Disparity in Intestine Disposition between Formed and Preformed Metabolites and Implications: A Theoretical Study

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ABSTRACT:

Metabolite in safety testing has been proposed for toxicity assessments. The question of how exposure of the synthetic metabolite compares with that of the formed metabolite was appraised kinetically by using physiologically based pharmacokinetic models, the (traditional) physiological model (TM), and segregated flow (SFM) models. The SFM differs from the TM and describes a partial (~10%) total intestinal flow that perfuses the absorptive, metabolic, and secretory enterocyte layer to account for the higher extent of metabolism observed with oral versus systemic dosing of drugs. Theoretical solutions for the areas under the curve (AUC) of the formed metabolite after oral and intravenous administration of the precursor (AUC(mi,P)) and preformed, synthetic metabolite (AUC(mi)) showed identical AUC(mi,P), AUC(pmi), and AUC(pmi) for both the TM and SFM, whereas a larger AUC(mi,P) existed for the SFM. The AUC(pmi) was influenced by metabolite parameters only: binding, absorptive ($k_a$), and luminal degradation ($k_d$) constants, intrinsic clearances for metabolism (CLint,met,$I_{mi}$), apical efflux (CLint,$sec,I_{mi}$), and basolateral transfer (CLint,$ap,I_{mi}$ and CLint,$ap,I_{pmi}$) for the metabolite. By contrast, the AUC(mi,P) was influenced additionally by precursor parameters: rate constants $k_a$ and $k_f$, and CLint,$met,I_{mi}$ and CLint,$sec,I_{mi}$ but not the basolateral transfer clearances. The drug parameters: CLint,$met,I_{mi}$ and $k_a$ increased whereas CLint,$sec,I_{mi}$ decreased AUC(mi,P), and the effect of secretion was counterbalanced by reabsorption with high $k_a$ values. The simulated time courses for the metabolite and the AUC(mi) and AUC(mi,P) resulting from intravenous and oral routes of administration of preformed metabolite and precursor differed, inferring that the kinetics of the preformed and formed metabolites are not identical.

Metabolite-in-safety testing and the attainment of safe and efficacious drug use are key concerns in the drug development/surveillance paradigm. There has been a resurgence of interest in the testing of metabolites that are mediators of drug activity and toxicity due to the improvement in analytical methods for their detection, isolation, and characterization (Bailie et al., 2002). Metabolite testing is recommended, especially when metabolites are unique and identified only in humans, or when the metabolite exists at disproportionately higher levels in humans (>10%) than the animal species that was used for standard, nonclinical toxicity testing (Naito et al., 2007) (February, 2008 FDA Guidance for Industry, Safety Testing of Drug Metabolites. Pharmacology and Toxicology; http://www.fda.gov/cder/guidance/). It has been proposed that a metabolite is considered to be a major metabolite when the metabolite represents >10% (Davis-Bruno and Atrakchi, 2006) or 25% exposure (or area under the curve) of the precursor (Bailie et al., 2002). Others have suggested the estimate to be based on unbound concentration in the circulation or amounts in the excreta (Smith and Obach, 2005). When the “% exposure” is based on total radioactivity, the parameter is time-dependent and may not be a reliable estimate when the metabolite in question is readily metabolized to other metabolites. Other studies suggest that the definition of the percentage exposure or amount may not be the key issue.

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ABBREVIATIONS: AUC, area under the concentration-time curve; (mi,P), metabolite formed from precursor; (pmi), preformed metabolite; PBPK, physiologically based pharmacokinetic model; TM, traditional model; SFM, segregated flow model; Qs, blood flow for the whole intestine; P, precursor; CLint,$ap,I$ and CLint,$ap,P$, basolateral, transfer intrinsic clearances between blood and tissue for the TM, or between blood and the enterocytes layer for the SFM; CLint,$met,I$, metabolic intrinsic clearance of precursor; CLint,$sec,I$, secretory intrinsic clearance of precursor at the apical membrane; $k_a$, rate constant of absorption for precursor; $k_g$, rate constant of luminal transit and degradation; $F_{abs}$, fraction of drug absorbed from the intestinal lumen into intestine tissue; $Q_{en}$, blood flow for the enterocyte layer; $Q_s$, blood flow for the serosal layer; CLint,$ap,I$ and CLint,$ap,P$, transfer intrinsic clearances between serosal blood and serosal tissue; (mi), qualifier for metabolite (formed and preformed); F(pmi), the bioavailability of the preformed metabolite.
and enterocyte region for TM, but there is only partial flow (Q\text{int,sec}) in regions. For the oral route of administration, the entire oral dose passes through the enterocyte region for both TM and SFM. The total flow (Q) is the sum of intestinal blood blood perfusing the enterocyte region, serosal blood, and serosal region, respectively. The absorptive, metabolic, and efflux activities within the villus tips of the mucosal layer are represented by the rate constant, k_{int,met}, and metabolic and secretory intrinsic clearances, CL\text{int,met,I} and CL\text{int,sec,I}, respectively. Gastrointestinal transit or degradation rate constant in the lumen is denoted by k_a. A similar set of parameters exist for the metabolite, qualified by {mi}.

In this communication, we tested the hypotheses that precursor and metabolite disposition has not been extended to the intestine, although there has been development on modeling of transporters and/or enzymes for drug absorption (Cong et al., 2000; Doherty and Pang, 2000). This concept was also adopted by SimCYP in the modeling of intestinal clearances with a villous flow (Pang et al., 2008). Subscripts enb, en, sb, and s denote enterocyte blood, enterocyte region, serosal blood, and serosal region, respectively. The absorptive, metabolic, and efflux activities within the villus tips of the mucosal layer are represented by the rate constant, k_{int,met}, and metabolic and secretory intrinsic clearances, CL\text{int,met,I} and CL\text{int,sec,I}, respectively. Gastrointestinal transit or degradation rate constant in the lumen is denoted by k_a. A similar set of parameters exist for the metabolite, qualified by {mi}.

1993) and kidney (Geng et al., 1999) after administrations of both the drug and preformed metabolite. Differences were found between the fates of the generated and preformed metabolites upon solving for the AUC of the formed versus the preformed metabolite when either the liver or kidney was involved as the metabolite formation and elimination organ (Pang et al., 2008). Transporters and enzymes of the precursor drug and metabolite were found to be important variables that determine the AUC of the formed metabolite, AUC\text{(mi,P)} (Pang et al., 2008). But the drug parameters were absent regarding the AUC of the preformed metabolite, AUC\text{(mi,P)} in both the liver and kidney.

The theoretical examination of metabolite disposition has not been extended to the intestine, although there has been development on modeling of transporters and/or enzymes for drug absorption (Cong et al., 2000; Doherty and Pang, 2000). In this tissue, apical absorptive transporters bring molecules into the enterocyte, whereas apical efflux transporters are capable of secreting the absorbed drug back to the lumen, and the drug in tissue is subject to biotransformation before it enters the circulation at the basolateral membrane, either passively or via efflux transporters (Pang, 2003). The net outcome of the events constitutes intestinal drug absorption.

In this communication, we tested the hypotheses that precursor and metabolite parameters influence the kinetics of the formed metabolite and that differences exist between preformed and formed metabolite kinetics in the intestine. Two physiologically based pharmacokinetic (PBPK) models are used. The traditional physiological model (TM) (Fig. 1A) has identified parameters of gastrointestinal transit, intestinal flow, and intestinal transporters and enzymes as variables that regulate the ultimate absorption of orally administered substrates. The segregated flow model (SFM) suggests that a minor proportion of the intestinal flow perfuses the enterocyte region that mediates absorption, metabolism, and efflux, and a larger flow perfuses a nonmetabolizing (inert) serosal tissue (Fig. 1B). Both models are presently extended to describe metabolite kinetics to identify parameters that would affect the AUC of the formed and preformed metabolites for oral and intravenous administration. The SFM is necessary to explain the greater extent of intestinal metabolism that is associated with oral dosing compared with systemic dosing. This route-dependent, intestinal metabolism was observed for morphine (Cong et al., 2000; Doherty and Pang, 2000) and other substrates in both animals and man (Pang, 2003). Not unlike the SFM, SimCYP, the simulation program, uses a strategy based on a reduced villous flow and not total intestinal flow for the prediction of intestinal clearances (Yang et al., 2007).

Materials and Methods

The TM and SFM models are physiologically based models that have been introduced to relate flow, volume, transporters that mediate absorption, distribution and secretion, and enzymes for metabolism (Fig. 1) (Cong et al., 2000). The need for comparing metabolite kinetics for the TM and SFM is based on the superiority of the SFM in explaining route-dependent intestinal metabolism, i.e., that less drug is metabolized with intravenous versus oral drug administration (Cong et al., 2000; Doherty and Pang, 2000). This concept was also adopted by SimCYP in the modeling of intestinal clearances with a villous flow (Q\text{villi}), which is lower compared with the total intestinal flow (Yang et al., 2007).

According to the TM, the total intestinal blood (Q) perfuses the entire intestinal tissue; the transfer intrinsic clearances for the transport of precursor (P) between blood and tissue at the basolateral membrane are described by CL_{int,met} and CL_{int,sec}, representing the summation of passive and transporter-mediated pathways. In tissue, metabolism (intrinsic clearance; CL_{int,met,I}) to form the primary metabolite, Mi, and apical secretion into the lumen (secretory
intrinsic clearance; CL\textsubscript{int,sec,I} are available elimination processes (Fig. 1A). In the lumen, the precursor drug may be absorbed (rate constant, $k_p$) or leave the lumen irreversibly via gastrointestinal transit or degradation (rate constant, $k_g$). The fraction of dose absorbed, $F_{abs}$, is given by $k_p/(k_p + k_g)$ and describes the net extent of intestinal absorption into the intestinal tissue.

For the SFM, the intestinal blood flow is segregated, with a lower flow ($Q_{en}$, 10% total intestinal flow) perfusing the enterocyte region that consists of apical and basolateral transporters and metabolic enzymes. The remaining, serosal flow ($Q_s$, 90% flow) perfuses a nonmetabolizing or inert region (serosa, submucosa, and part of the mucosa) (Cong et al., 2000) (Fig. 1B). Subscripts en, sb, and s, denoting enterocyte blood, enterocyte tissue, serosal blood, and serosal tissue, respectively, are used to describe parameters for flow and volume for the precursor, P, and the primary metabolite, Mi. The precursor drug exchanges between blood and the corresponding tissue layers with transfer clearances, i.e., CL\textsubscript{d1} and CL\textsubscript{d2} for the enterocyte region and CL\textsubscript{d3} and CL\textsubscript{d4} for the serosal region (Fig. 1). A similar set of parameters exists for the metabolite. The parameters of the precursor are not qualified, whereas those for the metabolite are qualified by [mi]; those pertaining specifically for the preformed and formed metabolites are further classified by [pmi] and [mi,P], respectively.

These models were used to build concepts on metabolite kinetics. For simplicity, the intestine was assumed to be the only tissue for metabolism, secretion, as well as absorption, and PBPK modeling was based on the perfused rat intestine preparation. Binding of the drug and metabolite was assumed as nonexistent (unbound fractions = unity), and it was assumed that the P formed only one primary metabolite (Mi), which underwent further metabolism and secretion in the intestine (Fig. 1). It was assumed that activities for absorption ($k_p$[mi]), luminal degradation and gastrointestinal transit ($k_g$[mi]), transport (CL\textsubscript{d1} [mi], CL\textsubscript{d2} [mi], CL\textsubscript{d3} [mi], and CL\textsubscript{d4} [mi]), secretion (CL\textsubscript{int,sec} [mi]) and metabolism (CL\textsubscript{int,met} [mi]) were identical for both the preformed and formed metabolites.

Rate-equations pertaining to the rates of change of precursor and metabolite were presented in the Appendix under linear conditions. The coefficients were used for matrix inversion by the Maple9 program (Waterloo Maple Inc., Waterloo, Canada). The solutions provided the areas under the concentration-time curve of the preformed (AUC\textsubscript{pmi}) and formed (AUC\textsubscript{mi,P}) primary metabolites after administration of the synthetic metabolite and precursor drug (doses of 100 units into a reservoir volume of 200 ml and luminal volume of 2 ml), respectively. Accordingly, the unit of metabolite concentration in the reservoir is dose unit/ml, and the unit of AUC is dose unit/ml/min. The solutions were in turn used to examine the influence of flow (total intestinal flow, $Q_{en} = 8$ ml/min for the TM, and $Q_{en} = 0.8$ ml/min and $Q_s = 7.2$ ml/min for the SFM), transfer (CL\textsubscript{d1} [mi] and CL\textsubscript{d2} [mi], from 0.01 to 2 ml/min), intestinal metabolism (CL\textsubscript{int,met} and CL\textsubscript{int,met} [mi], from 0 to 1 ml/min), secretion (CL\textsubscript{int,sec} and CL\textsubscript{int,sec} [mi], from 0 to 1 ml/min), absorption ($k_p$ and $k_g$ [mi], from 0.01 to 1 min\textsuperscript{-1}), and luminal degradation/transit ($k_p$ and $k_g$ [mi], from 0 to 1 min\textsuperscript{-1}) parameters of the precursor (not qualified) and metabolite (qualified by [mi]) on AUC\textsubscript{mi,P} and AUC\textsubscript{pmi}. The simulated AUCs highlighted differences between the preformed [mi] and formed [mi,P] metabolites with respect to different routes of administration of the precursor and preformed metabolite, and they identified the sensitivity of the AUC of the metabolite, formed and preformed, to the various parameters.

Numerous conditions may also be used to simulate concentration-time profiles of the formed and preformed metabolites. However, it was not feasible to display all of the conditions. Simulations were confined only to several examples to illustrate the important features (Table 1). The time course of preformed and formed metabolites was compared using the TM and SFM for both intravenous and oral dosing by varying the $k_p$ of the precursor and the transfer clearances CL\textsubscript{d1} [mi], CL\textsubscript{d2} [mi], CL\textsubscript{d3} [mi], and CL\textsubscript{d4} [mi], or CL\textsubscript{d3} [mi], CL\textsubscript{d4} [mi] and CL\textsubscript{d3} [mi], CL\textsubscript{d4} [mi], and CL\textsubscript{d3} [mi], and CL\textsubscript{d4} [mi] = 2 and 0.01 ml/min, respectively, denoting a high versus low value transfer clearance for the metabolite; the precursor transfer clearances at the basolateral membrane were assumed to be identical and were assigned the higher value (CL\textsubscript{d3} = 2 ml/min). The absorption rate constant of the precursor, $k_p$, was changed from 0.01 to 0.05 and 1 min\textsuperscript{-1}, and $k_g$ [mi] was changed from 0.05 to 0.01 and 0.000001 min\textsuperscript{-1}.

| Table 1 | Parameters for the simulation of time courses of preformed and formed metabolites with the TM and SFM (for simulating Figs. 8 and 9) |
|-----------------|-----------------|-----------------|-----------------|
| Parameters | Case 1 | Case 2 |
| Drug precursor | | | |
| CL\textsubscript{d1} (ml/min) | 2 | 2 |
| CL\textsubscript{d2} (ml/min) | 2 | 2 |
| CL\textsubscript{d3} (ml/min) | 2 | 2 |
| CL\textsubscript{d4} (ml/min) | 2 | 2 |
| $k_p$ (min\textsuperscript{-1}) | 0.01 and 1 | 0.01 and 1 |
| $k_g$ (min\textsuperscript{-1}) | 0.01 | 0.01 |
| CL\textsubscript{int,sec} (ml/min) | 0.5 | 0.5 |
| CL\textsubscript{int,met} (ml/min) | 0.5 | 0.5 |
| Metabolite | | | |
| CL\textsubscript{d1} [mi] (ml/min) | 2 | 0.01 |
| CL\textsubscript{d2} [mi] (ml/min) | 2 | 0.01 |
| CL\textsubscript{d3} [mi] (ml/min) | 2 | 0.01 |
| CL\textsubscript{d4} [mi] (ml/min) | 2 | 0.01 |
| $k_p$ [mi] (min\textsuperscript{-1}) | 0.05 | 0.05 |
| $k_g$ [mi] (min\textsuperscript{-1}) | 0.01 | 0.01 |
| CL\textsubscript{int,sec} [mi] (ml/min) | 0.5 | 0.5 |
| CL\textsubscript{int,met} [mi] (ml/min) | 0.5 | 0.5 |

Results

The following solutions for AUC\textsubscript{pmi} and AUC\textsubscript{mi,P} for intravenous (subscripted iv) or oral (subscripted po) administration of the precursor and preformed metabolite, respectively, for the TM and SFM were obtained from matrix inversion by Maple.

\[ AUC\textsubscript{TM,iv}\textsubscript{pmi} = \frac{Dose_\textsubscript{p} [mi] (1 - F_{abs}) [mi] CL\textsubscript{int,sec} [mi] (Q_s + CL\textsubscript{d1} [mi])}{Q_s CL\textsubscript{d1} [mi] (1 - F_{abs}) [mi] CL\textsubscript{int,sec} [mi] + CL\textsubscript{int,met} [mi]} \]

\[ AUC\textsubscript{po}\textsubscript{pmi} = \frac{Dose_\textsubscript{p} [mi] F_{abs} [mi] CL\textsubscript{d2} [mi]}{CL\textsubscript{d2} [mi] (1 - F_{abs}) [mi] CL\textsubscript{int,sec} [mi] + CL\textsubscript{int,met} [mi]} \]

The above equations for the formed metabolite were found to differ from those for the preformed metabolite when doses of the performed metabolite (Dose\textsubscript{p} [mi]) or precursor (Dose) were given intravenously.

\[ CL\textsubscript{d1} [mi] (1 - F_{abs}) [mi] CL\textsubscript{int,sec} [mi] + CL\textsubscript{int,met} [mi] \]

\[ F_{abs} = \frac{Dose_\textsubscript{p} [mi] CL\textsubscript{d2} [mi]}{CL\textsubscript{d2} [mi] (1 - F_{abs}) [mi] CL\textsubscript{int,sec} [mi] + CL\textsubscript{int,met} [mi]} \]
or orally (Table 2). It was also noted in all of the above solutions that the CL_{int,sec,I} and CL_{int,sec,I} terms were effectively reduced by (1-F_{abs}) and (1-F_{abs}[mi]), respectively, suggesting that intestinal re-absorption effectively reduced the apparent luminal, secretory activity. The equations on the AUCs for the precursor drug and formed metabolite may be used to predict changes in transporter and/or enzyme and the associated intrinsic clearances or activities, and they should be useful in predicting transporter-transporter and enzyme-transporter interplay and drug-drug interactions.

AUC_{iv}[mi,P], AUC_{po}[mi,P], and F_{[pi]} after Administration of Preformed Metabolite. The precursor drug parameters, CL_{int,met,I}, CL_{int,sec,I}, CL_{abs}, k_{a}, and k_{po}, were absent in the solutions for the preformed metabolite, AUC_{iv}[pi] and AUC_{po}[pi] (eqs. 1–3). The AUC_{po}[pi] for the TM and SFM was identical, but differences existed for the AUC_{iv}[pi] due to a difference in the flow terms: Q_{i} for TM and Q_{en} for the SFM (eqs. 1 and 2), as described previously by Cong et al. (2000). Because the flow terms were absent in the solution for AUC_{po}[pi], the same solution (eq. 3) existed for the TM and SFM.

Simulations that were based on eqs. 1 and 2 revealed similar profiles for AUC_{iv}[pi] for the TM and SFM, when CL_{int,met,I} and CL_{int,sec,I} were altered. The resulting AUC_{iv}[pi] was lower for the TM due to the higher flow rate (Q_{i} > Q_{en}) (Fig. 2). Increasing CL_{int,met,I} or CL_{int,sec,I} decreased the AUC_{iv}[pi], and the effect was greater for the TM than the SFM. Comparatively speaking, CL_{int,sec,I} reduced AUC_{iv}[pi] more so than CL_{int,sec,I} for both the TM (Fig. 2, A and C) and the SFM (Fig. 2, B and D). Values of AUC_{iv}[pi] were dramatically higher with lower transfer clearances of the metabolite that tended to bar the metabolite from entry into enterocytes (see CL_{abs}[mi] at 2 versus 0.01 ml/min). The rate constant k_{i}[mi] and the F_{abs}[mi] affected the AUC[pi] for both oral and intravenous metabolite dosing. The metabolite that was secreted into the lumen was subject to re-absorption, rendering higher AUC_{iv}[pi] with high k_{i}[mi], regardless of whether the preformed metabolite was given orally or intravenously (Fig. 3). This effect explained why there were greater changes associated with CL_{int,met,I} than with CL_{int,sec,I} for both intravenous (Fig. 2) and oral (Fig. 4A) administration of the metabolite. Secretion was effectively reduced by avid re-absorption (high k_{i}[mi]) to negate the effects of metabolite secretion (CL_{int,sec,I}[mi]) (Fig. 4B); these patterns were generally similar to that of AUC_{iv}[pi] (Figs. 2 and 3). The luminal removal constant of the metabolite, k_{i}[mi], tended to facilitate the removal of the secreted metabolite, thereby decreasing both AUC_{iv}[pi] and AUC_{po}[pi] (simulations not shown). Values of AUC_{po}[pi] changed proportionally with the ratio, CL_{abs}[mi]/CL_{abs}[mi], regardless of the absolute values of CL_{abs}[mi] and CL_{abs}[mi]: the greater the ratio, the greater the AUC_{po}[pi] (Fig. 4C).

Values of F_{[pi]} obtained as the ratio AUC_{po}[pi]/AUC_{iv}[pi], also displayed similar patterns as those of AUC_{[pi]}, with respect to the greater sensitivity to CL_{int,met,I} but less so with CL_{int,sec,I}. Values of F_{[pi]} were generally higher for the TM over the SFM for the same set of variables (compare Fig. 5, A and B), and re-absorption of the metabolite tended to neutralize the effect of secretion of the metabolite. At low basolateral transfer clearances, absorption was greatly affected, rendering much lower F_{[pi]} (Fig. 5, C and D). Higher values of F_{[pi]} were apparent with higher CL_{abs}[mi] and reduced CL_{abs}[mi] (simulations not shown).

AUC_{iv}[pi,P] and AUC_{po}[pi,P] after Administration of Precur- sor. Solutions for AUC_{iv}[pi,P], the formed metabolite for the TM, were identical to that of the SFM (eq. 4). The same comment also applied to AUC_{po}[pi,P] (eq. 5). These areas of the formed metabolite (AUC_{iv}[pi,P] and AUC_{po}[pi,P]) arising from precursor administration were further affected by precursor parameters for metabolism and secretion (CL_{int,met,I} and CL_{int,sec,I}) and the fraction of precursor dose absorbed (F_{abs}). In addition, AUC_{iv}[pi,P] and AUC_{po}[pi,P] were also modulated by metabolite parameters: k_{i}[mi] and k_{po}[mi] that affect F_{abs}[mi], the metabolite transfer clearances CL_{abs}[mi] and CL_{abs}[mi], and the metabolite intrinsic clearances CL_{int,met,I}[mi] and CL_{int,sec,I}[mi] (eqs. 4 and 5).

Expectedly, increasing CL_{int,met,I} values led to increased
AUC_{iv\{mi,P\}} (Fig. 6A) and AUC_{po\{mi,P\}} (Fig. 6B), whereas increasing the intrinsic activity of the competing, secretory pathway of the precursor (CL_{int,sec,I}) decreased both the AUC_{iv\{mi,P\}} and AUC_{po\{mi,P\}}. Again, the effects of precursor secretion could be alleviated by high \(k_a\) values (Fig. 6, C and D). Changes in CL_{int,met,I} affected the AUC_{iv\{mi,P\}} and AUC_{po\{mi,P\}} more than with CL_{int,sec,I} (Fig. 7, A and B). Changes in metabolite transport also affected the AUC\{mi,P\}; an increase in CL_{d2\{mi\}} or decrease in CL_{d1\{mi\}}, as well as an increase in \(k_a\) or \(k_j\), increased both AUC_{iv\{mi,P\}} and AUC_{po\{mi,P\}} (simulation on ratio of transfer clearances; shown in Fig. 7, C and D). It was noted that the ratio of the metabolite areas, AUC_{po\{mi,P\}}/AUC_{iv\{mi,P\}}, was the fraction of precursor absorbed, \(F_{abs}\) (Table 2).

Comparison of AUCs of the Metabolites. The solutions of the AUCs of the formed and preformed metabolites allowed a comparison of the areas for the TM and SFM with respect to different routes of administration (Table 2). The ratios of areas for the formed to preformed metabolite after intravenous and oral administration of precursor and preformed metabolite, \([AUC_{iv\{mi,P\}}/AUC_{iv\{pmi\}}]\) and \([AUC_{po\{mi,P\}}/AUC_{po\{pmi\}}]\), were not unity (Table 2). \(F_{pmi}\), given by the ratio \([AUC_{iv\{mi,P\}}/AUC_{iv\{pmi\}}]\), exhibited different sensitivities to the metabolite parameters for the TM and SFM; this was again due to the different flow terms, \(Q_I\) for the TM and \(Q_{en}\) for the SFM (eqs. 1 and 2). Note that AUC_{iv\{pmi\}} was strongly modulated by CL_{int,met,I} and not CL_{int,sec,I}.

For this simulation, CL_{d2\{mi\}} was set as identical to CL_{d1\{mi\}}, i.e., \(CL_{d2\{mi\}}/CL_{d1\{mi\}} = 1\).

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For this simulation, CL_{d2\{mi\}} was set as identical to CL_{d1\{mi\}}, i.e., \(CL_{d2\{mi\}}/CL_{d1\{mi\}} = 1\).

AUC_{iv\{mi,P\}} (Fig. 6A) and AUC_{po\{mi,P\}} (Fig. 6B), whereas increasing the intrinsic activity of the competing, secretory pathway of the precursor (CL_{int,sec,I}) decreased both the AUC_{iv\{mi,P\}} and AUC_{po\{mi,P\}}. Again, the effects of precursor secretion could be alleviated by high \(k_a\) values (Fig. 6, C and D). Changes in CL_{int,met,I} affected the AUC_{iv\{mi,P\}} and AUC_{po\{mi,P\}} more than with CL_{int,sec,I} (Fig. 7, A and B). Changes in metabolite transport also affected the AUC\{mi,P\}; an increase in CL_{d2\{mi\}} or decrease in CL_{d1\{mi\}}, as well as an increase in \(k_a\) or \(k_j\), increased both AUC_{iv\{mi,P\}} and AUC_{po\{mi,P\}} (simulation on ratio of transfer clearances; shown in Fig. 7, C and D). It was noted that the ratio of the metabolite areas, AUC_{po\{mi,P\}}/AUC_{iv\{mi,P\}}, was the fraction of precursor absorbed, \(F_{abs}\) (Table 2).

Comparison of AUCs of the Metabolites. The solutions of the AUCs of the formed and preformed metabolites allowed a comparison of the areas for the TM and SFM with respect to different routes of administration (Table 2). The ratios of areas for the formed to preformed metabolite after intravenous and oral administration of precursor and preformed metabolite, \([AUC_{iv\{mi,P\}}/AUC_{iv\{pmi\}}]\) and \([AUC_{po\{mi,P\}}/AUC_{po\{pmi\}}]\), were not unity (Table 2). \(F_{pmi}\), given by the ratio \([AUC_{iv\{mi,P\}}/AUC_{iv\{pmi\}}]\), exhibited different sensitivities to the metabolite parameters for the TM and SFM; this was again due to the different flow terms, \(Q_I\) for the TM and \(Q_{en}\) for the SFM (eqs. 1 and 2). Note that AUC_{iv\{pmi\}} was strongly modulated by CL_{int,met,I} and not CL_{int,sec,I}.

For this simulation, CL_{d2\{mi\}} was set as identical to CL_{d1\{mi\}}, i.e., \(CL_{d2\{mi\}}/CL_{d1\{mi\}} = 1\).
for the preformed metabolite after its intravenous administration revealed a faster decay profile for preformed metabolite for the TM compared with the SFM (Fig. 8A). This result is expected due to the greater intestinal clearance based on $Q_I$ and not $Q_{en}$, yielding a higher clearance and lower AUC_{iv} for the TM. With oral dosing, the peak concentration of the preformed metabolite was lower according to the SFM, and the peak time for the SFM occurred at almost the same time as that for the TM; the decay $t_{1/2}$ of the metabolite for the SFM was also longer. The TM metabolite profile crossed over that of the SFM, but the AUC_{iv} for the TM and SFM were identical. Poor basolateral transfer characteristics of the metabolite into the enterocyte transformed the decay profile to yield a protracted $t_{1/2}$ for the metabolites, showing the strong influence of the metabolite basolateral transfer clearances (see Fig. 8, A and B). Under the condition of poor metabolite entry, there was virtually no difference in the decay profiles of the preformed metabolite between the TM and SFM. Note also that the $t_{1/2}$s for the metabolite for the intravenous and oral cases for the TM or SFM were parallel (Fig. 8).

Profiles of the formed metabolite were found to alter with the absorption rate constant of the precursor, $k_a$, and the metabolite basolateral transfer clearance, $CL_{d1}$ (Fig. 9). At high $CL_d$ and low absorption rate constant of the precursor ($k_a = 0.01 \text{ min}^{-1}$), levels of the formed metabolite for the TM and the SFM after intravenous precursor administration were generally higher than those after oral dosing. At high $CL_d$ and high $k_a$ (1 min$^{-1}$), the concentration-time profiles of the metabolite edged closer for both intravenous and oral precursor doses, although the metabolite profiles after oral dosing peaked earlier than those for intravenous dosing (Fig. 9B). However, at low $CL_d$ and high $k_a$ (1 min$^{-1}$), only upswing profiles were observed for the simulated cases of 24 h at both the low (0.01 min$^{-1}$) and high (1 min$^{-1}$) $k_a$ values (Fig. 9, C and D). The metabolite profiles after intravenous precursor administration were comparatively higher than those for oral dosing of precursor that exhibited poor absorption ($k_a = 0.01 \text{ min}^{-1}$) (Fig. 9C). With good precursor absorption ($k_a = 1 \text{ min}^{-1}$), all of the metabolite profiles became comparable (Fig. 9D) when reabsorption reclaimed most of the secreted precursor. Generally speaking, the formed metabolite peaked earlier and higher with increasing $k_a$, because a faster precursor absorption rate not only...
Simulations Based on Equivalent Preformed Metabolite Dose.

One strategy was formulated to estimate the dose equivalent of the preformed metabolite that would yield an $\text{AUC}_{\text{p,mi}}$ identical to the $\text{AUC}_{\text{mi,P}}$ (precursor Dose = 100 units). From Table 2, the required doses, $\text{Dose}_{\text{v,mi}}$ and $\text{Dose}_{\text{po,mi}}$, can be calculated, as shown in eqs. 6 and 7.

For the TM, intravenous case

$$\text{Dose}_{\text{v,mi}} = \frac{\text{Dose}_{\text{v,mi}} Q_{\text{CL}_{\text{int,met,I}}} \text{CL}_{\text{d,2,mi}}}{((1 - F_{\text{abs,mi}}) \text{CL}_{\text{int,sec,I}} + \text{CL}_{\text{int,met,I}}) ((1 - F_{\text{abs,mi}}) \text{CL}_{\text{int,sec,I}})}$$

and for the SFM, intravenous case

$$\text{Dose}_{\text{v,mi}} = \frac{\text{Dose}_{\text{v,mi}} Q_{\text{CL}_{\text{int,met,I}}} \text{CL}_{\text{d,2,mi}}}{((1 - F_{\text{abs,mi}}) \text{CL}_{\text{int,met,I}} + \text{CL}_{\text{int,met,I}}) ((1 - F_{\text{abs,mi}}) \text{CL}_{\text{int,met,I}} + \text{CL}_{\text{int,sec,I}})}$$

For both the TM and SFM, oral cases

$$\text{Dose}_{\text{po,mi}} = \frac{\text{Dose}_{\text{po,mi}} F_{\text{abs,mi}} \text{CL}_{\text{int,met,I}}}{F_{\text{abs,mi}} (1 - F_{\text{abs,mi}}) \text{CL}_{\text{int,sec,I}} + \text{CL}_{\text{int,met,I}}}$$

Simulations of the most likely scenario of preformed metabolite administration, the oral case, required an estimate of $\text{Dose}_{\text{po,mi}}$, as shown in eq. 8 [$\text{AUC}_{\text{po,mi}} = \text{AUC}_{\text{po,mi,P}}$]. Simulations derived thereafter showed consistently lower profiles of the formed metabolite versus those for the preformed metabolite, especially at the earlier times for all of the cases simulated (Fig. 10). These patterns held regardless of $k_2$ (0.01 or 1 min$^{-1}$) at high or low $\text{CL}_{\text{d,2,mi}}$ (2 or 0.01 ml/min) (Fig. 10, A and B, versus C and D). The influence of $k_2$ was also examined. Upon decreasing $k_2$, the required $\text{Dose}_{\text{po,mi}}$ according to eq. 8, needed to be increased accordingly. The resulting simulations at low $k_2$ showed profiles that were similar to those in Fig. 10, although the metabolite concentrations were lower (simulations not shown).

**Discussion**

The aspect on drug safety affects the preclinical and clinical stages in drug development and extends to the time when the drug is in the market place. In vitro testing and in vivo pharmacokinetics either in animals or in man is of paramount importance. These include in silico testing, high-throughput systems that are enzyme- or cell-based, including microfluidic systems of multiple cells in culture (Leeder et al., 1989; Johansson et al., 2004; Khentani and Bhata, 2006; Li, 2007), and identification of mechanisms of on-target and off-target toxicity via covalent binding, alkylation protein, DNA (Bailie, 2006) or downstream adducts, biomarkers, or mechanism-based inactivation of cytochrome P450s (Jones et al., 2007). Animal models and surrogate animal testing are used for investigation of metabolite-mediated toxicity, genotoxicity studies, toxicity testing of embryonic-fetal development, and carcinogenicity studies in toxicological testing (Stevens,
In cases where metabolite exposure has been inadequately assessed, it is proposed that a synthesized reference standard may need to be prepared and the safety of the metabolite assessed using a route of administration whereby adequate exposure can be obtained in an appropriate species (2008 FDA Guidance for Industry).

The recent 2008 FDA guidance on metabolite safety testing defined a major metabolite as $>10\%$ AUC of the precursor. The method likely assumes similar volumes of distribution of the metabolite and precursor and may not hold. It must also be noted that the distribution volume of the formed metabolite may or may not be identical to that occupied by the preformed metabolite. In addition, direct dosing of the preformed metabolite may lead to formation of the metabolites other than those originating from precursor administration. All these factors would complicate the toxicity picture (Prueksaritanont et al., 2006; Pang et al., 2008). The estimate of metabolite amount in the excreta (Smith and Obach, 2006) is also imprecise, even with use of a radioactive dose. This is because the amount as metabolite in excreta is time-dependent and reliant on the eliminatory pathways of the metabolite. Hence, a reverberating theme that came through is that the safety assessment of metabolites should be a case-by-case approach (Hastings et al., 2003; Naito et al., 2007; 2008 FDA Guidance for Industry).

If metabolite testing is mandated in animal testing in man, an inevitable question is how to administer the metabolite, systemically or orally. From a theoretical standpoint, there is the need to consider the SFM in addition to the TM for intestinal modeling, because many drugs are shown to exhibit intestinal route-dependent metabolism.
A similar strategy was used by the SimCYP group to describe intestinal clearances (Yang et al., 2007). Therefore, we felt compelled to compare the results on AUC\textsubscript{mi,P} for both the TM and the SFM, expecting to see higher AUC\textsubscript{iv,pmi} for the SFM due to the flow differences, \(Q_e\) for the SFM and \(Q_i\) for the TM (Table 2). We showed that formed and preformed metabolite kinetics and time courses differed, and these were dependent on the route of administration and flow patterns (\(Q_e\) versus \(Q_i\)) of the intestine (Figs. 2–10). The AUC\textsubscript{po,pmi} were identical for both models. Moreover, identical AUC\textsubscript{mi,P}s were found for the TM and SFM for intravenous precurser administration, and the same was applied to oral administration as well. The time courses of the formed metabolite were found to differ with different \(k_a\) of the precursor (Fig. 9). Even when “AUC” preformed metabolite oral doses were given, the correspondence between the formed and preformed metabolite levels was poor, especially for the early time points (Fig. 10).

The results showed that, in consideration with intestinal elimination (Pang, 2003). A similar strategy was used by the SimCYP group to describe intestinal clearances (Yang et al., 2007). Therefore, we felt compelled to compare the results on AUC\textsubscript{mi,P} for both the TM and the SFM, expecting to see higher AUC\textsubscript{iv,pmi} for the SFM due to the flow differences, \(Q_e\) for the SFM and \(Q_i\) for the TM (Table 2). We showed that formed and preformed metabolite kinetics and time courses differed, and these were dependent on the route of administration and flow patterns (\(Q_e\) versus \(Q_i\)) of the intestine (Figs. 2–10). The AUC\textsubscript{po,pmi} were identical for both models. Moreover, identical AUC\textsubscript{mi,P}s were found for the TM and SFM for intravenous precurser administration, and the same was applied to oral administration as well. The time courses of the formed metabolite were found to differ with different \(k_a\) of the precursor (Fig. 9). Even when “AUC” preformed metabolite oral doses were given, the correspondence between the formed and preformed metabolite levels was poor, especially for the early time points (Fig. 10).

The results showed that, in consideration with intestinal elimination only, the resulting AUC\textsubscript{pmi} was affected only by the absorptive, distributional, and eliminatory characteristics of the metabolite, whereas AUC\textsubscript{mi,P} was additionally influenced by the absorptive and eliminatory characteristics of the precursor. Upon comparison, the AUC\textsubscript{mi,P} differed from AUC\textsubscript{pmi} for the oral and intravenous cases for both models (Table 2). The same was found in the liver and kidney. The areas under the curve of the formed metabolite were influenced by distributional and eliminatory characteristics of both the precursor and metabolite in the organ, suggesting that metabolite administration will lead to kinetic observations that deviate from those of the preformed metabolite (Pang et al., 2008). Indeed, evidence on different kinetics between the formed species was found to exist in the liver (Xu et al., 1990; de Lannoy et al., 1993), kidney (Geng et al., 1999), and intestine (Cong et al., 2000).

The time course of the toxic, formed metabolite and its AUC\textsubscript{mi,P} would be influenced by distributional and eliminatory characteristics of the metabolite in the intestinal tissue, and those for the precursor (Figs. 8–10), the route of administration, and whether a partial or total flow...
perfuses the enterocyte region (TM versus SFM). The notion that exposure of the metabolite is reproducible by administration of the preformed metabolite is not attainable, and there were practical limitations in this approach. The same was implied in a commentary by Smith and Obach (2006). However, the argument of percent exposure versus absolute amount (Baillie et al., 2002; Smith and Obach, 2005, 2006) is immaterial and irrelevant in providing any clearer answers. More relevant is how to mimic the kinetics and toxicity of the metabolite in question.

The theoretical examination has alluded to the dilemma in metabolite toxicity testing with testing of synthetic metabolites. There are additional features that can modulate metabolite AUCs presented in this theoretical treatise. First, the heterogeneous distribution of transporters and enzymes along the segment of the small intestine has been shown to influence drug bioavailability (Pang, 2003; Tam et al., 2003; Liu et al., 2006). In liver, heterogeneous distribution of enzymes is also known to result in dramatically different fates of administered and formed primary metabolites (Xu et al., 1990; Tan et al., 2001; Pang et al., 2008). Metabolite profiles will change when metabolite formation occurs in multiple organs (de Lannoy and Pang, 1993) or when there is sequential handling of the formed, primary metabolite in other downstream or parallel organs (e.g., intestinally formed, primary metabolites undergo hepatic metabolism or excretion before reaching the systemic circulation) (Chen and Pang, 1997). Transport characteristics of the primary metabolite in each of the organs involved in its formation or further metabolism could differ and result in differences in metabolite AUC.

Although metabolite administration/testing would not directly reflect the time course of the formed metabolite, the data may be used favorably. The available preformed metabolite data may be wisely incorporated into a combined PBPK model of the precursor-metabolite to improve the predictions on metabolite behavior resulting from drug administration. This kind of strategy is frequently used in risk assessment. The administration of a metabolite, intravenous or oral, had aided in the modeling and prediction of the formation of toxic metabolites as well as targeted toxicity outcomes. Examples of this elegant work includes modeling of the following: metabolism of styrene oxide from styrene on respiratory tract toxicity (Sarangapani et al., 2002); the metabolism and enterohepatic

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**Fig. 7.** Dependence of the areas under the curve of the formed metabolite on metabolite parameters: when drug was given intravenously $\text{AUC}_{iv}\{mi,P\}$ (A and C) and orally $\text{AUC}_{po}\{mi,P\}$ (B and D); against the $\text{CL}_{int,sec,1}\{mi\}$ and $\text{CL}_{int,met,1}\{mi\}$ when $\text{CL}_{d2}\{mi\}/\text{CL}_{d1}\{mi\} = 1$ (A and B), and $\text{CL}_{d2}\{mi\}/\text{CL}_{d1}\{mi\}$ and $k_a\{mi\}$ (C and D) of the metabolite. Note that increasing $k_a\{mi\}$ neutralized the effect of luminal secretion of the precursor.
FIG. 9. Simulation of the time courses of formed metabolite (profiles) for Cases 1 and 2 (Table 1): CL_{dmi} = 2 ml/min (A), or 0.01 ml/min (B), with doses of 100 units for the formed metabolite; k_{mi} = 0.05 min^{-1}; reservoir and lumen volumes of 200 and 2 ml, respectively. The initial concentration of formed metabolite in the dosing compartment (reservoir) is 0.5 (dose unit/ml) for the intravenous cases and 50 (dose unit/ml) in the intestinal lumen for the oral cases. Other parameters of the formed metabolite used for simulation were shown in Table 1. A, the graphs differed for CL_{dmi} = 2 ml/min (A) and CL_{dmi} = 0.01 ml/min (B). A, at high CL_{dmi}, the t_{1/2} for the TM (intravenous and oral) was faster than those for the SFM. B, at low CL_{dmi}, the graphs were identical for TM and SFM after intravenous and oral dosing of the formed metabolite.

FIG. 8. Simulation of the time courses of preformed metabolite (profiles) for Cases 1 and 2 (Table 1): CL_{dmi} = 2 ml/min (A), or 0.01 ml/min (B), with doses of 100 units for the preformed metabolite; k_{mi} = 0.05 min^{-1}; reservoir and lumen volumes of 200 and 2 ml, respectively. The initial concentration of preformed metabolite in the dosing compartment (reservoir) is 0.5 (dose unit/ml) for the intravenous cases and 50 (dose unit/ml) in the intestinal lumen for the oral cases. Other parameters of the preformed metabolite used for simulation were shown in Table 1. A, the graphs differed for CL_{dmi} = 2 ml/min (A) and CL_{dmi} = 0.01 ml/min (B). A, at high CL_{dmi}, the t_{1/2} for the TM (intravenous and oral) was faster than those for the SFM. B, at low CL_{dmi}, the graphs were identical for TM and SFM after intravenous and oral dosing of the preformed metabolite.

METABOLITE KINETICS IN INTESTINE

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A, k_{i} = 0.01 min^{-1}
B, k_{i} = 1 min^{-1}
C, k_{i} = 0.01 min^{-1}
D, k_{i} = 1 min^{-1}

Higher (formed) metabolite levels were observed with higher k_{i} and higher CL_{dmi}. The absorptive constant, k_{a}, also influenced the time course/profile of the formed metabolite by reducing the effective secretion intrinsic clearance (CL_{int,sec,I}), thereby increasing the rate of drug metabolism.
circulation of di-n-butylphthalate to the monobutylphthalate and the glucuronide; compounds that cause impairment of male reproductive tissues (Clewell et al., 2008); the metabolism and oral absorption and of atrazine on toxicity of the pituitary axis and enzyme inhibition (McMullin et al., 2007); the metabolism-associated toxicities of trichloroethylenes (Dobrev et al., 2002); the bioactivation of vinyl chloride to the DNA-reactive epoxide (Reitz et al., 1996; Clewell et al., 2001); and the inhalation toxicity of acrylate ester (Frederick et al., 1998, 2002; Sweeney et al., 2004). The in vitro/in vivo drug and metabolite transport/distribution and elimination behavior, especially in target organs, should be studied to add to the robustness of the PBPK model. In a recent study, involvement of the multidrug resistance-associated protein 1 for the efflux of rusovastatin in muscle, the target organ for rhadomyolosis, has been implicated as a plausible cause of toxicity of the statin (Dorajoo et al., 2008); similar transporters of metabolites may need to be identified in toxicity evaluations. Through the refinement of modeling approaches by the incorporation of transporter and enzymes, a better solution may be on hand for metabolite toxicity testing and for the prediction of metabolite toxicity.

Appendix

Mass Balance Equations and the Corresponding Matrix for Preformed and Formed Metabolite in TM and SFM Models

In the following section, P denotes the concentration of the precursor, whereas Mi denotes the concentration of the metabolite, either preformed ([pmi]) or formed from the administration of the precursor ([mi,P]). V denotes the volume in each compartment, $f_i$ the unbound fraction; subscripts: I, R, intb, int, and lumen denote the intestine, reservoir, intestinal blood, intestinal tissue, and intestine lumen, respectively. Subscripts s, sb, en, and enb describe the serosal, serosal blood, enterocyte, and enterocyte blood, respectively. $Q_I$ describes the total intestinal blood flow rate or sum of the blood flows perfusing the enterocyte layer ($Q_{enb}$, 10% of $Q_I$) and the serosal layer ($Q_{sb}$, 90% of $Q_I$). The parameters pertaining to the metabolite are qualified by $\{mi\}$. A definition of the kinetic parameters has been given under Materials and Methods.

I. TM: Preformed Metabolite

Rate of change of metabolite in reservoir, as shown in eq. 9.

$$ V_R \frac{dM_{ir}}{dt} = Q_I (M_{intb} - M_{ir}) $$

Rate of change of metabolite in intestinal blood, as shown in eq. 10.

$$ V_{intb} \frac{dM_{intb}}{dt} = Q_I (M_{ir} - M_{intb}) + f_s[mi] CL_{sb}[mi] M_{int} - f_s[mi] CL_{enb}[mi] M_{intb} $$

Fig. 10. Simulation of the time courses of formed metabolite (profiles) for Cases 1 and 2 (similar to those of Fig. 9). $CL_d[mi] = 2 \text{ ml/min}$ for Case 1A (A) and Case 1B (B), and $CL_d[mi] = 0.01 \text{ ml/min}$ for Case 2A (C) and Case 2B (D); and $k_s[mi] = 0.05 \text{ (dose unit/ml)}$. The precursor dose of 100 units was used, whereas the dose of the preformed metabolite was estimated with eq. 8, such that $AUC_{cpo}[mi,P] = AUC_{cpo}[pmi]$. Note again that higher levels of metabolites were observed with higher $k_s$ and higher $CL_d[mi]$. The concentration-time profiles of the preformed metabolite were consistently higher than those of the formed metabolite for the TM or SFM, especially at the earlier time points, although the AUC of the preformed and formed metabolites were identical.
Rate of change of metabolite in tissue, as shown in eq. 11.

\[
\frac{dM_{int}}{dt} = f_i(m_i)CL_{a1}(m_i)M_{int} + k_i(m_i)M_{lumen} V_{lumen} - f_i(m_i)(CL_{a2}(m_i) + CL_{int,sec1}(m_i) + CL_{int,sec2}(m_i))M_{int}
\]  

(11)

Rate of change of metabolite in intestine lumen, as shown in eq. 12.

\[
\frac{dM_{lumen}}{dt} = -(k_i(m_i) + k_i(m_i))M_{lumen} V_{lumen} + f_i(m_i)(CL_{int,sec1}(m_i)M_{int})
\]  

(12)

Matrix for the TM model: preformed metabolite, as shown in the equation below.

\[
\begin{pmatrix}
\frac{Q_i}{V_R} & \frac{Q_i}{V_{int}} & 0 & 0 \\
\frac{Q_i}{V_R} & \frac{Q_i}{V_{int}} + CL_{a1}(m_i) & -CL_{a1}(m_i) & 0 \\
0 & CL_{a1}(m_i) & \frac{CL_{int,sec1}(m_i) + CL_{int,sec2}(m_i)}{V_{int}} & -k_i(m_i) \\
0 & 0 & \frac{CL_{int,sec1}(m_i)}{V_{int}} & k_i(m_i) + k_i(m_i)
\end{pmatrix}
\]

II. TM: Formed Metabolite

Rates of change of precursor and metabolite in reservoir, as shown in eqs. 13 and 14.

\[
V_R \frac{dP_{R}}{dt} = \frac{Q_i}{V_{int}}(P_{int} - P_{R})
\]  

(13)

\[
V_R \frac{dM_{R}}{dt} = \frac{Q_i}{V_{int}}(M_{int} - M_{R})
\]  

(14)

Rates of change of precursor and metabolite in intestinal blood, as shown in eqs. 15 and 16.

\[
\frac{dP_{int}}{dt} = \frac{Q_i}{V_{int}}(P_{int} - P_{R}) + f_i(CL_{a2}(m_i) + CL_{int,sec1} + CL_{int,sec2})P_{int}
\]  

(15)

\[
\frac{dM_{int}}{dt} = \frac{Q_i}{V_{int}}(M_{int} - M_{R}) + f_i(m_i)(CL_{a1}(m_i)M_{int}) - f_i(m_i)(CL_{a2}(m_i) + CL_{int,sec1}(m_i) + CL_{int,sec2}(m_i))M_{int}
\]  

(16)

Rates of change of precursor and metabolite in intestine tissue, as shown in eqs. 17 and 18.

\[
\frac{dP_{lumen}}{dt} = \frac{Q_i}{V_{int}}(P_{lumen} - P_{R}) + k_i(m_i)P_{lumen}V_{lumen} - f_i(CL_{a1}(m_i) + CL_{int,sec1}(m_i) + CL_{int,sec2}(m_i))P_{int}
\]  

(17)

\[
\frac{dM_{lumen}}{dt} = \frac{Q_i}{V_{int}}(M_{lumen} - M_{R}) + k_i(m_i)M_{lumen}V_{lumen} + f_i(CL_{a1}(m_i) + CL_{int,sec1}(m_i) + CL_{int,sec2}(m_i))M_{int}
\]  

(18)

Rates of change of precursor and metabolite in intestine lumen, as shown in eqs. 19 and 20.

\[
\frac{dP_{lumen}}{dt} = \frac{Q_i}{V_{int}}(P_{lumen} - P_{R}) + \frac{Q_i}{V_{int}}(P_{lumen} - P_{int}) + f_i(CL_{a1}(m_i) + CL_{int,sec1}(m_i) + CL_{int,sec2}(m_i))P_{int}
\]  

(19)

\[
\frac{dM_{lumen}}{dt} = \frac{Q_i}{V_{int}}(M_{lumen} - M_{R}) + \frac{Q_i}{V_{int}}(M_{lumen} - M_{int}) + f_i(CL_{a1}(m_i) + CL_{int,sec1}(m_i) + CL_{int,sec2}(m_i))M_{int}
\]  

(20)

Matrix for the TM model: formed metabolite
III. SFM: Preformed Metabolite

Rate of change of metabolite in reservoir, as shown in eq. 21.

\[ V_R \frac{dM_{i_{ab}}}{dt} = Q_{ab}M_{i_{ab}} + Q_{cab}M_{i_{cab}} - Q_{Mi_R} \]  

(21)

Rate of change of metabolite in intestinal serosal blood, as shown in eq. 22.

\[ V_s \frac{dM_{i_{sh}}}{dt} = Q_{sb}(M_{i_R} - M_{i_{sh}}) + f_{1i}[mi]C_{L_{int,1}}[mi]M_{i_{sh}} - f_{1i}[mi]C_{L_{int,2}}[mi]M_{i_{sh}} \]  

(22)

Rate of change of metabolite in intestine serosal tissue, as shown in eq. 23.

\[ V_s \frac{dM_{i_{sb}}}{dt} = f_{1i}[mi]C_{L_{int,2}}[mi]M_{i_{sh}} - f_{5i}[mi]C_{L_{int,3}}[mi]M_{i_{sh}} \]  

(23)

Matrix for the SFM model: preformed metabolite

\[
\begin{pmatrix}
Q_s & Q_{en} & 0 & 0 \\
\frac{Q_s}{V_R} & 0 & C_{L_{int,1}}[mi] & 0 \\
0 & \frac{C_{L_{int,1}}[mi]}{V_s} & 0 & 0 \\
0 & 0 & 0 & \frac{C_{L_{int,2}}[mi]}{V_s} \\
0 & 0 & 0 & 0 \\
\end{pmatrix}
\]

IV. SFM: Formed Metabolite

Rates of change of precursor and metabolite in reservoir, as shown in eqs. 27 and 28.

\[ V_R \frac{dP_{i_{ab}}}{dt} = Q_{ab}P_{i_{ab}} + Q_{cab}P_{i_{cab}} - Q_{Pi_R} \]  

(27)

\[ V_R \frac{dM_{i_{ab}}}{dt} = Q_{ab}M_{i_{ab}} + Q_{cab}M_{i_{cab}} - Q_{Mi_R} \]  

(28)

Rates of change of precursor and metabolite in intestinal serosal blood, as shown in eqs. 29 and 30.

\[ V_s \frac{dP_{i_{sh}}}{dt} = Q_{sb}(P_{i_R} - P_{i_{sh}}) + f_{1i}[mi]C_{L_{int,1}}P_{i_{sh}} - f_{1i}[mi]C_{L_{int,2}}P_{i_{sh}} \]  

(29)

\[ V_s \frac{dM_{i_{sh}}}{dt} = Q_{sb}(M_{i_R} - M_{i_{sh}}) + f_{1i}[mi]C_{L_{int,1}}[mi]M_{i_{sh}} - f_{5i}[mi]C_{L_{int,2}}[mi]M_{i_{sh}} \]  

(30)

Rates of change of precursor and metabolite in intestine serosal tissue, as shown in eqs. 31 and 32.

\[ V_s \frac{dP_{i_{sb}}}{dt} = f_{1i}[mi]C_{L_{int,1}}P_{i_{sh}} - f_{6i}[mi]C_{L_{int,2}}P_{i_{sh}} \]  

(31)

Rate of change of metabolite in the blood perfusing the enterocyte layer, as shown in eq. 24.

\[ V_eh \frac{dM_{i_{sh}}}{dt} = Q_{eh}(M_{i_R} - M_{i_{sh}}) + f_{1i}[mi]C_{L_{int,2}}[mi]M_{i_{sh}} - f_{1i}[mi]C_{L_{int,3}}[mi]M_{i_{sh}} \]  

(24)

Rate of change of metabolite in enterocytes, as shown in eq. 25.

\[ V_{en} \frac{dM_{i_{sh}}}{dt} = f_{1i}[mi]C_{L_{int,2}}[mi]M_{i_{sh}} + k_{1i}[mi]M_{i_{lumen}}V_{lumen} \]  

(25)

Rate of change of metabolite in intestine lumen, as shown in eq. 26.

\[ V_{lumen} \frac{dM_{i_{sh}}}{dt} = -(k_{1i}[mi] + k_{1i}[mi])M_{i_{lumen}}V_{lumen} + f_{1i}[mi]C_{L_{int,3}}[mi]M_{i_{sh}} \]  

(26)

Rates of change of precursor and metabolite in the blood perfusing the enterocyte layer, as shown in eqs. 33 and 34.

\[ V_{eh} \frac{dP_{i_{sh}}}{dt} = f_{1i}[mi]C_{L_{int,1}}[mi]P_{i_{sh}} - f_{1i}[mi]C_{L_{int,2}}[mi]P_{i_{sh}} \]  

(32)

\[ V_{eh} \frac{dP_{i_{sh}}}{dt} = Q_{eh}(P_{i_R} - P_{i_{sh}}) + f_{i}[mi]C_{L_{int,2}}P_{i_{sh}} - f_{1i}[mi]C_{L_{int,2}}P_{i_{sh}} \]  

(33)

Rates of change of precursor and metabolite in enterocytes, as shown in eqs. 35 and 36.

\[ V_{en} \frac{dP_{i_{sh}}}{dt} = f_{1i}[mi]C_{L_{int,2}}[mi]M_{i_{sh}} + k_{i}[mi]M_{i_{lumen}}V_{lumen} \]  

(34)

\[ V_{en} \frac{dP_{i_{sh}}}{dt} = f_{1i}[mi]C_{L_{int,1}}P_{i_{sh}} + k_{i}[mi]M_{i_{lumen}}V_{lumen} + f_{i}[mi]C_{L_{int,2}}P_{i_{sh}} - f_{1i}[mi]C_{L_{int,2}}[mi] \]  

(35)

\[ V_{en} \frac{dM_{i_{sh}}}{dt} = f_{i}[mi]C_{L_{int,2}}[mi]M_{i_{sh}} + k_{i}[mi]M_{i_{lumen}}V_{lumen} + f_{i}[mi]C_{L_{int,2}}[mi]M_{i_{sh}} \]  

(36)
Rates of change of precursor and metabolite in intestine lumen, as shown in eqs. 37 and 38.

\[
V_{\text{lumen}} \frac{dP_{\text{lumen}}}{dt} = -\left(k_a + k_{\text{gi}}\right)P_{\text{lumen}} + f_{\text{CL}_{\text{int,occ}}}/P_{\text{en}} \quad (37)
\]

\[
V_{\text{lumen}} \frac{dM_{\text{lumen}}}{dt} = -(k_g \{\text{mi}\} + k_{\text{mi}} \{\text{mi}\})M_{\text{lumen}} + f_{\text{mi}}(\text{mi}) \frac{\text{CL}_{\text{int,occ}}(\text{mi})}{P_{\text{en}}} \quad (38)
\]

Matrix for the SFM model: formed metabolite

\[
\begin{pmatrix}
\frac{Q_a}{V_a} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & \frac{Q_{\text{en}}}{V_{\text{en}}} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & \frac{Q_{\text{en}}}{V_{\text{en}}} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\frac{Q_a}{V_a} & 0 & \frac{Q_{\text{en}}}{V_{\text{en}}} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & \frac{Q_{\text{en}}}{V_{\text{en}}} & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & \frac{Q_{\text{en}}}{V_{\text{en}}} & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & \frac{Q_{\text{en}}}{V_{\text{en}}} & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & \frac{Q_{\text{en}}}{V_{\text{en}}} & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{Q_{\text{en}}}{V_{\text{en}}} & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{Q_{\text{en}}}{V_{\text{en}}} & 0 \\
\end{pmatrix}
\]

References


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