Quantitative Prediction of Intestinal Metabolism in Humans from a Simplified Intestinal Availability Model and Empirical Scaling Factor

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ABSTRACT:

This study aimed to establish a practical and convenient method of predicting intestinal availability (Fint) in humans for highly permeable compounds at the drug discovery stage, with a focus on CYP3A4-mediated metabolism. We constructed a “simplified Fint model,” described using only metabolic parameters, assuming that passive diffusion is dominant when permeability is high and that the effect of transporters in epithelial cells is negligible. Five substrates for CYP3A4 (alprazolam, amlodipine, clonazepam, midazolam, and nifedipine) and four for both CYP3A4 and P-glycoprotein (P-gp) (nicardipine, quinidine, tacrolimus, and verapamil) were used as model compounds. Observed fraction of drug absorbed (Fint) values for these compounds were calculated from in vivo pharmacokinetic (PK) parameters, whereas in vitro intestinal intrinsic clearance (CLint,intestine) was determined using human intestinal microsomes. The CLint,intestine for the model compounds corrected with that of midazolam was defined as CLm,index and incorporated into a simplified Fint model with empirical scaling factor. Regardless of whether the compound was a P-gp substrate, the Fint could be reasonably fitted by the simplified Fint model, and the value of the empirical scaling factor was well estimated. These results suggest that the effects of P-gp on Fint and Fint are substantially minor, at least in the case of highly permeable compounds. Furthermore, liver intrinsic clearance (CLint,liver) can be used as a surrogate index of intestinal metabolism based on the relationship between CLint,liver and CLm,index. Fint can be easily predicted using a simplified Fint model with the empirical scaling factor, enabling more confident selection of drug candidates with desirable PK profiles in humans.

Given the substantial time and cost associated with drug discovery and development, increasing importance has been placed on the prediction of pharmacokinetics (PK) in humans of drug candidates at the discovery stage to avoid later termination of development because of an undesirable PK profile. Bioavailability (F) of an orally administered drug, which is the fraction of drug reaching systemic blood circulation, is expressed as the product of the fraction of the dose that enters the enterocyte (Fa), intestinal availability (Fint), and hepatic availability (Fh). Therefore, to effectively pass into systemic blood circulation, orally administered drugs must not only have high solubility and permeability in the gastrointestinal tract but also be stable against metabolizing enzymes in the gastrointestinal tract and liver.

At the drug discovery stage, compounds selected as drug candidates using high-throughput screening typically are those with high permeability because intestinal permeability is one of the most important factors in determining the F of orally administered drugs. Although P-glycoprotein (P-gp) is highly expressed in intestinal epithelial cells and has the potential to reduce drug absorption, the effect on drug absorption is not quantitatively as important as suggested, particularly for highly permeable compounds (Chiu et al., 2001; Lin, 2004; Cao et al., 2005). Therefore, for highly permeable compounds, a relatively high Fa can be expected when dissolution in the gastrointestinal tract is not the rate-limiting step.

CYP3A subfamily and P-gp are present in high levels in human intestinal epithelial cells as a metabolizing enzyme and an efflux transporter, respectively. Benet et al. (1999) proposed that the synergistic effects of CYP3A4-mediated metabolism and P-gp-mediated efflux in epithelial cells may result in an unexpectedly high first-pass metabolism in the intestine because of the overlapping substrate specificities of these proteins.

ABBREVIATIONS: PK, pharmacokinetic(s); Fa, fraction of drug absorbed; Fint, intestinal availability; Fh, hepatic availability; P-gp, P-glycoprotein; Papp, apparent influx clearance; Peff, efflux clearance; CLint, absorption clearance; CLm, metabolic clearance; Q, luminal flow rate; CLm,index, intestinal intrinsic clearance; CLm,index, intestinal intrinsic clearance corrected with that of midazolam; CLint,liver, liver intrinsic clearance; HIM, human intestinal microsome; HLM, human liver microsone; PAMPA, parallel artificial membrane permeability assay; DMSO, dimethyl sulfoxide; Papp, apparent permeability coefficient; LC/MS/MS, liquid chromatography/tandem mass spectrometry; P450, cytochrome P450; CLm, hepatic clearance; CLtot, total body clearance; CLr, renal clearance; Qh, hepatic blood flow; Rb, blood-to-plasma concentration ratio; SF, scaling factor; α, empirical scaling factor.
Ito et al. (1999) constructed a theoretical model for \( F_{g,F} \), that took into consideration the integrated process of permeability and metabolism in epithelial cells (eq. 1):

\[
F_{g,F} = \frac{CL_{ab}}{CL_{ab} + CL_{m}} \times \left( 1 - \exp \left( \frac{PS_{\text{eff}} \times CL_{ab} + CL_{m}}{Q \times PS_{\text{eff}} + CL_{ab} + CL_{m}} \right) \right)
\]

(1)

where \( PS_{\text{eff}} \) is the apparent influx clearance from the lumen to epithelial cells, \( PS_{\text{eff}} \) is the efflux clearance from cells to the lumen, \( CL_{ab} \) is the absorption clearance from cells to blood, \( CL_{m} \) is the metabolic clearance in cells, and \( Q \) is luminal flow rate. However, precise estimation of several parameters described in the theoretical model is difficult; therefore, application of the model has not been reported.

Yang et al. (2007) constructed a “Q_gut model” for predicting \( F_g \) (eqs. 2 and 3):

\[
F_g = \frac{Q_{\text{gut}}}{Q_{\text{gut}} + fu_3 \times CL_{\text{int,intestine}}} \quad (2)
\]

\[
Q_{\text{gut}} = \frac{Q_{\text{vill}} \times CL_{\text{perm}}}{Q_{\text{vill}} + CL_{\text{perm}}} \quad (3)
\]

where \( CL_{\text{perm}} \) is the clearance defining permeability through the enterocyte, \( Q_{\text{vill}} \) is villous blood flow, \( fu_3 \) is the fraction of unbound drug in the enterocyte, and \( Q_{\text{gut}} \) is a hybrid parameter of \( CL_{\text{perm}} \) and \( CL_{\text{int}} \). In their report, assuming \( fu_3 \) to be unity and a \( Q_{\text{vill}} \) value of 18 l/h, the accuracy in predicting \( F_g \) was better than that of the “well stirred” gut model. Therefore, if the values of \( CL_{\text{perm}} \) and intestinal intrinsic clearance (CL_{\text{int,intestine}}) can be estimated precisely and easily, the Q_gut model may be useful in predicting \( F_g \) at the drug discovery stage.

In the present study, we aimed to establish a more practical and convenient method for predicting \( F_g \) by simplifying the theoretical model under conditions of high permeability. We focused on CYP3A4-mediated metabolism and examined CL_{\text{int,intestine}} of five model compounds for CYP3A4 substrates (alprazolam, amlodipine, clonazepam, midazolam, and nifedipine) and four model compounds for both CYP3A4 and P-gp substrates (nicardipine, quinidine, tacrolimus, and verapamil). The obtained CL_{\text{int,intestine}} were corrected using the CL_{\text{int,intestine}} of midazolam (reference compound). These corrected values, defined as CL_{\text{m,index}} and CL_{\text{m,liver}}, were incorporated into the simplified model. We examined whether \( F_g \) values for highly permeable compounds can be explained satisfactorily by the simplified model. In addition, the relationship between CL_{\text{m,index}} and CL_{\text{int,liver}} was examined for 35 compounds, and a reliable procedure for predicting \( F_g \) was proposed.

Materials and Methods

Materials. All the compounds except midazolam and tacrolimus were purchased from either Sigma-Aldrich (St. Louis, MO) or Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Midazolam, tacrolimus, and in-house compounds were provided by Astellas Pharma Inc. (Tokyo, Japan). Pooled human intestinal microsomes (HIMs) were purchased from Xenotech, LLC (Lexena, KS), and individual HIMs were purchased from KAC Co., Ltd. (Kyoto, Japan). Pooled human liver microsomes (HLMs) were purchased from Xenotech, LLC. All the other chemicals and reagents were of analytical grade and purchased from commercial sources.

Permeability Study Using Artificial Membrane. Parallel artificial membrane permeability assays (PAMPA) conducted in this study used the PAMPA Evolution from pION Inc. (Woburn, MA). In a PAMPA, a “sandwich” is formed from a 96-well microtiter plate (pION Inc.) and a 96-well filter plate (polyvinylidene difluoride; Millipore Corporation, Billerica, MA), so that each composite well is divided into two chambers, with a donor at the bottom and an acceptor at the top, separated by a 125-μm-thick microfilter disc (0.45-μm pores) coated with a 20% (w/v) dodecanol solution of a lecithin mixture (pION Inc.). Drug samples were introduced via a 10 mM dimethyl sulfoxide (DMSO) stock solution in a 96-well polypyrrole microtiter plate. The robotic liquid handling system draws a 5-μl aliquot of the DMSO stock solution and mixes it into an aqueous buffer solution of 10% (v/v) DMSO to attain a final typical sample concentration of 50 μM. The drug solutions were filtered using a 96-well filter plate (polyvinylidene fluoride; Corning Life Sciences, Lowell, MA) and added to the donor compartments. The donor solution was adjusted to pH 6.5 (NaOH-treated universal buffer; pION Inc.), whereas the acceptor solution had a pH of 7.4 (pION Inc.). The plates were sandwiched together and incubated at 25°C for 2 h in a humidity-saturated atmosphere. After incubation, the sandwiched plates were separated, and both the donor and acceptor compartments were assayed for the amount of material present by comparison with the UV spectrum (270–400 nm) obtained from reference standards. Mass balance was used to determine the amount of material remaining in the membrane barrier. The apparent permeability coefficient (P_app) was calculated using PAMPA Evolution software (pION Inc.).

In Vitro Metabolism Study in HIMs. CL_{\text{int,intestine}} was calculated from the substrate disappearance rate in HIMs. Each compound was incubated with a reaction mixture (1000 μl) consisting of human intestinal microsomal protein in 100 mM potassium phosphate buffer, pH 7.4, and 0.1 mM EDTA. After preincubation for 5 min at 37°C, the enzyme reaction was initiated by adding 100 μl of 10 mM NADPH. The final concentration of each compound was 0.2 μM. The microsomal concentration used was 0.2 mg/ml except nicardipine, with the concentration of 0.02 mg/ml. The final concentration of the organic solvent (acetonitrile) in the reaction mixture was 0.5% (v/v). For all the compounds except tacrolimus and cyclosporine, 100 μl of the reaction mixture was moved into 200 μl of acetonitrile containing internal standard (diazepam, 100 ng/ml) at appropriate time points for stopping the reaction. The reaction mixture was then centrifuged at 10,000g for 5 min, and 10 μl of the aliquot was injected into a liquid chromatography/tandem mass spectrometry (LC/MS/MS) system. With regard to tacrolimus, 100 μl of the reaction mixture was moved into 100 μl of acetonitrile containing internal standard (ascomycin, 100 ng/ml) for stopping the reaction, and 500 μl of 0.01 M ammonium acetate buffer, pH 7.5, and 4.5 μl of tert-butyl methyl ether were added. The mixture was shaken for 20 s and centrifuged at 1870g for 10 min at 4°C. Four milliliters of the organic layer was evaporated to dryness at 40°C under a stream of nitrogen. The residue was dissolved with 200 μl of ammonium acetate (2 mM) in water with 75% methanol, and 10 μl of the aliquot was injected into LC/MS/MS. With regard to cyclosporine, 100 μl of the reaction mixture was moved into 100 μl of acetonitrile containing internal standard (in-house compound, derivatized cyclosporine), 100 μg/ml to stop the reaction, and 500 μl of distilled water and 4.5 μl of tert-butyl methyl ether were added. The mixture was shaken for 20 s and centrifuged at 1870g for 10 min at 4°C. Four milliliters of the organic layer was evaporated to dryness at 40°C under a stream of nitrogen. The residue was then dissolved with 200 μl of ammonium acetate (10 mM) in water with 90% methanol, and 10 μl of the aliquot was injected into LC/MS/MS.

Because of the activity of cytochrome P450 (P450) enzymes in HIMs depends on the preparation method (mucosal scraping and enterocyte elution) (Galetin and Houston, 2006), the use of absolute value of CL_{\text{int,intestine}} for \( F_g \) prediction is somewhat problematic. Therefore, in the present study, CL_{\text{int,intestine}} expressed as milliliters per minute per milligram of microsomal protein, was corrected for each compound with that of midazolam using eq. 4. This corrected value was defined as CL_{\text{m,index}}. The CL_{\text{m,index}} values were used for metabolic parameter in intestine.

\[
CL_{\text{m,index}} = \frac{CL_{\text{int,intestine}} \text{ of compound A}}{CL_{\text{int,intestine}} \text{ of midazolam}} \quad (4)
\]

In Vitro Metabolism Study in HLMs. CL_{\text{int,liver}} was calculated from the substrate disappearance rate in HLMs. For nicardipine and felodipine, the microsomal concentrations used were 0.02 and 0.05 mg/ml, respectively, whereas a concentration of 0.2 mg/ml was used for the remaining commercial compounds. For in-house compounds, the concentrations used ranged from 0.02 to 0.2 mg/ml. The other experimental conditions and sample preparation
methods were the same as those used in the HIM in vitro metabolism study. CL_int,liver, expressed as milliliters per minute per milligram of microsomal protein, was scaled to a whole-body clearance (milliliters per minute per kilogram) using physiological parameters of 32 mg of microsomal protein/g of liver (Barter et al., 2007) and 24.1 g of liver/kg b.w.t. (Davis and Morris, 1993).

**Analytical Method.** The compounds were measured by LC/MS/MS using a Quattro Ultima (Waters, Milford, MA) with an Alliance 2695 separation module (Waters). Multiple-reaction monitoring mode was used to monitor ions as follows (precursor ion/product ion): alprazolam (309,0/281,0), amiodipine (409,0/238,0), clonazepam (315,9/270,0), midazolam (326,0/291,1), nifedipine (347,1/315,1), nicardipine (480,0/315,0), quinidine (325,1/183,9), tacrolimus (821,5/768,3), verapamil (455,2/165,0), amitriptyline (278,0/91,0), cyclosporine (409,0/238,0), clonazepam (315,9/270,0), midazolam (326,0/291,1), nifedipine (1219,2/1202,7), felodipine (383,9/337,9), propafenone (342,1/116,0), propranolol (260,1/182,9), diazepam (284,9/154,0), inhouse compound (derivative of cyclosporine), and ascomycin (809,6/756,2). With regard to alprazolam, amiodipine, clonazepam, nicardipine, and felodipine, samples were injected into an XTerra MC C18 column (3.5 μm, 50 mm; Waters) warmed to 40°C. Elution was conducted at a flow rate of 0.2 ml/min with 10 mM ammonium acetate in water/methanol (10:90).

For highly permeable compounds, absorption is complete (F_a = 1) when dissolution in the gastrointestinal tract is not the rate-limiting step.

Equation 1 can thus be simplified to eq. 8 by Assumption 1.

\[
F_a F_g = \frac{CL_{ab}}{CL_{ab} + CL_{m}} = \frac{1}{1 + CL_m/CL_{ab}} \tag{8}
\]

According to eq. 8, \(F_a\) value is determined by the balance of \(CL_{ab}\) and \(CL_m\).

To estimate the \(F_a\) value for highly permeable compounds, Assumption 1 was made, and the following equation was obtained.

\[
F_a = \frac{1}{1 + \alpha \times CL_{m\text{,index}}} \tag{11}
\]

where \(\alpha\) is the empirical scaling factor (eq. 12).

\[
\alpha = A \times SF \tag{12}
\]

where \(A\) is the reciprocal value of \(CL_{ab}\) and assumed to be treated as a constant value.

**Assumption 3.** \(CL_m\) is proportional to \(CL_{m\text{,index}}\). As shown in eq. 4, \(CL_{m\text{,index}}\) is a metabolic parameter that has been corrected using reference compound (midazolam).

\[
CL_m = SF \times CL_{m\text{,index}} \tag{10}
\]

where SF is the scaling factor between \(CL_m\) and \(CL_{m\text{,index}}\).

Combining eqs. 9 and 10 gives eq. 11.

\[
F_a = \frac{1}{1 + \frac{\alpha}{\alpha}} \times \frac{1}{CL_{m\text{,index}}} \tag{11}
\]

where \(\alpha\) is defined as the empirical scaling factor (eq. 12).

In the present study, eq. 11 is referred to as the simplified \(F_a\) model. We examined whether \(F_a\) values for highly permeable compounds can be satisfactorily explained using this simplified \(F_a\) model.

**Estimation of the Empirical Scaling Factor (\(\alpha\)) of Simplified \(F_a\) Model.** Alprazolam, amiodipine, clonazepam, midazolam, nifedipine, nicardipine, quinidine, tacrolimus, and verapamil were used to establish the simplified \(F_a\) model. The value of \(\alpha\) was estimated from a fitting study between human \(F_a\) and the \(CL_{m\text{,index}}\) values of these model compounds using a nonlinear least-squares method, MULTI (Yamaoka et al., 1981).

**Results**

Initially, permeability of the model compounds (alprazolam, amiodipine, clonazepam, midazolam, nifedipine, nicardipine, quinidine, tacrolimus, and verapamil) was examined using an artificial membrane, and the values of \(P_{app,PAMPA}\) were compared with those of 25

<table>
<thead>
<tr>
<th>Reference Compounds</th>
<th>(P_{app}) (×10⁻⁶ cm/s)</th>
<th>(F_a^*)</th>
<th>Model Compounds</th>
<th>(P_{app}) (×10⁻⁶ cm/s)</th>
</tr>
</thead>
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<td>Acetaminophen</td>
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<td>0.21</td>
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<td>0.94</td>
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<tr>
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<tr>
<td>Warfarin</td>
<td>12.9</td>
<td>0.97</td>
<td>Warfarin</td>
<td>0.97</td>
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</table>

*Quoted from Zhu et al. (2002).*
reference compounds having known \( F_a \) in humans (Zhu et al., 2002) (Table 1). Figure 1 shows the correlation of \( P_{app,PAMPA} \) versus \( F_a \) of the reference compounds. Based on the correlation, high \( F_a \) can be expected when \( P_{app,PAMPA} \) value is \( >1.0 \times 10^{-6} \) cm/s. Because all the model compounds for CYP3A4 substrates showed high permeability (\( \geq 7.0 \times 10^{-6} \) cm/s), the \( F_aF_g \) values were assumed equal to \( F_g \) values (\( F_a = 1 \)).

Table 2 shows the \( CL_{int,intestine} \) of the model compounds obtained from in vitro metabolism study in HIMs. On comparison of \( CL_{int,intestine} \) for four HIMs from different lots, an approximately 3-fold variation in the \( CL_{int,intestine} \) was observed. \( CL_{int,intestine} \) ranged from 0.167 to 0.534 ml/min/mg for midazolam, 1.287 to 3.600 ml/min/mg for nicardipine, and 0.107 to 0.291 ml/min/mg for verapamil. However, similar values were observed after correction using eq. 4 to obtain \( CL_{int,index} \), with values ranging from 0.07 to 0.90 for alprazolam, 0.53 to 0.64 for nifedipine, 6.74 to 7.71 for nicardipine, 2.82 to 3.36 for tacrolimus, and 0.54 to 0.64 for verapamil. Therefore, in the present study, mean values of \( CL_{int,index} \) were used for the fitting study with simplified \( F_g \) model.

Table 3 summarizes the PK parameters of the model compounds in humans and values for \( F_F_g \) calculated from eqs. 5 through 7 under three different \( Q_h \) conditions. \( F_F_g \) values for the model compounds represented a wide range. For example, under high \( Q_h \) conditions (25.5 ml/min/kg), high \( F_F_g \) values were observed for alprazolam, amiodipine, clonazepam, nifedipine, and quinidine (0.730–0.932), middle-range values for midazolam and verapamil (0.482–0.509), and

**TABLE 2**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>( CL_{int,intestine} )</th>
<th>( CL_{int,index} )</th>
<th>( CL_{opt,intestine} )</th>
<th>( CL_{opt,index} )</th>
<th>( CL_{int,intestine} )</th>
<th>( CL_{int,index} )</th>
<th>( CL_{opt,intestine} )</th>
<th>( CL_{opt,index} )</th>
<th>( CL_{int,intestine} )</th>
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<td>N.D.</td>
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<td></td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>1.792</td>
<td>3.36</td>
<td>N.T.</td>
<td></td>
<td>0.107</td>
<td>0.64</td>
<td>N.T.</td>
<td></td>
<td>0.107</td>
<td>0.64</td>
<td>N.T.</td>
<td></td>
</tr>
<tr>
<td>Verapamil</td>
<td>0.291</td>
<td>0.54</td>
<td>N.T.</td>
<td></td>
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</table>

N.D. no depletion; N.T. not tested.
* Individual microsomes.
** Pooled microsomes.

**TABLE 3**

PK parameters, \( F_a, F_h, \) and \( F_F_g \) of nine model compounds in humans

PK parameters were quoted from references as follows: alprazolam (Smith et al., 1984); amiodipine (Faulkner et al., 1986); clonazepam (Crevoisier et al., 2003); midazolam (Kupferschmidt et al., 1995; Thummel et al., 1996; Tsunoda et al., 1999); nifedipine (Holtbecker et al., 1996); nicardipine (Higuchi and Shiobara, 1980); quinidine (Greenblatt et al., 1977; Rakhit et al., 1984); tacrolimus (Flore et al., 1997; Moller et al., 1999); verapamil (McAllister and Kirsten, 1982; Eichelbaum et al., 1984).
presented in Tables 5 and 6. For cyclosporine, mild metabolism in HIMs was observed. The observed F/Fg values were 0.886. The predicted values were slightly higher than the observed values.

The observed F/Fg and predicted Fg for felodipine and cyclosporine, which are CYP3A4 substrates with high permeability and outside the model compounds used to construct the simplified Fg model, are listed in Table 4. For felodipine, extensive metabolism in HIMs was observed (CLm/index was 2.80), and the predicted Fg ranged from 0.240 to 0.454. The predicted values were close to the observed F/Fg values (0.222–0.291). In contrast, for cyclosporine, mild metabolism in HIMs was observed (CLm/index was 0.30), and the predicted Fg ranged from 0.747 to 0.886. The predicted values were slightly higher than the observed F/Fg values (0.567–0.684).

Figure 3 shows the relationship between CLm/index and CLint,liver for 35 compounds containing the model compounds, additional 5 drug compounds (amitriptyline, felodipine, propafenone, propranolol, and timolol), and 21 in-house compounds. The results showed a good correlation between these parameters; for example, when CLint,liver was <100 ml/min/kg, CLm/index of almost all the compounds was <0.2. On the other hand, when CLint,liver was >100 ml/min/kg, most CLm/index values increased. Furthermore, when CLint,liver was >1000 ml/min/kg, CLm/index of all the compounds exceeded 1.0.

However, some compounds had CLm/index <0.2 despite having a CLint,liver >100 ml/min/kg.

**Discussion**

The importance of the intestine on drug metabolism has been well recognized (Wacher et al., 2001); however, it is difficult to estimate precisely all the important factors for intestinal metabolism at the drug discovery stage. Rats, dogs, and monkeys have been widely used in the prediction of PK in humans at the drug discovery stage, and evaluation using rats has been particularly useful for satisfactorily predicting Fg in humans (Zhao et al., 2003). However, expression levels and patterns for metabolizing enzymes in intestine differ distinctly between the two species (Cao et al., 2006). Furthermore, with regard to dogs, intestinal enzymes are generally less active than in humans (Prueksaritanont et al., 1996), and although monkeys are genetically similar to humans, some drugs have shown remarkably lower Fg values in monkeys than in humans, possibly because of activity of metabolizing enzymes and efflux transporters being higher in monkey intestine than in human intestine (Takahashi et al., 2009; Akabane et al., 2010). Therefore, these experimental animals are unsuitable for predicting Fg in humans.

In the present study, we aimed to develop a practical and convenient method for predicting Fg of highly permeable compounds using...
simplified $F_g$ model and empirical scaling factor, $\alpha$. Our results showed that, regardless of whether a compound was a P-gp substrate, $F_g F_g$ of the model compounds decreased as the CL\textsubscript{m,index} increased, and the $F_g F_g$ could be reasonably fitted using a simplified $F_g$ model (Fig. 2; Table 4). If P-gp-mediated efflux has a significant impact on $F_g$, and if synergistic effects of CYP3A4-mediated metabolism and P-gp-mediated efflux result in an unexpectedly low $F_g F_g$ of P-gp and non-P-gp substrates cannot be well fitted by the same curve obtained from the simplified $F_g$ model. This finding suggests that, at least for highly permeable compounds, the effect of P-gp on $F_g$ and the synergistic effects of CYP3A4 and P-gp on $F_g$ are substantially minor.

Furthermore, to verify the reliability of the simplified $F_g$ model and $\alpha$ values, $F_g$ prediction using simplified $F_g$ model was conducted for cyclosporine and felodipine, which are CYP3A4 substrates having high permeability (Tables 5 and 6). With regard to cyclosporine, the predicted $F_g$ value was slightly higher than the observed $F_g F_g$ value. This discrepancy may be because of the dissolution characteristics of cyclosporine being sufficiently poor to cause low $F_g$ (Varma and Panchagnula, 2005). With regard to felodipine, the predicted $F_g$ value was close to the observed $F_g F_g$ value.

According to the $Q_{\text{int}}$ model (Yang et al., 2007), when permeability is low ($Q_{\text{int}} >>$CL\textsubscript{perm}), eq. 2 can be transformed into eq. 14. $F_g$ values for low-permeability compounds are determined by the balance of the permeability and metabolic parameter.

$$F_g = \frac{Q_{\text{int}}}{\text{CL}_{\text{perm}} + fu_G \times \text{CL}_{\text{int,intestine}}}$$ (14)

In the present study, we examined the relationship between CL\textsubscript{int,index} and CL\textsubscript{int,liver} for 35 compounds (Fig. 3). Although results showed reasonably good correlation between these parameters, several compounds had CL\textsubscript{int,index} <0.2 despite having a CL\textsubscript{int,liver} >100 ml/min/kg. For example, whereas the model compounds for CYP3A4 substrates showed good correlation, propafenone, which is mainly metabolized by CYP2D6 (Dilger et al., 2000), had CL\textsubscript{int,index} <0.2 despite having a CL\textsubscript{int,liver} of approximately 700 ml/min/kg. Furthermore, in-house compound A, which is mainly metabolized by CYP1A2 and CYP2D6, had a CL\textsubscript{int,index} of 0.01 despite having a CL\textsubscript{int,liver} of approximately 600 ml/min/kg. Although Galetin and Houston (2006) reported that the rank order of CL\textsubscript{int} values for CYP3A4, CYP2C9, CYP2C19, and CYP2D6 substrates was consistent between HLMs and HIMs, Paine et al. (2006) reported minimal contribution of CYP2D6 for intestinal metabolism. For example, the CL\textsubscript{int} value for metoprolol, a substrate for CYP2D6, was much lower in HIMs compared with HLMs (0.7 versus 19.7 $\mu$/min/mg). Furthermore, Kato et al. (2003) reported that $F_g$ values of CYP3A4 substrates, but not non-CYP3A4 substrates, were reduced when CL\textsubscript{int,liver} exceeded 100 ml/min/kg. Therefore, these previous findings suggest that metabolism in the intestine is probably minor for non-CYP3A4 substrates, even if metabolism in the liver is extensive. To obtain a more reliable prediction of $F_g$, the value of CL\textsubscript{int,index} should be used rather than CL\textsubscript{int,liver}.

Based on the results, we proposed a reliable procedure for predicting $F_g$ in humans at the drug discovery stage. When CL\textsubscript{int,liver} of candidate compound is >100 ml/min/kg, CL\textsubscript{int,index} is typically >0.2, which results in a predicted $F_g$ of <0.814 when using a high $\alpha$ (1.13); therefore, an in vitro metabolism study in HIMs should be performed to predict $F_g$ from the simplified $F_g$ model. To avoid underestimating $F_g$ values of drug candidates, the use of a high $\alpha$ value (1.13) may be desirable at the drug discovery stage.

Although the present study focused on CYP3A4-mediated intestinal metabolism, conjugate enzymes such as UDP-glucuronosyltransferase and sulfotransferase have been found to be expressed in human intestine (Glatt et al., 2001; Cao et al., 2006). In fact, the intestinal conjugative metabolism exerts a measurable impact on $F$ in humans (Mizuma et al., 2005). If values of $F$ in humans of substrates for conjugate enzymes can be fully compiled, estimation of empirical scaling factor related to conjugation may be possible from the simplified $F_g$ model, as in CYP3A4 substrates.
In conclusion, we developed a practical and convenient method for predicting \( F_p \) of highly permeable compounds from a simplified \( F_g \) model and the results of an in vitro metabolism study in humans, focusing on CYP3A4-mediated metabolism. Using this method, \( F_g \) can be easily predicted at the drug discovery stage, enabling more confident selection of drug candidates with desirable PK profiles in humans.

References


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