and the microvilli) was used to estimate permeability clearance (Yang et al., 2007). If apparent permeability was used to estimate permeability clearance, an intestinal surface area of 200 m² was used.

**Midazolam as \( Q_{\text{Gut}} \) Calibrator.** To define a maximal value for \( Q_{\text{Gut}} \), that approaches mucusal blood flow, the use of midazolam as a calibrator was investigated. Midazolam was selected because this drug is characterized by high apparent permeability. The \( Q_{\text{Gut}} \) value of midazolam was estimated from the mean in vitro \( CL_{unb} \) data determined in this study and the mean \( F_G \) value in vivo determined from 16 intravenous/oral studies (Galezin et al., 2008) by rearranging eq. 2. The assumptions were that enterocytic binding is negligible \( (\text{fu}_{\text{Gut}} = 1) \) and that midazolam in vitro clearance is representative of its in vivo clearance. The \( F_G \) in vivo for midazolam ranged from 0.49 to 0.70 (Galezin et al., 2008) and the \( CL_{unb} \) ranged from 7.30 to 28.7 l/h (Table 1). The coefficient of variation on the mean \( Q_{\text{Gut}} \) value of midazolam was assessed by taking into account the variability of in vivo data \( (F_G) \) or both in vivo and in vitro clearance data.

**Determination of In Vitro Clearance.** In the in vitro clearance data were determined by depletion in three liver and one human intestinal microsomal pools in 0.1 M phosphate buffer (pH 7.4) containing 10 mM MgCl₂, 7.5 mM isocitric acid, 1.2 units/ml isocitric acid dehydrogenase, and 1 mM NADP. The human liver microsomal pools, HLM 1 \((n = 22)\), HLM 2 \((n = 50)\), and HLM 3 \((n = 33)\), were purchased from BD Gentest (Woburn, MA); HLM 2 \((n = 50)\) was kindly provided by Pfizer (Pharmacokinetics, Dynamics and Metabolism Department, Sandwich, Kent, UK). The human intestinal microsomal pool \((n = 10)\) was purchased from Xenotech, LLC (Lexena, KS), and microsomes were prepared by the elution method to ensure high enzyme activity, as reported previously (Galezin and Houston, 2006).

The final substrate concentration was 10-fold below the reported \( K_m \) values in the literature for the drugs investigated. For indinavir and saquinavir, a substrate concentration of 0.1 \( \mu \)M was used. The drug was added from methanol stock solution, 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 Eppendorf tubes at 37°C and 900 rpm in an Eppendorf Thermomixer. The metabolic reaction was initialized by adding warm NADP solution to warm incubation mixture, and samples were taken at six designated time points within 60 min of drug exposure. To monitor non-P450-dependent loss of drug over the incubation time additional samples were prepared in the absence of NADP. The metabolic reaction was terminated by addition of an equal volume of ice-cold acetonitrile containing the internal standard, samples were centrifuged at 1000g for 20 min at 4°C in a Mistral 3001 centrifuge (MSE, London, UK), and a 150-μl supernatant was removed from each Eppendorf vial and transferred to MVA glass vials (WVR International, Leicestershire, UK) before analysis on the LC-MS/MS system. Samples were embedded in the calibration curves upon LC-MS/MS analysis. \( CL_{unb} \) data were obtained for all drugs in the dataset, with the exception of alprazolam, quinidine, and triazolam; for these CYP3A substrates data were taken from a previous in-house study (Galezin and Houston, 2006).

**Microsomal Binding.** Nonspecific binding values of drugs to microsomal protein \( (\text{fu}_{\text{intra}}) \) were experimentally determined at different microsomal protein concentrations in HLM 1 using the microdialysis method (Gertz et al., 2008b). In addition to reported experimental values in Gertz et al. (2008b), \( \text{fu}_{\text{intra}} \) values for alfentanil, atorvastatin, cisapride, lovastatin, methadone, nisoldipine, rifabutin, sildenafil, trazodone, and zolpidem were determined at 0.1, 0.5, and 1.0 mg/ml microsomal protein concentrations over 8 h. The \( \text{fu}_{\text{intra}} \) values were fitted in Graft 5.0.10 (Erthacus Software Limited, Surrey, UK) against protein concentrations to determine the binding constant \( (K_b) \). The drug-specific \( K_b \) values are summarized at http://www.pharmacy.manchester.ac.uk/capkr/.

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PREDICTION OF HUMAN INTESTINAL FIRST-PASS METABOLISM

Comparison of CLint corrected for CYP3A abundance in liver and intestine for one HIM and three HLM pools

TABLE 1

<table>
<thead>
<tr>
<th>Substrate</th>
<th>HIM 1</th>
<th>HIM 2</th>
<th>HIM 3</th>
<th>HLM Mean (All)</th>
<th>CV</th>
</tr>
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<tbody>
<tr>
<td>Dosage</td>
<td>µM</td>
<td>µM</td>
<td>µM</td>
<td>µM</td>
<td></td>
</tr>
<tr>
<td>Alfentanil</td>
<td>0.950</td>
<td>0.550</td>
<td>0.737</td>
<td>1.33</td>
<td>37</td>
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<tr>
<td>Atorvastatin</td>
<td>0.272</td>
<td>0.279</td>
<td>0.296</td>
<td>0.573</td>
<td>41</td>
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<tr>
<td>Buspirone</td>
<td>2.17</td>
<td>1.60</td>
<td>1.47</td>
<td>2.12</td>
<td>19</td>
</tr>
<tr>
<td>Cisapride</td>
<td>2.74</td>
<td>1.77</td>
<td>1.56</td>
<td>2.45</td>
<td>26</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>0.396</td>
<td>0.228</td>
<td>0.598</td>
<td>0.675</td>
<td>43</td>
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<tr>
<td>Felodipine</td>
<td>23.4</td>
<td>8.40</td>
<td>13.5</td>
<td>16.6</td>
<td>33</td>
</tr>
<tr>
<td>Indinavir</td>
<td>6.00</td>
<td>2.60</td>
<td>3.90</td>
<td>4.70</td>
<td>41</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>48.8</td>
<td>18.8</td>
<td>31.6</td>
<td>42.2</td>
<td>37</td>
</tr>
<tr>
<td>Methadone</td>
<td>0.072</td>
<td>0.105</td>
<td>0.152</td>
<td>0.177</td>
<td>37</td>
</tr>
<tr>
<td>Midazolam</td>
<td>6.80</td>
<td>1.70</td>
<td>2.70</td>
<td>3.80</td>
<td>58</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>2.20</td>
<td>1.90</td>
<td>1.70</td>
<td>2.20</td>
<td>14</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>76.7</td>
<td>27.0</td>
<td>54.0</td>
<td>54.5</td>
<td>38</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>0.472</td>
<td>0.594</td>
<td>0.865</td>
<td>1.02</td>
<td>34</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>0.516</td>
<td>0.319</td>
<td>0.577</td>
<td>0.642</td>
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</tr>
<tr>
<td>Saquinavir</td>
<td>60.7</td>
<td>42.4</td>
<td>38.5</td>
<td>63.2</td>
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<tr>
<td>Sildenafil</td>
<td>1.12</td>
<td>0.87</td>
<td>1.05</td>
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<tr>
<td>Simvastatin</td>
<td>69.6</td>
<td>36.1</td>
<td>47.6</td>
<td>53.7</td>
<td>27</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>13.2</td>
<td>2.7</td>
<td>7.0</td>
<td>10.4</td>
<td>54</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>33.0</td>
<td>19.2</td>
<td>20.2</td>
<td>27.2</td>
<td>26</td>
</tr>
<tr>
<td>Trazodone</td>
<td>0.236</td>
<td>0.323</td>
<td>0.414</td>
<td>0.505</td>
<td>31</td>
</tr>
<tr>
<td>Verapamil</td>
<td>2.36</td>
<td>1.30</td>
<td>2.22</td>
<td>2.87</td>
<td>19</td>
</tr>
<tr>
<td>Zolpidem</td>
<td>0.022</td>
<td>0.070</td>
<td>0.104</td>
<td>0.144</td>
<td>61</td>
</tr>
</tbody>
</table>

CV, coefficient of variation.

closporine, the extent of nonspecific binding was predicted (Hallifax and Houston, 2006). CLint was calculated using eq. 4. Clearance values were corrected for the different organ abundance of CYP3A in liver and intestinal microsomes: 50 and 155 pmol of CYP3A/mg of protein for the small intestine and the liver, respectively (Paine et al., 1997; Rostami-Hodjegan and Tucker, 2007).

\[
\text{CL}_{\text{int}} = \frac{V}{k} \frac{1}{\text{protein}_{\text{microsomal}}} \frac{1}{f_{\text{ub}} \cdot \text{abundance}_{\text{CYP3A}}} \tag{4}
\]

where CLint is unbound intrinsic clearance (microliters per minute per picomole CYP3A), k is the depletion rate constant (minute \(^{-1}\)), V is the initial incubation volume (milliliters), protein_{microsomal} is the initial amount of protein (milligrams), and abundance_{CYP3A} is the abundance of CYP3A (picomoles of CYP3A per milligram of protein).

Testosterone 6β-Hydroxylation Activity, CYP3A enzyme activities of the HLM and HIM pools were determined at a testosterone concentration of 250 µM (equivalent to V_{max}), monitoring 6β-hydroxytestosterone formation. Incubation conditions were the same as those used in the depletion assay, and organic solvent content was 0.3% (v/v) methanol. Microsomal protein concentrations for the HLM and HIM pools were 0.25 and 0.50 mg/ml, respectively. On each occasion, three samples were taken after a 5-min incubation. Aliquots of 100 µl were removed into Eppendorf vials with 100 µl of ice-cold acetonitrile containing 1 µM progesterone as the internal standard. Activity was determined on three separate occasions to account for interday variability. Testosterone 6β-hydroxylation activities were 3.38 ± 0.323, 4.48 ± 0.218, and 6.09 ± 0.195 nmol/min/mg for HLM pool 1, 2, and 3, respectively, and 1.84 ± 0.173 nmol/min/mg for HIM.

Prediction of Intravenous and Oral Clearance. Oral and intravenous clearance values, fub, values, and blood/plasma distribution ratios (Rb) were respectively, and 1.84

1.74

H11006

available at http://www.mrc-bsu.cam.ac.uk/bugs/), assuming log-normal distributions of CLint and AUCoral. Criteria to exclude studies from analysis were non-white populations, nonlinear dose-AUC response, AUC reported over an insufficiently long time-course, analytical method inappropriate to determine the concentration of the drug of interest adequately, and studies performed in elderly or patient populations.

For the fixed-effects model, lnCLint, lnAUCoral ~ N(µ, σ²), where AUCoral represents the dose-normalized AUC, µ represents the log-transformed mean CLint, or mean AUCoral, and σ represents the variance (S.D.\(^2/N\)). The fixed-effects model was used for drugs for which sparse data were available; this model made no distributional assumption on ω. The mean and variance of the untransformed variables are exp(µ + 0.5 σ²) and exp(2µ + σ²)(exp(σ²) − 1), respectively. Otherwise random-effects models were used [lnCLint, lnAUCoral ~ N(µ, σ²), gamma distribution of ω] or a modified random-effects model [for midazolam CLint, only: lnCLint, lnAUCoral ~ N(µ, σ²)] accounting for the distribution of the true S.D. of study (S.D., ~ MS.D.mean, S.D. precision), where ω and the true precision followed gamma distributions].

CL_{int} values after intravenous and oral drug administration were obtained using eqs. 5 and 6, respectively (Pang and Rowland, 1977):

\[
\text{CL}_{\text{oral}} = \frac{\text{CL}_{\text{h}}}{f_{\text{ub}} \cdot \left(1 - \frac{\text{CL}_{\text{h}}}{\text{Q}_d}\right)} \tag{5}
\]

\[
\text{CL}_{\text{oral}} = \frac{\text{D}}{\text{AUC} \cdot f_{\text{ub}} \cdot F_{\text{a}} F_p} \tag{6}
\]

where CLh and D/AUC represent the hepatic blood clearance obtained from mean plasma data (Table 2) after correcting for renal clearance (where applicable) and Rp. The fub represents the fraction unbound in blood, Q_d represents the average hepatic blood flow of 20.7 ml/min/kg (Kato et al., 2003), D represents the oral drug dose (milligrams per kilogram), AUC represents the area under the drug concentration-time curve (milligram per minute per milliliter), and F_a represents the fraction absorbed.

Clearance data for cisapride, lovastatin, simvastatin, and terfenadine were only available after oral drug administration. Oral clearance data for cyclo-
sporine were available for two different oral formulations (Sandimmune and Neoral) and both were used in the assessment. Rb values were not available for lovastatin, nisoldipine, and trazodone. Given the very high structural similarity between simvastatin and lovastatin, the Rb value used for lovastatin was 0.57. The CL_{int,h} displayed a high sensitivity to changes in Rb. Dose-AUC response data for atorvastatin, nisoldipine, and trazodone were assessed where available to avoid any bias in CL_{int,h} estimates from oral data.

**Determination of In Vitro Permeability.** Drug permeability experiments in Caco-2 and MDCK-MDR1 cells were performed at Pfizer (Pharmacokinetics, Dynamics and Metabolism Department). The permeability experiments were performed during cell passages 11 to 32 for the MDCK-MDR1 cells and 25 to 35 for the Caco-2 cells. To determine the passive permeability of drugs with efflux ratios greater than 2, the P-gp inhibitor CP-100356 (Wandel et al., 1999) was coincubated at a concentration of 10 μM. MDCK-MDR1 cells (250 μl, density 2.5 × 10^5 cells/ml in MDCK-MDR1 cell media) were added to the apical side of Costar HTS 24-well Transwell plates and MDCK-MDR1 cell media (1 ml) was added to the basolateral side. Plates were incubated for 4 days in a LECC Research Incubator (37°C under 5% CO2 in air) before cell permeability experiments. One day before the experiment, the cell media in the apical and basolateral sides of the plate were replaced by fresh media. The incubation buffer was prepared from HEPES (2.38 g) solubilized in HLM and HIM, respectively, and triazolam (51.2 and 4.73 μl/min/mg in HLM and HIM, respectively).

### TABLE 2

<table>
<thead>
<tr>
<th>Substrate</th>
<th>(f_u)</th>
<th>(R_b)</th>
<th>In Vivo Plasma Clearance</th>
<th>(n)</th>
<th>Oral Plasma Clearance</th>
<th>(n)</th>
<th>CL_{int,h}</th>
<th>Predicted</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(\text{ml/min/kg})</td>
<td></td>
<td>(\text{ml/min/kg})</td>
<td></td>
<td>(\text{ml/min/kg})</td>
<td></td>
</tr>
<tr>
<td>Alfentanil</td>
<td>0.086</td>
<td>0.63</td>
<td>4.19 (3.93, 4.46)</td>
<td>4</td>
<td>11.4 (10.7, 12.2)</td>
<td>4</td>
<td>71.8</td>
<td>73.9</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>0.29</td>
<td>0.85</td>
<td>0.76 (0.71, 0.82)</td>
<td>2</td>
<td>0.99 (0.90, 1.10)</td>
<td>14</td>
<td>7.6</td>
<td>8</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>0.02</td>
<td>0.55</td>
<td>8.93</td>
<td>1</td>
<td>226 (211, 241)</td>
<td>7</td>
<td>2070</td>
<td>2170</td>
</tr>
<tr>
<td>Buspirone</td>
<td>0.05</td>
<td>0.81</td>
<td>28.3</td>
<td>1</td>
<td>1,170 (797, 1670)</td>
<td>14</td>
<td>3,020</td>
<td>4,920</td>
</tr>
<tr>
<td>Cisapride</td>
<td>0.02</td>
<td>1.0</td>
<td>7.85</td>
<td>4</td>
<td>7.0 (7.08, 8.69)</td>
<td>14</td>
<td>216</td>
<td>256</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>0.06</td>
<td>1.36</td>
<td>3.99 (3.50, 4.59)</td>
<td>7</td>
<td>12.3 (9.92, 14.9)</td>
<td>8</td>
<td>110</td>
<td>97</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>0.06</td>
<td>1.36</td>
<td>3.89 (3.50, 4.59)</td>
<td>7</td>
<td>25.4 (20.4, 31.0)</td>
<td>7</td>
<td>110</td>
<td>201</td>
</tr>
<tr>
<td>Felodipine</td>
<td>0.004</td>
<td>0.70</td>
<td>11.9 (11.4, 12.4)</td>
<td>4</td>
<td>110 (88, 137)</td>
<td>11</td>
<td>17,020</td>
<td>12,300</td>
</tr>
<tr>
<td>Indinavir</td>
<td>0.36</td>
<td>0.84</td>
<td>18.4</td>
<td>1</td>
<td>14.2 (13.1, 15.3)</td>
<td>6</td>
<td>440</td>
<td>30.4</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>0.043</td>
<td>0.57</td>
<td>—</td>
<td>—</td>
<td>329 (293, 368)</td>
<td>5</td>
<td>535</td>
<td>4,100</td>
</tr>
<tr>
<td>Methadone</td>
<td>0.21</td>
<td>0.75</td>
<td>1.66 (1.49, 1.85)</td>
<td>5</td>
<td>2.13 (1.94, 2.34)</td>
<td>6</td>
<td>7.48</td>
<td>7.03</td>
</tr>
<tr>
<td>Midazolam</td>
<td>0.031</td>
<td>0.55</td>
<td>6.16 (5.64, 6.72)</td>
<td>30</td>
<td>24.2 (20.5, 28.5)</td>
<td>14</td>
<td>440</td>
<td>402</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>0.044</td>
<td>0.67</td>
<td>7.55 (7.00, 8.14)</td>
<td>14</td>
<td>15 (12.0, 17.5)</td>
<td>14</td>
<td>378</td>
<td>245</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>0.003</td>
<td>1.0†</td>
<td>14.4 (13.2, 15.6)</td>
<td>4</td>
<td>319 (280, 361)</td>
<td>5</td>
<td>15,900</td>
<td>11,700</td>
</tr>
<tr>
<td>Quinidine</td>
<td>0.26</td>
<td>0.87</td>
<td>3.86 (3.42, 4.35)</td>
<td>2</td>
<td>5.64 (5.18, 6.13)</td>
<td>3</td>
<td>11.6</td>
<td>15.0</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>0.015</td>
<td>0.60</td>
<td>7.76</td>
<td>1</td>
<td>13.7 (12.8, 14.8)</td>
<td>21</td>
<td>1,380</td>
<td>815</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>0.29</td>
<td>0.60</td>
<td>3.46</td>
<td>1</td>
<td>16.2 (12.8, 20.5)</td>
<td>8</td>
<td>16.6</td>
<td>11.5</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>0.028</td>
<td>0.74</td>
<td>12.9 (10.3, 16.0)</td>
<td>4</td>
<td>3.440 1 3.216 1 3.929 1 6.490 1 6.390 1 4.410 1 8.360</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sildenafil</td>
<td>0.04</td>
<td>0.64</td>
<td>8.97</td>
<td>1</td>
<td>9.42 (8.72, 10.2)</td>
<td>4</td>
<td>694</td>
<td>127</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>0.06</td>
<td>0.57</td>
<td>—</td>
<td>—</td>
<td>387 (317, 469)</td>
<td>18</td>
<td>903</td>
<td>80,370</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>0.13</td>
<td>0.35</td>
<td>0.64* (0.57, 0.73)</td>
<td>4</td>
<td>4.93* (4.09, 5.83)</td>
<td>8</td>
<td>179</td>
<td>186</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>0.03</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>1,260 1 1,680 1 2,940 1 2,290 1 2,360</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trazodone</td>
<td>0.07</td>
<td>1.0†</td>
<td>2.14 (1.94, 2.34)</td>
<td>3</td>
<td>2.27 (2.18, 2.37)</td>
<td>8</td>
<td>34.1</td>
<td>26.9</td>
</tr>
<tr>
<td>Triazolam</td>
<td>0.10</td>
<td>0.62</td>
<td>2.94 (2.77, 3.13)</td>
<td>7</td>
<td>6.36 (5.92, 6.83)</td>
<td>9</td>
<td>38.2</td>
<td>47.2</td>
</tr>
<tr>
<td>Verapamil</td>
<td>0.093</td>
<td>0.89</td>
<td>11.7 (11.0, 12.5)</td>
<td>6</td>
<td>43.3 (39.1, 47.8)</td>
<td>6</td>
<td>347</td>
<td>303</td>
</tr>
<tr>
<td>Zolpidem</td>
<td>0.08</td>
<td>0.76</td>
<td>4.25 (3.96, 4.55)</td>
<td>2</td>
<td>5.14 (4.33, 6.06)</td>
<td>11</td>
<td>71.8</td>
<td>50.8</td>
</tr>
</tbody>
</table>

### Notes

a Intravenous and oral clearance data represent systemic plasma clearances. Observed CL_{int,h} values after intravenous and oral administration were calculated using eqs. 5 and 6, respectively, after plasma clearances were corrected for renal excretion and blood/plasma ratio (CL_{int,h} = (CL_{int} - CL_{Rb}))/Rb).

b CL_{int,h} predictions based on the mean in vitro clearance data from three HLM pools.

c Data were supplemented from Galetin and Houston (2006) for alprazolam (8.46 and 0.515 ml/min/mg in HLM and HIM, respectively), quinidine (6.60 and 2.13 ml/min/mg in HLM and HIM, respectively)
d Blood clearance data.

e Oral clearance of cyclosporine (Neoral).

f Fraction absorbed: 0.9 and 0.3 for cyclosporine and saquinavir, respectively; otherwise complete absorption was assumed.

g Oral clearance of cyclosporine (Sandimmune).

h Provided by Pfizer, Global Research and Development.

i Evidence for saturation in vivo (Yeh et al., 1999).

j Assumed to be the same as for simvastatin.

k Assumed to be 1.
in a final substrate concentration of 0.1 μM (final concentration of dimethyl sulfoxide 0.03%, v/v). For each plate, metoprolol and talinolol were co-incubated as positive controls for passive permeability and active efflux, respectively; both were incubated at a final substrate concentration of 2 μM. The substrates and the positive controls were incubated in three separate wells in both directions. The plates were placed in a LEEC Research Incubator (37°C under 5% CO₂ in air) on a shaker at 150 rpm for 2 or 2.5 h for the Caco-2 and MDCK-MDR1 cell permeability experiment, respectively. The setup and sampling were performed on a Tecan Genesis RSP 150 Robot. Samples and calibration curves were added to acetonitrile and centrifuged for 20 min at 3000 rpm before analysis on an LC-MS/MS system.

**Sample Analysis.** Quantification of samples was performed with Analyst version 1.4.1 software (Applied Biosystems, Foster City, CA). Permeability values were calculated in both directions using eq. 7; drug recovery was between 75% and 130% except where noted in Table 3. Apparent permeability (P_{app}) and efflux ratio (ER) data, defined as P_{app} (B-A)/P_{app} (A-B), for metoprolol and talinolol were assessed with every plate. P_{app} (A-B) for metoprolol >200 nm/s and an ER value for talinolol of >5 were used as an indicator for a functional cell monolayer.

\[
P_{\text{app}} = \frac{V_{\text{fs}} \cdot dC}{(A-C_{0}) \cdot dt}
\]

where V_{fs} represents volume of the receiver chamber, A represents surface area of the cell monolayer (0.33 cm²), C_{0} represents the initial substrate concentration (micromolar concentration), and dC/dt represents the change of concentration over time.

Metoprolol P_{app} data were available for 11 and 9 occasions for the assessment of interday variability of P_{app} in MDCK-MDR1 and Caco-2 cells, respectively. Permeability data for the drugs investigated were normalized for the mean metoprolol permeability to account for interday variability. The mean ± S.D. P_{app} (A-B) for metoprolol in MDCK-MDR1 cells was 341 ± 92 nm/s and in Caco-2 cells was 281 ± 42 nm/s. No permeability data could be determined for nisoldipine, terfenadine, and atorvastatin, and literature values were taken.

**TABLE 3**

<table>
<thead>
<tr>
<th>Substrate (^{a})</th>
<th>PSA</th>
<th>HBD</th>
<th>MDCK-MDR1</th>
<th>Caco-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P_{app} (A-B) mm/s</td>
<td>ER</td>
<td>P_{app} (A-B) mm/s</td>
<td>ER</td>
</tr>
<tr>
<td>Alfenilat</td>
<td>85.0</td>
<td>376</td>
<td>0.7</td>
<td>293</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>43.1</td>
<td>369</td>
<td>0.9</td>
<td>255</td>
</tr>
<tr>
<td>Buspirone</td>
<td>69.6</td>
<td>398</td>
<td>1.0</td>
<td>254</td>
</tr>
<tr>
<td>Cisapride</td>
<td>86.1</td>
<td>299</td>
<td>0.6</td>
<td>299</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>278.5</td>
<td>6</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Felodipine</td>
<td>64.6</td>
<td>139</td>
<td>0.5</td>
<td>42</td>
</tr>
<tr>
<td>Indinavir</td>
<td>118</td>
<td>19</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>72.8</td>
<td>261</td>
<td>1.1</td>
<td>145</td>
</tr>
<tr>
<td>Methadone</td>
<td>20.3</td>
<td>259</td>
<td>1.4</td>
<td>219</td>
</tr>
<tr>
<td>Midazolam</td>
<td>30.2</td>
<td>369</td>
<td>0.8</td>
<td>324</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>107.8</td>
<td>389</td>
<td>1.0</td>
<td>235</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>108</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Quinidine</td>
<td>45.6</td>
<td>176</td>
<td>4.4</td>
<td>85</td>
</tr>
<tr>
<td>Repaglinide</td>
<td>78.9</td>
<td>282</td>
<td>1.0</td>
<td>241</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>206</td>
<td>52</td>
<td>6.0</td>
<td>95</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>166.8</td>
<td>8</td>
<td>75</td>
<td>4</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>113</td>
<td>262</td>
<td>2.0</td>
<td>256</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>72.8</td>
<td>215</td>
<td>1.3</td>
<td>68</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>178.4</td>
<td>105</td>
<td>4.6</td>
<td>131</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>43.7</td>
<td>2</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Trizodone</td>
<td>42.3</td>
<td>295</td>
<td>1.1</td>
<td>242</td>
</tr>
<tr>
<td>Triazolam</td>
<td>43.1</td>
<td>350</td>
<td>0.9</td>
<td>280</td>
</tr>
<tr>
<td>Verapamil</td>
<td>64</td>
<td>318</td>
<td>1.0</td>
<td>138</td>
</tr>
<tr>
<td>Zolpidem</td>
<td>37.6</td>
<td>365</td>
<td>1.0</td>
<td>319</td>
</tr>
</tbody>
</table>

P_{app}, polar surface area; HBD, hydrogen bond donor; N.D., not determined.

\(^{a}\) P_{app} (A-B) data were supplemented from the literature for atorvastatin: 60 nm/s in Caco-2 cells (Wu et al., 2000; Hochman et al., 2004); terfenadine: 106 nm/s in MDCK-MDR1 cells (Polli et al., 2001; Doan et al., 2002); and nisoldipine: >200 nm/s, given its structural similarity to nifedipine and nitrendipine, both of which show high permeability (Polli et al., 2001).
Prediction of $P_{eff}$ from Physicochemical Data. In addition to in vitro data, the physicochemical parameters, hydrogen bond donors, and polar surface area were collated for all drugs investigated on http://pubchem.ncbi.nlm.nih.gov/. With use of eq. 11, $P_{eff}$ data were predicted from these physicochemical parameters, as described previously (Wininwarter et al., 1998). The application of $P_{eff}$ values using this approach for the prediction of $F_{oc}$ was assessed in comparison to other permeability approaches described above.

$$\log P_{eff} = -2.546 - 0.011\text{PSA} - 0.278\text{HBD}$$  \hspace{1cm} (11)

where PSA is polar surface area and HBD is hydrogen bond donor.

Bias and precision in estimating $CL_{int,h}$ and $F_{oc}$ were calculated as geometric fold error (gmfe) in eq. 12 and root mean squared error (rmse) (units of the parameter investigated) in eq. 13 (Sheiner and Beal, 1981; Fahimi et al., 2008). The gmfe does not allow over- and underpredictions to cancel each other out and indicates therefore an absolute deviation from the line of unity.

$$\text{gmfe} = 10^{\log 10(\text{predicted})/\text{observed}}$$  \hspace{1cm} (12)

$$\text{rmse} = \sqrt{\frac{1}{n}\sum (\text{predicted} - \text{observed})^2}$$  \hspace{1cm} (13)

where $n$ is number of observations.

Results

In Vitro Clearance. In vitro clearance data were obtained in pooled human intestinal microsomes ($n = 10$) and three liver microsomal pools (in total, $n = 105$ donors). The $CL_{int}$ values covered four orders of magnitude and ranged from 1.10 to 3840 µL/min/mg protein for zolpidem and nisoldipine, respectively, for the HIM pool. The correlation between the HIM and the mean HLM clearance was strong ($R^2 = 0.98$). The $CL_{int}$ values (expressed per milligram of protein) determined in HIM represented between 32 and 47% of the average HLM $CL_{int}$. However, tacrolimus and midazolam showed higher than average $CL_{int}$ values in HIM representing 63 and 79% of the HLM clearance, respectively.

A direct comparison of $CL_{int}$ obtained in HLM and in HIM after the correction for the tissue-specific abundance of CYP3A is shown in Table 1. Corrected $CL_{int}$ values in the HIM pool ranged from 0.022 to 76.7 µL/min/mmol of CYP3A for zolpidem and nisoldipine, respectively, and from 0.106 to 48.1 µL/min/mmol of CYP3A in the HLM pools for zolpidem and saquinavir, respectively. Figure 2 illustrates comparison of the $CL_{int}$ values of the drugs investigated normalized for the population CYP3A abundance in the intestine and liver. Good agreement between the estimates was observed with 50% of the clearance values within 1.5-fold and 14% outside 2-fold of unity. The most pronounced discrepancy in the HLM and HIM clearance was observed for midazolam ($CL_{int}$ in HIM represented 246% of mean $CL_{int}$ in HLM) and zolpidem ($CL_{int}$ in HIM represented 21% of mean $CL_{int}$ in HLM). After correction for tissue-specific CYP3A abundance, the $CL_{int}$ determined in the HIM pool represented 100 to 144% of the $CL_{int}$ in the HLM pools and was not statistically different at a significance level of 5% (Student’s $t$ test).

The $CL_{int}$ data obtained from the three different liver pools were compared and found to be significantly different ($p < 0.05$). The highest clearance values were determined in the HLM 3 pool, whereas clearance in HLM pools 1 and 2 represented 56 to 75% of the $CL_{int}$ determined in this pool. The microsomal activity toward 6β-hydroxytestosterone was determined in all microsomal pools and ranged from 1.84 to 6.09 nmol/min/mg in the HIM and the HLM batch 3, respectively. The activity determination for each microsomal batch was associated with low interday variability ($\leq 10\%$). The coefficient of variation between the activities of the different HLM batches was 29%, comparable to the 31% average interbatch variability in clearance for the drugs investigated. Most of the $CL_{int}$ variability between different microsomal pools could be attributed to the differences in 6β-hydroxytestosterone activity ($R^2 = 0.99$). For midazolam, nifedipine, nisoldipine, and tacrolimus only 49 to 73% of the changes in clearance could be attributed to differences in testosterone 6β-hydroxylation activity.

Prediction of Hepatic Intrinsic Clearance from Intravenous and Oral Data. A large body of clinical clearance data after intravenous drug administration was collated for the drugs investigated, with up to 30 clinical studies investigating 469 individuals available for midazolam (Table 2). The systemic plasma clearance of midazolam was estimated to be 6.16 ml/min/kg ($5.64, 6.72, 95\%$ credible interval) using meta-analysis of literature data, which corresponded to an $CL_{int,h}$ of 440 ml/min/kg ($364, 533, 95\%$ credible interval). In total, the database consisted of 21 drugs, because no intravenous clearance data were available for cisapride, lovastatin, simvastatin, and terfenadine, with a range of in vivo systemic plasma clearance, varying from 0.76 to 28.3 ml/min/kg for the intravenous data of alprazolam and buspirone, respectively (Table 2). The $fu_r$ ranged from 0.3 to 36% for nisoldipine and indinavir, respectively, and the $CL_{int}$ ranged from 4 to ~100% of $Q_h$ for tacrolimus and buspirone, respectively. Overall, 11 of the investigated drugs showed $CL_{int}$ ≥50% of $Q_h$. The highest blood clearance values were observed for buspirone, indinavir, and saquinavir (all >80% of $Q_h$).

A moderate correlation existed between the observed and the predicted $logCL_{int,h}$ values when the well stirred liver model was used ($R^2 >0.65$); 43 and 76% of the predictions were within 2- and 5-fold of unity, respectively. The most significant $CL_{int,h}$ underpredictions ($\leq 20\%$ of observed) were noted for atorvastatin (2.5%), buspirone (7.6%), regaplinide (7.9%), felodipine (10%), and sildenafil (20%). The use of the average HLM clearance data and a mean microsomal recovery value of 40 mg/g resulted in a median underprediction of 19%, a 3.1-fold bias (gmfe), and a precision (rmse) of 4140 (Fig. 3A). The bias was notably decreased with increasing microsomal CYP3A4 activity of the different liver pools; however, the precision of $CL_{int,h}$ predictions was not affected by the use of different HLM pools.

A substantial number of clinical studies were available after oral administration; for most drugs investigated data from ≥3 clinical studies were used in the meta-analyses. For midazolam, 14 separate studies with 262 individuals in total were considered in the analysis. The oral clearance of midazolam was estimated to be 24.2 ml/min/kg (20.5, 28.5, 95% credible interval), which corresponded to an $CL_{int,h}$
of 402 ml/min/kg (340, 473, 95% credible interval). Clinical studies with an oral dose of midazolam exceeding 10 mg were excluded from the analysis because of a possible nonlinear response in midazolam AUC. For the entire database, the oral clearance values ranged from 0.99 to 3440 ml/min/kg for alprazolam and saquinavir, respectively (Table 2). The contribution of the small intestine to oral clearance was incorporated using the C_lint,h values listed in Table 5, as shown in eq. 6. Consequently, C_lint,h values ranged from 3.22 to 16,800 ml/min/kg for alprazolam and terfenadine, respectively. After correction for the drug-specific F_C, the C_lint,h values estimated from oral data corresponded to 94% of the intravenous estimate on average. Particular differences between intravenous and oral estimates were apparent for indinavir (where oral C_lint,h represented 7% of intravenous data) and sildenafil (18%). For indinavir, this was attributed to enzyme saturation/inhibition at the high dose at which this drug is generally administered, and the data were subsequently excluded from the analysis.

The predictability of oral clearance from in vitro data generated in the current study was investigated. Figure 3B displays the comparison of the predicted to the observed C_lint,h from oral data; 38 and 65% of the predictions were within 2- and 5-fold, respectively. The median prediction success was 93% of the observed values (interquartile range 33–204%). Accounting for minor differences between intravenous and oral estimates was apparent for indinavir (where oral C_lint,h represented 7% of intravenous data) and sildenafil (18%). For indinavir, this was attributed to enzyme saturation/inhibition at the high dose at which this drug is generally administered, and the data were subsequently excluded from the analysis.

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alone was accounted for and 61% when combined variability of the in vivo \( F_G \) estimate and the in vitro CL\(_{int,\text{all}} \) data was accounted for. This value approached the \( Q_{\text{Gut}} \) value of 18 l/h used in this study, suggesting no permeability limitations and supporting the use of midazolam as a \( Q_{\text{Gut}} \) calibrator.

**Predictions of Intestinal Availability.** Permeability and in vitro clearance data were used for \( F_G \) predictions using the \( Q_{\text{Gut}} \) model by eight different methods (Fig. 4). First, \( P_{\text{app}} \) data determined across Caco-2 and MDCK-MDR1 cell monolayers were used directly. In addition, \( P_{\text{eff}} \) data were estimated from the regression analysis to \( P_{\text{app}} \) values from either Caco-2 or MDCK-MDR1 and from physicochemical properties. In the latter approaches, \( Q_{\text{Gut}} \) data were investigated before and after calibration with midazolam \( Q_{\text{Gut}} \). The results of the prediction bias and accuracy of different approaches for the entire dataset and for the subset of drugs with in vivo \( F_G < 0.5 \) are summarized in Table 4. The best \( F_G \) prediction success was apparent from the direct input of \( P_{\text{app}} \) data as shown by the lowest bias and interquartile range of all approaches investigated (Table 4, 1). The use of permeability data obtained in the different cell lines investigated in the current study resulted in minor differences in \( F_G \) prediction success. In contrast, the input of the \( P_{\text{eff}} \) data from the regression analysis to \( P_{\text{app}} \) data resulted in a larger bias regardless of the cell line used (Fig. 4A; Table 4); however, this underprediction trend was corrected after adjustment for midazolam \( Q_{\text{Gut}} \). The analysis has also indicated that the use of these empirical regression equations for drugs with \( P_{\text{app}} < 10 \) nm/s is problematic, as highlighted by considerable scatter in this area (Fig. 2). The use of polar surface area and hydrogen bonding potential resulted in the most biased \( F_G \) predictions and significant underprediction of \( F_G \) (\( p < 0.05 \)). Adjustment for midazolam as a calibrator of \( Q_{\text{Gut}} \) had a negligible impact on the \( F_G \) predictions from physicochemical properties and the underprediction trend remained (Fig. 4; Table 4, 4).

Figure 4B illustrates the high degree of prediction accuracy for drugs with in vivo \( F_G \) values > 0.5. In contrast, a subset of 11 drugs with in vivo \( F_G < 0.5 \) is comparatively poorly predicted, and the degree of imprecision is considerably increased in comparison to drugs with low to moderate intestinal extraction (Fig. 4C; Table 4). Consistent over the entire set, the direct input of \( P_{\text{app}} \) data resulted in the lowest bias and interquartile range in \( F_G \) predictions. For indinavir, significant underprediction was observed regardless of permeability parameter input. The choice to perform permeability experiments under isotonic conditions might have biased the \( P_{\text{app}} \) (A-B) values for certain drugs in the dataset, because the intestinal pH in the duodenum and jejunum is < 7 (Fallingborg et al., 1989). Considering that a significant impact of permeability to \( F_G \) predictions was only apparent at drug permeability < 100 nm/s, the chosen in vitro conditions might have biased subsequent \( F_G \) predictions of indinavir and saquinavir (\( P_{\text{app}} < 100 \) nm/s) and to a minor extent quinidine (\( P_{\text{app}} < 100 \) nm/s in Caco-2 cells).

![Figure 4](image-url)

**Fig. 4.** Prediction success of \( F_G \) using eight different permeability inputs in the \( Q_{\text{Gut}} \) model. The average CL\(_{int,\text{all}} \) data from HLM and HIM were used. A, log(predicted \( F_G \)/observed \( F_G \)) of the complete dataset of 25 drugs. B and C, prediction success of drugs with in vivo \( F_G \) values above \( n = 14 \) and below 0.5 \( n = 11 \), respectively. Zero indicates unity and negative and positive values represent under- and overpredictions, respectively; *, significant underpredictions (\( p < 0.05 \)); cal., denotes calibration for midazolam \( Q_{\text{Gut}} \); PSA, polar surface area; HBD, hydrogen bond donor.
Different permeability approaches were used as follows: 1, \( P_{ap} \) (A-B) data from Caco-2 (data in parentheses: MDCK-MDR1); 2, \( P_{app} \) data from correlation to \( P_{app} \) (A-B) from Caco-2 (data in parentheses: MDCK-MDR1); 3, \( P_{app} \) data from correlation to \( P_{app} \) (A-B) from Caco-2 (data in parentheses: MDCK-MDR1) calibrated for midazolam \( Q_{Gut} \); and 4, \( P_{app} \) data from correlation to in silico data (data in parentheses: calibrated for midazolam \( Q_{Gut} \)).

\[
\begin{array}{c|cccc}
\text{Substrate} & \text{Predicted}^a & \text{Observed} & \text{\( Q_{Gut} \)} & \text{Estimated} \\
\hline
\text{Alfentanil} & 0.82 \pm 0.06 & 0.60 & 16.6 \\
\text{Alprazolam} & 0.99 \pm 0.01 & 0.94 & 16.4 \\
\text{Atorvastatin} & 0.90 \pm 0.04 & 0.24 & 12.7 \\
\text{Buspirone} & 0.68 \pm 0.04 & 0.21 & 16.4 \\
\text{Cisapride} & 0.65 \pm 0.06 & 0.55 & 16.6 \\
\text{Cyclosporine} & 0.82 \pm 0.07^b & 0.44 & 8.6^b \\
\text{Felodipine} & 0.20 \pm 0.07 & 0.45 & 14.5 \\
\text{Indinavir} & 0.25 \pm 0.07 & 0.93 & 5.7^b \\
\text{Lovastatin} & 0.10 \pm 0.04 & 0.07 & 15.4 \\
\text{Methadone} & 0.97 \pm 0.01 & 0.78 & 16.2 \\
\text{Midazolam} & 0.54 \pm 0.14 & 0.51 & 16.6 \\
\text{Nifedipine} & 0.66 \pm 0.03 & 0.74 & 16.3 \\
\text{Nisoldipine} & 0.08 \pm 0.03 & 0.11 & 16.3 \\
\text{Quinidine} & 0.99 \pm 0.001 & 0.90 & 13.9^b \\
\text{Repaglinide} & 0.84 \pm 0.05 & 0.89 & 16.3 \\
\text{Rifabutin} & 0.87 \pm 0.03 & 0.21 & 14.3^b \\
\text{Saquinavir} & 0.01 \pm 0.003 & 0.18^a & 2.4^b \\
\text{Sildenafil} & 0.78 \pm 0.02 & 0.54 & 16.4 \\
\text{Simvastatin} & 0.06 \pm 0.02 & 0.14 & 13.2 \\
\text{Tacrolimus} & 0.34 \pm 0.16 & 0.14 & 15.1 \\
\text{Teronafenedin} & 0.11 \pm 0.02 & 0.40 & 11.9 \\
\text{Tramazoline} & 0.91 \pm 0.03 & 0.83 & 16.3 \\
\text{Trizazolam} & 0.95 \pm 0.04 & 0.75 & 16.5 \\
\text{Verapamil} & 0.67 \pm 0.07^b & 0.65 & 15.2^b \\
\text{Zolpidem} & 0.98 \pm 0.01 & 0.79 & 16.6 \\
\end{array}
\]

Superscript letters indicate the different methods used for estimation.

**TABLE 4**

Description of bias (gmfe) and percentage within 1.5-fold of unity for predictions of the \( F_G \) for either total set of 25 drugs or for a subset of 11 drugs for which in vivo \( F_G \) < 0.5

**TABLE 5**

Predictions of \( F_G \) and \( Q_{Gut} \) using the \( Q_{Gut} \) model from in vitro clearance and permeability data for 25 drugs

**Figure 5.** \( F_G \) predictions obtained using in vitro clearance (Table 1) and \( P_{app} \) (A-B) data obtained in either Caco-2 (*) or MDCK-MDR1 cells (+). The dashed lines represent 1.5-fold deviation from unity, and error bars represent the S.D. associated with the \( F_G \) predictions using different HLM and HIM pools. Outliers identified are the following: 1, rifabutin; 2, atorvastatin; 3, buspirone; 4, tacrolimus; 5, simvastatin; 6, saquinavir; 7, terfenadine; 8, felodipine; and 9, indinavir.

Figure 5 and Table 5 illustrate the comparison between predicted and observed \( F_G \) values based on \( P_{app} \) data from both cell lines; each outlier is identified by a number. Overprediction was apparent for rifabutin (1), atorvastatin (2), buspirone (3), and tacrolimus (4). The average predicted tacrolimus \( F_G \) was associated with a high degree of variability (indicated by the large error bars) driven by the highly variable in vitro clearance of this drug. Underprediction was observed for simvastatin (5), saquinavir (6), terfenadine (7), felodipine (8), and indinavir (9). The degree of underprediction was dependent on the use of permeability input and was particularly high for indinavir (17–33% of observed), saquinavir (5–12%), and terfenadine (16–29%). The \( Q_{Gut} \) value, a hybrid parameter reflecting both drug permeability and mucosal blood flow, ranged from 2.4 to 16.6 l/h for saquinavir and midazolam, respectively (Table 5).

**Discussion**

This study has evaluated the use of various sources of metabolism and permeability data for predicting \( F_G \) using the \( Q_{Gut} \) model. A group of 25 structurally diverse CYP3A4 substrates was used for this investigation with corresponding \( F_G \) values in vivo ranging from 0.07 to 0.94 for Lovastatin and Alprazolam, respectively.

**In Vitro Clearance Data.** A high degree of comparability was observed between in vitro clearance from HIM and three HLM pools for the dataset investigated, as illustrated in Fig. 2. This suggests that in vitro clearance between hepatic and intestinal microsomes can be extrapolated if enzyme abundance data are available and the contribution of P450 enzyme to drug metabolism is known. The current findings support our previous work (Galetin and Houston, 2006), in which good agreement between normalized hepatic and intestinal clearances was observed for a limited number of substrates for a range of P450 enzymes. No significant difference in the hepatic and intestinal clearances seen once normalized for the tissue-specific CYP3A abundance supports their interchangeable use, as illustrated here in the \( Q_{Gut} \) model. CLint data were normalized using reported population enzyme abundance data based on a meta-analysis of 241 liver samples (Rowland-Yeo et al., 2004; Rostami-Hodjegan and Tucker, 2007). Abundance data for both CYP3A and CYP3A4 in the liver (155 and 111 pmol/mg, respectively) are associated with large coefficients of variation (67–119%). In the current study, actual CYP3A4 abundance was available only for the HLM pool 2 (138 pmol/mg), which was within the reported population limits. Furthermore, the reported pop-
ulation abundance data are based on liver microsomes of white origin and consequently the HLM pools with a high white donor percentage should be selected to allow appropriate scaling with population estimates. In contrast to the liver data, intestinal data are characterized to a lesser extent, as the CYP3A and CYP3A4 abundance data are available from 31 individual of mixed ethnicity (Paine et al., 2006).

This study showed a very strong linear correlation between clearance values determined in different microsomal pools and their respective testosterone 6β-hydroxylation activity. The differences observed in microsomal enzyme activity between the pools contributed considerably to the prediction success of clearance. Considering the aforementioned, pools with small donor sizes might not be representative of the true population mean. In addition, different substrate binding sites associated with the CYP3A4 enzyme probably explain this discrepancy (Galetin et al., 2003), as well as differing contributions of CYP3A4/CYP3A5 to drug clearance (Galetin et al., 2004; Huang et al., 2004) in comparison with the marker substrate testosterone (e.g., tacrolimus and saquinavir). However, although prediction bias was affected by the choice of microsomal pools, it had only a marginal effect on the precision of clearance predictions. The current analysis included three known inhibitors of CYP3A, namely indinavir, saquinavir, and verapamil (Eagling et al., 1997; Wang et al., 2004; Ernest et al., 2005). To avoid biased clearance estimates, incubations were performed at a substrate concentration below \( K_{\text{c}} \) (for competitive inhibitors) or \( K_{\text{t}} \) (time-dependent inhibitors) and over a short (\( \leq 30 \) min) time period. No apparent inhibition was evident in the depletion plots; however, the possibility that enzyme inhibition in vitro might have biased the extrapolation of in vivo clearance cannot be ruled out.

**Prediction of Hepatic Intrinsic Clearance from Intervenous and Oral Data.** A variable degree of \( \text{CL}_{\text{int,h}} \) prediction success from microsomal data has been reported in the literature (Obach, 1999; Ito and Houston, 2005; Riley et al., 2005). The current study found a low degree of underprediction for the CYP3A4 substrates investigated. The prediction success of \( \text{CL}_{\text{int,h}} \) from oral data was considerably improved in the current study when drug-specific \( F_G \) values were incorporated; this was particularly evident for atorvastatin, buspirone, cyclosporin, felodipine, and nisoldipine. The \( \text{CL}_{\text{int,h}} \) estimate from oral data represented 98% (79, 117, 95% CI) of the \( \text{CL}_{\text{int,h}} \) estimate for these drugs. Likewise, for indinavir poor prediction success for oral clearance was observed. This drug is administered at a high dose, resulting in a corresponding hepatic inlet concentration of 4 \( \mu \text{M} \) after oral administration; therefore, saturation/inhibition of systemic indinavir metabolism might occur, given its low \( K_{\text{m}} \) (Chiba et al., 1997; Koudriakova et al., 1998) and \( K_{\text{t}} \) values <1 \( \mu \text{M} \) (Eagling et al., 1997).

Permeability experiments were performed in MDCK-MDR1 and Caco-2 cells at a low substrate concentration (0.1 \( \mu \text{M} \)) and in the presence of a P-gp inhibitor for the drugs with apparent drug efflux mediated by P-gp (ER \( \geq 2 \)). Permeability data were of considerable importance for the \( F_G \) predictions of drugs with \( P_{\text{app}} \) (A-B) <100 nm/s; in contrast, if drug permeability exceeded this value, \( F_G \) predictions were mainly driven by in vitro clearance because the \( Q_{\text{int}} \) model was reduced to a perfusion rate-limited model. Because single concentrations below the anticipated luminal concentration were used in the permeability assessment, consequently an overestimation of the contribution of P-gp might have occurred. A more comprehensive in vitro assessment of P-gp-mediated transport (e.g., full kinetic profiles and differential pH to account for variability in vivo) for the P-gp substrates in the current study would be beneficial to fully understand the contribution of P-gp to intestinal first-pass metabolism.

**Predictions of Intestinal First-Pass Metabolism.** The \( Q_{\text{int}} \) model accounts for the fact that a drug with low permeability will have a longer exposure to the metabolizing enzymes in the enterocytes (Rostami-Hodjegan and Tucker, 2004; Yang et al., 2007). For drugs with in vitro permeability exceeding 100 nm/s, the hybrid function \( Q_{\text{int}} \) (as defined in eq. 9) is reduced to \( Q_{\text{int}} \) resulting in simple perfusion rate-limited processes. Considering this and the large availability of in vivo intestinal first-pass metabolism data, midazolam, a highly permeable CYP3A substrate, was explored as a \( Q_{\text{int}} \) calibrator. However, because no in vivo measure of \( Q_{\text{int}} \) or \( \text{CL}_{\text{int,g}} \) is available, this approach is based on the assumption that predicted \( \text{CL}_{\text{int,g}} \) represents an adequate measure for midazolam in vivo intestinal clearance (as seen for hepatic data). The variability associated with \( \text{CL}_{\text{int,g}} \) and \( F_G \) values propagates into the estimation of \( Q_{\text{int}} \) estimates, resulting in a considerable uncertainty associated with this parameter.

Application of the \( Q_{\text{int}} \) model resulted in high \( F_G \) prediction success for drugs with low intestinal first-pass metabolism (\( F_G >0.5 \)) with indinavir representing the only significant outlier (Fig. 4B). In contrast, the prediction success was considerably reduced for the subset of drugs with \( F_G <0.5 \) (Fig. 4C). This trend was also observed for the prediction success of \( Q_{\text{int}} \) (data not shown) with comparable bias and imprecision (gmfe 2.9 versus 2.5 for \( F_G \) and \( F_H \), respectively). In general, direct input of \( P_{\text{app}} \) (A-B) data from Caco-2 or MDCK-MDR1 cells resulted in the highest \( F_G \) prediction success and is therefore recommended. Both cell models were considered in the present analysis because they represent common tools in the pharmaceutical industry to determine permeability of new chemical entities. In contrast, the use of a regression equation based on physicochemical properties and \( F_{\text{eff}} \) should be avoided because it resulted in significant underprediction of \( F_G \) for the current dataset. This may partly be explained by a number of drugs with very high polar surface area (>100 Å²) (e.g., indinavir, saquinavir, and tacrolimus), as the validity of the existing regression equation (Winiwarter et al., 1998) was not established for drugs with those properties. Because in vivo \( F_G \) data were available for cyclosporin and verapamil (Lennermøø, 2007), these data were used in the current \( F_G \) predictions.

The \( F_G \) overestimations observed for buspirone and atorvastatin are consistent with the underprediction of hepatic clearance (<8% of observed). Furthermore, for both drugs \( F_H \) approaches \( Q_{\text{app}} \), which impedes accurate estimation of in vivo \( F_G \), bearing in mind that this parameter is indirectly assessed from intervenous/oral data. Indeed, whereas intervenous/oral data for atorvastatin suggested an \( F_G \) value of 0.24, grapefruit juice (GFJ) interaction data suggested a less extensive intestinal contribution to atorvastatin first-pass metabolism: \( F_{G,\text{GFJ}} = 0.56 \) (Gertz et al., 2008a). In the case of tacrolimus, \( F_G \) predictions were highly variable among the different microsomal pools used within this study, showing a general overprediction trend. In addition to CYP3A, tacrolimus undergoes UGT-mediated metabolism, and the underestimation of intestinal clearance might also be attributed to conjugative metabolism (Strassburg et al., 2001). However, because no absolute UGT abundance data to allow incorporation of UGT metabolism into \( F_G \) predictions currently exist, the impact of this contributing pathway could not be assessed.

Underprediction of \( F_G \) was observed for a number of drugs, including terfenadine, saquinavir, and indinavir. Terfenadine dis-
played considerable nonspecific binding even at a low protein concentration, and erroneous \( f_{uu} \) determination might have subsequently affected the in vitro estimate of its clearance. To minimize any issues associated with the nonspecific binding, low microsomal protein concentrations were used, and \( f_{uu} \) values were experimentally determined for all the drugs using microdialysis (Gertz et al., 2008b), with the exception of cyclosporine for which this value was predicted (Halifax and Houston, 2006). In addition, the use of substrate concentrations 10-fold below \( K_m \) may overestimate intestinal clearance, given the high anticipated drug concentration in the enterocytes during the absorption phase. Potential saturation of CYP3A and P-glycoprotein in vivo (e.g., saquinavir) and the region of the intestine in which drug is absorbed also need to be considered when observed \( F_G \) underprediction is interpreted. Some of the substrates for which \( F_G \) is underpredicted represent either time-dependent or reversible inhibitors of CYP3A (i.e., indinavir and saquinavir), which may affect their in vivo estimates of \( F_G \). Finally, one must not forget that the in vivo estimates of \( F_G \) represent indirect assessments of the intestinal first-pass metabolism liable to several assumptions. In particular, for drugs with low and variable bioavailability where \( CL_{AUC}/Q_v \) approaches 1 (buspirone, felodipine, indinavir, lovastatin, and saquinavir), delineation of the intestinal and hepatic contribution to first-pass metabolism is virtually impossible using the intravenous/oral approach.

In conclusion, this study has comprehensively investigated the suitability of the \( Q_{out} \) model to predict \( F_G \) for drugs with differential clearance and permeability characteristics. Although drugs with low intestinal extraction were generally well predicted, the prediction success for drugs with high intestinal extraction (\( F_G \leq 0.5 \)) was considerably less accurate and requires further refinement.

Acknowledgments. We acknowledge the assistance of Dr. David Halifax and Sue Murby with the LC-MS/MS analysis, Dr. In-Sun Nam Knutsdon for guidance with the meta-analyses (University of Manchester), and Dr. Katherine Fenner, Sarah Kempshall, Rebecca Greenstreet, and Charles Malloy (Pfizer, Pharmacokinetics, Dynami- cks and Metabolism Department, Sandwich, UK).

References


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