A Commentary: Theoretical Predictions of Flow Effects on Intestinal ($F_I$)

and Systemic Availability ($F_{sys}$) in PBPK Intestine Models:

The Traditional Model (TM), Segregated Flow Model (SFM) and $Q_{Gut}$ Model

K. Sandy Pang and Edwin C.Y. Chow

Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario,
Canada, M3S 3M2
Running Title: Intestinal flow models in PBPK modeling

Correspondence: Dr. K. Sandy Pang
Leslie Dan Faculty of Pharmacy, University of Toronto
144 College Street, Toronto
Ontario, Canada M5S 3M2
TEL: 416-978-6164
FAX: 416-978-8511
E-mail: ks.pang@utoronto.ca

Abstract: 242 words
Introduction: 745 words
Discussion: 891 words
References: 58
Figures: 5
Table: 0
**Glossary**

AUC  
area under the curve

C_A  
arterial concentration

\( \overline{C_{PV}} \)  
flow-averaged portal venous concentration

CL_{d1}, CL_{d2}, CL_{d3} and CL_{d4}  
influx and effect intrinsic clearances

CL_{int,1}  
total, intestinal intrinsic clearance

CL_{int,met1} and CL_{int,met2}  
metabolic intrinsic clearances

CL_{int,sec}  
intrinsic clearance for secretion

CL_{perm}  
drug permeability clearance

E  
extraction ratio

F_I, F_H, F_{sys}  
intestinal, hepatic and systemic availability

f_B, f_I and f_H  
unbound fraction in blood, intestine, and liver

f_Q  
fractional flow rate to the enterocyte region

GIT  
gastrointestinal tract

k_{a} and k_{g}  
absorption and luminal degradation rate constant

P_{app}  
apparent permeability

P_{eff}  
effective permeability

PBPK  
physiologically based pharmacokinetic

po and iv  
oral and intravenous administration

Q_{gut}  
effective flow

Q_{villi}  
villous flow

SFM  
segregated flow model

SSFM  
segmental segregated flow model

STM  
segmental traditional model

TM  
traditional model

v_I and v_H  
rate of intestinal and hepatic removal
Abstract

PBPK models for the intestine, comprising of different flow rates perfusing the enterocyte region, were revisited in the appraisal of flow affects on the intestinal availability (F_I) and in turn, the systemic availability (F_sys) and intestinal vs. liver contribution to the first-pass effect during oral drug absorption. The traditional model (TM), the segregated flow model (SFM) and the Q_Gut model, respectively, stipulate that 1.0x, ~0.1x to 0.3x and ≤ 0.484x of the total intestinal flow, respectively, reach the enterocyte region which houses metabolically-active and transporter-enriched enterocytes. The fractional flow rate to the enterocyte region, f_Q, when examined under varying experimental conditions, was found to range from 0.024 to 0.2 for the SFM and 0.065 to 0.43 for the Q_Gut model. Appraisal of these flow intestinal models, when used in combination with whole body-physiological-based pharmacokinetic (PBPK) models, showed the ranking was SFM < Q_Gut model < TM in the description of F_I, and the same ranking existed for the contribution of the intestine to first-pass removal. But the ranking for the predicted contribution of hepatic metabolism to first-pass removal was opposite: SFM > Q_Gut model > TM. The findings suggest that the f_Q value strongly influences the rate of intestinal metabolism, F_I and F_sys, and indirectly affects the rate of liver metabolism due to substrate sparing effect. Thus, the f_Q value in the intestinal flow model poses serious implications on the interpretation of data on the first-pass effect and oral absorption of drugs.
INTRODUCTION

Compartmental models are no longer adequate to address effects of permeability barriers (de Lannoy and Pang, 1986; de Lannoy and Pang, 1987), intestinal and liver transporters and enzymes (Suzuki and Sugiyama, 2000a; 2000b), and sequential metabolism within the intestine and liver (Pang and Gillette, 1979; Sun and Pang, 2010) during oral drug absorption (for reviews, see Pang, 2003; Pang et al., 2008; Fan et al., 2010; Pang and Durk, 2010; Chow and Pang, 2012). These aspects are especially pertinent when intestinal metabolic activity is substantial relative to that in the liver, and when different extents of induction/inhibition of intestinal and hepatic enzymes or transporters result upon treatment of the culprit compound, usually showing a higher induction/inhibition effect with oral administration (Fromm et al., 1996; Paine et al., 1996; Thummel et al., 1996; Eeckhoudt et al., 2002; Mouly et al., 2002; Fang and Zhang, 2010; Liu et al., 2010; Lledó-García et al., 2011; Zhu et al., 2011).

Over the past decade, there have been exciting advances made towards the development of physiologically-relevant PBPK intestinal models to inter-relate intestinal transporters, enzymes and blood flow in the appraisal of their influence on intestinal (FI), liver (FH), and oral systemic (Fsys or FabsFI FH) availability. In this commentary, we revisited several physiologically-based intestinal models that are associated with differential flow patterns: the traditional model, TM, in which the entire intestinal flow perfuses the enterocyte region, the segregated flow model, SFM, in which a low enterocyte flow, Qen, perfuses the enterocyte region (fractional flow, fQ or Qen/QPV is ≤ 0.3) (Cong et al., 2000), and the QGut model, in which the effective flow QGut that perfuses the enterocyte region is at best half the intestinal flow and close in value to the villous flow, Qvilli (Yang et al., 2006; Yang et al., 2007; Gertz et al., 2010). These three intestinal models are viewed as competent to describe the immediate removal of the formed metabolite by excretion or
sequential metabolism within the intestine and/or further processing by liver, for drugs and metabolites exhibiting varying permeability properties (Cong et al., 2000; Yang et al., 2006; 2007; Gertz et al., 2010; Sun and Pang, 2010). The models are more prepared to supply mechanistic insight on the pharmacokinetics of drugs and their metabolites and allow inclusion of transporters into different organ components (apical or basolateral membranes) to discriminate between the permeability properties of the drug and its formed metabolite in permitting or delimiting influx and efflux in drug and metabolite processing (Pang et al., 2008; Darwich et al., 2010; Galetin et al., 2010; Gertz et al., 2010; Rowland Yeo et al., 2010; Chow and Pang, 2012). By virtue of inclusion of transport and eliminatory events, these physiologically-based models are able to describe more accurately the net appearance of the formed metabolite into the systemic circulation, since metabolite levels can be drastically reduced due to sequential metabolism (Pang and Gillette, 1979).

Intestinal PBPK models have been incorporated into whole body PBPK modeling. The semi-PBPK (SPBPK) model proposed by Hall and colleagues (Quinney et al., 2008; Zhang et al., 2009; Quinney et al., 2010) resembles the TM-PBPK and features the intestine and liver tissues separately while minimizing the number of other tissues involved, retaining characteristics of the intestine and liver to describe metabolism, transport and binding. The SPBPK model has been used to describe midazolam inhibition by intestinal and hepatically formed metabolites, N-desmethyldiltiazem from diltiazem in humans (Zhang et al., 2009) and hydroxyitraconazole from itraconazole in rats (Quinney et al., 2008), and in the estimation of the contribution of the intestine (~30 to 40%) in furamidine formation from pafuramidine in a prodrug-drug relationship in rats, then humans (Yan et al., 2012). Chow et al. (2011) used the combined TM-PBPK and SFM-PBPK models to predict the 1.8-fold and 2.6-fold induction of brain and kidney P-gp
protein expression with the vitamin D receptor ligand, 1α,25-dihydroxyvitamin D₃, respectively, and demonstrated a superior fit with the SFM-PBPK model in explaining the P-gp mediated excretion of digoxin. In the perfused rat intestine preparation in which the intestine is the only eliminating tissue, the SFM was found to be superior than the TM in describing morphine glucuronidation (Cong et al., 2000) and digoxin excretion by the P-glycoprotein under induced- and non-induced states (Liu et al., 2006). In this commentary, we appraised how these intestinal flow models differed by examining the effects of enterocytic flow on F₁ and in turn, Fsys and the extents of intestinal and liver first-pass removal with use of simulations.
THEORETICAL: THE FLOW INTESTINAL MODELS

The TM and SFM. Historically, the TM and SFM were first compared by Cong et al. (2000) to offer an explanation of the higher extent of intestinal metabolism of erythromycin (Lown et al., 1995) and midazolam (Paine et al., 1996) in humans, and enalapril hydrolysis (Pang et al., 1985) and morphine glucuronidation in the vascularly perfused rat intestine preparation (Doherty and Pang, 2000) between oral (po) vs. intravenous (iv) dosing of drugs. Both models describe the effects of protein binding, enzymes for parallel and sequential pathways, and passive diffusion and/or transporter-driven permeation in metabolically- and transport-competent enterocytes (Cong et al., 2000). In this model, one or more metabolic pathways, denoted as the metabolic intrinsic clearances, $CL_{int,met1,I}$ and $CL_{int,met2,I}$, for the intestine, may exist for precursor drug, P, and similarly, $CL_{int,met1,H}$ and $CL_{int,met2,H}$ denote parallel metabolic pathways for the liver (Fig. 1). Drug secretion is represented by the $CL_{int,sec,I}$ for the intestinal secretion intrinsic clearance and $CL_{int,sec,H}$ for the liver biliary intrinsic clearance. Figure 1A denotes intestinal removal only, whereas Figure 1B, both intestinal and liver removal; there is no elimination from other organs and tissues, which are lumped as highly and poorly perfused tissues and blood. The influx and efflux clearances are denoted as $CL_{d1}$, $CL_{d2}$, $CL_{d3}$ and $CL_{d4}$ for the intestine (superscript I) and liver (superscript H); the unbound fractions in blood, intestine, and liver are denoted as $f_B$, $f_I$ and $f_H$, respectively (though not shown in Fig. 1 for the sake of simplification). The single, significant difference between these models, TM and SFM, is the flow pattern for perfusion of tissue regions of the small intestine. The SFM emphasizes a small flow ($f_Q \approx 0.1-0.3\times$ total intestinal flow) that perfuses the enterocyte region (en), and the remaining flow $[(1-f_Q)Q_{PV}]$ is shunted to the serosal (s) or non-active region (Fig. 1). This segregated flow pattern contrasts with the TM that
describes the entire flow being able to reach the enterocyte or the total intestinal tissue (int), that is, \( f_Q = 1 \) (Cong et al., 2000).

Explicit solutions for the area under the curves (AUCs) for the TM- and SFM-PBPK models that feature the intestine as the only eliminating organ (Fig. 1A) were provided by Sun and Pang (2009; 2010). These AUCs could be further modified by consideration of protein binding (unbound fractions \( f_B \) and \( f_I \)) for po and iv dosing.

\[
\text{AUC}_{\text{po}} = \frac{F_{\text{abs, po}} \text{Dose}_{\text{po}} \text{CL}_{d1}}{f_B \text{CL}_{d1} \left[ \text{CL}_{\text{int,met,1}, \text{int,met,2}, \text{int,sec,1}} + (1 - F) \text{CL}_{\text{int,sec,1}} \right]}
\]

and

\[
\text{AUC}_{\text{iv}} = \frac{\text{Dose}_{\text{iv}} \left( f_Q \text{Q}_{\text{PV}} \text{CL}_{d2} + \left( f_B \text{CL}_{d1} + f_Q \text{Q}_{\text{PV}} \right) \left[ \text{CL}_{\text{int,met,1}, \text{int,met,2}, \text{int,sec,1}} + (1 - F) \text{CL}_{\text{int,sec,1}} \right] \right)}{f_Q \text{Q}_{\text{PV}} f_B \text{CL}_{d1} \left[ \text{CL}_{\text{int,met,1}, \text{int,met,2}, \text{int,sec,1}} + (1 - F) \text{CL}_{\text{int,sec,1}} \right]}
\]

In the above equations, \( f_B \) is found to appear next to \( \text{CL}_{d1} \). It is also recognized that tissue binding effects are non-operative since the \( f_I \) term cancels out in both the numerator and denominator. The difference in flow between the TM and SFM is denoted by \( f_Q \), the fraction of \( Q_{\text{PV}} \) that perfuses the enterocyte region; for TM, \( f_Q = 1 \), whereas for SFM, \( f_Q = 0.05 \) to 0.3. The flow term is absent for AUC_{po} but present in AUC_{iv}.

Accordingly, the \( F_I \) and \( F_{\text{sys}} \) is,

\[
\frac{\text{AUC}_{\text{po}}}{\text{Dose}_{\text{po}}} = F_{\text{sys}} = F_{\text{abs}} F_I = F_{\text{abs}} \frac{f_Q \text{Q}_{\text{PV}} \text{CL}_{d2}}{f_Q \text{Q}_{\text{PV}} \text{CL}_{d2} + \left( f_Q \text{Q}_{\text{PV}} + f_B \text{CL}_{d1} \right) \left[ \text{CL}_{\text{int,met,1}, \text{int,met,2}, \text{int,sec,1}} + (1 - F) \text{CL}_{\text{int,sec,1}} \right]}
\]

Similarly, the AUCs for the TM- and SFM-PBPK models that feature both the intestine and liver as eliminating organs (Fig. 1B) have been solved (Sun and Pang 2010), and their ratio, after consideration given to protein binding, is,
Again, tissue binding effects are non-operative since $f_I$ and $f_H$, or the tissue unbound fractions for the intestine and liver, cancel out in both the numerator and denominator. In above equation, the $f_B$ term appears next to the influx clearances for the intestine and liver, $I_{d1}CL_{d1}$ and $H_{d1}CL_{d1}$. Increases in $f_B$ would generally lower $F_I$ according to Eqs. 3 and 4.

These solutions for $F_I$ (Eqs. 3 and 4) revealed the blunting effect due to drug reabsorption, or the factor (1-$F_{abs}$), where $F_{abs}$ is $k_d/(k_a+k_g)$ [where $k_g$ is the luminal degradation constant that comprises of gastrointestinal transit and degradation] (Lin et al., 1999; Sun and Pang, 2009; Sun and Pang, 2010). The $F_{abs}$ term has been reported to be highly correlated to the permeability of drug, $P_{app}$ (Zhu et al., 2002; Corti et al., 2006; Kadono et al., 2010). Apical secretion mediated via the secretory intrinsic clearance, $CL_{int,sec,I}$, was nullified when the fraction absorbed ~1, rendering the conclusion that $F_I$ is affected more by $CL_{int,met,I}$ and not so much by $CL_{int,sec,I}$ (Sun and Pang, 2009; Sun and Pang, 2010; Chow and Pang, 2012). As emphasized for the SFM, the partial flow suggests a bypass of enterocytes for drugs entering the intestinal tissue from the systemic circulation, whereas by design, drug given orally necessitates passage of the entire absorbed amount through the enterocyte region. This scenario would lead to a greater extent of intestinal removal for the drug given orally vs. when the drug is given intravenously (Cong et al., 2000), rendering “route-dependent intestinal removal”.

The $Q_{Gut}$ Model. Yang et al. (2007) constructed the “$Q_{Gut}$ model” based on an effective flow, $Q_{Gut}$, to the enterocyte region, by relating this effective $Q_{Gut}$ flow to the intestinal availability, $F_I$, [Equation (4)].
or \( F_G \) in their terminology. The equation for \( F_I \) or \( F_G \) is based analogously to the equation for hepatic availability (\( F_H \)), according to the well-stirred liver model (Pang and Rowland, 1977), where \( f_i \) is the unbound fraction of drug in intestinal tissue and \( CL_{int,I} \), the total, intestinal intrinsic clearance that encompasses both secretion and metabolism.

\[
F_I \text{ or } F_G = \frac{Q_{Gut}}{Q_{Gut} + f_i CL_{int,I}} \tag{5}
\]

The effective flow, \( Q_{Gut} \), is a hybrid term derived from the actual villous blood flow (\( Q_{villi} \)) [18 l/h or 300 ml/min (Gertz et al., 2010), representing ~48.4% of the total intestinal flow (assumed to equal the portal venous flow or \( Q_{PV} \), ~620 ml/min) (Valentin, 2002; Yang et al., 2006; Yang et al., 2007)] and drug permeability clearance (\( CL_{perm} \)), a parameter that is normally estimated as the area x effective permeability (\( P_{eff} \)) assessed from perfused (human) jejunal studies, from Caco-2 cell apparent permeability (\( P_{app} \)), or based on physicochemical data such as hydrogen bond donors and polar surface area. The \( Q_{Gut} \) value of midazolam, a drug with high apparent permeability, was estimated to be 16.6 l/h, a value that is 92% of the value of \( Q_{villi} \) (Gertz et al., 2010).

\[
Q_{Gut} = \frac{Q_{villi} CL_{perm}}{Q_{villi} + CL_{perm}} \tag{6}
\]

For a drug that is highly permeable, \( CL_{perm} \gg Q_{villi} \), it may be deduced that \( Q_{Gut} \equiv Q_{villi} \).

Upon substitution of Eq. 6 into Eq. 5, one obtains

\[
F_I \text{ or } F_G = \frac{Q_{villi} CL_{perm}}{Q_{villi} + CL_{perm} + f_i CL_{int,I}} = \frac{Q_{villi} CL_{perm}}{Q_{villi} + CL_{perm} + f_i CL_{int,I}} \tag{7}
\]
As originally conceived by Yang et al. (2007), the CL\textsubscript{perm} term stands collectively for $\text{CL}_{d1}^{I}$ and $\text{CL}_{d2}^{I}$ but should be replaced appropriately by either $\text{CL}_{d1}^{I}$ or $\text{CL}_{d2}^{I}$. Upon comparison of Eq. 7 with Eq. 3, the CL\textsubscript{perm} terms for the Q\textsubscript{Gut} model could now be assigned. By analogy to Eq. 3, it is further recognized that $f_{C_{\text{Int,1,I}}}$ is equivalent to the composite term, $f_{C_{\text{I}}}[\text{CL}_{\text{Int,met1,I}} + \text{CL}_{\text{Int,met2,I}} + (1-F_{\text{abs}})\text{CL}_{\text{Int,sec,I}})]$. The term, $f_{C\text{Int,1,I}}$ that represents the summed unbound metabolic and secretory intrinsic clearances, fails to consider the intestinal secretion followed by reabsorption of the secreted material in the lumen. Upon consideration of all these missed events,

$$F_{I} \text{ or } F_{\text{Gut}} = \frac{Q_{\text{villi}}\text{CL}_{\text{perm}}}{Q_{\text{villi}}\text{CL}_{\text{perm}} + (Q_{\text{villi}} + \text{CL}_{\text{perm}})f_{C\text{Int,I}}}$$

or

$$\frac{Q_{\text{villi}}\text{CL}_{\text{d2}}^{I}}{Q_{\text{villi}}\text{CL}_{\text{d2}}^{I} + (Q_{\text{villi}} + f_{b}\text{CL}_{\text{d1}}^{I})[\text{CL}_{\text{Int,met1,I}} + \text{CL}_{\text{Int,met2,I}} + (1-F_{\text{abs}})\text{CL}_{\text{Int,sec,I}}]} (8)$$

one obtains Eq. 8 for the Q\textsubscript{Gut} model that appears in an equivalent format as that for the TM and SFM (Eq. 3). One sees similarities between the SFM/TM and the Q\textsubscript{Gut} model. The $f_{Q_{PV}}$ term for the SFM is equivalent to the $Q_{\text{villi}}$ term of the Q\textsubscript{Gut} model (300 ml/min), which describes a partial flow ($f_{Q} = 0.484$) perfusing the enterocyte region.
RESULTS

Comparison of \( f_Q \). A proper comparison of these models has not been made in any rigorous fashion, especially in regard to \( f_Q \) on \( F_I \). The starting point of the comparison is \( f_Q \), being of a low value (~0.1 to 0.3) for the SFM, ~0.5 (\( Q_{\text{villi}}/Q_{PV} = 0.484 \)) for the \( Q_{\text{Gut}} \) model, and high (1.0) for the TM. We felt that the \( f_Q \) term could serve as an important variable for selection of the most appropriate model to best describe the intestine. Upon perusal of the literature, estimates of \( Q_{\text{Gut}} \) according to Eq. 2 for various drugs varied from 2.4, 5.7, 8.6 to 16.6 l/h, corresponding to 6.5 to 43% of the total intestinal flow, with good predictions for midazolam but poor estimation of \( F_I \) (or \( F_G \)) for saquinavir \textit{in vivo} (Gertz et al., 2010). Some of these \( f_Q \) values for the \( Q_{\text{Gut}} \) model were higher than the \( f_Q \) values of 0.07, 0.024, and 0.2 estimated from fits of the SFM to the data on benzoic acid (Cong et al., 2001), morphine (Cong et al., 2000) and digoxin (Liu et al., 2006), respectively, from vascularly perfused rat small intestine preparations. For digoxin, which is mainly excreted unchanged in the mouse \textit{in vivo}, a value of 0.16 was found for \( f_Q \) (Chow et al., 2011). The \( f_Q \) terms, whether for the SFM or for \( Q_{\text{Gut}} \) model, were both less than unity (Gertz et al., 2010; Chow and Pang, 2012), with \( f_Q \) values being higher than 0.3 for the \( Q_{\text{Gut}} \) model. Values of \( f_Q \) for the SFM were lower and corresponded better with published evidence that suggests segregated flows for the small intestine, and that a small fraction of flow (5-30%) perfuses the active, mucosal region (Granger et al., 1980).

Simulation of \( F_I \). Eq. 1 for the TM and SFM which considers the intestine as the only eliminating organ, lacks any of the flow terms and suggests that \( \text{AUC}_{po} \) is identical among the TM, \( Q_{\text{Gut}} \) model, and SFM, whereas the \( \text{AUC}_{iv} \) intended for the TM/SFM (Eq. 2) consisted of the flow term, \( f_Q Q_{PV} \) for SFM and TM, and \( Q_{\text{villi}} \) for the \( Q_{\text{Gut}} \) model, by analogy. Thus, different \( \text{AUC}_{iv} \) values for the \( Q_{\text{Gut}} \) model and the SFM resulted when the flow term was replaced by the
appropriate flow rate, $f_0Q_{PV}$ or $Q_{villi}$. Since the rate of intestinal metabolism is dependent on the flow rate for delivery of substrate, it may be concluded that, when a smaller flow reaches the enterocyte region, a smaller intestinal removal rate results with systemic delivery, supplying a ranking of the intestinal removal rate as $SFM < Q_{Gut \ model} < TM$ after intravenous dosing. The lower flow rate stipulated by the SFM in bringing the substrate into enterocyte region yielded a higher $AUC_{iv}$ (ranking for $AUC_{iv}$: $SFM > Q_{Gut \ model} > TM$) and consequently a lower $F_I$ for the SFM compared to the $Q_{Gut \ model}$ and TM for given $CL_{int,met,I}$ values. This view was supported by the simulations (Fig. 2A). The ranking of $F_I$ was $SFM < Q_{Gut \ model} < TM$.

It was further observed that the expressions for the $F_I$ terms were identical for the scenario in which the intestine is the only eliminating organ (Eq. 3) and when the intestine and liver are both eliminating organs (Eq. 4). These patterns for $F_I$ (Fig. 2A) were translated into $F_{sys}$ for any given $F_{H} (= 0.1, 0.5$ or $0.9$, Fig. 2B). Again, the simulated patterns are consistent with the view that a decreased intestinal extraction ratio is accompanied an increase in mesenteric flow (Chen and Pang, 1997; Chalasani et al., 2001; Yang et al., 2007; Chow and Pang, 2012); the lower intestinal removal rate due to lower intestinal flows would result in a higher hepatic processing, as observed experimentally by Chen and Pang (1997).

**Changing $CL_{d1}$ or $CL_{d2}$ on $F_I$.** When we further examined effects of the basolateral influx ($CL_{d1}$) or efflux ($CL_{d2}$) transport clearances for drugs that exhibit varying degrees of absorption (described by $F_{abs} = 0.1, 0.5$ and $1.0$), all models showed that $F_I$ was attenuated when $CL_{d1}$ was increased or when $CL_{d2}$ was decreased (Fig. 3). Increasing the influx basolateral clearance ($CL_{d1}$) from low to high (left column from 1x to 5x and 20x blood flow, Fig. 3) would lead to lower $F_I$ values, whereas upon increasing values of $CL_{d2}$ from low to higher values (from 1x to 5x flow, middle column, then 20x flow, right column, Fig. 3), higher $F_I$ were attained due to ability of the
influxed drug to escape intestinal enzymes intracellularly. The $f_Q$ effects from the flow models were apparent again with the simulations, and the ranking for $F_I$ values was $\text{SFM} < Q_{\text{Gut model}} < \text{TM}$ (Fig. 3).

**Contributions from Intestine and Liver to First-Pass Effect.** To assess the contributions from the intestine vs. the liver in first-pass removal among these flow-intestinal models, we further simulated the rates predicted from the mass equations shown below (Eqs. 9 and 10) that described the rates of intestinal ($v_I$) and hepatic ($v_H$) removal. For estimation of the rates, there exists the need to define the flow-averaged portal venous concentration, $\bar{C}_{PV}$, to account for the partial flow entering the enterocyte region and for accurate prediction of the intestinal removal rate, $v_I$.

\[
\bar{C}_{PV} = \frac{f_Q Q_{PV} F_I C_A + (1 - f_Q) Q_{PV} C_A}{Q_{PV}} = C_A [f_Q F_I + (1 - f_Q)]
\]  

\[
v_I = Q_{PV} C_A - Q_{PV} \bar{C}_{PV} = Q_{PV} C_A \left(1 - [f_Q F_I + (1 - f_Q)]\right) = f_Q Q_{PV} C_A (1 - F_I)
\]  

and

\[
v_H = Q_{PV} \bar{C}_{PV} E_H + Q_{HA} C_A E_H = C_A E_H \left(Q_{PV} [f_Q F_I + (1 - f_Q)] + Q_{HA}\right)
\]

Here, $E$ is the extraction ratio for the intestine or liver that equals $(1 - F)$, and $C_A$ is the arterial concentration. The fractional contributions by the intestine and liver were then calculated.

The fractional contribution by intestine to the first-pass effect is

\[
\frac{v_I}{v_I + v_H} = \frac{f_Q Q_{PV} (1 - F_I)}{f_Q Q_{PV} (1 - F_I) + E_H \left(Q_{PV} [f_Q F_I + (1 - f_Q)] + Q_{HA}\right)}
\]
and the fractional contribution by liver to the first-pass effect is

$$\frac{v_h}{v_l + v_h} = \frac{E_H \left( Q_{PV} \left[ f_Q F_1 + (1 - f_Q) \right] + Q_{HA} \right)}{f_Q Q_{PV} (1 - F_1) + E_H \left( Q_{PV} \left[ f_Q F_1 + (1 - f_Q) \right] + Q_{HA} \right)}$$

(Q_villi replaces $f_Q Q_{PV}$ in Eqs. 9, 10, 12, and 13 for the QGut model, with $f_Q = 0.484$. Again, substitution of $f_Q (=1, 0.484$ and $0.1$, respectively, for TM, QGut model and SFM) embedded in F_I or E_I (Eq. 3) yielded the corresponding fractional removal estimates. Accordingly, the lower intestinal removal rate ($v_I$) predicted by the SFM due to the reduced flow rate resulted in a correspondingly higher contribution by the liver due to the substrate sparing effect of the intestine (Fig. 4). Whereas for TM, the greater intestinal contribution in removing the drug led to a lesser removal contribution by the liver due to a substrate depleting effect of the intestine (Fig. 4). Predictions from the QGut model on the intestinal and liver contributions to first-pass removal fell in between those for the SFM and TM, and the patterns were similar when $F_{abs} = 0.1$ or $0.9$ (Fig. 4).

Again, the predictions revealed that the $f_Q$ values in different intestinal models affected the contributions of the intestine and liver in the first-pass effect. For any given $CL_{int, met, I}$, this difference translates to ranking for the intestinal contribution to the first-pass effect as $TM > QGut > SFM$, and for the liver, the ranking is $TM < QGut < SFM$. These opposite trends in intestinal vs. hepatic contributions to first-pass have been discussed by Xu et al. (1989) and Chen and Pang (1997), attributing their observations to the anterior positioning of the intestine without recognizing the segregated flow effects. It must be commented that the value of $F_{abs}$ was not very apparent to affect the contributions of the intestine or liver in first-pass removal in these simulations; the $F_{abs}$ term affected only the reabsorption of the intestinally secreted drug (Eqs. 3

16
and 4), which was, for all intent and purpose, a minor pathway (CL_{int,sec,1} was set as 200 ml/min) relative to values of CL_{int,met1,1} examined.

**Effects of Binding**

The mathematical manipulation revealed that tissue binding effects are non-operative since the unbound fraction terms in intestine (f_I) or liver (f_H) canceled out in both the numerator and denominator. As seen from Eqs. 3 and 4, only the f_B term persisted in the equations and was associated with the influx clearances, CL_{di}, for the intestine and liver (superscripted I and H, respectively). Upon changing f_B at three sets of CL_{int,met1,1} values for the various models (Fig. 5), it could be seen that increased values of f_B generally lowered F_I (Fig. 5). Exceedingly similar patterns were observed for F_{abs} = 0.1 and 0.9.
DISCUSSION

This examination reveals that $f_Q$ is the key issue in the prediction of $F_I$ and contribution of both the intestine and liver to first-pass removal. The $Q_{\text{Gut}}$ model is similar to the SFM in many respects, except that a higher limit exists for $f_Q$. The simulations, based on the various $f_Q$ values, show that the predicted intestinal availability of the $Q_{\text{Gut}}$ model falls between those of the TM and SFM models under varying conditions of efflux and influx clearances (Fig. 3). Decreased intestinal availabilities are expected with lower $f_Q$ values (Fig. 2), and this contributes to a greater proportion of first-pass extraction by the liver, the posterior organ (Fig. 4).

A major issue for the prediction of $F_I$ is the choice of the correct $f_Q$ value for intestinal models, especially for the $Q_{\text{Gut}}$ model. The problem, that the intended $Q_{\text{Gut}}$ term is a hybrid function of $Q_{\text{villi}}$ and $CL_{\text{perm}}$ (as shown in Eq. 7), could now be circumvented with use of Eq. 8. Although literature reports for the $Q_{\text{Gut}}$ model suggest that $f_Q$ varies between 0.07 and 0.43, we suggest use of the unambiguous $Q_{\text{villi}}$ term or $f_QQ_{PV}$ ($f_Q = 0.484$) for the $Q_{\text{Gut}}$ model, with inclusion of the $CL_{et}$ and $CL_{st}$ terms in lieu of $CL_{\text{perm}}$ in a format similar to those for the SFM and TM (Eq. 8) to define the fractional flow and the transport intrinsic clearances. This revelation implies that the effective flow rate to the enterocyte region ($f_Q = 0.484$) for the $Q_{\text{Gut}}$ model is higher than that for the SFM. Another revelation is that $f_{\text{CL_{int,l}}}$ in $Q_{\text{Gut}}$ model falls short of the more comprehensive term, $[CL_{\text{int,met,l}} + (1-F_{\text{abs}})CL_{\text{int,sec,l}}]$, in the prediction of $F_G$ (or $F_I$ in our terms). This may be another reason why poor prediction prevails for some drugs that are P-gp substrates (Gertz et al., 2010). Indeed, improved estimation of $P_{\text{eff}}$ with use of a P-gp inhibitor seemed to improve the $F_I$ prediction of saquinavir (Gertz et al., 2011). The need for $f_I$ in the equation for the $Q_{\text{Gut}}$ model is questionable since the term cancels out even when the binding effects of intestinal tissue on efflux, metabolism or excretion are taken into consideration.
Other theoretical modeling that considers heterogeneity of transporters and enzymes along the length of the small intestine, as in the segmental traditional (STM) and segmental segregated flow (SSFM) models (counterparts of TM and SFM), has revealed that metabolic heterogeneity strongly impacts $F_I$ (Tam et al., 2003). Wu (2011) has recently commented, in a theoretical examination, that heterogeneity matters in predicting $F_{sys}$ after comparison of simulations from the TM-PBPK and SSFM-PBPK models on the systemic availability of the parent aglycone during the process of enterohepatic circulation of biliarily excreted glucuronides. The consideration of heterogeneity of transporters and enzymes on intestinal modeling in vivo surfaced much later, possibly due to the difficulty in obtaining population and length-averaged estimates on physiological dimensions of the lumen, surface area, flow, and enzymes and transporters in humans and animals (Badhan et al., 2009; Bruyère et al., 2010). Other compartmental models, when coupled with a refined description on the linear transfer kinetics of state properties of the drug (unreleased or solid form, undissolved or aggregate form, and dissolved or solution form), physicochemical properties (pKa, solubility, particle size, particle density, and permeability), physiological properties (gastric emptying, intestinal transit rate, intestinal metabolism, and luminal transport), and dosage factors (dosage form and dose) in the GIT, show much improved predictions of drug kinetics (Agoram et al., 2001; Hendriksen et al., 2003), especially with inclusion of heterogeneity factors in the modeling (Bolger et al., 2009; Abuasal et al., 2012). The ability of many of the present models to fully describe metabolite kinetics, however, remains uncertain. We have noted that heterogeneity models such as the SSFM and STM (Tam et al., 2003), whether necessary or not, are more pertinent in cases of enzyme heterogeneity among the segments. In absence of metabolism by the intestine, we found that the STM and SSFM perform equally well as the TM and SFM, as found for studies on the
absorption of benzoic acid (Cong et al., 2001) and digoxin absorption and efflux by P-gp (Liu et al., 2006) in the vascu larly perfused intestine preparation. The presence of metabolite data is an absolutely necessity for the discrimination between the SFM and TM.

It can be concluded that the designated flow rate to the enterocyte region of the intestine, defined according to the different intestinal flow models, strongly impacts $F_I$ and $F_{sys}$, and the proportions of intestinal and liver in first-pass removal. With the solved equations for the AUCs, it is apparent that predictions on the interplay between intestine and hepatic transporters and enzymes are readily attainable (Pang et al., 2009; Sun and Pang, 2010). Key issues for proper intestinal modeling are the accurate definition of $f_Q$ and improved estimates of the transport clearances. The proper definition of $f_Q$ is of paramount importance and this awaits use of sophisticated tools to properly estimate the enteroctye vs. the total intestinal flow rate. Notwithstanding the deficiencies persisting in all of the mentioned models, it is rewarding to see how the theoretical refinement in intestinal modeling has advanced our activity and knowledge towards how transporter- and enzyme heterogeneity as well as segregated flow patterns affect drug metabolism and excretion by the small intestine and liver in first-pass removal during oral drug absorption.
AUTHORSHIP CONTRIBUTIONS

Participated in research design: Pang, K.S. and Chow, E.C.
Conducted experiments: Chow, E.C. Pang, K.S.
Performed data analysis: Chow, E.C. Pang, K.S.
Wrote or contributed to the writing of the manuscript: Pang, K.S. and Chow, E.C.
References


Chow EC and Pang KS (2013) Why we need PBPK modeling to examine intestine and liver oral drug absorption. *Curr Drug Metab* accepted

Chow EC, Durk MR, Cummins CL, and Pang KS (2011) 1α,25-Dihydroxyvitamin D₃ up-regulates P-glycoprotein via the vitamin D receptor and not farnesoid X receptor in both fxr(-/-) and fxr(+/+) mice and increased renal and brain efflux of digoxin in mice *in vivo*. *J Pharmacol Exp Ther* **337**:846-859.


itraconazole, controls the time course of hepatic CYP3A inhibition and the bioavailability of itraconazole in rats. *Drug Metab Dispos* **36**:1097-1101.


FOOTNOTES

This work was supported by the Canadian Institute for Health Research, CIHR (K.S.P.) and the Alexander Graham Bell NSERC fellowship, National Science and Engineering Research Council of Canada, NSERC (E.C.C.)
Legends

Figure 1. Whole body PBPK, with the liver and other lumped compartments (highly perfused, poorly perfused) being connected to the intestine model (TM and SFM), depicting the intestine (A) and intestine and liver (B) as the eliminating tissue(s)/organs (s). The intestine subcompartments are: for TM, subscripts int and intB denote intestinal tissue and intestinal blood, respectively; for SFM, subscripts en and enB denote enterocyte and enterocyte blood, respectively; s and sB denote serosal tissue and serosal blood, respectively. For the liver, subscripts L and LB represent liver tissue and liver blood, respectively; subscript R denotes the reservoir or blood compartment. For TM, the intestine represents a well-mixed enterocyte region and receives the entire intestinal blood flow, $Q_I$ or $Q_{PV}$. For SFM, the blood flow is segregated to perfuse the enterocyte and serosal regions; the flow to the enterocyte region is denoted as $f_Q Q_{PV}$, and the serosal region, $(1-f_Q)Q_{PV}$. At the basolateral membrane, the drug influx and efflux clearances into or out of the intestine or enterocyte are characterized by the transport clearance parameters, $CL_{d1}^{I}$ and $CL_{d2}^{I}$ respectively. For SFM, additional influx and efflux clearance into or out of the serosal tissue compartment are characterized by the transport clearance parameters, $CL_{d1}^{L}$ and $CL_{d2}^{L}$. The liver receives blood from hepatic blood artery ($Q_{HA}$) arising from the blood compartment and venous flow, $Q_{PV}$, from the intestine; the summed blood flow exits the liver as $Q_H$. The influx and efflux clearances of the drug into or out of the liver are $CL_{d1}^{H}$ and $CL_{d2}^{H}$, respectively. Intrinsic metabolic clearance of parent drug (P) to form the primary metabolites in the intestine are denoted as $CL_{int,met1,I}$ and $CL_{int,met2,I}$ and those in liver are $CL_{int,met1,H}$ and $CL_{int,met2,H}$, the intestine and liver secrete P out via secretory intrinsic clearances, $CL_{int,sec,I}$ and $CL_{int,sec,H}$, respectively. The bile flow rate is denoted as $Q_{bile}$. Drug administrated orally (solution form) is administered into the lumen, and may be either absorbed into intestine with the rate constant, $k_a$, or degraded in lumen by the rate constant, $k_g$; drug given intravenously directly enters the blood compartment.

Figure 2  Effects of changing $CL_{int,met1,I}$ (e.g. induction or inhibition of enzymes) on $F_I$ according to the TM, $Q_{Gut}$ model and SFM with Eq. 4 (A) and $F_{sys}$ with the $F_I$ values shown in (A) under varying conditions of $F_H = 0.1, 0.5$ and $0.9$ (B). In this simulation, $CL_{int,sec,I}$ was set as 200 ml/min; $Q_{PV} = 620$ ml/min; $f_Q = 1.0$ (TM), or 0.484 ($Q_{Gut}$ model) and 0.1 (SFM); $F_{abs} = 0.1, 0.5$, or $0.9$; $CL_{int,met2,I} = 0$; $CL_{d1}^{I}$ = $CL_{d2}^{I}$ = 20x $Q_{PV}$, denoting a highly permeable drug.

Figure 3. Effects of varying basolateral transport clearances, $CL_{d1}^{I}$ and $CL_{d2}^{I}$, on $F_I$ according to Eq. 3, for drugs which are highly absorbed ($F_{abs} = 0.9$). For these simulations, $CL_{int,sec,I}$ was set as 200 ml/min; $Q_{PV}$ was set as 620 ml/min, and $f_Q = 1.0$ (for TM), or 0.484 (for $Q_{Gut}$ model) and 0.1 (for SFM). Values of $CL_{d1}^{I}$ and $CL_{d2}^{I}$ were altered from 1x, 5x, and 20x $Q_{PV}$. The value of $CL_{int,met2,I}$, the intrinsic clearance for the alternate metabolic pathway, was set as 0.
Figure 4. **Simulation for the fractional contributions of the intestine,** $\frac{v_i}{v_i+v_{hi}}$ (Eq. 12) (A) and liver, $\frac{v_{hi}}{v_i+v_{hi}}$ (Eq. 13), (B). The designated drug examples vary from being poorly to highly absorbed ($F_{abs} = 0.1$ and 0.9), whose hepatic availability ($F_H$) vary from 0.1 to 0.9, $Q_{HA} = 300$ ml/min and $CL_{di}^I = CL_{d2}^I = 20x Q_{PV}$, with an excretion component $CL_{int,sec,I} = 200$ ml/min and a non-existent, alternate metabolic pathway ($CL_{int,met2,I} = 0$); the assigned flow rates were: $Q_{PV} = 620$ ml/min; $f_Q = 1.0$ (TM) or 0.1 (or $Q_{en}/Q_{PV}$ for SFM). Simulations for the $Q_{Gut}$ model ($f_Q = 0.484$) were intermediate of those for the SFM and TM. See text for details.

Figure 5. **Effects of changing $f_B$ on $F_I$ according to the TM, $Q_{Gut}$ model and SFM with Eq. 3 at $F_{abs} = 0.1$ (A) or 0.9 (B) at $CL_{int,met1,I} = 100$, 1000, or 2000 ml/min.** In this simulation, $CL_{int,sec,I}$ was set as 200 ml/min; $Q_{PV} = 620$ ml/min; $f_Q = 1.0$ (TM), 0.484 ($Q_{Gut}$ model) or 0.1 (SFM); $CL_{int,met2,I} = 0; CL_{d1}^I = CL_{d2}^I = 20x Q_{PV}$, denoting a highly permeable drug. See text for details.
Figure 1

(B)

TM-PBPK

SFM-PBPK

Reservoir/Body

Highly perfused tissue

Poorly perfused tissue

Liver blood

Intestine blood

Bile

Reservoir/Body

Highly perfused tissue

Poorly perfused tissue

Liver blood

Intestine blood

Bile

Reservoir/Body

Highly perfused tissue

Poorly perfused tissue

Liver blood

Intestine blood

Bile

This article has not been copyedited and formatted. The final version may differ from this version.
Figure 2

(A) CL_{int,met1,I} (ml/min) vs. F_{sys}

(B) F_{sys} for different F_{H} values

<table>
<thead>
<tr>
<th>F_{abs}</th>
<th>f_Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>0.1</td>
<td>0.484</td>
</tr>
<tr>
<td>0.5</td>
<td>0.484</td>
</tr>
<tr>
<td>0.9</td>
<td>0.484</td>
</tr>
<tr>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>0.9</td>
<td>0.1</td>
</tr>
</tbody>
</table>

FH = 0.1

FH = 0.5

FH = 0.9
Figure 4

(A) % Contribution of Intestine to First-Pass Effect

- $F_{abs} = 0.1$

- $F_{abs} = 0.9$

(B) % Contribution of Liver to First-Pass Effect

- $F_{abs} = 0.1$

- $F_{abs} = 0.9$
Figure 5

(A) F_{abs} = 0.1

(B) F_{abs} = 0.9

Unbound Blood Fraction (f_B)