Pharmaceutical Research, Vol. 27, No. 7, July 2010 (© 2010)
DOI: 10.1007/s11095-010-0049-2

Research Paper

Physiological Modeling to Understand the Impact of Enzymes and Transporters on Drug and Metabolite Data and Bioavailability Estimates

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Received October 5, 2009; accepted January 4, 2010; published online April 6, 2010

Purpose. To obtain mathematical solutions that correlate drug and metabolite exposure and systemic bioavailability (F_{sys}) with physiological determinants, transporters and enzymes.
Methods. A series of physiologically-based pharmacokinetic (PBPK) models that included renal excretion and sequential metabolism within the intestine and/or liver as metabolite formation organs were developed. The area under the curve for drug (AUC) and formed metabolite (AUC{mi,P}) were solved by matrix inversion.
Results. The PBPK models revealed that AUC{mi,P} was dependent on dispositional parameters (transport and elimination) for the drug and metabolite. The solution was unique for each metabolite formation organ and was dependent on the type of drug and metabolite elimination organs. The AUC ratio of the formed metabolite after oral and intravenous drug dosing was useful for determination of the fraction absorbed (F_{abs}) and not the systemic bioavailability (F_{sys}) when either intestine or liver was the only drug elimination organ.
Conclusions. The AUC ratio of the formed metabolite after oral and intravenous drug dosing differed from that for drug and would not provide F_{sys}. However, the AUC ratio of the formed metabolite for oral and intravenous drug dosing furnished the estimate of F_{abs} when intestine or liver was the only drug metabolic organ.

KEY WORDS: area under the curve of metabolite; AUC ratios; bioavailability; drug disposition; fraction absorbed; metabolic enzymes; metabolite kinetics; PBPK modeling; transporters.

INTRODUCTION

Bioavailability (BA) and bioequivalence (BE) are important issues in the evaluation of oral drug absorption and comparison of the rate and extent of drug absorption between drug formulations. Modern bioequivalence evaluation is enhanced with the biopharmaceutics drug classification system (BCS) (1) that was adopted as guidelines of BE by the Food and Drug Administration (FDA). Generally, the target entity in BA and BE studies is the parent drug; the comparison of the maximum plasma concentration (C_{max}) and the oral and intravenous area under the concentration-time profile (AUC from time zero to infinity) provide the rate and extent of absorption and oral bioavailability (2–5), respectively. Riegelman and Rowland (6) and Rescigno (7) emphasized that BA cannot be determined by AUC ratios unless the AUC is proportional to the fraction absorbed and the clearance is constant. For drugs that are highly metabolized and distributed, however, low levels of the drug result and render poor estimation of BA/BE. The anticipated difference in the extent of portal absorption between formulations is revealed by the fraction of dose absorbed, F_{abs}, the ratio of the absorption rate constant, k_a divided by (k_a+k_g), where k_g is the rate constant of drug degradation or loss in the gastrointestinal tract, GIT. Generic equivalents that differ in k_a due to formulation differences would show variations in F_{abs}. First pass removal is denoted as F_I and F_H for the fractions available of the intestine and liver, respectively. Their product, $F_{abs}F_IF_H$, yields the systemic bioavailability, F_{sys}.

A point of departure among oral dosage forms is the fraction absorbed (F_{abs}). Different excipients from drug formulations can confer variability and affect k_a and therefore F_{abs}, since many excipients are able to modulate intestinal drug permeability via complexation, hydrogen bonding, ion-dipole, dipole-dipole and van der Waals interactions and modify the physicochemical, pharmacological or pharmacokinetic behavior of the medication. Generally speaking, excipients are important determinants of disintegration and dissolution of the solid formulation and the biopharmaceutical processes in gastrointestinal tract that can affect k_a. Moreover, it is recognized that excipients are modulators of drug transporters and can affect drug absorption and first-pass removal. Many compounds are substrates of the P-glycoprotein (P-gp), whose activities are shown to be impacted by various excipients (8). Pluroic block copolymers are found to inhibit the P-gp excretion of antineo-

Electronic supplementary material The online version of this article (doi:10.1007/s11095-010-0049-2) contains supplementary material, which is available to authorized users.

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plastic agents such as doxorubicin (9–12). Inhibition of P-gp activity has been noted for cremophor EL, Tween 20 (13,14), Tween 80 or polysorbate 80 (15,16), Pecol (17), PEG-300 (18), and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS 1000) (19). Moreover, Tween 80 is shown to inhibit the oligopeptide transporter, PEPT1, and cremophor EL, the monocarboxylic acid transporter, MCT, thus changing k_a and F_{abs} (20). Surfactants (21) and excipients such as sodium taurodeoxycholate, sodium glycodeoxycholate and sodium lauryl sulfate could increase the stability and transport (22) of the modalities. N-Trimethyl chitosan chloride acts as a potential absorption enhancer by opening tight junctions of intestinal epithelial cells to allow increased transport of hydrophilic compounds through the paracellular transport pathway (23,24). In other instances, excipients are found to reduce hepatocyte uptake (25,26) and/or the activity of enzymes and decrease the metabolic intrinsic clearance (27,28). Therefore, important components of pharmaceutical formulations that affect improvement of formulation characteristics can reduce the effectiveness of some preparations and result in variations in BA and BE with use of different excipients.

The Guidance for Industry issued by the FDA (29) stated that "For bioequivalence (BE) studies, measurement of only the parent drug released from the dosage form is generally recommended. The rationale of this recommendation is that the concentration-time profile of the parent drug is more sensitive to changes in formulation performance than a metabolite, which is more reflective of metabolite formation, distribution, and elimination." Consideration of metabolite data in bioequivalence studies has been proposed. One of the most common reasons is the case of the inactive prodrug where the active metabolite is the appropriate species for the assessment of BE. Another justification for use of metabolite area-under-the-curve data (AUC{mi,P}) exists when (a) the distribution volume of the parent drug is large and/or the clearance of the drug is high such that the vascular (plasma or blood) concentrations of the parent drug are too low to yield meaningful or reliable bioavailability parameters, (b) data for the parent drug is highly variable (30,31), and (c) the metabolite, in addition to the parent drug, exerts therapeutic activities and is present in higher concentrations than the parent drug. Some proposals are made to consider metabolite data for BA estimates of drugs that are highly metabolized in liver ($CL_{int,H} >> Q_H$) when the renal clearance of drug (CL_r) is low (32). Usually, the data on the parent drug is preferred for drugs that are poorly cleared (intrinsic clearance, $CL_{int,H} << $ hepatic blood flow rate, Q_H). Midha *et al.* (33) further commented that many of the above arguments lacked weight and came to the conclusion that the correct answers were unknown and that there was no simple generalization that could be made such that each drug/metabolite combination must be examined individually.

Recently, there is a resurgence of the use of physiologically-based pharmacokinetic (PBPK) models. PBPK models describe the essential physiological (blood flow rate, flow pattern, and intestinal transit) and biochemical (protein binding, transporters, and enzymes) factors that influence rate processes of transfer and removal of the drug and its metabolites and, in turn, highlight the disposition of these chemical species. The models describe compartments of discrete volumes that are connected to the blood compartment by flow; the unbound species traverses across the membranes and is the species that is eliminated. In the PBPK model, drug and metabolite are taken up by passive diffusion and/or via transporters and are metabolized intracellularly by enzymes or subject to excretion by apical transporters within the eliminating organs, and automatically account for sequential metabolism/excretion of the formed metabolite if such pathways exist. Analytical solutions of the AUCs (or exposure) and clearances based on the PBPK models have been solved for the liver, intestine, and kidney (34). These solutions are presently extended to the formed metabolites and are paramount to the understanding of the roles of each of the underlying variables in determining the exposure and fates of the drug and the formed metabolites. Hence, the usefulness of PBPK modeling surpasses the existing compartmental approaches (35,36). Moreover, the AUC ratios readily provide the systemic bioavailability or F_{sys}, the BA estimate, and reveal the variables affecting estimation of this parameter. However, the AUC solutions for the formed metabolite are usually based on the presence of only one eliminating organ, e.g., the intestine, liver, or kidney (34), and the AUC solutions based on a PBPK model that encompasses more than one eliminatory organ for the handling of the parent drug and metabolite are lacking. There is no doubt that such solutions on F_{sys} and drug/metabolite disposition are dependent on the transporters and enzymes among the different organs.

In this theoretical study, we revisited the question and investigated whether metabolite data might be useful or act as a surrogate for bioavailability estimates. We used pharmacokinetic theory based on PBPK modeling to understand what variables alter the AUC of the parent drug and of the formed metabolite (AUC{mi,P}) following the administration of the parent drug. With the PBPK models, we showed that the AUC of the formed metabolite (AUC{mi,P}) depended on both the metabolism and excretion characteristics and basolateral transport and efflux of the precursor drug as well as those that impact the metabolite. The usefulness of the ratio of AUC of the formed metabolite in F_{sys} and F_{abs} was discussed.

METHODS

Physiologically-Based Pharmacokinetic (PBPK) Models

Several PBPK models were described: Cases 1 to 4 showcase the intestine and/or the liver as the first-pass metabolite formation organ. In these models, the kidney is included for the excretion of drug and metabolite (Figs. 1, 2 and 3). For the sake of simplicity, the renal clearances for the excretion of drug (CL_r) and metabolite (CL_r{mi}) are assigned to the blood compartment. Other organs are lumped as highly perfused (subscripted HP) or poorly perfused (subscripted PP) tissues. The uptake and efflux intrinsic clearances for the intestine are denoted CL^I_{d1} and CL^I_{d2}, respectively, and CL^H_{d1} and CL^H_{d2} for the liver. These transfer clearances represent the sum of both the passive diffusion (CL_{diff}) and the transfer intrinsic clearances that are composed of V_{max}s, the maximum uptake velocities, and K_ms, the Michaelis-Menten constants or $\Sigma(V_{max}/K_m)$ of the carrier-mediated processes under linear condition. In the first two cases, we only considered the simplest case in which the intestine (Case 1) or the liver (Case 2) is the only formation organ for the

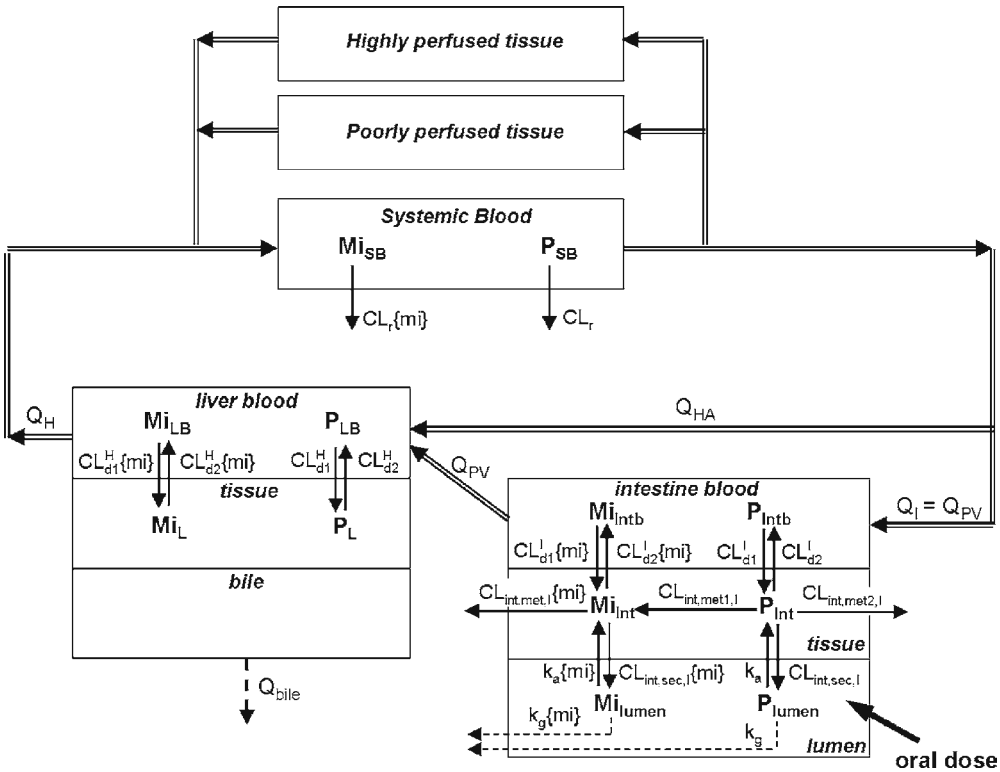

Fig. 1. A PBPK model depicting the intestine as the only tissue for metabolite formation and sequential metabolism. Metabolism of drug to other metabolites also occurs; both drug and metabolite are secreted by the intestine. The drug and metabolite distribute into the liver, but no elimination occurs within this organ; both may be excreted by the kidney (Case 1). See text for details.

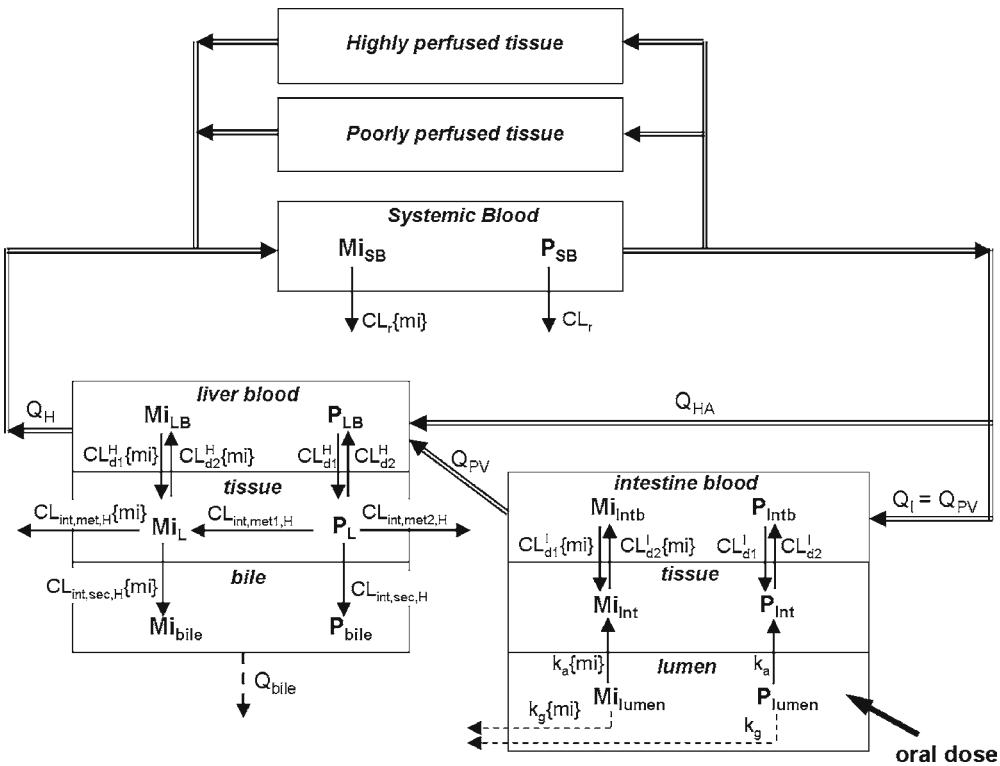

Fig. 2. A PBPK model depicting the liver as the only tissue for metabolite formation and sequential metabolism. Metabolism of drug to other metabolites also occurs; both drug and metabolite are secreted by the liver. The drug and metabolite distribute into the intestine, but no elimination occurs within this tissue; both may be excreted by the kidney (Case 2). See text for details.

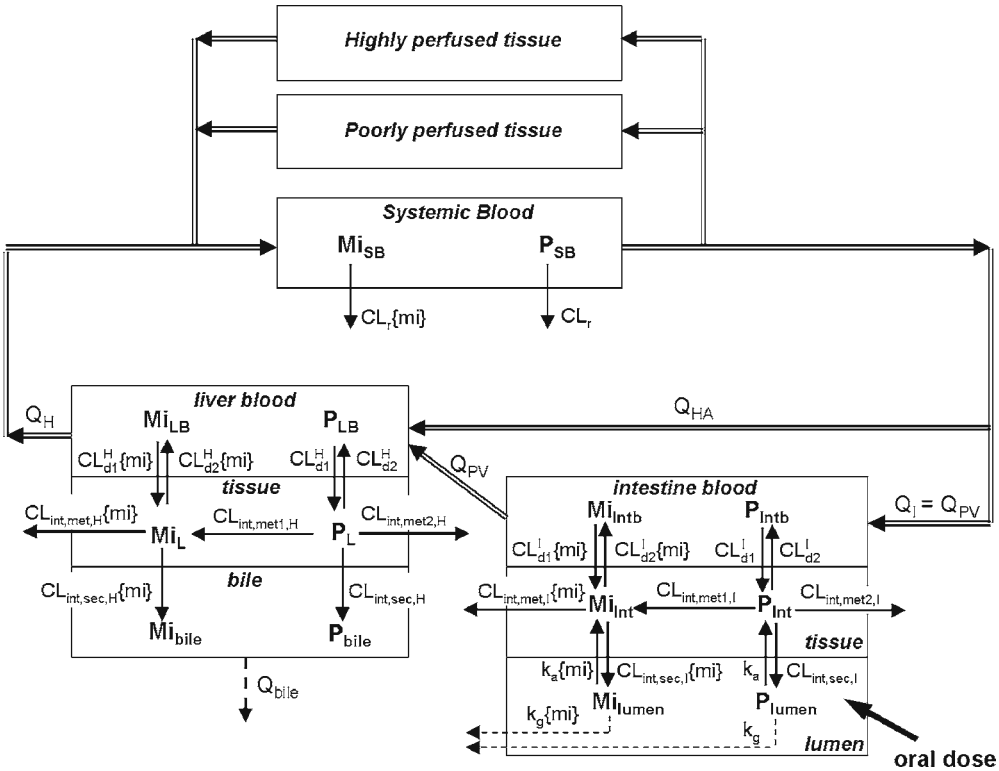

Fig. 3. A PBPK model depicting the intestine and liver as tissues for metabolite formation and sequential metabolism. For Case 3, separate metabolites are formed by the intestine and liver, respectively, and the formed metabolites can distribute into the alternate organ, but is only eliminated by the formation organ and kidney. For Case 4, the same primary metabolite is formed in both liver and intestine. For both Cases 3 and 4, both the drug and metabolite may be excreted by the kidney. See text for details.

metabolite in question, and the metabolite may be further metabolized within its formation organ but not within other eliminating organs. In the third scenario, the intestine forms one particular metabolite, whereas the liver forms another, different metabolite; the metabolite formed would only be metabolized or excreted within its organ of formation (Case 3). The last example describes that both the intestine and liver form a common metabolite; the metabolite is further metabolized in the intestine and liver (Case 4).

Case 1

PBPK models involving the intestine that consider transporters and enzymes have been used previously to describe intestinal metabolism (37). One is the traditional model (TM) and the second is the segregated flow model (SFM) (37). The latter is more appropriate, since many compounds are observed to exhibit a greater extent of metabolism with the oral (po) route *vs.* the intravenous (iv) route, or route-dependent intestinal metabolism (38). The original models are somewhat restrictive, since the drug forms only one metabolite, and the formed metabolite is further metabolized. The areas under the curve of the formed metabolite (AUC{mi,P}) from po dosing for TM and SFM are the same, whereas that from iv dosing differs in the flow term, Q_I or Q_{PV} for TM and Q_{en} (enterocyte flow, or a small portion of Q_I) for SFM (37).

Here we described a more extensive PBPK intestinal model that further considers the presence of competing, metabolic pathways for the drug and for the metabolite within the intestine (Fig. 1). The metabolite (Mi_{Int}) is formed via intestinal metabolism with $CL_{int,met1,I}$, and the drug may be metabolized to other products with intrinsic clearance, $CL_{int,met2,I}$, or is secreted with the intrinsic clearance, $CL_{int,sec,I}$. The intestinally formed metabolite (Mi_{Int}) may undergo further metabolism or secretion by the intestine, with metabolic ($CL_{int,met,I}\{mi\}$) and secretory ($CL_{int,sec,I}\{mi\}$) intrinsic clearances, respectively. The secretory intrinsic clearance represents the sum of both passive and carrier-mediated processes, denoted by $CL_{diff} + \Sigma(V_{max,sec,I}/K_{m,sec,I})$ of the secretory pathways. The drug and metabolite may be absorbed with the respective rate constants, k_a and $k_a\{mi\}$, and luminally removed by k_g and $k_g\{mi\}$. There is no hepatic elimination of either drug or metabolite.

Case 2

The liver, the most important drug and metabolite metabolizing organ, is considered as the only formation and metabolizing organ for the metabolite in question, Mi_L (Fig. 2). The drug forms the designated metabolite (Mi_L) in the liver with metabolic intrinsic clearance, $CL_{int,met1,H}$, and other metabolites with the metabolic intrinsic clearance, $CL_{int,met2,H}$, and undergoes biliary excretion with the secretory intrinsic clearance, $CL_{int,sec,H}$ (Fig. 2). There is no metabolism or excretion of either drug or metabolite by the intestine (Fig. 2). The hepatically formed metabolite (Mi_L) is further metabolized in the liver (with intrinsic clearance, $CL_{int,met,H}\{mi\}$) or excreted into bile (with intrinsic clearance, $CL_{int,sec,H}\{mi\}$).

Case 3

Case 3 brings into perspective that when the drug forms different metabolites within the intestine and liver, Mi_{Int} and Mi_L, respectively (Fig. 3), each metabolite may be metabolized and/or excreted within its organ of formation but not formed or metabolized in other tissues. With this scenario, Mi_{Int} may enter the liver but not for further processing. The same comment applies to Mi_L; this metabolite will not be eliminated by the intestinal tissue.

Case 4

Case 4 describes a more common occurrence in which the same metabolite is formed in both the intestine and the liver (Fig. 3). For Case 4, the intestine and liver both form the same metabolite, and Mi_{Int} and Mi_L are chemically identical. The metabolite formed in the intestine may enter the liver for further processing; the same applies to the hepatically formed metabolite which can enter the intestine for further processing.

Method Used to Solve AUC

The method of Sun and Pang (37) was used. The rate equations for each model were written, providing the coefficients for square matrix inversion to obtain the area under the curve of the drug and the metabolite (see Appendix). Matrix inversion was conducted with the program Maple9™ (MapleSoft, Waterloo, ON), and the solutions obtained were further simplified by LiveMath® (MathMonkeys, Cambridge, MA) to render these in a presentable format.

RESULTS

Physiologically Based Pharmacokinetic (PBPK) Models

Case 1: Metabolite Formation (with $CL_{int,met1,I}$) and Sequential Metabolism Within the Intestine Only

Upon matrix inversion, the area under the curve for the drug after po (AUC_{po}) and iv (AUC_{iv}) administration with doses $Dose_{po}$ and $Dose_{iv}$, respectively, are summarized in Table I (Case 1a). The solutions showed that the AUC_{iv} was influenced by the drug transport clearances, CL^I_{d1} and CL^I_{d2}, intestinal flow (Q_{PV}), the renal clearance (CL_r) and the intestinal metabolic ($CL_{int,met1,I}$ and $CL_{int,met2,I}$) and the secretory clearance ($CL_{int,sec,I}$), a parameter that was influenced by reabsorption and appeared as a product with the fraction $(1-F_{abs})$; the AUC_{po} was further affected by F_{abs}. Upon comparison, the dose-corrected AUC_{po}/AUC_{iv} yielded F_{sys} or F_{abs} F_I. The intestinal availability, F_I, was a complex fraction of CL^I_{d1}, CL^I_{d2}, $CL_{int,met1,I}$, $CL_{int,met2,I}$, $(1-F_{abs})$ $CL_{int,sec,I}$ and Q_{PV} (for TM) or Q_{en} (for SFM), equaled $\frac{Q_{PV}CL^I_{d2}}{Q_{PV}CL^I_{d2}+\left(Q_{PV}+CL^I_{d1}\right)\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]}$ for the TM and $\frac{Q_{en}CL^I_{d2}}{Q_{en}CL^I_{d2}+\left(Q_{en}+CL^I_{d1}\right)\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]}$ for the SFM; the renal clearance term (CL_r) was absent in the dose-corrected AUC_{po}/AUC_{iv}.

The areas under the curve of the formed metabolite following po ($AUC_{po}\{mi,P\}$) (same for TM and SFM) and iv ($AUC_{iv}\{mi,P\}$) dosing of drug were influenced by all of the parent drug parameters for transport, metabolism, and excretion, and those of the metabolite: the basolateral influx and efflux clearances of metabolite, $CL^I_{d1}\{mi\}$ and $CL^I_{d2}\{mi\}$, the metabolic ($CL_{int,met1,I}\{mi\}$) and the net secretory $[(1-F_{abs}\{mi\})$ $CL_{int,sec,I}\{mi\}]$ intrinsic clearances of the metabolite; $(1-F_{abs}\{mi\})$ equaled $[k_g\{mi\}/(k_a\{mi\} + k_g\{mi\})]$, a term, which when multiplied to $CL_{int,sec,I}\{mi\}$, denoted the net secretory intrinsic clearance of Mi_{Int}. The metabolite AUC ratio $[AUC_{po}\{mi,P\}/Dose_{po}]/[AUC_{iv}\{mi,P\}/Dose_{iv}]$ yielded the product of F_{abs} and $\left[1+\frac{CL_r\left(CL^I_{d1}+Q_{PV}\right)}{Q_{PV}CL^I_{d1}}\right]$ for the TM and $\left[1+\frac{CL_r\left(CL^I_{d1}+Q_{en}\right)}{Q_{en}CL^I_{d1}}\right]$ for the SFM. Note the presence of the term CL_r, the renal clearance of the drug, in the metabolite area ratio (Case 1a, Table I).

The solutions were much simplified in absence of renal excretion for the drug or the metabolite (CL_r and $CL_r\{mi\} = 0$; Case 1b Table I). Again, the AUC_{iv} was affected by the drug transfer and intrinsic clearances and the intestinal flow; AUC_{po} was additionally influenced by F_{abs}. Interestingly, the ratio of dose-corrected AUC_{po}/AUC_{iv} or F_{sys} ($=F_{abs}F_I$) of the drug remained the same as that in Case 1a. The AUC for the metabolite, $AUC_{po}\{mi,P\}$ and $AUC_{iv}\{mi,P\}$, were further influenced by the transfer clearances, together with the intrinsic clearances for metabolism and excretion of the metabolite, and $(1-F_{abs}\{mi\})$. The dose-corrected ratio of $AUC_{po}\{mi,P\}/AUC_{iv}\{mi,P\}$ yielded F_{abs} neatly for Case 1b and was the same for the TM and SFM. It was further deduced that $([AUC_{po}/AUC_{iv}]/[AUC_{po}\{mi,P\}/AUC_{iv}\{mi,P\}]$ furnished F_I. This condition (Case 1b) emphasized the usefulness of the metabolite area ratio in the estimation of F_{abs} and F_I for both the TM and SFM. Note, in the solutions for Case 1a and Case 1b, that the basolateral transport clearances of both the drug and metabolite in the liver (CL^H_{d1}, CL^H_{d2}, $CL^H_{d1}\{mi\}$, and $CL^H_{d2}\{mi\}$), the non-eliminating organ, were absent.

Case 2: Metabolite Formation (with $CL_{int,met1,H}$) and Sequential Metabolism in Liver Only

The solutions for AUC_{po} and AUC_{iv} of a hepatically and renally cleared drug were given in Table II (Case 2a). It was observed that the AUC_{iv} was influenced by the total liver blood flow, Q_H, the liver transport clearances, CL^H_{d1} and CL^H_{d2}, the total hepatic intrinsic clearance, $CL_{int,H}$ or sum of $CL_{int,met1,H}$, $CL_{int,met2,H}$ and $CL_{int,sec,H}$, and CL_r. AUC_{po} was further influenced by F_{abs}. The dose-corrected AUC_{po}/AUC_{iv} or the systemic availability, F_{sys}, was given by $F_{abs}F_H$, where F_H was $\left[Q_H\left(CL^H_{d2}+CL_{int,H}\right)\right]/\left[Q_H\left(CL^H_{d2}+CL_{int,H}\right)+CL^H_{d1}CL_{int,H}\right]$. For the areas under the curve for the metabolite after po and iv drug doses, additional parameters for the metabolite as well as $(1-F_{abs}\{mi\})$ appeared in the solutions (Table II for Case 2a). The ratio of the dose-corrected $AUC_{po}\{mi,P\}/AUC_{iv}\{mi,P\}$ yielded the simple product of F_{abs} and $(Q_H + CL_r)/Q_H$. Hence, if the drug renal clearance, CL_r, and Q_H are known, F_{abs} may be readily estimated.

In absence of the renal excretion of the drug ($CL_r=0$), the solutions for AUC_{po} and AUC_{iv} were simplified (Case 2b in Table II). The AUC_{iv} was influenced by the total liver blood flow, Q_H, the liver transport clearances, CL^H_{d1} and CL^H_{d2}, and $CL_{int,H}$, whereas AUC_{po} was further influenced by F_{abs}. The dose-corrected AUC ratio remained equal to $F_{abs}F_H$. For the metabolite, given that both CL_r and $CL_r\{mi\}$ equaled zero,

Table I. Solutions for AUCs and Ratio of AUCs in PBPK Case 1 (Intestinal Metabolism) Model (Fig. 1)

PBPK modeling, Case 1a - CL_r and $CL_r\{mi\} > 0$; for SFM, Q_{PV} becomes Q_{en}

$$AUC_{po} = \frac{F_{abs}Dose_{po}Q_{PV}CL^{I}_{d2}}{CL_rQ_{PV}CL^{I}_{d2}+\left[CL_rQ_{PV}+CL^{I}_{d1}(CL_r+Q_{PV})\right]\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]}$$

$$AUC_{iv} = Dose_{iv}\frac{Q_{PV}CL^{I}_{d2}+\left(Q_{PV}+CL^{I}_{d1}\right)\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]}{CL_rQ_{PV}CL^{I}_{d2}+\left[CL_rQ_{PV}+CL^{I}_{d1}(CL_r+Q_{PV})\right]\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]}$$

$$\frac{AUC_{po}/Dose_{po}}{AUC_{iv}/Dose_{iv}} = F_{sys} = F_{abs}F_I = F_{abs}\frac{Q_{PV}CL^{I}_{d2}}{Q_{PV}CL^{I}_{d2}+\left(Q_{PV}+CL^{I}_{d1}\right)\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]}$$

$$AUC_{po}\{mi,P\} = \frac{F_{abs}Dose_{po}CL_{int,met1,I}\left[CL^{I}_{d1}(CL_r+Q_{PV})+CL_rQ_{PV}\right]}{CL_rQ_{PV}CL^{I}_{d2}+\left[CL_rQ_{PV}+CL^{I}_{d1}(CL_r+Q_{PV})\right]\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]}\times\frac{Q_{PV}CL^{I}_{d2}\{mi\}}{CL_r\{mi\}Q_{PV}CL^{I}_{d2}\{mi\}+\left[CL_r\{mi\}Q_{PV}+CL^{I}_{d1}\{mi\}(CL_r\{mi\}+Q_{PV})\right]\left[CL_{int,met,I}\{mi\}+(1-F_{abs}\{mi\})CL_{int,sec,I}\{mi\}\right]}$$

$$AUC_{iv}\{mi,P\} = \frac{Dose_{iv}CL_{int,met1,I}Q_{pv}CL^{I}_{d1}}{CL_rQ_{PV}CL^{I}_{d2}+\left[CL_rQ_{PV}+CL^{I}_{d1}(CL_r+Q_{PV})\right]\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]}\times\frac{Q_{pv}CL^{I}_{d2}\{mi\}}{CL_r\{mi\}Q_{PV}CL^{I}_{d2}\{mi\}+\left[CL_r\{mi\}Q_{PV}+CL^{I}_{d1}\{mi\}(CL_r\{mi\}+Q_{PV})\right]\left[CL_{int,met,I}\{mi\}+(1-F_{abs}\{mi\})CL_{int,sec,I}\{mi\}\right]}$$

$$\frac{AUC_{po}\{mi,P\}/Dose_{po}}{AUC_{iv}\{mi,P\}/Dose_{iv}} = F_{abs}\frac{\left[CL^{I}_{d1}(CL_r+Q_{PV})+CL_rQ_{PV}\right]}{Q_{PV}CL^{I}_{d1}} = F_{abs}\left[1+\frac{CL_r\left(CL^{I}_{d1}+Q_{PV}\right)}{Q_{PV}CL^{I}_{d1}}\right]$$

PBPK modeling, Case 1b - CL_r and $CL_r\{mi\} = 0$; for SFM, Q_{PV} becomes Q_{en}

$$AUC_{po} = \frac{F_{abs}Dose_{po}CL^{I}_{d2}}{CL^{I}_{d1}\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]}$$

$$AUC_{iv} = \frac{Dose_{iv}\left(Q_{PV}CL^{I}_{d2}+\left(CL^{I}_{d1}+Q_{PV}\right)\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]\right.}{Q_{PV}CL^{I}_{d1}\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]}$$

$$\frac{AUC_{po}/Dose_{po}}{AUC_{iv}/Dose_{iv}} = F_{sys} = F_{abs}F_I = F_{abs}\frac{Q_{PV}CL^{I}_{d2}}{Q_{PV}CL^{I}_{d2}+\left(CL^{I}_{d1}+Q_{PV}\right)\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]}$$

$$AUC_{po}\{mi,P\} = \frac{F_{abs}Dose_{po}CL_{int,met1,I}CL^{I}_{d2}\{mi\}}{CL^{I}_{d1}\{mi\}\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]\left[CL_{int,met,I}\{mi\}+(1-F_{abs})CL_{int,sec,I}\{mi\}\right]}$$

$$AUC_{iv}\{mi,P\} = \frac{Dose_{iv}CL_{int,met1,I}CL^{I}_{d2}\{mi\}}{CL^{I}_{d1}\{mi\}\left[CL_{int,met,I}\{mi\}+(1-F_{abs})CL_{int,sec,I}\{mi\}\right]\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]}$$

$$\frac{AUC_{po}\{mi,P\}/Dose_{po}}{AUC_{iv}\{mi,P\}/Dose_{iv}} = F_{abs}$$

[a] F_{abs}, the fraction of dose absorbed intestinally, equals $k_a/(k_a + k_g)$, where k_a and k_g are the rate constant of absorption and luminal degradation/transit, respectively.

Table II. Solutions for AUCs and Ratio of AUCs in PBPK Case 2 (Liver Metabolism) Model (Fig. 2)

PBPK modeling, Case 2a - CL_r and $CL_r\{mi\} > 0$

$$AUC_{po} = \frac{F_{abs}Dose_{po}Q_H\left(CL_{d2}^H + CL_{int,H}\right)}{CL_rQ_H\left(CL_{d2}^H + CL_{int,H}\right) + CL_{d1}^H CL_{int,H}(CL_r + Q_H)}$$

$$AUC_{iv} = Dose_{iv}\frac{Q_H\left(CL_{d2}^H + CL_{int,H}\right) + CL_{d1}^H CL_{int,H}}{CL_rQ_H\left(CL_{d2}^H + CL_{int,H}\right) + CL_{d1}^H CL_{int,H}(CL_r + Q_H)}$$

$$\frac{AUC_{po}/Dose_{po}}{AUC_{iv}/Dose_{iv}} = F_{sys} = F_{abs}F_H = F_{abs}\frac{Q_H\left(CL_{d2}^H + CL_{int,H}\right)}{Q_H\left(CL_{d2}^H + CL_{int,H}\right) + CL_{d1}^H CL_{int,H}}$$

$$AUC_{po}\{mi,P\} = \frac{F_{abs}Dose_{po}Q_HCL_{d1}^H CL_{int,met1,H}(CL_r + Q_H)}{CL_rQ_H\left(CL_{d2}^H + CL_{int,H}\right) + CL_{d1}^H CL_{int,H}(CL_r + Q_H)} \times \frac{CL_{d2}^H\{mi\}}{CL_r\{mi\}Q_H\left(CL_{d2}^H\{mi\} + CL_{int,H}\{mi\}\right) + CL_{d1}^H\{mi\}CL_{int,H}\{mi\}(CL_r\{mi\} + Q_H)}$$

$$AUC_{iv}\{mi,P\} = \frac{Dose_{iv}Q_HCL_{d1}^H CL_{int,met1,H}}{CL_rQ_H\left(CL_{d2}^H + CL_{int,H}\right) + CL_{d1}^H CL_{int,H}(CL_r + Q_H)} \times \frac{Q_HCL_{d2}^H\{mi\}}{CL_r\{mi\}Q_H\left(CL_{d2}^H\{mi\} + CL_{int,H}\{mi\}\right) + CL_{d1}^H\{mi\}CL_{int,H}\{mi\}(CL_r\{mi\} + Q_H)}$$

$$\frac{AUC_{po}\{mi,P\}/Dose_{po}}{AUC_{iv}\{mi,P\}/Dose_{iv}} = \frac{F_{abs}(Q_H + CL_r)}{Q_H} = F_{abs}\left(1 + \frac{CL_r}{Q_H}\right)$$

PBPK modeling, Case 2b - CL_r and $CL_r\{mi\} = 0$

$$AUC_{po} = \frac{F_{abs}Dose_{po}\left(CL_{d2}^H + CL_{int,H}\right)}{CL_{d1}^H CL_{int,H}}$$

$$AUC_{iv} = \frac{Dose_{iv}\left[Q_H\left(CL_{d2}^H + CL_{int,H}\right) + CL_{d1}^H CL_{int,H}\right]}{CL_{d1}^H Q_H CL_{int,H}}$$

$$\frac{AUC_{po}/Dose_{po}}{AUC_{iv}/Dose_{iv}} = F_{sys} = F_{abs}F_H = \frac{F_{abs}Q_H\left(CL_{d2}^H + CL_{int,H}\right)}{Q_H\left(CL_{d2}^H + CL_{int,H}\right) + CL_{d1}^H CL_{int,H}}$$

$$AUC_{po}\{mi,P\} = \frac{F_{abs}Dose_{po}CL_{int,met1,H}CL_{d2}^H\{mi\}}{CL_{d1}^H\{mi\}CL_{int,H}CL_{int,H}\{mi\}}$$

$$AUC_{iv}\{mi,P\} = \frac{Dose_{iv}CL_{int,met1,H}CL_{d2}^H\{mi\}}{CL_{d1}^H\{mi\}CL_{int,H}CL_{int,H}\{mi\}}$$

$$\frac{AUC_{po}\{mi,P\}/Dose_{po}}{AUC_{iv}\{mi,P\}/Dose_{iv}} = F_{abs}$$

[a] The total hepatic blood flow (Q_H) is the sum of the blood flow from hepatic artery (Q_{HA}) and portal vein (Q_{PV}).
[b] The total hepatic intrinsic clearance ($CL_{int,H}$) is the sum of metabolic intrinsic clearances ($CL_{int,met1,H} + CL_{int,met2,H}$) and the secretory intrinsic clearance ($CL_{int,sec,H}$).

the solutions showed that additional parameters for the metabolite affected the areas under the curve for the metabolite, AUC{mi,P} after po and iv drug dosing (Case 2b in Table II). Note that the influx and efflux clearance of the parent drug at the basolateral membrane, CL^{H}_{d2} and CL^{H}_{d1}, were absent in the solutions of AUC{mi,P}s. The ratio of the dose-corrected AUC_{po}{mi,P}/AUC_{iv}{mi,P} yielded neatly F_{abs}. Again, F_H may be ascertained from [AUC_{po}/AUC_{iv}]/[AUC_{po}{mi,P} / AUC_{iv}{mi,P}]. Note in these solutions for Case 2a and Case 2b, the drug and metabolite transfer clearances in the intestine (CL^{I}_{d1}, CL^{I}_{d2}, CL^{I}_{d1}{mi}, and CL^{I}_{d2}{mi}), the non-eliminating organ, were absent.

Case 3: Intestinal (with $CL_{int,met1,I}$) and Hepatic (with $CL_{int,met1,H}$) Formation of Different Metabolites, Mi_{Int} and Mi_L, Respectively

For Case 3, each metabolite formed by the intestine or liver was metabolized or excreted within its organ of formation and in other tissues. The solutions were indeed very complex. Among the solutions, there was a clear indication that the AUC{mi,P} after oral and intravenous drug dosing was under the influence of all drug and metabolite parameters. Due to the lengthiness of the solution, only the dose-corrected AUC ratios of the drug and metabolite were presented (Table III). The ratio of AUC_{po}/AUC_{iv} yielded the systemic availability, F_{sys}, which equaled $F_{abs}F_IF_H$ (Table III), with the F_I and F_H terms being identical to those solved previously, with the intestine (Case 1) and liver (Case 2) being the only organ for metabolism (Tables I and II), respectively. The AUC_{po}{mi,P}/AUC_{iv}{mi,P} for the intestinally formed metabolite, Mi_{Int}, was modified by F_{abs}, the blood flows (Q_{PV} and Q_{HA}), and surprisingly, by the intrinsic clearances in the liver, the alternate metabolizing organ, and the influx drug intrinsic clearances for the liver (CL^{H}_{d1} and CL^{H}_{d2}) and intestine (CL^{I}_{d1} and CL^{I}_{d2}), as well as the renal clearance (CL_r). Similarly the ratio for the hepatically formed metabolite, AUC_{po}{mii,P} /AUC_{iv}{mii,P}, was modulated by F_{abs}, Q_{PV} and Q_{HA}, the intrinsic metabolic clearance of the intestine, the influx drug intrinsic clearances for the intestine (CL^{I}_{d1} and CL^{I}_{d2}) and liver (CL^{H}_{d1} and CL^{H}_{d2}) as well as the renal clearance (CL_r). The solutions for the AUC_{po} and AUC_{iv} became much simpler if the renal clearance was zero, as shown in the lower section of Table III (Case 3b); the AUC_{po}/AUC_{iv} ratio or F_{sys} was the same as for Case 3a, and $AUC\{mi,P\}_{po}/AUC\{mi,P\}_{iv}$ and AUC_{po}{mii,P} /AUC_{iv}{mii,P} were similar to those of Case 3a, with the exception that CL_r was absent. These solutions for the metabolite ratio for Case 3 differed from those of Case 1 and Case 2.

Case 4: Intestinal (with $CL_{int,met1,I}$) and Hepatic (with $CL_{int,met1,H}$) Formation Giving Rise to the Same Metabolite (Mi_{Int} is Mi_L)

For Cases 4a and 4b, the solutions for the AUCs for drug and metabolite (po and iv) were too bulky to be simplified as presentable forms (Table IV). The AUC ratio of the precursor, F_{sys}, the product of F_{abs}, F_I, and F_H, was found to be identical to that for Case 3 (Table III). For the drug whose renal elimination is negligible (CL_r=0), the AUCs for the precursor may be further simplified. However, AUC_{po}{mi,P}/AUC_{iv}{mi,P} was distinct from those for Cases 1, 2, and 3 and was very complicated.

Ratio of Area Ratios of Metabolite to Drug for po or iv Administration

The AUC ratio of formed primary metabolite to that of the precursor is often used in drug-drug interaction (DDI) studies to suggest the mechanism of interaction, whether reversible inhibition or enzyme-based DDI. Solutions for such ratios clearly showed that for Case 1 for intestinal metabolism only, $CL_{int,met1,I}$, the metabolite formation intrinsic clearance was present in the numerator, and hence the ratio was most sensitive to changes in $CL_{int,met1,I}$ caused either by a competitive inhibitor or enzyme inducer (Table V). The AUC ratio of metabolite to drug was found to be modulated by parameters pertaining to the formed metabolite and some parameters of the precursor, including the renal clearance (CL_r), influx and efflux intrinsic clearances in the intestine as well as the alternate drug elimination clearances, including the alternate pathways of intestinal metabolism ($CL_{int,met2,I}$) and secretion [$(1-F_{abs})CL_{int,sec,I}$]. For Case 2, in which metabolism only occurred in the liver, solutions for AUC ratio of metabolite to precursor again showed the formation intrinsic clearance, $CL_{int,met1,H}$, in the numerator, and hence the ratio would be most sensitive to changes in $CL_{int,met1,H}$ as modified by competitive inhibition or enzyme induction. However, the ratio was also affected by all parameters related to the formed metabolite, and the influx, efflux, and alternate metabolic and secretory intrinsic clearances for the precursor. It was noted that the renal clearance term was present in the AUC{mi,P}/AUC ratio after an oral not iv drug administration. Therefore, caution should be taken when the AUC ratio of metabolite/precursor is used to interpret data in Cases 1 and 2. The AUC ratios of metabolite/precursor were too lengthy to be presented for Cases 3 and 4. The composite findings suggest that the AUC{mi,P}/AUC would not necessarily reflect changes in $CL_{int,met1,I}$ or $CL_{int,met1,H}$ only.

DISCUSSION

Several methods have been adopted to estimate the contribution of intestinal absorption (F_{abs}), intestinal metabolism (F_I), and hepatic elimination (F_H) to the first-pass removal of drugs. One method is to compare the drug exposure (AUC) following the dosing into the intestine lumen (po), the superior mesenteric artery, portal vein, and peripheral vein (iv) (39). Alternately, blood samples may be taken from the peripheral vein, artery and portal vein following iv and po dosing to construct the various AUCs in the estimation of F_{abs}, F_I, and F_H (40). With the use of isotopically labeled drug, one is also able to administer the drug labeled with the stable isotope via one route and the unlabeled drug via another route simultaneously to yield the AUCs of labeled and unlabeled drug from the same subject so as to minimize the interindividual and intraindividual variation (40). However, this method involves the complex experimental design and is ordinarily not used in the clinical setting.

Table III. AUC and AUC Ratios for Case 3 (Fig. 3, Different Metabolites from Intestine and Liver)

PBPK modeling, Case 3a - CL_r and $CL_r\{mi\} > 0$

$$\frac{AUC_{po}/Dose_{po}}{AUC_{iv}/Dose_{iv}} = F_{sys} = F_{abs}F_I F_H = F_{abs}\left[\frac{Q_{PV}CL^I_{d2}}{Q_{PV}CL^I_{d2}+\left(Q_{PV}+CL^I_{d1}\right)\left[CL_{int,met1,I}+CL_{int,met2,I}+CL_{int,sec,I}(1-F_{abs})\right]}\right]\left[\frac{Q_H\left(CL^H_{d2}+CL_{int,H}\right)}{Q_H\left(CL^H_{d2}+CL_{int,H}\right)+CL^H_{d1}CL_{int,H}}\right]$$

For the intestinally formed metabolite,

$$\frac{AUC_{po}\{mi,P\}/Dose_{po}}{AUC_{iv}\{mi,P\}/Dose_{iv}} = F_{abs}\frac{CL^H_{d1}\left(CL^I_{d1}+Q_{PV}\right)(CL_r+Q_H)CL_{int,H}+Q_H\left[Q_{PV}\left(CL^I_{d1}+CL_r\right)+CL^I_{d1}CL_r\right]\left(CL^H_{d2}+CL_{int,H}\right)}{CL^I_{d1}Q_{PV}\left[CL^H_{d1}CL_{int,H}+Q_H\left(CL^H_{d2}+CL_{int,H}\right)\right]}$$

For the hepatically formed metabolite,

$$\frac{AUC_{po}\{mii,P\}/Dose_{po}}{AUC_{iv}\{mii,P\}/Dose_{iv}} = F_{abs}\frac{CL^I_{d2}Q_{PV}(Q_H+CL_r)}{Q_{PV}Q_H\left[CL^I_{d2}+CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]+Q_{HA}CL^I_{d1}\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]}$$

PBPK modeling, Case 3b - CL_r and $CL_r\{mi\} = 0$

$$AUC_{po} = \frac{F_{abs}Dose_{po}Q_{PV}\left(CL^H_{d2}+CL_{int,H}\right)CL^I_{d2}}{CL^H_{d1}Q_{PV}CL_{int,H}CL^I_{d2}+\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]\left[CL^I_{d1}\left[Q_{PV}\left(CL^H_{d2}+CL_{int,H}\right)+CL^H_{d1}CL_{int,H}\right]+CL^H_{d1}Q_{PV}CL_{int,H}\right]}$$

$$AUC_{iv} = \frac{Dose_{iv}}{Q_H}\frac{\left[Q_H\left(CL^H_{d2}+CL_{int,H}\right)+CL^H_{d1}CL_{int,H}\right]\left[Q_{PV}CL^I_{d2}+\left(Q_{PV}+CL^I_{d1}\right)\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]\right]}{CL^H_{d1}Q_{PV}CL_{int,H}CL^I_{d2}+\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]\left[CL^I_{d1}\left[Q_{PV}\left(CL^H_{d2}+CL_{int,H}\right)+CL^H_{d1}CL_{int,H}\right]+CL^H_{d1}Q_{PV}CL_{int,H}\right]}$$

$$\frac{AUC_{po}/Dose_{po}}{AUC_{iv}/Dose_{iv}} = F_{sys} = F_{abs}F_I F_H = F_{abs}\left[\frac{Q_{PV}CL^I_{d2}}{Q_{PV}CL^I_{d2}+\left(Q_{PV}+CL^I_{d1}\right)\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]}\right]\left[\frac{Q_H\left(CL^H_{d2}+CL_{int,H}\right)}{Q_H\left(CL^H_{d2}+CL_{int,H}\right)+CL^H_{d1}CL_{int,H}}\right]$$

For the intestinally formed metabolite, Mi_{Int}

$$AUC_{po}\{mi,P\} = \frac{F_{abs}Dose_{po}CL_{int,met1,I}\left[CL^I_{d1}CL^H_{d2}Q_{PV}+CL_{int,H}\left[CL^H_{d1}\left(CL^I_{d1}+Q_{PV}\right)+CL^I_{d1}Q_{PV}\right]\right]}{CL^H_{d1}Q_{PV}CL_{int,H}CL^I_{d2}+\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]\left[CL^I_{d1}\left[Q_{PV}\left(CL^H_{d2}+CL_{int,H}\right)+CL^H_{d1}CL_{int,H}\right]+CL^H_{d1}Q_{PV}CL_{int,H}\right]}\times$$
$$\frac{CL^I_{d2}\{mi\}}{CL_r\{mi\}Q_{PV}CL^I_{d2}\{mi\}+\left[CL_r\{mi\}Q_{PV}+CL^I_{d1}\{mi\}(CL_r\{mi\}+Q_{PV})\right]\left[CL_{int,met,I}\{mi\}+(1-F_{abs}\{mi\})CL_{int,sec,I}\{mi\}\right]}$$

$$AUC_{iv}\{mi,P\} = \frac{Dose_{iv}CL_{int,met1,I}Q_{pv}CL^I_{d1}\left[Q_H\left(CL^H_{d2}+CL_{int,H}\right)+CL^H_{d1}CL_{int,H}\right]}{CL^H_{d1}Q_{PV}CL_{int,H}CL^I_{d2}+\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]\left[CL^I_{d1}\left[Q_{PV}\left(CL^H_{d2}+CL_{int,H}\right)+CL^H_{d1}CL_{int,H}\right]+CL^H_{d1}Q_{PV}CL_{int,H}\right]}\times$$
$$\frac{Q_{PV}CL^I_{d2}\{mi\}}{CL_r\{mi\}Q_{PV}CL^I_{d2}\{mi\}+\left[CL_r\{mi\}Q_{PV}+CL^I_{d1}\{mi\}(CL_r\{mi\}+Q_{PV})\right]\left[CL_{int,met,I}\{mi\}+(1-F_{abs}\{mi\})CL_{int,sec,I}\{mi\}\right]}$$

$$\frac{AUC_{po}\{mi,P\}/Dose_{po}}{AUC_{iv}\{mi,P\}/Dose_{iv}} = F_{abs}\frac{Q_H\left[CL^I_{d1}CL^H_{d2}Q_{PV}+CL_{int,H}\left[CL^H_{d1}\left(CL^I_{d1}+Q_{PV}\right)+CL^I_{d1}Q_{PV}\right]\right]}{Q_{pv}CL^I_{d1}\left[Q_H\left(CL^H_{d2}+CL_{int,H}\right)+CL^H_{d1}CL_{int,H}\right]}$$

For the hepatically formed metabolite, Mii_L, its individual AUC{mii,P} [AUC_{po}\{mii,P\} and AUC_{iv}\{mii,P\}] are more complex than AUC_{po}\{mi,P\} and AUC_{iv}\{mi,P\}, and are not readily simplified. Only the solution of the ratio is provided below.

$$\frac{AUC_{po}\{mii,P\}/Dose_{po}}{AUC_{iv}\{mii,P\}/Dose_{iv}} = F_{abs}\frac{CL^I_{d2}Q_{PV}Q_H}{Q_{PV}Q_H\left[CL^I_{d2}+CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]+Q_{HA}CL^I_{d1}\left[\left(CL_{int,met1,I}+CL_{int,met2,I}\right)+(1-F_{abs})CL_{int,sec,I}\right]}$$

Table IV. Solutions for AUCs and Ratio of AUCs in PBPK Model, Case 4 (Fig. 3, the Same Metabolite is Formed in the Intestine and the Liver)

PBPK modeling, Case 4a - CL_r and $CL_r\{mi\} > 0$

All of the solutions for AUCs (AUC_{iv}, AUC_{po}, $AUC_{iv}\{mi,P\}$, and $AUC_{po}\{mi,P\}$) and the AUC ratio ($AUC_{po}\{mi,P\}$ /$AUC_{iv}\{mi,P\}$) were too bulky to be presented, except for F_{sys}

$$\frac{AUC_{po}/Dose_{po}}{AUC_{iv}/Dose_{iv}} = F_{sys} = F_{abs}\,F_I\,F_H = F_{abs}\left[\frac{Q_{PV}CL^I_{d2}}{\left[Q_{PV}CL^I_{d2}+\left(Q_{PV}+CL^I_{d1}\right)\right]\left[CL_{int,met1,1}+CL_{int,met2,1}+CL_{int,sec,I}\left(1-F_{abs}\right)\right]}\right]\left[\frac{Q_H\left(CL^H_{d2}+CL_{int,H}\right)}{Q_H\left(CL^H_{d2}+CL_{int,H}\right)+CL^H_{d1}CL_{int,H}}\right]$$

PBPK modeling, Case 4b - CL_r and $CL_r\{mi\} = 0$

All of the solutions for AUCs (AUC_{iv}, AUC_{po}, $AUC_{iv}\{mi,P\}$, and $AUC_{po}\{mi,P\}$) and the AUC ratio ($AUC_{po}\{mi,P\}$ /$AUC_{iv}\{mi,P\}$) were too bulky to be presented, except for F_{sys}

$$\frac{AUC_{po}/Dose_{po}}{AUC_{iv}/Dose_{iv}} = F_{sys} = F_{abs}\,F_I\,F_H = F_{abs}\left[\frac{Q_{PV}CL^I_{d2}}{\left[Q_{PV}CL^I_{d2}+\left(Q_{PV}+CL^I_{d1}\right)\right]\left[CL_{int,met1,1}+CL_{int,met2,1}+CL_{int,sec,I}\left(1-F_{abs}\right)\right]}\right]\left[\frac{Q_H\left(CL^H_{d2}+CL_{int,H}\right)}{Q_H\left(CL^H_{d2}+CL_{int,H}\right)+CL^H_{d1}CL_{int,H}}\right]$$

The deconvolution of F_{abs}, F_I, and F_H from the systemic bioavailability (F_{sys}) may be made from the inference of the presented PBPK models (41). As we had learnt from compartmental modeling, the dose-corrected ratio of AUC_{po}/AUC_{iv} provides F_{sys}, the systemic bioavailability, the product of F_{abs} and the available fractions of the first-pass organs in question, F_I and F_H. The solutions showed that use of the dose-corrected AUC{mi,P} ratio even in these simple metabolic schemes of the precursor and metabolite as surrogate indices is uncertain (Fig. 1A and B). The compartmental model offers little physiological significance and may not relate to first-pass metabolism of the intestine or liver in the formation of the metabolite, sequential metabolism of the metabolite (42), nor describe the utilization of transporters. In these compartmental cases, the ambivalence as well as ambiguity in the application of metabolite data in BA is attributed to the over-simplistic models, which do not clarify whether the formed metabolite is sequentially metabolized within its organ/tissue of formation. Rather, the formed metabolite is eliminated by an organ distinct from the organ of metabolite formation. In reality, these simple examples are rarely the case. Multiple metabolite formation is likely present, and the formed, phase I metabolite usually undergoes sequential, phase II metabolism. Sequential handling of the metabolite occurring during its time of genesis, either by metabolism or excretion, or sequential elimination of the formed metabolite, will affect the area under the curve of the formed metabolite (42). Moreover, there is the need to consider uptake or efflux transporters at the basolateral and apical membranes that enhance entry and efflux as well as excretion of drugs and their metabolites within the eliminating organs. These factors will also affect the concentration-time profiles and area under the curves. In view of the multiplicity of enzymes and transporters for the drug and the metabolite, there is the need for a better understanding of the underlying assumptions of the removal characteristics of the drug and the metabolite, and expanding the understanding to factors which affect the AUC{mi,P}, giving consideration to what are the elimination organs for drug and formation organs of the metabolite. Hence, compartmental modeling fails to elucidate the specific organ involved in metabolite formation and sequential elimination or to identify the variables that affect AUCs and organ availability of the intestine and liver.

In contrast, these variables are readily identified by PBPK modeling that aptly adds to our understanding of the extent of absorption, though not on the rate of absorption (C_{max}) at this stage. PBPK modeling reveals, with great certainty, the roles of influx and efflux transporters at the basolateral and apical membranes, enzymes, flow, and binding, as well as renal clearance of the drug in determining the AUCs. Expectedly, more complex solutions are provided for the AUC{mi,P}s that depend not only on metabolite parameters but also those for the drug, and PBPK modeling again confirms that metabolite formation is highly dependent on physiological parameters for transport, secretion and metabolism of both drug and metabolite (Tables I, II, III and IV). Hence, by definition, the AUC{mi,P} is more variable, as commented by Midha *et al.* (33), and prone to characteristics of intrinsic clearance parameters for metabolite handling. Renal drug excretion will also impact the AUC{mi,P}; when

Table V. Meanings of AUC{mi,P}/AUC for po and iv Dosing of Drug Under Different Conditions for the Renal Clearances

Case 1a: CL_r and $CL_r\{mi\} > 0$

$$\frac{AUC\{mi,P\}_{po}}{AUC_{po}} = \frac{CL_{int,met1,I}\left[CL^{I}_{d1}(CL_r+Q_{PV})+CL_rQ_{PV}\right]CL^{I}_{d2}\{mi\}}{CL^{I}_{d2}\left(CL_r\{mi\}Q_{PV}CL^{I}_{d2}\{mi\}+\left[CL_r\{mi\}Q_{PV}+CL^{I}_{d1}\{mi\}(CL_r\{mi\}+Q_{PV})\right]\left[CL_{int,met,I}\{mi\}+(1-F_{abs}\{mi\})CL_{int,sec,I}\{mi\}\right]\right)}$$

$$\frac{AUC\{mi,P\}_{iv}}{AUC_{iv}} = \frac{CL_{int,met1,I}Q_{pv}CL^{I}_{d1}}{Q_{PV}CL^{I}_{d2}+(Q_{PV}+CL^{I}_{d1})\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]} \times \frac{Q_{pv}CL^{I}_{d2}\{mi\}}{CL_r\{mi\}Q_{PV}CL^{I}_{d2}\{mi\}+\left[CL_r\{mi\}Q_{PV}+CL^{I}_{d1}\{mi\}(CL_r\{mi\}+Q_{PV})\right]\left[CL_{int,met,I}\{mi\}+(1-F_{abs}\{mi\})CL_{int,sec,I}\{mi\}\right]}$$

Case 1b: CL_r and $CL_r\{mi\} = 0$

$$\frac{AUC\{mi,P\}_{po}}{AUC_{po}} = \frac{CL_{int,met1,I}CL^{I}_{d1}CL^{I}_{d2}\{mi\}}{CL^{I}_{d2}CL^{I}_{d1}\{mi\}\left[CL_{int,met,I}\{mi\}+(1-F_{abs}\{mi\})CL_{int,sec,I}\{mi\}\right]}$$

$$\frac{AUC\{mi,P\}_{iv}}{AUC_{iv}} = \frac{CL_{int,met1,I}Q_{pv}CL^{I}_{d1}}{Q_{PV}CL^{I}_{d2}+\left(Q_{PV}+CL^{I}_{d1}\right)\left[CL_{int,met1,I}+CL_{int,met2,I}+(1-F_{abs})CL_{int,sec,I}\right]} \times \frac{CL^{I}_{d2}\{mi\}}{CL^{I}_{d1}\{mi\}\left[CL_{int,met,I}\{mi\}+(1-F_{abs}\{mi\})CL_{int,sec,I}\{mi\}\right]}$$

Case 2a: CL_r and $CL_r\{mi\} > 0$

$$\frac{AUC\{mi,P\}_{po}}{AUC_{po}} = \frac{CL^{H}_{d1}CL_{int,met1,H}(CL_r+Q_H)}{\left(CL^{H}_{d2}+CL_{int,H}\right)} \times \frac{CL^{H}_{d2}\{mi\}}{CL_r\{mi\}Q_H\left(CL^{H}_{d2}\{mi\}+CL_{int,H}\{mi\}\right)+CL^{H}_{d1}\{mi\}CL_{int,H}\{mi\}(CL_r\{mi\}+Q_H)}$$

$$\frac{AUC_{iv}\{mi,P\}}{AUC_{iv}} = \frac{Q_HCL^{H}_{d1}CL_{int,met1,H}}{Q_H\left(CL^{H}_{d2}+CL_{int,H}\right)+CL^{H}_{d1}CL_{int,H}} \times \frac{Q_HCL^{H}_{d2}\{mi\}}{CL_r\{mi\}Q_H\left(CL^{H}_{d2}\{mi\}+CL_{int,H}\{mi\}\right)+CL^{H}_{d1}\{mi\}CL_{int,H}\{mi\}(CL_r\{mi\}+Q_H)}$$

Case 2b: CL_r and $CL_r\{mi\} = 0$

$$\frac{AUC_{po}\{mi,P\}}{AUC_{po}} = \frac{CL^{H}_{d1}CL_{int,met1,H}}{\left(CL^{H}_{d2}+CL_{int,H}\right)} \times \frac{CL^{H}_{d2}\{mi\}}{CL^{H}_{d1}\{mi\}CL_{int,H}\{mi\}}$$

$$\frac{AUC\{mi,P\}_{iv}}{AUC_{iv}} = \frac{Q_HCL^{H}_{d1}CL_{int,met1,H}}{Q_H\left(CL^{H}_{d2}+CL_{int,H}\right)+CL^{H}_{d1}CL_{int,H}} \times \frac{CL^{H}_{d2}\{mi\}}{CL^{H}_{d1}\{mi\}CL_{int,H}\{mi\}}$$

Table VI. Use of the Dose-Corrected Ratio of $AUC_{po}\{mi,P\}$ and $AUC_{iv}\{mi,P\}$ to Estimate F_{abs} (Case 2)

Precursor/Metabolite pair	Species	$AUC_{po}\{mi,P\}$	$AUC_{iv}\{mi,P\}$	CL_r (l/h/kg)	F_{abs}[a] (equations from Table II)	F_{abs}[b] (according to Weiss)	F_{abs}[c] (determined experimentally)	References
PNU96391 and the N-despropyl metabolite (M1)	Rat	18 μM·h (10 mg/kg)	10 μM·h (5 mg/kg)	0.55	0.77	0.78	0.95	(40)
Amiodarone (AM) and active metabolite, desethylamiodarone (DEA)	Rat	0.35 μM·h (10 mg/kg)	0.46 μM·h (10 mg/kg)	0	0.75	0.75		(47)
Enrofloxacin and ciprofloxacin	Horse	5.40 μM·h (5 mg/kg)	6.70 μM·h (5 mg/kg)	0.00022	0.80	0.78		(48)
Lidocaine and MEGX (monoethylglycine-xylidide)[d]	Human	33.6 $mg\cdot l^{-1}\cdot min^{-1}$ (100 mg)	34.6 $mg\cdot l^{-1}\cdot min^{-1}$ (100 mg)	~0	0.97	0.97		(49)
Tilidine (prodrug) and active metabolite, nortilidine	Human	375 $ng\cdot ml^{-1}\cdot h^{-1}$ (50 mg)	364 $ng\cdot ml^{-1}\cdot h^{-1}$ (50 mg)	0.015	1.02	1.01		(51)
Losartan and EXP3174	Human	1,915 $ng\cdot ml^{-1}\cdot h^{-1}$ (50 mg)	744 $ng\cdot ml^{-1}\cdot h^{-1}$ (20 mg)	0.056	0.98	0.95		(52)
Nomifensine and 4-hydroxy-nomifensine	Human	0.46 μM·h (100 mg)	0.41 μM·h (100 mg)	0.065	1.06	1.05		(53,54)

[a] $F_{abs} = \left(\left(AUC_{po}\{mi,P\}/Dose_{po}\right)/\left(AUC_{iv}\{mi,P\}/Dose_{iv}\right)\right)/(1 + CL_r/Q_H)$, as shown in Table II. Liver blood flow rate (Q_H) for the rat and human were set as 13.8 and 1,450 ml/min, respectively, from Davis and Morris (45), and horse liver blood flow rate was set as 23.8 ml/min/kg, from Dyke *et al.* (46).

[b] $F_{abs} = F_{sys} + f_m\left(\left(AUC_{po}\{mi,P\}/Dose_{po}\right)/\left(AUC_{iv}\{mi,P\}/Dose_{iv}\right) - F_{sys}\right)$, according to Weiss (43,44).

[c] F_{abs} was determined experimentally by comparison of AUC of drug concentrations in the portal vein *vs.* time following iv and po dosing.

[d] The definition of F_{abs} may not be very accurate; intestinal metabolism of lidocaine was found in the rabbit and may exist in human (50).

the renal excretion of drug is present, the solutions for AUC{mi,P} are more complex (Tables I, II, III and IV).

These simple cases used in the illustration highlight the need to consider the handling of the drug and metabolite in eliminating organs prior to their use in BA estimates. It is clear that, for different drugs and metabolites whose elimination characteristics differed, the solutions also differed. This is the basic tenet and the most important aspect in the consideration of metabolite kinetics, since the fundamental pharmacokinetic properties differ individually and will affect the AUCs and AUC{mi,P}s differentially. As can be seen from the solutions (Tables I, II, III and IV), the AUC comparison of drug for Cases 1 to 4 yields the systemic bioavailability, F_{sys}, which equals $F_{abs}F_I$ for Case 1, F_{abs} F_H for Case 2, and $F_{abs}F_IF_H$ for Cases 3 and 4. The AUC_{po}/AUC_{iv} comparison is independent of renal drug excretion but would not reveal F_{abs} directly (Tables I, II, III and IV). Since one of our goals in developing the PBPK models has been to ascertain the usefulness of AUC{mi,P} in BA estimates, a comparison of the AUC{mi,P} after iv and po dosing is thus made. In order to consider AUC{mi,P} as a metric for comparison of BA or BE, however, we need to first consider whether renal excretion of the drug exists and whether there are other organs that form as well as metabolize the metabolite, factors that would affect the AUC{mi,P} comparisons. The ratio of AUC_{po}{mi,P}/AUC_{iv}{mi,P} tends to be less complex especially when renal drug clearance is absent (see Tables I, II, III and IV). For the intestinally or hepatically formed metabolite, the CL_r would affect the dose-corrected AUC_{po}{mi,P}/AUC_{iv}{mi,P}, whereas when CL_r is absent, the ratio neatly yields the fraction absorbed, F_{abs} for Cases 1 and 2 (Tables I, II, III and IV). Since, for Cases 1 and 2, $F_{abs}F_{organ}$ is given by AUC_{po}/AUC_{iv}, one may be able to make inferences on F_{organ} (F_I or F_H) from $(AUC_{po}/AUC_{iv})/(AUC\{mi,P\}_{po}/AUC\{mi,P\}_{iv})$ when CL_r is absent (Tables I, II, III and IV). This holds when the drug and metabolite conform to the stipulation that the metabolite sequential elimination occurs within the organ for formation, and no other metabolizing organ exists.

Thus, it is possible to implement rules on the potential use of metabolite data in BA studies when one is able to identify that the intestine or the liver is the only metabolizing organ. We surmise that that greatest use rests with Case 2, in which the liver is the predominant organ for drug metabolism. Even with renal drug excretion, F_{abs} may be obtained from AUC_{po}{mi,P}/AUC_{iv}{mi,P} or $[F_{abs}(1 + CL_r/Q_H)]$ (Table II), when the renal drug clearance is estimated from the urinary and plasma data of the parent drug and hepatic blood flow is known. With AUC_{po}/AUC_{iv} that renders F_{sys} or $F_{abs}F_H$, and armed with the metabolite area ratio, one may estimate F_H readily as F_{sys}/F_{abs}. In other cases, when the intestine and liver are both able to metabolize the drug, either to different metabolites (Case 3, Table III) or the same metabolite (Case 4, Table IV), the situation becomes extremely complex, and $[AUC_{po}/AUC_{iv}]/[AUC_{po}\{mi,P\}/AUC_{iv}\{mi,P\}]$ would not provide any clean-cut estimates. For Case 4, the relationship AUC_{po}{mi,P}/AUC_{iv}{mi,P} fails to yield useful results.

In several theoretical communications, Weiss (43,44) showed derivations of the ratio of AUC{mi,P}s following oral and intravenous drug administration using a non-compartment approach to come up with Eq. 1. The inherent assumption must have involved hepatic but not intestinal metabolism.

$$\frac{AUC\{mi,P\}_{po}/Dose_{po}}{AUC\{mi,P\}_{iv}/Dose_{iv}} = F_{abs}[F_H + (1 - F_H)/f_m] \quad (1)$$

In Eq. 1, f_m is the fraction of the intravenous dose that is eliminated hepatically, or $(1-CL_r/CL_{total})$ since $CL_{total} = CL_r + CL_H$, and F_H or $1-E_H = 1-CL_H/Q_H$. Substitution of the above relationships into Eq. 1 yields a solution which is identical to that reported for Case 2a (see Table II), suggesting that the outcomes are surprisingly similar regardless of the initial modeling approaches.

$$\begin{aligned}\frac{AUC\{mi,P\}_{po}/Dose_{po}}{AUC\{mi,P\}_{iv}/Dose_{iv}} &= F_{abs}[F_H + (1 - F_H)/f_m] \\ &= F_{abs}\left[1 - \frac{CL_H}{Q_H} + \frac{\frac{CL_H}{Q_H}}{1-\frac{CL_r}{CL_r+CL_H}}\right] \\ &= F_{abs}\left[1 + \frac{CL_r}{Q_H}\right]\end{aligned} \quad (2)$$

Weiss further predicted that, in absence of renal excretion, the ratio of metabolites furnished F_{abs} (44). A perusal of examples in the literature (Table VI) pointed to the similarity in F_{abs} values derived from the present equations shown in Table II and Weiss's method. However, Weiss's method fails to reveal how the AUC of parent drug and the formed metabolite are being influenced by transporters and enzymes. By contrast, our method for Case 2 clearly shows the influence of transporters and enzymes and considers the renal excretion of the drug. With sampling only in the blood compartment, the ratio of the metabolite areas, AUC_{po}{mi,P}/AUC_{iv}{mi,P}, directly yields F_{abs} for Case 2b and $F_{abs}\left[1 + \frac{CL_r}{Q_H}\right]$ for Case 2a; for the latter, F_{abs} may be estimated when CL_r and Q_H are known. Our method offers another distinct advantage in that the additional estimation of F_H (Case 2b) or F_I (Case 1b) may be obtained from the ratio, $[AUC_{po}/AUC_{iv}]/[AUC_{po}\{mi,P\}/AUC_{iv}\{mi,P\}]$.

One may question the usefulness of the AUC ratio of formed primary metabolite *vs.* the precursor that is often used in DDI studies to reveal or verify the mechanism of DDI, particularly enzyme-based DDI. The solutions for the ratio reveal that, although the ratio is most sensitive to changes in metabolic intrinsic clearance for formation of the primary metabolite of interest, the ratio is also modulated by many other factors (Table V). Particularly, the alternative eliminatory pathways, such as the compensatory metabolism, secretion, and renal elimination, are found to be determinants of the ratio and should be taken into account when DDI data are analyzed and interpreted.

CONCLUSIONS

In summary, the PBPK models on elimination organs provide solutions on AUC and AUC{mi,P} pursuant to po and iv drug dosing, and show that the blood flow, binding, transporters, and enzymes as important factors affect the AUCs of the drug and metabolite. The solutions stipulate the need to consider the organ of formation and elimination for the metabolite when the area ratio of the formed metabolite is used for data interpretation. The AUC{mi,P}

ratio after oral and intravenous drug dosing furnishes an estimate of the fraction absorbed (F_{abs}) when intestine or liver is the only drug-elimination organ. For these simplified conditions, the organ availability of the intestine (F_I) and liver (F_H) may be ascertained. However, the AUC ratio of the formed metabolite after oral and intravenous drug dosing differed from that of the parent drug and would not provide estimates of F_{sys}.

ACKNOWLEDGMENT

This work was supported by the Canadian Institutes for Heath Research, MOP89850.

APPENDIX

Mass balance equations and the corresponding matrices for the physiologically based pharmacokinetic model (case 1 to case 4, as shown in Figs. 1, 2 and 3)

Definition of Terminologies

Q	blood flow rate
V	blood or tissue volume
P	parent drug
Mi	the primary metabolite of interest
SB	systemic blood, used as subscripts
HP	highly perfused organ, used as subscript
PP	poorly perfused organ, used as subscript
Intb	intestinal blood, used as subscript
Int	intestinal tissue, used as subscript
Lumen	intestinal lumen, used as subscript
LB	liver blood, used as subscript
L	liver tissue, used as subscript
PV	portal vein, used as subscript
HA	hepatic artery, used as subscript
CL_r, $CL_r\{mi\}$	apparent renal clearances of the parent drug and the metabolite, Mi, respectively
CL^I_{d1}, CL^I_{d2}	basolateral influx and efflux clearances of enterocytes, respectively
$CL_{int,met1,I}$	metabolic intrinsic clearance for formation of the Mi in the intestinal tissue
$CL_{int,met2,I}$	metabolic intrinsic clearance for formation of other metabolites in the intestinal tissue
$CL_{int,sec,I}$	secretory intrinsic clearance for drug in the intestinal tissue
k_a	rate constant of drug absorption in the intestine
k_g	rate constant of intestinal transit and degradation
CL^H_{d1}, CL^H_{d2}	basolateral influx and efflux clearances of the hepatocyte, respectively
$CL_{int,met1,H}$	metabolic intrinsic clearance for formation of the metabolite of interest in liver
$CL_{int,met2,H}$	metabolic intrinsic clearance for formation of other metabolites in the liver
$CL_{int,sec,I}$	secretory intrinsic clearance of drug in the liver
{mi} and {mii}	symbols used to qualify the parameters for primary metabolites formed in intestine and other primary metabolite formed in liver for case 3

(1) Case 1 (see Fig. 1 for the model scheme)

In systemic blood (denoted by the subscript, SB),

$$V_{SB}\frac{dP_{SB}}{dt} = Q_{HP}\frac{P_{HP}}{K_{HP}} + Q_{PP}\frac{P_{PP}}{K_{PP}} + (Q_{HA}+Q_{PV})P_{LB} - (CL_r + Q_{HP} + Q_{PP} + Q_{HA} + Q_{PV})P_{SB} \quad (1)$$

$$V_{SB}\frac{dMi_{SB}}{dt} = Q_{HP}\frac{Mi_{HP}}{K_{HP}\{mi\}} + Q_{PP}\frac{Mi_{PP}}{K_{PP}\{mi\}} + (Q_{HA}+Q_{PV})Mi_{LB} - (CL_r\{mi\} + Q_{HP} + Q_{PP} + Q_{HA} + Q_{PV})Mi_{SB} \quad (2)$$

In highly perfused organs (denoted by the subscript, HP),

$$V_{HP}\frac{dP_{HP}}{dt} = Q_{HP}\left(P_{SB} - \frac{P_{HP}}{K_{HP}}\right) \quad (3)$$

$$V_{HP}\frac{dMi_{HP}}{dt} = Q_{HP}\left(Mi_{SB} - \frac{Mi_{HP}}{K_{HP}\{mi\}}\right) \quad (4)$$

In poorly perfused organs (denoted by the subscript, PP),

$$V_{PP}\frac{dP_{PP}}{dt} = Q_{PP}\left(P_{SB} - \frac{P_{PP}}{K_{PP}}\right) \quad (5)$$

$$V_{PP}\frac{dMi_{PP}}{dt} = Q_{PP}\left(Mi_{SB} - \frac{Mi_{PP}}{K_{PP}\{mi\}}\right) \quad (6)$$

In intestinal blood (denoted by the subscript, Intb),

$$V_{Intb}\frac{dP_{Intb}}{dt} = Q_{PV}P_{SB} + CL^I_{d2}P_{Int} - \left(Q_{PV} + CL^I_{d1}\right)P_{Intb} \quad (7)$$

$$V_{Intb}\frac{dMi_{Intb}}{dt} = Q_{PV}Mi_{SB} + CL^I_{d2}\{mi\}Mi_{Int} - \left(Q_{PV} + CL^I_{d1}\{mi\}\right)Mi_{Intb} \quad (8)$$

In intestinal tissue (denoted by the subscript, Int),

$$V_{Int}\frac{dP_{Int}}{dt} = CL^I_{d1}P_{Intb} + k_aP_{lumen}V_{lumen} - \left(CL^I_{d2} + CL_{int,met1,I} + CL_{int,met2,I} + CL_{int,sec,I}\right)P_{Int} \quad (9)$$

$$V_{Int}\frac{dMi_{Int}}{dt} = CL^I_{d1}\{mi\}Mi_{Intb} + k_a\{mi\}Mi_{lumen}V_{lumen} + CL_{int,met1,I}P_{Int} - \left(CL^I_{d2}\{mi\} + CL_{int,met,I}\{mi\} + CL_{int,sec,I}\{mi\}\right)Mi_{Int} \quad (10)$$

In intestinal lumen (denoted by the subscript, lumen),

$$V_{lumen}\frac{dP_{lumen}}{dt} = CL_{int,sec,I}P_{Int} - \left(k_a + k_g\right)P_{lumen}V_{lumen} \quad (11)$$

$$V_{lumen}\frac{dMi_{lumen}}{dt} = CL_{int,sec,I}\{mi\}Mi_{Int} - \left(k_a\{mi\} + k_g\{mi\}\right)Mi_{lumen}V_{lumen} \quad (12)$$

In liver blood (denoted by the subscript, LB),

$$V_{LB}\frac{dP_{LB}}{dt} = Q_{PV}P_{Intb} + Q_{HA}P_{SB} + CL_{d2}^{H}P_L - \left(Q_{PV} + Q_{HA} + CL_{d1}^{H}\right)P_{LB} \quad (13)$$

$$V_{LB}\frac{dMi_{LB}}{dt} = Q_{PV}Mi_{Intb} + Q_{HA}Mi_{SB} + CL_{d2}^{H}\{mi\}Mi_L - \left(Q_{PV} + Q_{HA} + CL_{d1}^{H}\{mi\}\right)Mi_{LB} \quad (14)$$

In liver tissue (denoted by the subscript L),

$$V_L\frac{dP_L}{dt} = CL_{d1}^{H}P_{LB} - CL_{d2}^{H}P_L \quad (15)$$

$$V_L\frac{dMi_L}{dt} = CL_{d1}^{H}\{mi\}Mi_{LB} - CL_{d2}^{H}\{mi\}Mi_L \quad (16)$$

(2) Case 2 (see Fig. 2 for the model scheme)

In systemic blood (denoted by the subscript, SB),

$$V_{SB}\frac{dP_{SB}}{dt} = Q_{HP}\frac{P_{HP}}{K_{HP}} + Q_{PP}\frac{P_{PP}}{K_{PP}} + (Q_{HA} + Q_{PV})P_{LB} - (CL_r + Q_{HP} + Q_{PP} + Q_{HA} + Q_{PV})P_{SB} \quad (17)$$

$$V_{SB}\frac{dMi_{SB}}{dt} = Q_{HP}\frac{Mi_{HP}}{K_{HP}\{mi\}} + Q_{PP}\frac{Mi_{PP}}{K_{PP}\{mi\}} + (Q_{HA} + Q_{PV})Mi_{LB} - (CL_r\{mi\} + Q_{HP} + Q_{PP} + Q_{HA} + Q_{PV})Mi_{SB} \quad (18)$$

In highly perfused organs (denoted by the subscript, HP),

$$V_{HP}\frac{dP_{HP}}{dt} = Q_{HP}\left(P_{SB} - \frac{P_{HP}}{K_{HP}}\right) \quad (19)$$

$$V_{HP}\frac{dMi_{HP}}{dt} = Q_{HP}\left(Mi_{SB} - \frac{Mi_{HP}}{K_{HP}\{mi\}}\right) \quad (20)$$

In poorly perfused organs (denoted by the subscript, PP),

$$V_{PP}\frac{dP_{PP}}{dt} = Q_{PP}\left(P_{SB} - \frac{P_{PP}}{K_{PP}}\right) \quad (21)$$

$$V_{PP}\frac{dMi_{PP}}{dt} = Q_{PP}\left(Mi_{SB} - \frac{Mi_{PP}}{K_{PP}\{mi\}}\right) \quad (22)$$

In intestinal blood (denoted by the subscript, Intb),

$$V_{Intb}\frac{dP_{Intb}}{dt} = Q_{PV}P_{SB} + CL_{d2}^{I}P_{Int} - \left(Q_{PV} + CL_{d1}^{I}\right)P_{Intb} \quad (23)$$

$$V_{Intb}\frac{dMi_{Intb}}{dt} = Q_{PV}Mi_{SB} + CL_{d2}^{I}\{mi\}Mi_{Int} - \left(Q_{PV} + CL_{d1}^{I}\{mi\}\right)Mi_{Intb} \quad (24)$$

In intestinal tissue (denoted by the subscript, Int),

$$V_{Int}\frac{dP_{Int}}{dt} = CL_{d1}^{I}P_{Intb} + k_aP_{lumen}V_{lumen} - CL_{d2}^{I}P_{Int} \quad (25)$$

$$V_{Int}\frac{dMi_{Int}}{dt} = CL_{d1}^{I}\{mi\}Mi_{Intb} - CL_{d2}^{I}\{mi\}Mi_{Int} \quad (26)$$

In intestinal lumen (denoted by the subscript, lumen),

$$V_{lumen}\frac{dP_{lumen}}{dt} = -\left(k_a + k_g\right)P_{lumen}V_{lumen} \quad (27)$$

In liver blood (denoted by the subscript, LB),

$$V_{LB}\frac{dP_{LB}}{dt} = Q_{PV}P_{Intb} + Q_{HA}P_{SB} + CL_{d2}^{H}P_L - \left(Q_{PV} + Q_{HA} + CL_{d1}^{H}\right)P_{LB} \quad (28)$$

$$V_{LB}\frac{dMi_{LB}}{dt} = Q_{PV}Mi_{Intb} + Q_{HA}Mi_{SB} + CL_{d2}^{H}\{mi\}Mi_L - \left(Q_{PV} + Q_{HA} + CL_{d1}^{H}\{mi\}\right)Mi_{LB} \quad (29)$$

In liver tissue (denoted by the subscript, L),

$$V_L\frac{dP_L}{dt} = CL_{d1}^{H}P_{LB} - \left(CL_{d2}^{H} + CL_{int,met1,H} + CL_{int,met2,H} + CL_{int,sec,H}\right)P_L \quad (30)$$

$$V_L\frac{dMi_L}{dt} = CL_{d1}^{H}\{mi\}Mi_{LB} + CL_{int,met1,H}P_L - \left(CL_{d2}^{H}\{mi\} + CL_{int,met,H}\{mi\} + CL_{int,sec,H}\{mi\}\right)Mi_L \quad (31)$$

(3) Case 3 (see Fig. 3 for the model scheme, different metabolites were formed in intestine (as metabolite mi) and liver (as metabolite mii))

In systemic blood (denoted by the subscript, SB),

$$V_{SB}\frac{dP_{SB}}{dt} = Q_{HP}\frac{P_{HP}}{K_{HP}} + Q_{PP}\frac{P_{PP}}{K_{PP}} + (Q_{HA} + Q_{PV})P_{LB} - (CL_r + Q_{HP} + Q_{PP} + Q_{HA} + Q_{PV})P_{SB} \quad (32)$$

$$V_{SB}\frac{dMi_{SB}}{dt} = Q_{HP}\frac{Mi_{HP}}{K_{HP}\{mi\}} + Q_{PP}\frac{Mi_{PP}}{K_{PP}\{mi\}} + (Q_{HA}+Q_{PV})Mi_{LB} - (CL_r\{mi\}+Q_{HP}+Q_{PP}+Q_{HA}+Q_{PV})Mi_{SB} \quad (33)$$

$$V_{SB}\frac{dMii_{SB}}{dt} = Q_{HP}\frac{Mii_{HP}}{K_{HP}\{mii\}} + Q_{PP}\frac{Mii_{PP}}{K_{PP}\{mii\}} + (Q_{HA}+Q_{PV})Mii_{LB} - (CL_r\{mii\}+Q_{HP}+Q_{PP}+Q_{HA}+Q_{PV})Mii_{SB} \quad (34)$$

In highly perfused organs (denoted by the subscript, HP),

$$V_{HP}\frac{dP_{HP}}{dt} = Q_{HP}\left(P_{SB}-\frac{P_{HP}}{K_{HP}}\right) \quad (35)$$

$$V_{HP}\frac{dMi_{HP}}{dt} = Q_{HP}\left(Mi_{SB}-\frac{Mi_{HP}}{K_{HP}\{mi\}}\right) \quad (36)$$

$$V_{HP}\frac{dMii_{HP}}{dt} = Q_{HP}\left(Mii_{SB}-\frac{Mii_{HP}}{K_{HP}\{mii\}}\right) \quad (37)$$

In poorly perfused organs (denoted by the subscript, PP),

$$V_{PP}\frac{dP_{PP}}{dt} = Q_{PP}\left(P_{SB}-\frac{P_{PP}}{K_{PP}}\right) \quad (38)$$

$$V_{PP}\frac{dMi_{PP}}{dt} = Q_{PP}\left(Mi_{SB}-\frac{Mi_{PP}}{K_{PP}\{mi\}}\right) \quad (39)$$

$$V_{PP}\frac{dMii_{PP}}{dt} = Q_{PP}\left(Mii_{SB}-\frac{Mii_{PP}}{K_{PP}\{mii\}}\right) \quad (40)$$

In intestinal blood (denoted by the subscript, Intb),

$$V_{Intb}\frac{dP_{Intb}}{dt} = Q_{PV}P_{SB} + CL^{I}_{d2}P_{Int} - \left(Q_{PV}+CL^{I}_{d1}\right)P_{Intb} \quad (41)$$

$$V_{Intb}\frac{dMi_{Intb}}{dt} = Q_{PV}Mi_{SB} + CL^{I}_{d2}\{mi\}Mi_{Int} - \left(Q_{PV}+CL^{I}_{d1}\{mi\}\right)Mi_{Intb} \quad (42)$$

$$V_{Intb}\frac{dMii_{Intb}}{dt} = Q_{PV}Mii_{SB} + CL^{I}_{d2}\{mii\}Mii_{Int} - \left(Q_{PV}+CL^{I}_{d1}\{mii\}\right)Mii_{Intb} \quad (43)$$

In intestinal tissue (denoted by the subscript, Int),

$$V_{Int}\frac{dP_{Int}}{dt} = CL^{I}_{d1}P_{Intb} + k_aP_{lumen}V_{lumen} - \left(CL^{I}_{d2}+CL_{int,met1,I}+CL_{int,met2,I}+CL_{int,sec,I}\right)P_{Int} \quad (44)$$

$$V_{Int}\frac{dMi_{Int}}{dt} = CL^{I}_{d1}\{mi\}Mi_{Intb} + k_a\{mi\}Mi_{lumen}V_{lumen} + CL_{int,met1,I}P_{Int} - \left(CL^{I}_{d2}\{mi\}+CL_{int,met,I}\{mi\}+CL_{int,sec,I}\{mi\}\right)Mi_{Int} \quad (45)$$

$$V_{Int}\frac{dMii_{Int}}{dt} = CL^{I}_{dl}\{mii\}Mii_{Intb} - CL^{I}_{d2}\{mii\}Mii_{Int} \quad (46)$$

In intestinal lumen (denoted by the subscript, lumen),

$$V_{lumen}\frac{dP_{lumen}}{dt} = CL_{int,sec,I}P_{Int} - \left(k_a+k_g\right)P_{lumen}V_{lumen} \quad (47)$$

$$V_{lumen}\frac{dMi_{lumen}}{dt} = CL_{int,sec,I}\{mi\}Mi_{Int} - \left(k_a\{mi\}+k_g\{mi\}\right)Mi_{lumen}V_{lumen} \quad (48)$$

In liver blood (denoted by the subscript, LB),

$$V_{LB}\frac{dP_{LB}}{dt} = Q_{PV}P_{Intb} + Q_{HA}P_{SB} + CL^{H}_{d2}P_{L} - \left(Q_{PV}+Q_{HA}+CL^{H}_{d1}\right)P_{LB} \quad (49)$$

$$V_{LB}\frac{dMi_{LB}}{dt} = Q_{PV}Mi_{Intb} + Q_{HA}Mi_{SB} + CL^{H}_{d2}\{mi\}Mi_{L} - \left(Q_{PV}+Q_{HA}+CL^{H}_{d1}\{mi\}\right)Mi_{LB} \quad (50)$$

$$V_{LB}\frac{dMii_{LB}}{dt} = Q_{PV}Mii_{Intb} + Q_{HA}Mii_{SB} + CL^{H}_{d2}\{mii\}Mii_{L} - \left(Q_{PV}+Q_{HA}+CL^{H}_{d1}\{mii\}\right)Mii_{LB} \quad (51)$$

In liver tissue (denoted by the subscript, L),

$$V_{L}\frac{dP_{L}}{dt} = CL^{H}_{d1}P_{LB} - \left(CL^{H}_{d2}+CL_{int,met1,H}+CL_{int,met2,H}+CL_{int,sec,H}\right)P_{L} \quad (52)$$

$$V_L \frac{dMi_L}{dt} = CL_{d1}^{H}\{mi\}Mi_{LB} - CL_{d2}^{H}\{mi\}Mi_L \quad (53)$$

$$V_L \frac{dMii_L}{dt} = CL_{d1}^{H}\{mii\}Mii_{LB} + CL_{int,met1,H}P_L - \left(CL_{d2}^{H}\{mii\} + CL_{int,met,H}\{mii\} + CL_{int,sec,H}\{mii\}\right)Mii_L \quad (54)$$

(4) Case 4 (see Fig. 3 for the model scheme, the same metabolite was formed in intestine and liver)

In systemic blood (denoted by the subscript, SB),

$$V_{SB} \frac{dP_{SB}}{dt} = Q_{HP}\frac{P_{HP}}{K_{HP}} + Q_{PP}\frac{P_{PP}}{K_{PP}} + (Q_{HA} + Q_{PV})P_{LB} - (CL_r + Q_{HP} + Q_{PP} + Q_{HA} + Q_{PV})P_{SB} \quad (55)$$

$$V_{SB} \frac{dMi_{SB}}{dt} = Q_{HP}\frac{Mi_{HP}}{K_{HP}\{mi\}} + Q_{PP}\frac{Mi_{PP}}{K_{PP}\{mi\}} + (Q_{HA} + Q_{PV})Mi_{LB} - (CL_r\{mi\} + Q_{HP} + Q_{PP} + Q_{HA} + Q_{PV})Mi_{SB} \quad (56)$$

In highly perfused organs (denoted by the subscript, HP),

$$V_{HP} \frac{dP_{HP}}{dt} = Q_{HP}\left(P_{SB} - \frac{P_{HP}}{K_{HP}}\right) \quad (57)$$

$$V_{HP} \frac{dMi_{HP}}{dt} = Q_{HP}\left(Mi_{SB} - \frac{Mi_{HP}}{K_{HP}\{mi\}}\right) \quad (58)$$

In poorly perfused organs (denoted by the subscript, PP),

$$V_{PP} \frac{dP_{PP}}{dt} = Q_{PP}\left(P_{SB} - \frac{P_{PP}}{K_{PP}}\right) \quad (59)$$

$$V_{PP} \frac{dMi_{PP}}{dt} = Q_{PP}\left(Mi_{SB} - \frac{Mi_{PP}}{K_{PP}\{mi\}}\right) \quad (60)$$

In intestinal blood (denoted by the subscript, Intb),

$$V_{Intb} \frac{dP_{Intb}}{dt} = Q_{PV}P_{SB} + CL_{d2}^{I}P_{Int} - \left(Q_{PV} + CL_{d1}^{I}\right)P_{Intb} \quad (61)$$

$$V_{Intb} \frac{dMi_{Intb}}{dt} = Q_{PV}Mi_{SB} + CL_{d2}^{I}\{mi\}Mi_{Int} - \left(Q_{PV} + CL_{d1}^{I}\{mi\}\right)Mi_{Intb} \quad (62)$$

In intestinal tissue (denoted by the subscript, Int),

$$V_{Int} \frac{dP_{Int}}{dt} = CL_{d1}^{I}P_{Intb} + k_a P_{lumen}V_{lumen} - \left(CL_{d2}^{I} + CL_{int,met1,I} + CL_{int,met2,I} + CL_{int,sec,I}\right)P_{Int} \quad (63)$$

$$V_{Int} \frac{dMi_{Int}}{dt} = CL_{d1}^{I}\{mi\}Mi_{Intb} + k_a\{mi\}Mi_{lumen}V_{lumen} + CL_{int,met1,I}P_{Int} - \left(CL_{d2}^{I}\{mi\} + CL_{int,met,I}\{mi\} + CL_{int,sec,I}\{mi\}\right)Mi_{Int} \quad (64)$$

In intestinal lumen (denoted by the subscript, lumen),

$$V_{lumen} \frac{dP_{lumen}}{dt} = CL_{int,sec,I}P_{Int} - \left(k_a + k_g\right)P_{lumen}V_{lumen} \quad (65)$$

$$V_{lumen} \frac{dMi_{lumen}}{dt} = CL_{int,sec,I}\{mi\}Mi_{Int} - \left(k_a\{mi\} + k_g\{mi\}\right)Mi_{lumen}V_{lumen} \quad (66)$$

In liver blood (denoted by the subscript, LB),

$$V_{LB} \frac{dP_{LB}}{dt} = Q_{PV}P_{Intb} + Q_{HA}P_{SB} + CL_{d2}^{H}P_L - \left(Q_{PV} + Q_{HA} + CL_{d1}^{H}\right)P_{LB} \quad (67)$$

$$V_{LB} \frac{dMi_{LB}}{dt} = Q_{PV}Mi_{Intb} + Q_{HA}Mi_{SB} + CL_{d2}^{H}\{mi\}Mi_L - \left(Q_{PV} + Q_{HA} + CL_{d1}^{H}\{mi\}\right)Mi_{LB} \quad (68)$$

In liver tissue (denoted by the subscript, L),

$$V_L \frac{dP_L}{dt} = CL_{d1}^{H}P_{LB} - \left(CL_{d2}^{H} + CL_{int,met1,H} + CL_{int,met2,H} + CL_{int,sec,H}\right)P_L \quad (69)$$

$$V_L \frac{dMi_L}{dt} = CL_{d1}^{H}\{mi\}Mi_{LB} + CL_{int,met1,H}P_L - \left(CL_{d2}^{H}\{mi\} + CL_{int,met,H}\{mi\} + CL_{int,sec,H}\{mi\}\right)Mi_L \quad (70)$$

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