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Physiologically-based pharmacokinetic modeling of intestinal first-pass metabolism of CYP3A substrates with high intestinal extraction

Michael Gertz, J. Brian Houston and Aleksandra Galetin

Centre for Applied Pharmacokinetic Research, School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester, M13 9PT, United Kingdom (M.G., J.B.H. and A.G.)

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Running title: Mechanistic prediction of human intestinal first-pass metabolism

Corresponding author: Dr A. Galetin

School of Pharmacy and Pharmaceutical Sciences,

University of Manchester, Stopford Building

Oxford Road,

Manchester, M13 9PT, UK

Tel: (+) 44 161 275 6886

Fax: (+) 44 161 275 8349

Email: Aleksandra.Galetin@manchester.ac.uk

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Abbreviations: F_G, intestinal availability; CL_{oral}, oral clearance; CLu_{int}, unbound intrinsic clearance; fu_p, fraction unbound in plasma; R_b, blood to plasma concentration ratio; k_a, absorption rate constant; HLM, human liver microsomes; HIM, human intestinal microsomes; HJM, human jejunal microsomes; CYP3A, cytochrome P450 3A; Q_{Gut}, hybrid parameter of enterocytic blood flow and drug permeability; $logP_{o:w}$, partitioning coefficient between octanol and water; pK_a, dissociation constant; FaSSIF, fasted simulated small intestinal fluids; gmfe, geometric mean fold error; rmse, rooted mean squared error

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Abstract

Prediction of intestinal availability (F_G), in conjunction with hepatic metabolism, is of considerable importance in drug disposition in order to assess oral clearance and liability to drug-drug interactions. In the current study, F_G predictions were performed using a physiologically-based pharmacokinetic (PBPK) model utilizing in vitro permeability and clearance data. The prediction success was assessed in comparison to the Q_{Gut} model. In addition, apparent oral clearance, predicted using the PBPK model, was compared to in vivo observations from meta-analyses. Lastly, unbound intrinsic clearance values (CLu_{int}) were determined for 12 CYP3A substrates in eight individual human jejunal microsomal samples (HJM) to assess inter-individual variability in intestinal intrinsic clearance and subsequent F_G predictions. Overall, the PBPK model improved F_G predictions in comparison to the Q_{Gut} model; this was apparent by a reduced bias and increased precision. In particular, F_G predictions of indinavir, saquinavir and terfenadine were model-dependent. The predicted oral clearance values of the drugs investigated ranged from 8.79 to 6,320l/h for tacrolimus and simvastatin, respectively and were overall within 3-fold of the observed data with the exception of indinavir, atorvastatin and buspirone. The individual HJM CLu_{int} values ranged from 17 to 14,000µl/min/mg for atorvastatin and saquinavir, respectively and corresponding inter-individual variability in CLu_{int} estimates ranged from 41 to 67%. These in vitro data resulted in predicted F_G values ranging from 0.03 to 0.94 for simvastatin and indinavir, respectively. The largest inter-individual variability of F_G was predicted for terfenadine (65%) in contrast to the low variability in the case of indinavir (3%).

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Introduction

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

The importance of intestinal metabolism may be delineated from clinical studies after i.v. and oral drug administration or alternatively from grapefruit juice interaction studies under appropriate conditions. However, both in vivo methods are flawed by a number of inherent limitations discussed elsewhere (Galetin et al., 2008; Gertz et al., 2008a). The intestinal availability (F_G) for drugs like cyclosporine, midazolam, felodipine and tacrolimus is based on a considerable number of studies, in contrast to very limited and variable data for buspirone, statins, saquinavir and terfenadine; hence the in vivo F_G estimates for the latter drugs have to be regarded with caution. Additionally, in vitro data may be utilized to make predictions of intestinal metabolism based on either in vitro clearance alone (Thummel et al., 1997) or in combination with permeability data (Yang et al., 2007; Gertz et al., 2010). For the prediction of intestinal metabolism, the enterocytic, rather than the total intestinal blood flow, needs to be incorporated (Tam et al., 2003). The rationale for using enterocytic blood flow in the F_G predictions is that drugs are absorbed across enterocytes which express the majority of

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metabolically active enzymes in the small intestine (Kolars et al., 1994). In general, no binding to enterocytic proteins is assumed, although this has not been satisfactorily investigated and is in contrast to the assumptions of the well-stirred model for the liver. Advancement in computational power has allowed incorporation of intestinal transit times and heterogeneous expression levels of CYP3A into mechanistic predictions of intestinal absorption and metabolism. Complex models to predict intestinal absorption generally represent adaptations of the compartmental absorption and transit model (Yu and Amidon, 1999) and may account for drug dissolution, solubility, permeability, transport and metabolism (Agoram et al., 2001; Tam et al., 2003; Jamei et al., 2009). Limiting the current trend towards holistic and mechanistic data interpretation is the need for extensive in vitro studies (e.g., full kinetic characterization of drug affinity for metabolizing enzymes and transporters) and formulation-specific data (e.g., dissolution, solubility) in conjunction with the quality of physiological information (e.g., abundance data of relevant enzymes and transporters) to support these models.

To reduce the necessary in vitro input data, our previous work investigated the F_G prediction success using the Q_{Gut} model, which simplifies prediction of intestinal metabolism to the input of in vitro clearance and permeability data (Yang et al., 2007; Gertz et al., 2010). The Q_{Gut} model has the basic structure of the well-stirred model; however, the blood flow term is modified to represent a hybrid parameter, Q_{Gut}, consisting of physiological flow (i.e., enterocytic blood flow) and drug permeability (Chalasani et al., 2002; Rostami-Hodjegan and Tucker, 2002). However, our analysis highlighted considerable bias and imprecision for drugs with in vivo F_G values below 0.5 (Gertz et al., 2010). Particularly the Q_{Gut} model predictions of F_G for indinavir, saquinavir and terfenadine were under-predicted by up to 96%. Consequently, a physiologically-based pharmacokinetic (PBPK) model was developed, which retains the use of in vitro clearance and permeability data, but overcomes some of the limitations of the Q_{Gut} model. The PBPK model takes into account heterogeneity of

metabolizing enzymes, drug concentration in the enterocytes and any potential saturation of intestinal metabolism. The latter in particular was assumed to be responsible for some of the under-prediction trends observed previously. Prediction success of the PBPK model is assessed in comparison to the Q_{Gut} model using 12 CYP3A drugs with low intestinal availability in vivo. In addition, apparent oral clearance is predicted for the selected drugs using the PBPK modeling approach and compared to in vivo observations from meta-analyses. Finally, eight individual human jejunal microsomal samples (HJM) are utilized to determine in vitro intrinsic clearance data of 12 selected drugs in order to assess inter-individual variability in F_G predictions.

Materials and Methods

Determination of intestinal clearance in vitro. In vitro clearance data were determined by substrate depletion in eight individual human jejunal microsomal samples prepared by an elution method from white donors. The microsomes from individual jejunal donors were purchased from BD Gentest (Woburn, MA), TABLE 1. Substrate depletion experiments were performed in 0.1M phosphate buffer (pH 7.4) containing 10mM $MgCl_2$, 7.5mM isocitric acid, 1.2units/mL isocitric acid dehydrogenase and 1mM NADP. The microsomal protein concentrations ranged from 0.025 to 1.5mg/mL for lovastatin/ simvastatin and cyclosporine, respectively. The final substrate concentration was 10-fold below the reported K_m values in the literature for the drugs investigated. The drugs were added from methanol stock solutions resulting in a final concentration of organic solvent in the incubation of 0.1%v/v. Clearance incubations were prepared as replicates of two in Eppendorf tubes at 37°C and 900rpm in an Eppendorf Thermomixer. The metabolic reaction was initialized by the addition of NADP solution to the incubation mixture and samples were taken at 6 designated time points within 60 minutes. Non-P450 dependent loss of drug over the incubation time was monitored by preparing additional samples in the absence of NADP.

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Metabolic reactions were terminated by removal of aliquots into an equal volume of ice-cold acetonitrile containing internal standard. Samples were centrifuged at 1,000g for 20 minutes at 4°C in a Mistral 3001 centrifuge (MSE) and 150µL supernatant was removed from each Eppendorf vial and transferred to glass vials prior to analysis on the LC-MS/MS system. Detailed information on LC-MS/MS analysis and the list of chemical suppliers have been reported in our previous publication (Gertz et al., 2010). Clearance values were corrected for experimentally determined nonspecific binding values to microsomal protein (fu_{inc}) (Gertz et al., 2008b; Gertz et al., 2010). In the case of cyclosporine, binding was predicted using drug lipophilicity data. The unbound intrinsic clearance values, CLu_{int}, were calculated using Eq. 1. Clearance values were corrected for the mean population CYP3A abundances in the intestine of 50 pmolCYP3A/mg protein (Paine et al., 2006) prior to F_G predictions.

$$CLu_{int} = \frac{V \cdot k}{protein_{microsomal}} \cdot \frac{1}{fu_{inc}} \qquad \text{Eq. 1}$$

where k represents the depletion rate constant, V, initial incubation volume; $protein_{microsomal}$, initial amount of protein

Testosterone 6β-hydroxylation activity. CYP3A enzyme activities of the HJM were determined at 250 µM testosterone concentration (substrate concentration sufficient to achieve V_{max} conditions) monitoring 6β-hydroxytestosterone formation over 10 minutes at a microsomal protein concentration of 0.5 mg/mL. Aliquots of 100 µL were removed into 100 µL ice cold acetonitrile containing progesterone (1 µM) as internal standard. The appearance of 6β-hydroxytestosterone (m/z, 305.15>269.3, cone voltage 80 V and collision energy 15 eV at maximum cone gas flow) was monitored. The LC-MS/MS system used consisted of a Waters 2795 with a Micromass Quattro Ultima triple quadruple mass spectrometer (Waters, Milford, MA). A Luna C18 column (3 µm, 50 x 4.6 mm) was used for chromatographic separation of the analytes using a gradient of acetonitrile and water containing 0.05%v/v formic acid. Composition of cofactors was the same as in the substrate depletion assay;

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organic solvent content was 0.3%v/v methanol. Activity determination was performed on three separate occasions to assess inter-day variability.

Determination of K_m values. Michaelis-Menten constants were determined for 4 drugs where the literature indicated low and variable K_m values. Substrate depletion clearance was determined at the following substrate concentration ranges; felodipine: 0.5–10 µM (6 concentration); indinavir: 0.025–5.5 µM (10 concentrations); nisoldipine: 0.25–5.0 µM (6 concentrations); and saquinavir: 0.05–5.0 µM (6 concentrations). The depletion profiles at the different substrate concentrations were performed in one representative microsomal preparation, HJM 6, (chosen for its high testosterone 6β-hydroxylation activity and low between day variability). The CLu_{int} and K_m values together with associated standard errors were determined using Eq. 2 (Obach and Reed-Hagen, 2002). The equation reported by the original investigators was modified by multiplying both sides of the equation with the volume of incubation to transform depletion rate constants to intrinsic (at [S] approaching 0) and apparent intrinsic clearance (at any [S]). The fitting was performed in Grafit 5.0.10 (Erithacus Software Limited).

$$CLu_{int,app} = CLu_{int}\left(1 - \frac{[S]}{[S]+K_m}\right) \qquad \text{Eq. 2}$$

where CLu_{int} represents the unbound intrinsic clearance; $CLu_{int,app}$, apparent intrinsic clearance; [S], substrate concentration and K_m, Michaelis-Menten constant

Preliminary assessment of enterocytic drug concentration and possible saturation of first-pass metabolism. Indinavir, saquinavir and terfenadine F_G values were previously considerably under-predicted by 75 – 96% using the Q_{Gut} model (Gertz et al., 2010). Unlike the PBPK model, the Q_{Gut} model predictions did not take enterocytic concentrations (C_{ent}) into account. An initial assessment of enterocytic drug concentration was performed based on Eq. 3.

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$$C_{ent} = \frac{D \cdot k_a \cdot F_a}{Q_{ent}} \qquad \text{Eq. 3}$$

The doses (D), absorption rate constants (k_a) and fractions absorbed (F_a) considered for the preliminary analyses are summarized in TABLE 2; an enterocytic blood flow (Q_{ent}) of 18 l/h was assumed. The selection of doses and formulations was based on the studies from which in vivo F_G estimates were obtained. The investigated drugs were ranked for their likelihood that saturation may have biased previous F_G estimates by assuming linearity in the Q_{Gut} model. From this analysis, a minor bias of intestinal metabolism can be anticipated by neglecting saturation of intestinal metabolism (C_{ent}/K_m <1) for atorvastatin, felodipine, midazolam and tacrolimus. In contrast, saturation was anticipated to affect the assessment of intestinal metabolism for cyclosporine, indinavir, saquinavir and terfenadine to a greater extent (C_{ent}/K_m > 10). Additionally, for indinavir saturation of hepatic first-pass metabolism was anticipated. In fact, saturation of systemic clearance of indinavir (radiolabeled i.v. dose) was shown with concomitant administration of 400-800 mg indinavir orally (Yeh et al., 1999). Eq. 3 does not allow assessment of regional differences in C_{ent} and subsequent effects on F_G.

Re-assessment of atorvastatin F_G after i.v./oral administration. The in vivo estimate of atorvastatin F_G was re-assessed based on available blood-to-plasma concentration ratio data (R_b 0.61, TABLE 2). The initial assessment of atorvastatin F_G was based on the assumption of R_b being equivalent to 1 (Lennernas, 2003), which resulted in a lower hepatic extraction ratio in comparison to the current analysis (0.42 vs. 0.63) and subsequently a lower estimate of F_G (0.14 vs. 0.38). The current F_G estimate of 0.38 assumes complete absorption of atorvastatin (Lennernas, 2003). The re-evaluation of atorvastatin F_G from i.v./oral data compared favorably to data from grapefruit juice interaction studies (Gertz et al., 2008a).

Re-assessment of intrinsic clearance of cyclosporine and tacrolimus. In comparison to Gertz et al. (2010), the unbound fractions in plasma of cyclosporine and

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tacrolimus were re-evaluated from available literature. Different methods have been employed to determine both cyclosporine and tacrolimus fraction unbound in plasma. The current study favored fu_p measurements determined in stainless steel equilibrium dialysis chambers for cyclosporine (1.9%) and tacrolimus (1.3%) given their high reproducibility. The use of these fu_p values resulted in changed estimates of in vivo $CL_{int,h}$ in comparison to our previous publication (TABLE 2).

PBPK model to estimate F_G and CL_{oral} from in vitro data. A PBPK model was constructed in which tissues were connected by an arterial blood supply and a collective venous return to the lungs (Nestorov, 2003). All tissues were considered to be well-stirred compartments, i.e. that the unbound tissue concentration is at equilibrium with the unbound concentration in the emergent blood (Eq. 4).

$$V_T \cdot \frac{dC_T}{dt} = Q_T\left(C_{b,A} - \frac{C_T}{K_{b,T}}\right) \qquad \text{Eq. 4}$$

where V_T, C_T, Q_T and $K_{b,T}$ represent the volume, concentration, blood flow and tissue to blood concentration ratio of the different tissues; $C_{b,A}$, arterial blood concentration

The physiological values for blood flows and tissue volumes were taken from the literature (Brown et al., 1997; ICRP, 2002). The selected tissues accounted for >95% of total body weight; an additional compartment representing the rest of the body was included. Tissue to blood concentration ratios were either collated from the literature or predicted using mechanistic equations (Rodgers et al., 2005; Rodgers and Rowland, 2006) utilizing human tissue composition data (Poulin and Theil, 2002). In case where no human data were available, rat tissue composition data were used. Concentration-time profiles, as well as F_G and CL parameters, were taken into consideration in the model development using midazolam and alfentanil as test compounds due to availability of K_b data in rat (data not shown). However, subsequent analysis focused on the prediction of F_G, $CL_{i.v.}$ and CL_{oral}.

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

$$V_{b,Li} \cdot \frac{dC_{b,Li}}{dt} = C_{b,A} \cdot Q_{b,HA} + C_{b,PV} \cdot Q_{b,PV} - C_{b,Li} \cdot Q_{b,HV} - PS_{Li} \cdot \left(fu_b \cdot C_{b,Li} - fu_{Li} \cdot C_{Li} \right) \quad \text{Eq. 5}$$

$$V_{Li} \cdot \frac{dC_{Li}}{dt} = PS_{Li} \cdot \left(fu_b \cdot C_{b,Li} - fu_{Li} \cdot C_{Li} \right) - \frac{V_{max} \cdot A_{CYP3A,Li} \cdot fu_{Li} \cdot C_{Li}}{K_m + fu_{Li} \cdot C_{Li}} \quad \text{Eq. 6}$$

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

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

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

$$\frac{dA_{St}}{dt} = -A_{St} \cdot Kt_{St} \qquad \text{Eq. 7}$$

$$\frac{dA_{G,1}}{dt} = A_{St} \cdot Kt_{St} - Kt_{G,1} \cdot A_{G,1} - ka_{G1} \cdot A_{G,1} \qquad \text{Eq. 8}$$

$$\frac{dA_{G,n}}{dt} = Kt_{G,n-1} \cdot A_{G,n-1} - Kt_{G,n} \cdot A_{G,n} - ka_{Gn} \cdot A_{G,n} \quad n = 2-7 \qquad \text{Eq. 9}$$

$$\frac{dA_{Co}}{dt} = A_{G,7} \cdot Kt_{G,7} - A_{Co} \cdot Kt_{Co} \qquad \text{Eq. 10}$$

$$V_{ent,n} \frac{dC_{ent,n}}{dt} = ka_{G,n} \cdot A_{G,n} - Q_{Gut,n} \cdot C_{ent,n} - \frac{V_{max} \cdot A_{CYP3A_{ent,n}} \cdot fu_{ent,n} \cdot C_{ent,n}}{K_m + fu_{ent,n} \cdot C_{ent,n}} \qquad \text{Eq. 11}$$

where A denotes the amounts of drug in either the stomach (ST), the intestinal segments (G,n) or the colon (Co); Kt_{St}, $Kt_{G,n}$ and Kt_{Co} refer to the transit rate constants of stomach, intestinal lumen and colon, k_a represents the absorption rate constant determined using Eq.15; fu_{ent}, $C_{ent,n}$, $V_{ent,n}$, $A_{CYP3A,ent,n}$ and $Q_{Gut,n}$ refer to the unbound fraction, concentration, volume, amount of CYP3A, and the hybrid parameter of blood flow and permeability in the enterocytes of the nth intestinal compartment.

Drug permeability was incorporated as a hybrid parameter of enterocytic blood flow and permeability, as in the Q_{Gut} model (Chalasani et al., 2002; Rostami-Hodjegan and Tucker, 2002). Absorption was considered to occur from the small intestinal compartments with the exception of saquinavir where colonic absorption was also incorporated, as investigated previously (Agoram et al., 2001). The blood flow to the small intestine represents approximately 10% of the cardiac output (39 l/h (ICRP, 2002)) and the enterocytic blood flow represents approximately 50% (i.e., 18 l/h) of the small intestinal blood flow (Granger et al., 1980). The cardiac output in the current assessment was 6.5 l/h, based on male subjects aged

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20-35 (Brown et al., 1997; ICRP, 2002). Effect of age on cardiac output (Brown et al., 1997) was taken into account for tacrolimus F_G predictions.

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

$$CL_{app} = \frac{Dose}{\int_0^{t-last} C(t) \cdot dt} \qquad \text{Eq. 12}$$

$$F_G = 1 - \frac{\sum AM_{ent,n}}{Dose_{oral} \cdot F_a} \qquad \text{Eq. 13}$$

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

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

Parameters for PBPK modeling. The metabolism in the liver was scaled using the standard human microsomal recovery of 40 mg/g and average liver weight of 21.4 g/kg. The total CYP3A contents in the duodenum, jejunum and ileum were 9.7, 38.4 and 22.4 nmol,

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respectively (Paine et al., 1997). The regional weights of the enterocytes and the transit rate constants in the duodenum, jejunum and ileum were 18.2 g and 4.3 h^{-1}, 65.8 g and 1.7 h^{-1}, 38.3 g and 2.1 h^{-1}, respectively (Yu et al., 1996; Paine et al., 1997). Differential blood supply to the duodenum, jejunum and ileum was accounted for (Jamei et al., 2009; Darwich et al., 2011).

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

$$log\ P_{eff} = 0.829 \cdot log\ P_{app(A-B)} - 1.30 \qquad \text{Eq. 14}$$

$$k_a = \frac{2 \cdot P_{eff}}{r_{SI}} \qquad \text{Eq. 15}$$

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

$$\frac{dA_{un,n}}{dt} = A_{un,n-1} \cdot Kt_{n-1} - A_{un,n} \cdot Kt_n - \frac{3D}{\rho \cdot r \cdot h} \cdot A_{un,n} \cdot \left(C_{S,n} - \frac{A_{dis,n}}{V_n} \right) \qquad \text{Eq. 16}$$

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$$\frac{dA_{dis,n}}{dt} = A_{dis,n-1} \cdot Kt_{n-1} + \frac{3D}{\rho \cdot r \cdot h} \cdot A_{un,n} \cdot \left(C_{S,n} - \frac{A_{dis,n}}{V_n} \right) - A_{dis,n} \cdot Kt_n - A_{dis,n} \cdot k_{a,n} \qquad \text{Eq. 17}$$

where A_{un} and A_{dis} represent respectively the undissolved and dissolved amount in the intestinal compartment n (including stomach and colon); Kt_n and ka_n represent the transit rate and absorption rate constants of the nth compartment; D, diffusion coefficient; ρ, density; r, particle radius; h, diffusion layer thickness; $C_{S,n}$, drug solubility in the nth luminal compartment; V_n, luminal volume of the different intestinal segments (default values in Gastro Plus® v.7)

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

Michaelis-Menten constants for CYP3A metabolism were collated from the literature except for felodipine, indinavir, nisoldipine and saquinavir, for which K_m data were determined in the current study. A summary of K_m data, physicochemical properties, and drug permeability and clearance data utilized in the current analysis is provided in TABLE 2. The unbound CL_{int} data in intestinal (HIM) and liver microsomes (HLM) have been taken from our previous publication (Gertz et al., 2010).

In vitro intrinsic clearance data utilized for predictions were determined in either pooled intestinal and liver microsomes (Gertz et al., 2010) or eight individual HJM reported in the current study. The clearance data obtained in the intestinal and liver pools were used for a comparison of model performance between the F_G predictions by the Q_{Gut} and the PBPK model and for the prediction of apparent i.v. and oral clearance using the PBPK model. On

the other hand, the individual HJM data were used to assess inter-individual variability in F_G predictions using the PBPK model alone.

Measurement of prediction success and comparison to in vivo data and Q_{Gut} predictions. The predictions of F_G, apparent i.v. and oral clearance data were compared to corresponding in vivo data reported elsewhere (Gertz et al., 2010). Bias and precision of F_G and clearance predictions were assessed by geometric fold error (gmfe) and root mean squared error (rmse).

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Results

Small intestinal metabolism was assessed for 12 drugs in 8 individual human jejunal microsomal preparations (6 male and 2 female white donors, TABLE 1). The mean CYP3A activity was 4.12 nmol/min/mg, ranging from 2.09 to 5.88 nmol/min/mg as measured by formation of 6β-hydroxytestosterone at 250µM testosterone concentration. The mean CYP3A activity was associated with a coefficient of variation of 36%; a 2.8-fold difference was observed between the donors with the lowest and highest CYP3A activity. The between day variability in testosterone 6ß-hydroxylation activity determinations was low, ranging from 2 to 14%.

The individual intrinsic clearance values determined in the current study showed a more than 800-fold difference ranging from 17.0 to 14,000 µl/min/mg for atorvastatin and saquinavir, respectively (Figure 2). HJM 1 showed the lowest CYP3A activity and resulted, on average, in the lowest intrinsic clearance values for the drugs investigated. In contrast, HJM 6 generally resulted in the highest clearance values whilst displaying the second highest CYP3A activity. The mean HJM intrinsic clearance values ranged from 33.5 to 7,220 µl/min/mg for atorvastatin and saquinavir, respectively, while the coefficient of variation ranged from 41 to 67% for simvastatin and tacrolimus, respectively (TABLE 4). Overall, the testosterone 6β-hydroxylation activity was a reasonable predictor for intrinsic clearance values between different individual jejunal microsomal preparations (R^2 = 0.72 combined data of all 12 substrates). The intrinsic clearance values of atorvastatin and saquinavir were poorly correlated with testosterone 6β-hydroxylation activity (R^2 < 0.30), in contrast to the good correlation seen for buspirone, midazolam and simvastatin (R^2 > 0.85).

In order to assess the K_m values of indinavir, felodipine, nisoldipine and saquinavir, intrinsic clearance values were measured via a substrate depletion approach across a range of substrate concentrations. The determined K_m values were estimated using Eq. 2 at 0.1 ±0.01

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(mean and SE from the fit), 0.3 ±0.02, 2.1 ±0.3 and 5.3 ±0.4 μM for indinavir, saquinavir, nisoldipine and felodipine, respectively.

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

The F_G predictions obtained by the PBPK model were compared to the predictions obtained by the Q_{Gut} model (Figure 3). To allow a direct comparison between the models our previously published in vitro intrinsic clearance and permeability data were utilized (TABLE 5). The F_G predictions of atorvastatin, felodipine, midazolam, nisoldipine and simvastatin were marginally affected by the choice of model. However, considerable differences were observed in the F_G predictions of indinavir, saquinavir and terfenadine for which the PBPK model predicted higher F_G values - more in line with the observed data. The F_G predictions for indinavir, saquinavir and terfenadine using the PBPK model were 0.98, 0.56 and 0.32, respectively. Differences in the F_G estimates were due to the ability of the PBPK model to account for saturation of intestinal metabolism by using V_{max} and K_m data rather than CL_{int}. The predicted F_G for saquinavir and terfenadine were very sensitive to changes in the K_m values used in contrast to indinavir. A reduction in prediction accuracy was observed for buspirone and cyclosporine when the PBPK model was compared to the Q_{Gut} model. For these drugs F_G over-prediction observed using the Q_{Gut} model increased further in the PBPK model (e.g., in the case of cyclosporine from 93% to 110% over-prediction). In the case of tacrolimus, a reduction in enterocytic blood flow in response to reduction in cardiac output with age resulted in lower F_G estimates to those reported previously (clinical data of tacrolimus were generally reported in individuals aged 50 or older). This was accurately

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accounted for by both models. An overall improvement in F_G predictions was observed using the PBPK model as measured by the decrease in gmfe (from 2.4 to 1.8) and rmse value (from 0.32 to 0.28) in comparison to the Q_{Gut} model.

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

Due to a combination of under-prediction of hepatic and intestinal clearance, a particularly low prediction success of oral clearance was observed for atorvastatin and buspirone (both < 2% of the observed value). In contrast, over-predictions of oral clearance were observed for indinavir and simvastatin. This was due to an approximately 2-fold over-prediction of hepatic intrinsic clearance (TABLE 2) for both drugs. In addition, for indinavir the incorporation of drug solubility data predicted incomplete absorption (61%) which contributed to the over-prediction of CL_{oral}. Saturation of hepatic first-pass metabolism of indinavir on the other hand was predicted by the model, as illustrated by the ratio of the AUCs in venous blood and portal vein (E_H = 32% vs. 11% under linear conditions). For felodipine, over-prediction of intestinal metabolism (observed and predicted F_G were 0.45 and 0.20, respectively) and under-prediction of apparent hepatic clearance (TABLE 5) resulted in an under-prediction of apparent CL_{oral} by 1.8-fold. Overall, the average bias was 4.7-fold for the

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predictions of apparent oral clearance, largely driven by the extensive under-predictions for buspirone and atorvastatin CL_{oral}; removal of these two drugs from the dataset reduced the bias to 2.9-fold.

Predictions of F_G values from individual human jejunal microsomes. F_G predictions were also performed using the individual data from 8 human jejunal microsomal preparations (Figure 5). In accordance with the generally higher in vitro clearance data obtained in the individual HJM microsomes in comparison to the HIM microsomal pool, the predicted F_G values were overall lower than those obtained from data in pooled microsomes. Mean F_G predictions ranged from 0.03 to 0.94 for simvastatin and indinavir, respectively (TABLE 6). The coefficient of variation in F_G predictions from the individual HJM clearance data ranged from 3 to 65% for indinavir and terfenadine, respectively. The F_G predictions of terfenadine showed the highest CV of all drugs investigated resulting in an 8.5-fold difference between the lowest and highest estimate. The remaining drugs showed less than 5-fold differences with the exception of lovastatin and tacrolimus (both 5.5-fold). The lowest F_G values (~2%) were predicted for nisoldipine from the HJM preparations 5, 6 and 7, which also showed the highest intrinsic clearance values of nisoldipine (Figure 2 and Figure 5). Higher CLu_{int} values were determined for saquinavir; however, this did not result in lower F_G values due to the prediction of saturation of intestinal first-pass for this drug. The highest F_G values and lowest CV were predicted for indinavir (0.92 to 0.98). This was not due to low metabolic clearance, the average CLu_{int} value of indinavir being 645µl/min/mg (greater than midazolam intrinsic clearance), but due to the prediction of extensive saturation of intestinal metabolism. Consistent with the predictions based on the data from microsomal pools, considerable F_G over-predictions were apparent for atorvastatin and buspirone from the individual microsomal preparations (2- to 3-fold, respectively).

Intrinsic clearance data from eight individual HJM were utilized to assess inter-individual variation of F_G. In the literature, very few clinical studies report individual data to

allow an estimation of inter-individual variability of this parameter. In the case of felodipine, individual AUC data before and after grapefruit juice coadministration have been reported in four separate studies for a total of 45 individuals, summarized in Gertz et al. (2008a). The mean F_G of felodipine was 0.45, this estimate was associated with a between individual variability of 41% and ranged from 0.12 to 1.0 (8.3-fold between highest and lowest F_G). Utilizing the HJM clearance data, the predicted F_G of felodipine was 0.11 (range: 0.06 to 0.17) and the CV of 42% reflected the variability observed in vivo. In the case of midazolam, individual data after i.v. and oral dose have been reported in 20 individuals (Thummel et al., 1996). This study indicated a 42% inter-individual variability of F_G which ranged from 0.18 to 0.99 (5.4-fold ratio between highest and lowest F_G). Utilizing the HJM clearance data, the predicted F_G of midazolam was 0.33 (range: 0.19 to 0.52) and a CV of 36% which reflected the variability observed in vivo.

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Discussion

Determination of intestinal clearance in vitro. The in vitro clearance values from eight individual human jejunal microsomal samples compared well with our previous data generated in pooled human intestinal microsomes (TABLE 4). The CYP3A activity was considerably greater than in the HIM pool, despite the fact that elution methods were used in both cases to obtain the microsomes (1.84 and 4.12 nmol/min/mg for HIM pool and HJMs, respectively). The mean activity of the HJM was comparable to the mean of the three HLM pools (4.65 nmol/min/mg). The between individual variability of 36% was lower than anticipated from reports on tacrolimus and cyclosporine in 14 small intestinal samples, where the between subject variability was 54-70%; 4.6- to 11-fold difference between highest and lowest activity in contrast to 2.8-fold in the current study (Lampen et al., 1995; Lampen et al., 1996). Considering the relatively small sample size, one can question whether the current HJM selection accurately reflects the true population variability of intestinal CYP3A activity. However, to our knowledge this is the largest dataset of intrinsic clearance data obtained in individual intestinal microsomes in the current literature.

Prediction of F_G using PBPK modeling. In this study a PBPK model was applied to make predictions of intestinal availability using in vitro clearance and permeability data for 12 selected drugs. The analysis focused on drugs with high intestinal extraction in vivo, as F_G prediction accuracy for these drugs based on the Q_{Gut} model was lower in comparison to drugs with F_G>0.5 (Gertz et al., 2010). Use of the PBPK model improved F_G prediction resulting in lower bias and increased precision. In contrast to the Q_{Gut} model, the PBPK model accounted for substrate concentrations at the enzyme site, Michaelis-Menten constants and potential saturation of metabolism. Additionally, the PBPK model accommodated any potential regional differences in intestinal availability due to heterogeneous expression of CYP3A enzymes along the small intestine (Paine et al., 1997). Solubility of cyclosporine, indinavir, saquinavir and terfenadine in simulated intestinal fluids under fasted conditions were taken

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

In addition to the need for more informative data on intestinal transporters, refinement of regional P450 contents based on a larger number of individuals and preferably determined by a less destructive preparation technique will provide greater confidence in the predictions of intestinal metabolism. The current data presented by Paine (1997) are based on mucosal scraping technique and a relatively small sample size, in contrast to hepatic CYP3A content data obtained from a meta-analysis of a large number of individuals (Rostami-Hodjegan and Tucker, 2007).

Prediction of apparent i.v. and oral clearance. Successful predictions of apparent oral clearance (inside 3-fold) were obtained using pooled microsomal intrinsic clearance data for 6 out of 12 drugs. In contrast, large under-predictions of oral clearance were observed for atorvastatin and buspirone (consistent with under-prediction of $CL_{i.v.}$). Metabolism of buspirone has been suggested to be mainly mediated by CYP3A and a similar metabolite pattern in HLM was reported in comparison to in vivo (Jajoo et al., 1989; Zhu et al., 2005). Previously reported metabolite formation data resulted in marginally higher CLu_{int} values

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(correction for fu_{inc} applied) compared to our substrate depletion data (460 vs. 268 µl/min/mg (Zhu et al., 2005; Gertz et al., 2010)). Buspirone clinical data after i.v. administration in eight volunteers were highly variable (i.e., CL_p = 28.3 ±10.3 ml/min/kg, F = 3.9 ±4.3%) with negligible renal excretion of unchanged drug (Gammans et al., 1986). However, neither the variability in the in vivo data nor the differences in the in vitro data are sufficient to explain the extensive under-prediction of intrinsic hepatic clearance, as the in vitro data would classify buspirone as low to moderate extraction drug (E_H = 38%, TABLE 5) while the in vivo data show clearance values in excess of hepatic blood flow.

A number of factors may contribute to the extensive under-prediction of both i.v. and oral clearance of atorvastatin. Firstly, in vitro data have highlighted drug affinity for uptake transporters (Kameyama et al., 2005) and studies in polymorphic OATP1B1 populations confirmed the clinical relevance of at least OATP1B1 to atorvastatin disposition (Pasanen et al., 2007). It has been suggested that inter-conversion between acid and lactone forms may represent the initial step in atorvastatin metabolism (Jacobsen et al., 2000); this may occur directly from the parent or from the acyl glucuronide metabolite (Prueksaritanont et al., 2002). In human liver microsomes, the lactone displays on average a 70-fold greater intrinsic clearance than the hydroxy-acid form (Jacobsen et al., 2000). Incorporation of the higher intrinsic clearance of the lactone form into the current analysis resulted in apparent i.v. and oral clearance values of 54.4 and 786 l/h, respectively and better agreement with the observed data (37.5 and 949 l/h, respectively). The acid-lactone conversion was assumed to occur in plasma and therefore only CLu_{int} for hepatic metabolism was modified in the PBPK model, while intestinal intrinsic clearance remained unaltered. However, given the large variability in the ratio of CL_{int} of the lactone and the acid form (14 to 160-fold reported from four individual HLM (Jacobsen et al., 2000)) more data are required to investigate the rate limiting step in atorvastatin metabolism.

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In the case of cyclosporine, the reason for a ~4-fold under-prediction of intrinsic clearance is unclear. Although UGT mediated metabolism in HLM has been reported (Strassburg et al., 2001), we were unable to confirm this by a substrate depletion assay performed in alamethicin activated microsomes in the absence and presence of bovine serum albumin (data not shown). The under-estimation of $CL_{int,h}$ of cyclosporine was masked in the reasonably well predicted oral clearance by the fact that cyclosporine absorption was also under-estimated (F_a of 24% and 16% for Neoral® and Sandimmune®, respectively).

F_G predictions using individual jejunal microsomal samples. Intrinsic clearance data from eight individual HJM were utilized to predict F_G and associated inter-individual variability. Individual in vivo F_G values were reported for terfenadine, tacrolimus, midazolam and felodipine in 6, 12, 20 and 45 individuals respectively. Use of individual HJM clearance data in the PBPK model resulted in an under-prediction of felodipine F_G (consistent with the pooled data); however, the predicted variability in F_G reflected the variability seen in vivo. Considering extensive binding of felodipine to plasma proteins (>99%; TABLE 2), binding to enterocytic proteins during absorption cannot be ruled out. Any binding to the enterocytic proteins would lead to reduced apparent intestinal clearance and therefore increased F_G predictions. Midazolam F_G was well predicted using the pooled microsomal CLu_{int} data but somewhat under-predicted using the individual HJM. While the coefficient of variation was predicted reasonably well, the F_G range was under-predicted. Particularly, the ability to predict the upper limit of in vivo F_G values was poor; suggesting that selected individual jejunal donors did not cover the full spectrum of CYP3A activity present in the small intestine in vivo.

Other examples for which individual estimates of F_G were available in the literature include tacrolimus (Floren et al., 1997; Hebert et al., 1999) and terfenadine (Clifford et al., 1997). The inter-individual variability of in vivo F_G estimates for tacrolimus and terfenadine were 36 and 54%, respectively. Both the CV and the fold-difference between the highest and

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lowest F_G values were over-predicted for terfenadine 65% and 8.5-fold, respectively. Additionally, the mean predicted F_G of terfenadine was under-estimated using the individual HJM data. Extensive binding to microsomal proteins has been observed for terfenadine using equilibrium dialysis (Gertz et al., 2008b). Any inaccuracies in the fu_{inc} estimate will bias the estimate of intrinsic clearance and also the estimate of unbound K_m used in the PBPK model. In contrast, prediction of the mean tacrolimus F_G was more successful (0.08 vs. 0.14 for the predicted and observed F_G, respectively); however, both the CV and the fold-difference were somewhat over-predicted using the intrinsic clearance data from eight individual HJM (CV=58% and 5.5-fold). Alternatively, a propagation of the variability in CYP3A in the small intestine (von Richter et al., 2004) using Monte Carlo simulations may be employed to predict inter-individual variability of intestinal metabolism and F_G. However, it has to be emphasized that CYP3A abundance of a larger population needs to be assessed in order to capture the inter-individual variability correctly.

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

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Authorship contribution

Participated in research design: Galetin, Gertz and Houston

Conducted experiments: Gertz

Performed data analysis: Gertz

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

Other: Galetin and Houston acquired funding for the research

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Footnotes

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Legends of Figures

Figure 1: Illustration of the absorption model applied for the current analyses. First (grey) and second levels represent undissolved and dissolved drug (after immediate release) in the different segments of the intestinal lumen, respectively; third level: enterocytes; St, refers to stomach; D, duodenum; J1-2, jejunal segments; I1-4, ileal segments; Kt, transit rate constants; k_a, absorption rate constants; CL, apparent clearance in the different intestinal segments, Q_{Gut}, hybrid function of regional enterocytic blood flows and drug permeability and Q_{ent}, total enterocytic blood flow

Figure 2: Individual unbound intrinsic clearance values from 8 HJM preparations for atorvastatin (ATV), buspirone (BSP), cyclosporine (CYS), felodipine (FDP), indinavir (IDV), lovastatin (LVS), midazolam (MDZ), nisoldipine (NSP), saquinavir (SQV), simvastatin (SVS), tacrolimus (TCL) and terfenadine (TFD); HJM 1 (△), HJM 2 (□), HJM3 (▽), HJM 4 (○), HJM 5 (▲), HJM 6 (■), HJM 7 (▼) and HJM 8 (●); in the case of tacrolimus, CLu_{int} data in HJM preparation 7 were not included, as the no-NADP-incubation showed extensive drug depletion.

Figure 3: Drug-specific F_G prediction success using the Q_{Gut} (Gertz et al., 2010) or the PBPK model (black and dotted bars, respectively) of atorvastatin (ATV), buspirone (BSP), cyclosporine (CYS), felodipine (FDP), indinavir (IDV), lovastatin (LVS), midazolam (MDZ), nisoldipine (NSP), saquinavir (SQV), simvastatin (SVS), tacrolimus (TCL) and terfenadine (TFD); dashed lines indicate a 2-fold deviation from unity. In vitro clearance and permeability data used are listed in **TABLE 2**.

Figure 4: Comparison of observed and predicted apparent clearance after i.v. (□) and oral (■) administration. In all instances data represent plasma clearance, with the exception of

cyclosporine and tacrolimus (blood clearance). Predictions were performed using the in vitro data (**TABLE 2**) and the current PBPK model. The clinical data were determined by meta-analyses (Gertz et al., 2010); the error bars on the x- and y-axis indicate the range in the clinical data and in the predictions using different microsomal pools, respectively. The dashed lines indicate a 3-fold deviation from unity; outliers are highlighted: 1, cyclosporine; 2, atorvastatin; 3, buspirone; 4, saquinavir; 5, simvastatin and 6, indinavir.

Figure 5: Individual F_G predictions using the mechanistic first-pass model and intrinsic clearance data determined in 8 individual HJM preparations (**Figure 2**) for atorvastatin (ATV), buspirone (BSP), felodipine (FDP), indinavir (IDV), lovastatin (LVS), midazolam (MDZ), nisoldipine (NSP), saquinavir (SQV), simvastatin (SVS), tacrolimus (TCL) and terfenadine (TFD); HJM 1 (△), HJM 2 (□), HJM3 (▽), HJM 4 (○), HJM 5 (▲), HJM 6 (■), HJM 7 (▼) and HJM 8 (●)

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TABLE 1

Summary of individual human jejunal microsomal preparations

Donor[1]	**Gender**	**Age**	**CYP3A activity ± SD**[2]
		years	*nmol/min/mg*
HJM 1	male	24	2.09 ±0.176
HJM 2	male	59	3.08 ±0.085
HJM 3	male	26	2.63 ±0.151
HJM 4	male	35	3.38 ±0.485
HJM 5	male	68	5.15 ±0.313
HJM 6	male	60	5.56 ±0.122
HJM 7	female	39	5.20 ±0.198
HJM 8	female	65	5.88 ±0.393

[1] Microsomal preparation by elution method from the jejunum
[2] Testosterone 6ß-hydroxylation activity was determined at a testosterone concentration of 250 μM

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TABLE 2

Summary of drug related parameters used in the current PBPK model for 12 drugs investigated[1]

	Dose	k_a[2]	C_{ent}	$logP_{o:w}$	pK_a	fu_p	R_b	P_{eff}[3]	K_m[4]	in vivo $CL_{int,h}$[5]	in vitro $CL_{int,h}$[6]
	mg	h^{-1}	*μM*					*μm/s*	*μM*	*l/h*	*l/h*
Atorvastatin	40	3.7	15	4.07	4.46	0.051	0.61	1.72	33	1,990	213
Buspirone	20	5.0	14	2.63	7.32, 4.12	0.05	0.81	7.17	8.0	20,700[8]	963
Cyclosporine[7]	380 570	2.0 1.1	32 14	3.45	neutral	0.019[9]	1.36	3.30	1.4	1,450	279
Felodipine	10	2.8	4.0	3.86	neutral	0.0048	0.70	3.00	5.3	39,100	7,150
Indinavir[7]	400	1.8	57	2.92	5.9, 3.7	0.36	0.84	2.07[10]	0.1	1,120	2,090
Lovastatin	20	0.8	9.0	4.26	neutral	0.017	0.57	5.05	7.8	5,750[8]	17,200
Midazolam	3	4.2	2.2	3.25	6.1	0.031	0.55	6.73	3.3	1,610	1,540
Nisoldipine	20	3.0	8.6	3.80	neutral	0.0041	1.0	4.05	2.1	38,900	25,200
Saquinavir[7]	600	2.5	37	4.10	8.2	0.028	0.74	3.33[10]	0.3	7,660[8]	26,800
Simvastatin	40	2.0	10	4.71	neutral	0.014	0.57	4.30	3.4	14,300[8]	25,500
Tacrolimus	0.05 mg/kg	2.6	0.7	3.26	neutral	0.013[9]	35	5.95[10]	2.6	7,530	3,750
Terfenadine	120	2.8	47	5.62	9.7	0.03	1.0	5.43[10]	1.0	70,600	12,400

[1] References for $logP_{o:w}$, pK_a, fu_p, k_a and K_m in the Supplementary material (Supplemental Table 1) provided at http://www.pharmacy.manchester.ac.uk/capkr/

[2] Estimation of k_a based on clinical data using $T_{max} = \frac{ln\left(\frac{k_a}{k_{el}}\right)}{k_a - k_{el}}$

[3] Estimated from $P_{app\ (A-B)}$ data in MDCK-MDR1 cells (Gertz et al., 2010); in the case of cyclosporine in vivo P_{eff} data were used (Supplemental Table 1)

[4] Represent the unbound K_m values (if original studies reported protein content)

[5] Values reported in Gertz et al. (2010); updated values fu_p values of atorvastatin, cyclosporine, felodipine, lovastatin, nisoldipine, simvastatin and tacrolimus in comparison to Gertz et al. (2010)

[6] Data represent mean from three human liver microsomal pools (Gertz et al., 2010)

[7] Fraction absorbed of cyclosporine (Sandimmune® and Neoral® 0.9 and 0.5, respectively), indinavir and saquinavir (Invirase®) 0.8 and 0.3, respectively

[8] In vivo intrinsic clearance was estimated from oral data; in the case of buspirone i.v. clearance exceeded hepatic blood flow and showed very large inter-individual variability (Gammans et al., 1986)

[9] Based on equilibrium dialysis experiments in stainless steel chambers

[10] Based on P_{app} (A-B) data in the presence of a P-gp inhibitor

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TABLE 3

Drug solubility data obtained in fasted simulated small intestinal fluids and particle size for cyclosporine, indinavir, saquinavir and terfenadine[1]

	Solubility	***Particle radius***
	μM	*μm*
Cyclosporine	9.1 (13.3) [2]	0.018[3]
		1.87[4]
Indinavir	90[5]	25[6]
Saquinavir	64[5]	25[6]
Terfenadine	38[7]	25[6]

[1] Additional references (Supplemental Table 1)
[2] Value in brackets, fasted human intestinal fluids
[3, 4] Cyclosporine Neoral® and Sandimmune®, respectively
[5] Refer to indinavir sulphate and saquinavir mesylate; solubility data of indinavir and saquinavir over pH range were collated from the literature and solubility was limited to the highest reported value
[6] assumed
[7] Henderson-Hasselbalch equation for a monoprotic base was used to estimate pH dependent solubility; maximum solubility was limited to 848μM

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TABLE 4

Individual CLu_{int} values of 12 drugs determined by a substrate depletion method in 8 individual HJM preparations and comparison to previously published data in human intestinal and hepatic microsomal pools

	CLu_{int}			CV[2]
	HIM[1]	HLM[1]	HJM mean[2]	
		µl/min/mg		*%*
Atorvastatin	13.6	59.3	33.5	47
Buspirone	108	268	142	60
Cyclosporine	27.7	79.5	53.5	66
Felodipine	1,170	1,990	1,610	51
Indinavir	298	582	645	47
Lovastatin	2,440	4,790	2,740	59
Midazolam	340	429	418	47
Nisoldipine	3,840	7,000	6,180	47
Saquinavir	3,030	7,460	7,220	65
Simvastatin	3,480	7,100	5,840	41
Tacrolimus	658	1,040	1,970	67
Terfenadine	1,650	3,440	3,690	59

[1] CLu_{int} data from HIM pool (n=10 donors) and three HLM pools (n=105 donors in total) reported in Gertz et al. (2010)
[2] Data from the present study; in the case of tacrolimus CLu_{int} value represents the mean and CV of 7 individual intrinsic clearance values

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TABLE 5

Comparison of predicted F_G, apparent i.v. and oral clearance data for 12 drugs and observed data. Predictions were performed using either the Q_{Gut} or PBPK modeling approach

	Observed			Predicted			
	F_G[1]	$CL_{i.v.}$[1]	CL_{oral}[1]	$F_G - Q_{Gut}$[2]	F_G – PBPK[2]	$CL_{i.v.}$[2]	CL_{oral}[2]
	l/h					*l/h*	
Atorvastatin	0.38[3]	37.5	949	0.90	0.90	9.05[4]	12.0[4]
Buspirone	0.21	119	4,910	0.69	0.73	29.7	70.6
Cyclosporine							
Neoral	0.44	16.8[5]	51.7[5]	0.85	0.92	3.71[5]	17.1[5] (F_a = 24%)
Sandimmune	0.44	16.8[5]	107[5]	0.85	0.92	3.70[5]	24.6[5] (F_a = 16%)
Felodipine	0.45	50.0	462	0.21	0.20	22.5	254
Indinavir	0.93	77.3	59.6	0.31	0.98	82.0	547 (F_a = 61%)
Lovastatin	0.07	-	1,380	0.11	0.10	45.9	4,110
Midazolam	0.51	25.9	102	0.54	0.53	24.7	150
Nisoldipine	0.11	60.5	1,340	0.08	0.08	49.0	1,990
Saquinavir, inclufe	0.18	54.2	14,400	0.02	0.56[6]	65.6	3,620[6] (F_a = 41%)
Simvastatin	0.14	-	1,630	0.07	0.07	48.0	6,320
Tacrolimus	0.14	2.69[5]	20.7[5]	0.28	0.28	1.36[5]	8.79[5]
Terfenadine	0.40	-	5,290	0.11	0.32	76.9	2,720 (F_a = 52%)

[1] Summary from Gertz et al. (2010)

[2] Predictions were performed using the clearance data from HIM and HLM and apparent permeability data determined in MDCK-MDR1 cells reported previously (Gertz et al., 2010)

[3] Reassessed from the i.v. clearance data provided in Lennernäs (2003) and R_b value of 0.61 (see Methods)

[4] Acid-lactone conversion was not considered. Predictions based on 70-fold higher intrinsic clearance values of the lactone (Jacobsen et al., 2000) resulted in apparent i.v. and oral clearance values of 54.4 and 786l/h, respectively

[5] Apparent blood clearance values

[6] Absorption in the proximal colon (Agoram et al., 2001) was incorporated in the current model

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TABLE 6

Summary of the average, range and CV of F_G values predicted from individual clearance data determined in 8 human jejunal microsomal preparations using the PBPK modeling approach

	F_G	range	CV
			%
Atorvastatin	0.83	0.73 - 0.90	8
Buspirone	0.65	0.48 - 0.85	24
Cyclosporine	0.84	0.71 - 0.94	11
Felodipine	0.11	0.06 - 0.17	42
Indinavir[1]	0.94	0.92 - 0.98	3
Lovastatin	0.08	0.03 - 0.16	59
Midazolam	0.33	0.19 - 0.52	36
Nisoldipine	0.04	0.02 - 0.07	53
Saquinavir[1]	0.36	0.19 - 0.55	39
Simvastatin	0.03	0.02 - 0.06	45
Tacrolimus[1]	0.08	0.03 - 0.15	58
Terfenadine[1]	0.12	0.03 - 0.23	65

[1] based on P_{app} (A-B) data in the presence of a P-gp inhibitor

Figure 1

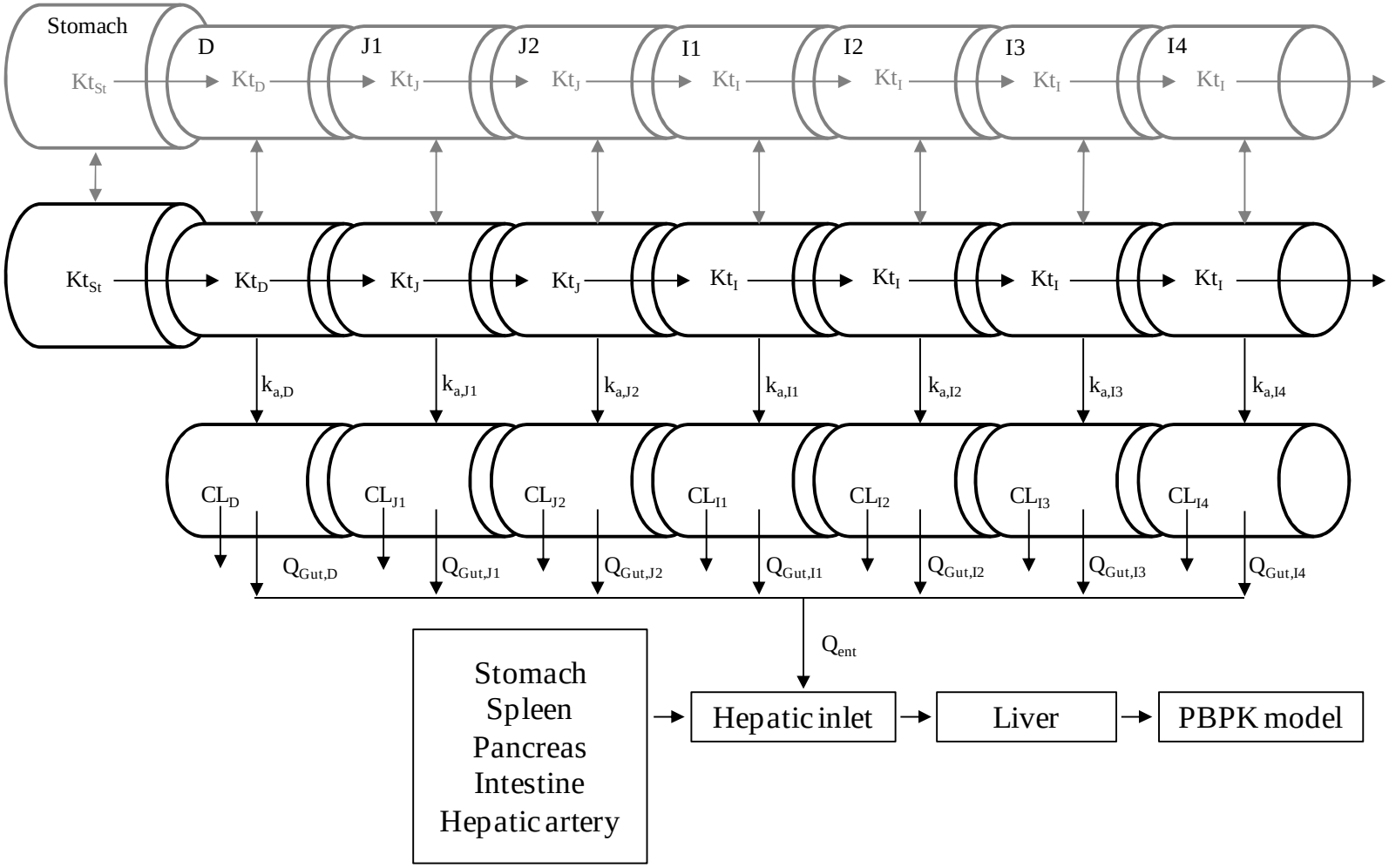

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Figure 2

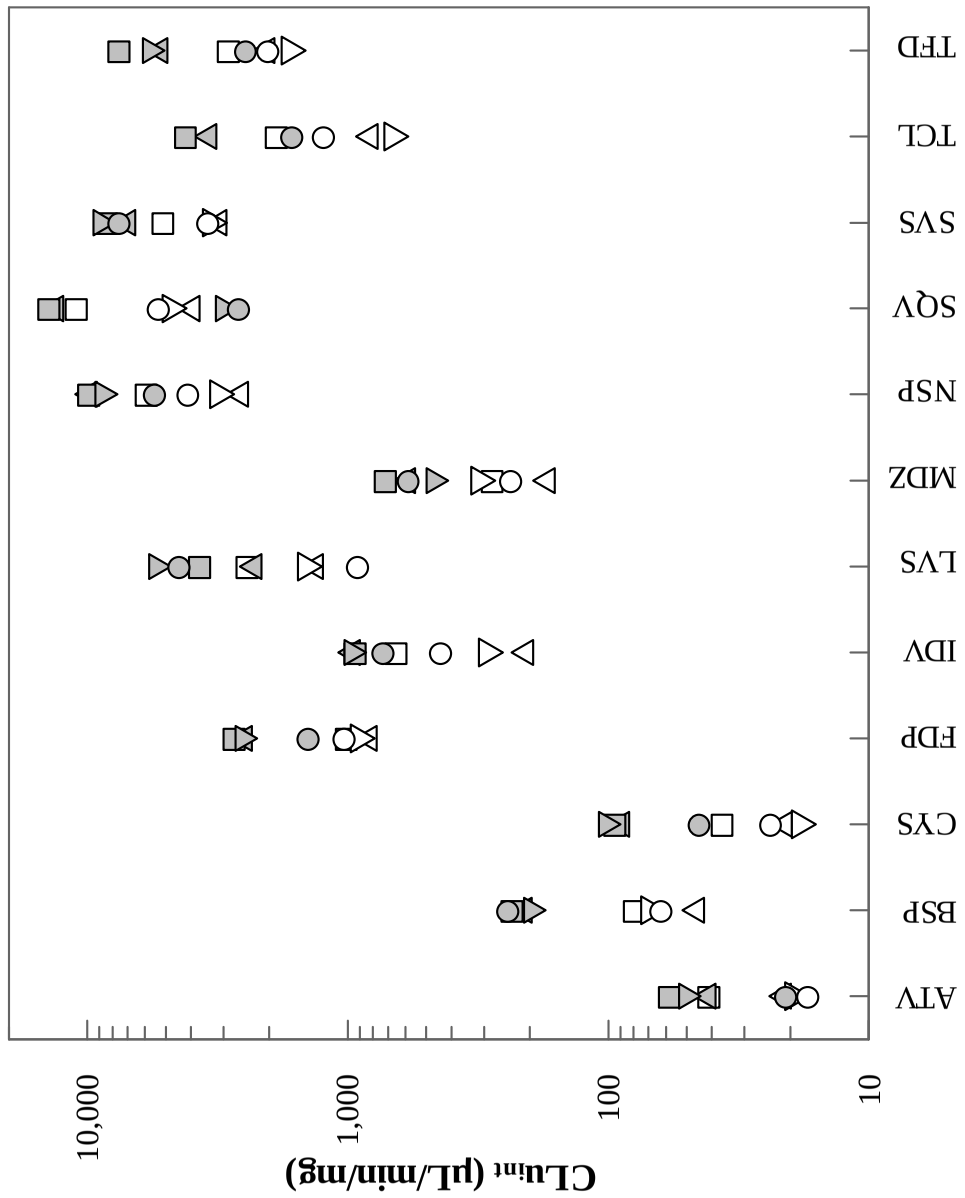

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Figure 3

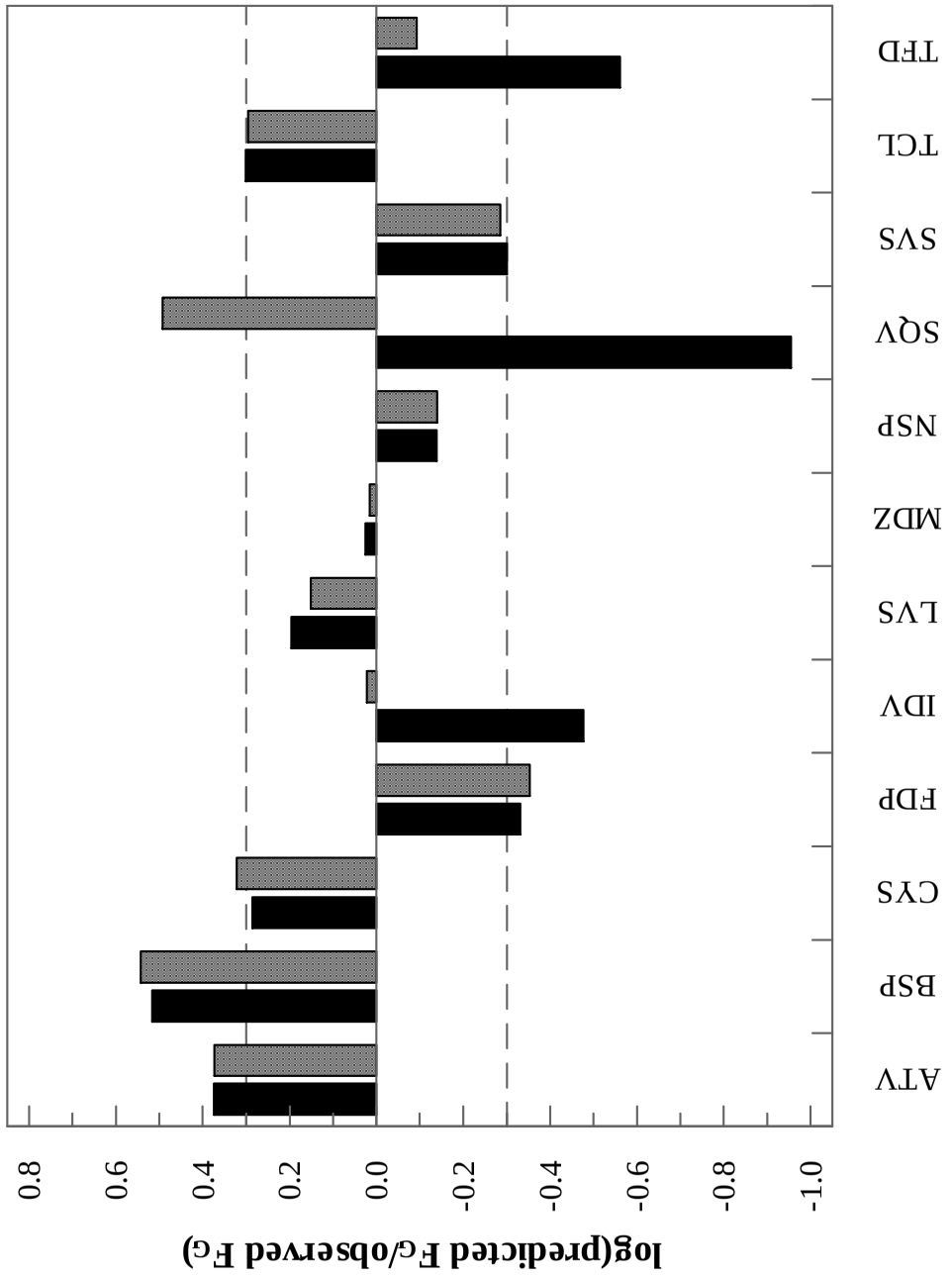

Figure 4

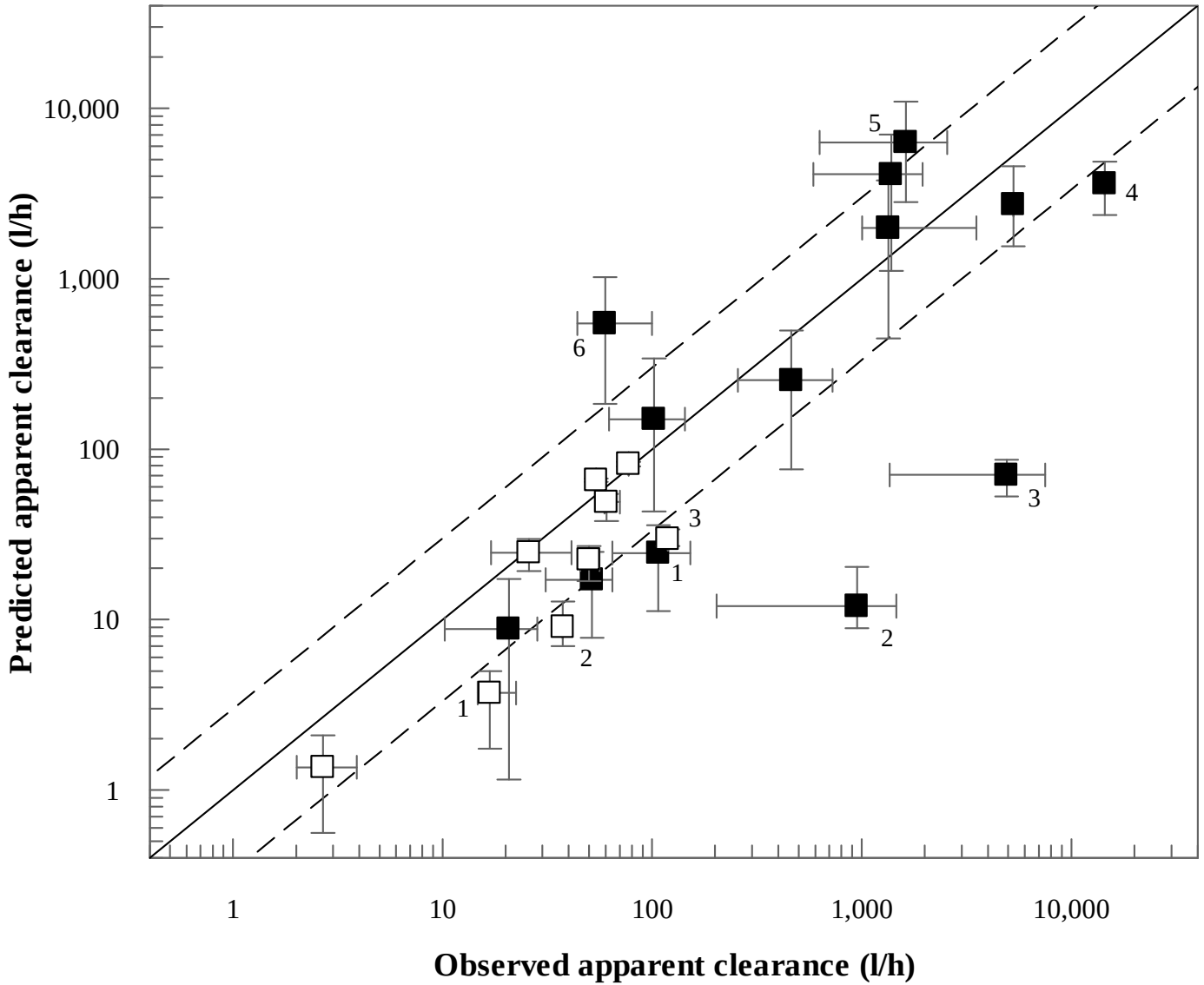

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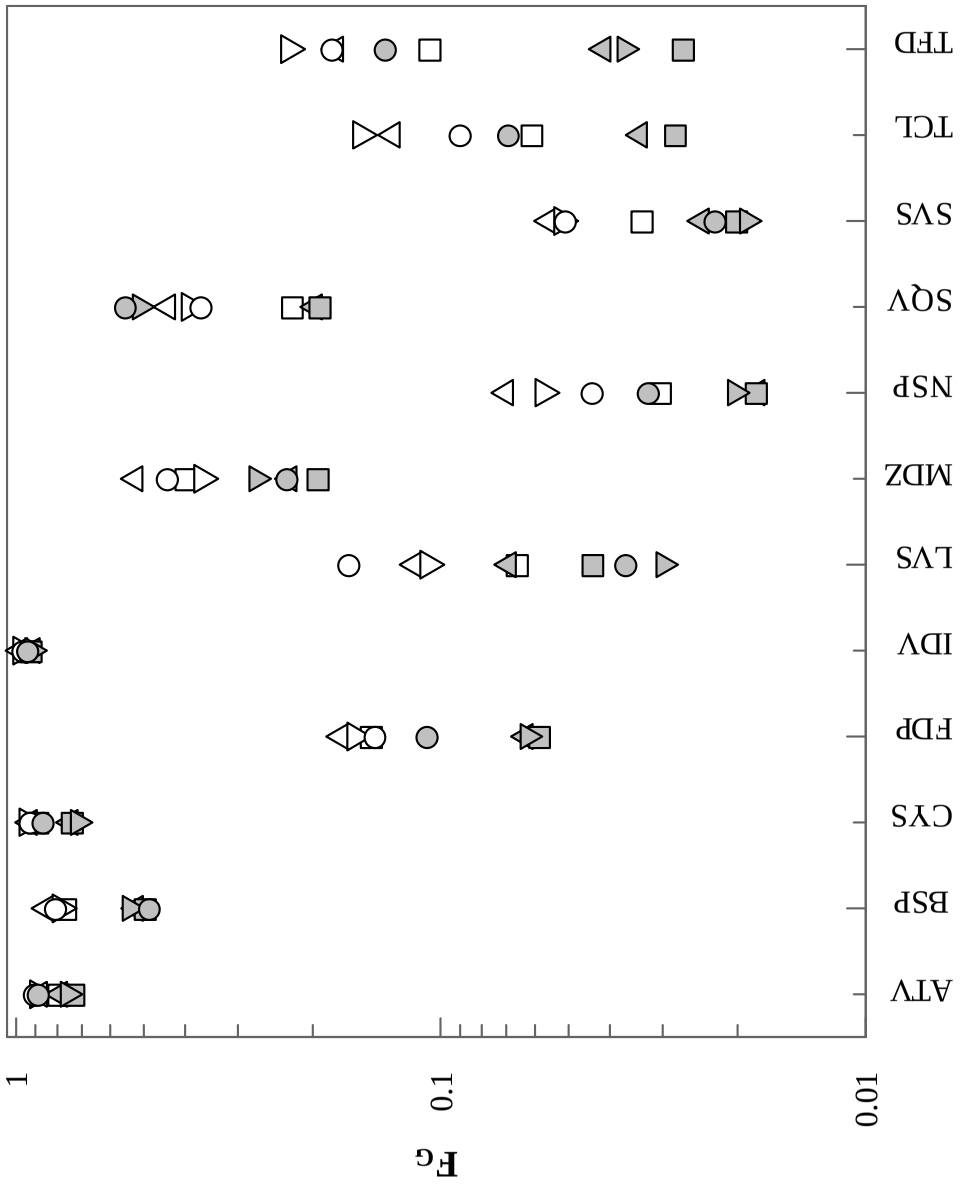
Figure 5



Physiologically-based pharmacokinetic modeling of intestinal first-pass metabolism of CYP3A substrates with high intestinal extraction – supplementary material - Drug metabolism and Disposition (DMD #39248)

Michael Gertz, J. Brian Houston and Aleksandra Galetin

TABLE 1

Summary of drug related parameters for used in the current PBPK model for 12 drugs investigated

	$logP_{o:w}$	pK_a	fu_p	R_b	k_a	K_m	Solubility	Particle radius	P_{eff}
					h^{-1}	μM	μM	μm	$\mu m/s$
Atorvastatin	4.07[1]	4.46[2]	0.051[3]	0.61[4]	3.7 ±1.8[5]	33[6]	-	-	Predicted
Buspirone	2.63[1]	7.32, 4.12[2]	0.05[3]	0.81[4]	5.0 ±0.71[5] 2.3 ±1.3[6]	8[7]	-	-	Predicted
Cyclosporine	3.45[1]	neutral	0.019[2]	1.36[3]	2.0 ±0.30[4] 1.1 ±0.44[5]	1.4[6]	9.1 (13.3) [7]	0.018[8] 1.87[8]	3.30[9]
Felodipine	3.86[1]	neutral	0.0048[2]	0.70[3]	2.8 ±0.9[4]	5.3[5]	-	-	Predicted
Indinavir	2.92[1]	5.9, 3.7[2]	0.36[3]	0.84[4]	1.8 ±0.4[5]	0.1[6]	90[7]	-	Predicted
Lovastatin	4.26[1]	neutral	0.017[2]	0.57[3]	0.8[4]	7.8[5]	-	-	Predicted
Midazolam	3.25[1]	6.1[2]	0.031[3]	0.55[4]	4.2 ±0.7[5]; 2.2 ±0.6[6]	3.3[7]	-	-	Predicted
Nisoldipine	3.80[1]	neutral	0.0041[2]	1.0[3]	3.0 ±1.3[4]	2.1[5]	-	-	Predicted
Saquinavir	4.10[1]	8.2[2]	0.028[3]	0.74[4]	2.5[5]	0.3[6]	64[7]	-	Predicted

Simvastatin	4.71[1]	neutral	0.014[2]	0.57[3]	2.0 ±0.9[4]	3.4[5]	-	-	Predicted
Tacrolimus	3.26[1]	neutral	0.013[2]	35[3]	2.6 ±0.7[3]	2.6[4]	-	-	Predicted
Terfenadine	5.62[1]	9.7[2]	0.03[3]	1.0[4]	2.8[5]	1[6]	38[5]	-	Predicted

Effective permeability (P_{eff}) was predicted from P_{app}(A-B) data (Gertz et al., 2010) and diffusion coefficents for cyclosporine, indinavir, saquinavir and terfenadine were predicted from molecular weights (Avdeef et al., 2004)

Atorvastatin: [1] (Ishigami et al., 2001; Lennernas, 2003); calculated from $logD_{7.0}$ = 1.63; [2] (Lennernas, 2003); [3, 4] (Watanabe et al., 2010); [5] (Kantola et al., 1998a; Lilja et al., 1999; Mazzu et al., 2000; Fukazawa et al., 2004; Ando et al., 2005); [6] (Jacobsen et al., 2000)

Buspirone: [1] http://chem.sis.nlm.nih.gov/chemidplus/ (experimental); [2] (Mahmood and Sahajwalla, 1999); [3] (Gammans et al., 1986); [4] as in Gertz (2010); [5] solution: (Gammans et al., 1985) [6] tablet: (Barbhaiya et al., 1994; Kivisto et al., 1997; Lamberg et al., 1998a; Lamberg et al., 1998c; Lamberg et al., 1998b; Lilja et al., 1998a; Dockens et al., 2006); [7] (Zhu et al., 2005)

Cyclosporine: [1] (el Tayar et al., 1993; Lauerma et al., 1997; Lucangioli et al., 2003); [2] (Henricsson, 1987; Lindholm et al., 1988; Yang and Elmquist, 1996; Akhlaghi et al., 1999); [3] as in Gertz (2010); [4] Neoral®: (Mueller et al., 1994a; Mueller et al., 1994b; Ku et al., 1998; Lee et al., 2001; Schwarz et al., 2006); [5] Sandimmune®: (Mueller et al., 1993; Mueller et al., 1994a; Mueller et al., 1994b; Ducharme et al., 1995; Edwards et al., 1999); [6] (Lampen et al., 1995); [7] (Persson et al., 2005); [8] (Andrysek, 2003); [9] current study supplemented by (Chiu et al., 2003)

Felodipine: [1] http://chem.sis.nlm.nih.gov/chemidplus/ (experimental); [2] (Valle et al., 1996; Kochansky et al., 2008); [3] as in Gertz (2010); [4] (Blychert et al., 1991); [5] current study

Indinavir: [1] (Lin et al., 1995; Glynn and Yazdanian, 1998); [2] (Lin et al., 1995); [3] (Obach et al., 2008); [4] as in Gertz (2010) provided by Pfizer Pharmacokinetics, Dynamics and Metabolism department (Sandwich, UK); [5] (Ferry et al., 1998; Hsu et al., 1998; Sandhu et al., 2003); [6] current study; [7] current study supplemented by (Lin et al., 1995; Pathak et al., 2010)

Lovastatin: [1] http://chem.sis.nlm.nih.gov/chemidplus/ (experimental); [2] (Duggan et al., 1989); [3] as in Gertz (2010) provided by Pfizer Pharmacokinetics, Dynamics and Metabolism department (Sandwich, UK); [4] (Kantola et al., 1998c); [5] (Jacobsen et al., 1999)

Midazolam: [1, 2] (Gueorguieva et al., 2004; Rodgers and Rowland, 2006); [3] (Gertz et al., 2010); meta-analysis; [4] (Obach, 1999); [5] from solution: (Tsunoda et al., 1999; Rogers et al., 2003; Kharasch et al., 2004; Kharasch et al., 2007); [6] from tablets: (Olkkola et al., 1993; Kupferschmidt et al., 1995; Backman et al., 1996; Olkkola et al., 1996; Palkama et al., 1999; Saari et al., 2006; Farkas et al., 2007); [7] (Galetin and Houston, 2006)

Nisoldipine: [1] (Sakamoto et al., 1993); [2] (Boelaert et al., 1988); [3] assumed to be 1; [4] (van Harten et al., 1988a; van Harten et al., 1988b; van Harten et al., 1988c; van Harten et al., 1989a; van Harten et al., 1989b; van Harten et al., 1989c; van Harten et al., 1989d; Baksi et al., 1991; Bailey et al., 1993; Heinig et al., 1999; Takanaga et al., 2000); [5] current study

Saquinavir: [1] (Glynn and Yazdanian, 1998; Williams and Sinko, 1999); [2] (Chen and Venkatesh, 2004); [3] (Holladay et al., 2001) ; [4] as in Gertz (2010); [5] (Kupferschmidt et al., 1998); [6] current study; [7] current study supplemented by (Lin et al., 1995; Pathak et al., 2010)

Simvastatin: [1] (Corsini et al., 1999); [2] (Vickers et al., 1990); [3] assumed to be the same as for lovastatin; [4] (Kantola et al., 1998b; Lilja et al., 1998b; Neuvonen et al., 1998; Backman et al., 2000; Kyrklund et al., 2000; Lilja et al., 2000; Mousa et al., 2000; Hsyu et al., 2001; Sugimoto et al., 2001; Dingemanse et al., 2003; Bergman et al., 2004; Jacobson, 2004; Lilja et al., 2004; Ucar et al., 2004; Jerling et al., 2005; Chung et al., 2006; McKenney et al., 2006; Ayalasomayajula et al., 2007; Sunkara et al., 2007; Neuvonen et al., 2008); [5] (Prueksaritanont et al., 1997);

Tacrolimus: [1] (Lauerma et al., 1997; Lucangioli et al., 2003); [2] (Zahir et al., 2001); [3] (Jusko et al., 1995; Venkataramanan et al., 1995); [4] (Venkataramanan et al., 1995; Floren et al., 1997; Hebert et al., 1999; Moller et al., 1999; Wallemacq and Verbeeck, 2001; Hebert et al., 2004; Itagaki et al., 2004; Hebert et al., 2005; Dowell et al., 2007; Xin et al., 2007); [5] (Lampen et al., 1995)

Terfenadine: [1, 2] (Avdeef and Berger, 2001); [3] (Benet et al., 1996); [4] assumed to be 1; [5] (Lalonde et al., 1996); [6] (Brown et al., 2007); [7] current study supplemented by (Avdeef, 2007)

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