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Interplay of Drug Metabolism and Transport: A Real Phenomenon or an Artifact of the Site of Measurement?

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Abstract: The interdependence of both transport and metabolism on the disposition of drugs has recently gained heightened attention in the literature, and has been termed the "interplay of transport and metabolism". Such "interplay" is observed when inhibition of biliary clearance of a drug results in an "apparent" increase in the metabolic clearance of the drug or vice versa. In this manuscript, we derived and explored through simulations a physiological-based pharmacokinetic model that integrates both transport and metabolism and explains the "apparent" dependence of hepatic clearance on both these processes. In addition, we show that the phenomenon of hepatic "transport−metabolism interplay" is a result of using the plasma concentration as a point of reference when calculating metabolic or biliary clearance, and this interplay is maximal when the drug is actively transported into the hepatocytes (i.e., hepatocyte sinusoidal influx clearance is greater than the sinusoidal efflux clearance). When the hepatic drug concentration is used as a reference point to calculate metabolic or biliary clearance, this interplay ceases to exist. A mechanistic understanding of this interplay phenomenon can be used to explain the somewhat paradoxical results that may be observed in drug−drug interaction studies when a drug is cleared by both metabolism and biliary excretion. That is, when one of these two pathways is inhibited, the other pathway appears to be induced or activated. This interplay results in an increase in hepatic drug concentrations and therefore has implications for the hepatic efficacy and toxicity of a drug.

Keywords: Drug metabolism; drug transport; interplay; hepatic clearance models; drug interactions; PBPK

Introduction

The development of the well-stirred model of hepatic drug elimination[1] was a landmark in the field of pharmacokinetics. This model mechanistically explained the relationship between the intrinsic capacity of the liver to eliminate drug (intrinsic clearance) and the actual capacity of the liver to eliminate drug (hepatic clearance).[2] Although this model has been subsequently refined (parallel tube and dispersion model), the well-stirred model has withstood the test of time and is routinely used in predicting *in vivo* drug clearance and drug interactions.[3−5] A crucial assumption made in the development of various hepatic clearance models is that the drug is assumed to instantaneously distribute between the

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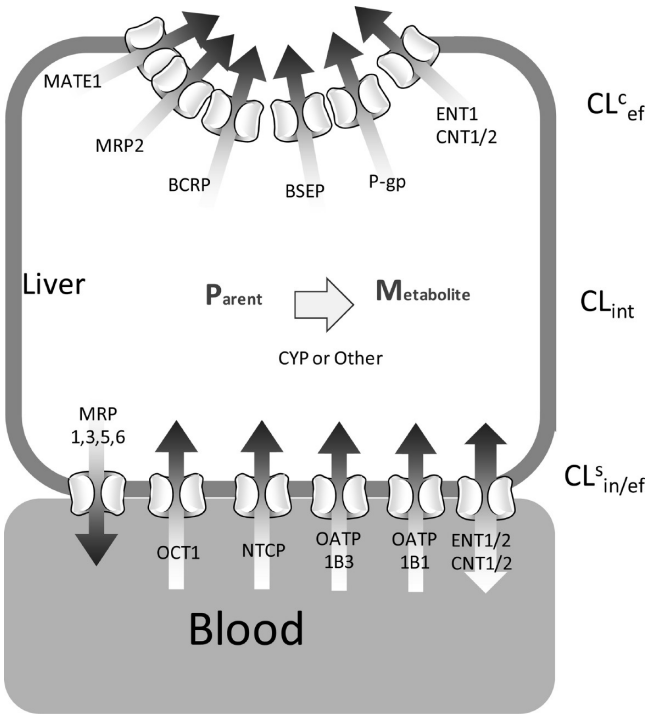

Figure 1. Cellular localization of hepatic drug transporters and drug metabolizing enzymes. Schematic of cellular localization of hepatic drug transporters and drug metabolizing enzymes. Transporters mediate the hepatic distribution into (CL^{s}_{in}) and out of (CL^{s}_{ef}) the sinusoidal membrane and biliary excretion (CL^{c}_{ef}), whereas drug metabolizing enzymes mediate hepatic metabolism (CL_{int}).

portal blood and the hepatocytes, where it becomes "available" to be eliminated by drug metabolizing enzymes. In addition, the unbound drug concentration in the hepatocytes is assumed to be equal to the unbound drug concentration in the blood perfusing the liver. With increasing awareness of the role of transporters in the influx and efflux of drugs into and out of the hepatocytes (Figure 1), it is clear that these assumptions are often violated. For example, active transport of a drug into the hepatocytes could result in hepatocyte drug concentration that is many-fold higher than blood concentration perfusing the liver. Likewise, high biliary and/or sinusoidal efflux clearance will produce hepatocyte concentration that is substantially lower than the blood concentration perfusing the liver. The consequences for violating these assumptions have begun to be addressed by various groups.[6−10] Indeed, there is now considerable reference made to the "interplay" between transporters and metabolic enzymes in the clearance of a drug.[11−18] This interplay should not be interpreted as change in "intrinsic capacity" of an enzyme to metabolize a drug when the activity of the transporter effluxing the drug out of the hepatocytes is modulated or vice versa. In this report, through simulations, we show that this "interplay" between enzymes and transporters is an artifact of the point of reference (plasma vs hepatic drug concentrations). In addition we show when this artifact is likely to result in an "apparent" change in the intrinsic capacity of an enzyme or a transporter that could be erroneously interpreted as activation or induction of these processes. Finally, we address the implication of this artifact on hepatic drug efficacy and toxicity.

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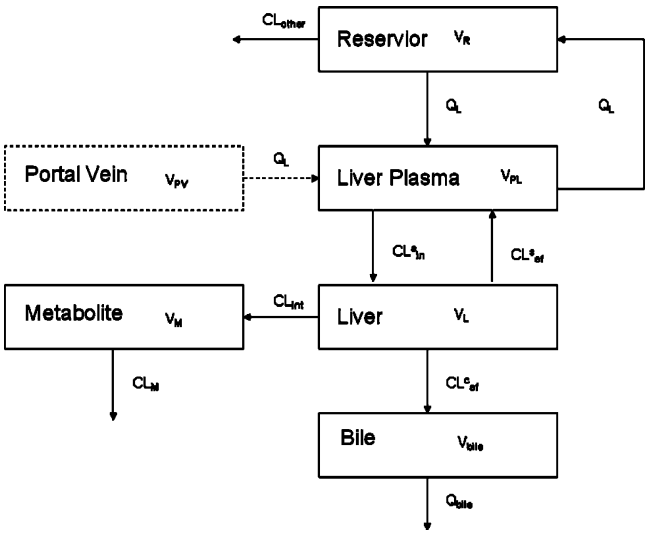

Figure 2. Physiologically based pharmacokinetic model of hepatic drug disposition. Drug enters the system as a bolus dose (D) into either the reservoir or the portal vein. Drug is eliminated from the reservoir by the nonhepatic clearance pathway (CL_{other}), or distributes into or out of the hepatic plasma by a hepatic blood flow (Q_L) limited pathway. Unbound (f_p) drug in the hepatic plasma distributes into and out of the liver by sinusoidal influx and efflux clearances (CL^s_{in} and CL^s_{ef}), which themselves are composed of a transport and a diffusional mediated component.

Experimental Section

Theory. A multicompartment PBPK model that integrates the effects of drug transport and diffusion at the sinusoidal and canalicular membrane, hepatic drug metabolism and nonhepatic drug elimination on parent drug and metabolite concentrations was derived (Figure 2). In this model, drug is administered to the reservoir into the portal vein as a bolus. Drug is eliminated either by a nonhepatic clearance pathway (CL_{other}), hepatic intrinsic metabolic clearance (CL_{int}) or hepatic intrinsic biliary efflux (CL^c_{ef}). Q_L represents hepatic blood flow. This model is an extension of the model first proposed by Sirianni and Pang.[8] The extension is an addition of a nonhepatic clearance pathway, a metabolite compartment and a portal vein dosing compartment. The inclusion of nonhepatic clearance allows for determination of various parameters (compartmental AUCs and clearance) in situations where a portion of drug elimination is mediated by a nonhepatic pathway. This is often the case, as many drugs have at least a minor component of renal clearance, and the inclusion of this integrates the contribution of nonhepatic clearance pathway on drug disposition. Additionally, the inclusion of a metabolite compartment allows prediction of hepatic metabolite concentrations, metabolic clearance and fraction metabolized, which are the primary means by which metabolic clearance is investigated *in vivo*. Finally, the inclusion of a portal vein compartment allows determination of the first-pass availability by the liver (F_H). Derivations and expressions for the dependence of F_H on the transport and metabolic intrinsic clearances are provided in the Supporting Information but, for brevity, are not discussed further.

Model Derivation. The rate equations for the model were written in the form of the fundamental pharmacokinetic parameters volume of distribution (V) and clearance (CL) in matrix form, and solved for the concentration−time profile and the AUC matrix (Supporting Information A). Mechanistically, the intrinsic distributional clearances for sinusoidal influx (CL^s_{in}) and efflux (CL^s_{ef}) and canalicular (biliary) efflux (CL^c_{ef}) can be thought of being composed of diffusional (CL_{dif}) and saturable (and potentially multiple) transporter mediated components

$$CL^s_{in} = \sum_i \frac{V_{max,i}}{C_{PL} + K_{M,i}} + CL_{dif} \quad (1)$$

$$CL^s_{ef} = \sum_i \frac{V_{max,i}}{C_L + K_{M,i}} + CL_{dif} \quad (2)$$

$$CL^c_{ef} = \sum_i \frac{V_{max,i}}{C_L + K_{M,i}} + CL_{dif} \quad (3)$$

where $\sum_i ...$ is the sum of the individual transporter mediated components, $V_{max,i}$ and $K_{M,i}$ are the V_{max} and K_M of each (ith) transporter component, and C_{PL} and C_L are the drug concentrations in the portal vein and liver respectively. Under nonsaturating conditions, eqs 1 to 3 simplify to

$$CL^s_{in} = \sum_i \frac{V_{max,i}}{K_{M,i}} + CL_{dif} \quad (4)$$

$$CL^s_{ef} = \sum_i \frac{V_{max,i}}{K_{M,i}} + CL_{dif} \quad (5)$$

$$CL^c_{ef} = \sum_i \frac{V_{max,i}}{K_{M,i}} + CL_{dif} \quad (6)$$

The intrinsic metabolic clearance is often mediated by multiple enzymes

$$CL_{int} = \sum_i \frac{V_{max,i}}{C_L + K_{M,i}} \quad (7)$$

which, also under nonsaturating conditions, simplifies to

$$CL_{int} = \sum_i \frac{V_{max,i}}{K_{M,i}} \quad (8)$$

For simplicity, we developed this model in terms of intrinsic clearances (i.e., CL^s_{in}, CL^s_{ef}, CL^c_{ef} and CL_{int}). Because of this, the general solutions are presented in terms of these parameters.

Derivation of Clearance Relationships. The clearance from the reservoir (CL_R, equivalent to systemic clearance) was determined using the definition

$$CL_R = \frac{D}{AUC_R} \quad (9)$$

where D is the dose, and AUC_R is the reservoir AUC, and was

$$CL_R = \frac{Q_L f_p CL^s_{in}(CL^c_{ef} + CL_{int})}{f_p CL^s_{in}(CL^c_{ef} + CL_{int}) + Q_L(CL^c_{ef} + CL_{int} + CL^s_{ef})} + CL_{other} \quad (10)$$

where f_p is the plasma free-fraction (reservoir and portal vein). Equation 10 can be simplified to the relationship for hepatic clearance (CL_H) developed by Sirianni and Pang:[8]

$$CL_H = \frac{Q_L f_p CL^s_{in}(CL^c_{ef} + CL_{int})}{f_p CL^s_{in}(CL^c_{ef} + CL_{int}) + Q_L(CL^c_{ef} + CL_{int} + CL^s_{ef})} \quad (11)$$

plus the additive nonhepatic clearance (CL_{other})

$$CL_R = CL_H + CL_{other} \quad (12)$$

This expression of hepatic clearance (CL_H) simplifies to the well-stirred model when there is only metabolic elimination (i.e., no biliary efflux or nonhepatic clearance), and instantaneous distribution between the hepatic plasma and the liver (i.e., the sinusoidal influx and efflux clearances are equal and well exceed intrinsic clearance, i.e., $CL^s_{in} = CL^s_{ef} \gg CL_{int}$). Because of this, the use of the well-stirred model is valid only when these conditions are met, otherwise the more general description of hepatic clearance presented here is appropriate.

Derivation of Metabolic and Biliary Clearance. The hepatic metabolic and biliary clearances were determined using two different methods. The apparent hepatic metabolic and biliary clearance were calculated by dividing the total amount metabolized or excreted by the plasma AUC, whereas the true and the more proximate hepatic metabolic and biliary clearance were calculated by dividing the same by the hepatic AUC (Supporting Information). The apparent metabolic clearance and biliary clearances were

$$CL^{app}_{metabolic} = \frac{Q_L f_p CL^s_{in} CL_{int}}{f_p CL^s_{in}(CL^c_{ef} + CL_{int}) + Q_L(CL^c_{ef} + CL_{int} + CL^s_{ef})} \quad (13)$$

$$CL^{app}_{biliary} = \frac{Q_L f_p CL^s_{in} CL^c_{ef}}{f_p CL^s_{in}(CL^c_{ef} + CL_{int}) + Q_L(CL^c_{ef} + CL_{int} + CL^s_{ef})} \quad (14)$$

and depended on f_p, Q_L and the transport (CL^s_{in}, CL^s_{ef} and CL^c_{ef}) and metabolic (CL_{int}) clearances, whereas the true metabolic

$$CL_{metabolic} = f_L CL_{int} \quad (15)$$

and true biliary clearance

$$CL_{biliary} = f_L CL^c_{ef} \quad (16)$$

depend only on the fraction unbound in the liver (f_L) and CL_{int} or CL^c_{ef}.

Simulations. All simulations were performed using Mathematica 6.0 (Wolfram Research Inc.). Surface plots were generated to show the relationship of systemic clearance (*Z*-axis) on metabolic intrinsic (CL_{int}, *X*-axis, 0 to 5 mL/min per g), and canalicular efflux clearance (CL^c_{ef}), *Y*-axis, 0 to 5 mL/min per g), at various values of sinusoidal influx and efflux (0.1, 1, 10, and 100 mL/min per g) clearance. For simplicity, hepatic blood flow was fixed at 1 mL/min per g, the plasma and hepatic free-fractions (f_p and f_L) were fixed at 1, and the nonhepatic clearance was fixed at 0 mL/min per g. Similar plots were generated to illustrate the relationship of apparent metabolic and biliary clearance with metabolic intrinsic and canalicular efflux clearance. Finally, surface plots were generated to show the relationship of true metabolic and biliary clearance with metabolic intrinsic and canalicular efflux clearance.

Results

The systemic (reservoir) clearance is a function of hepatic blood flow (Q_L), the plasma free fraction (f_p), and the transport (CL^s_{in}, CL^s_{ef}, CL^c_{ef}), metabolic (CL_{int}) intrinsic and nonhepatic (CL_{other}) clearances (eq 10). Liu and Pang have previously reported that in the absence of nonhepatic clearance (i.e., $CL_{other} = 0$) the apparent hepatic clearance is dependent only on f_L, CL_{int} and CL^c_{ef} [9] (eq 11). It is interesting to note that, in the presence of a nonhepatic elimination pathway, the apparent hepatic, metabolic and biliary clearances become dependent on the sinusoidal distributional clearances (CL^s_{in} and CL^s_{ef}), whereas in the absence of nonhepatic clearance, these clearances are independent of these parameters. However, irrespective of the value of CL_{other}, the systemic clearance is dependent on both sinusoidal uptake and efflux clearance, the canalicular efflux clearance and the intrinsic metabolic clearance.

The dependence of the systemic clearance on various values of the sinusoidal influx and efflux clearance were simulated and plotted (Figure 3) in the absence of nonhepatic clearance. The shape and maximum values of the systemic clearance surface plots depend on the distributional clearance at the sinusoidal membrane of the hepatocytes. When CL^s_{in} and CL^s_{ef} are equal and large relative to the hepatic blood flow (100-fold greater; Figure 3A), the maximal systemic clearance approaches hepatic blood flow (1.0 mL/min per g). Interestingly, CL_{sys} will be equal to Q_L only when CL^s_{in} and CL^s_{ef} are equal and much greater than Q_L.

The "plateau," or maximum systemic clearance, depends on both the sinusoidal influx and efflux clearance. As the relative magnitude of the sinusoidal influx and efflux clearance decreases with respect to hepatic blood flow (Figure 3B), the plateau of systemic clearance decreases. This is because distribution into the liver becomes the rate-limiting step in hepatic elimination. Additionally, the shape of the systemic clearance surface plot depends on the relative magnitude of CL^s_{in} and CL^s_{ef} to each other. When there is

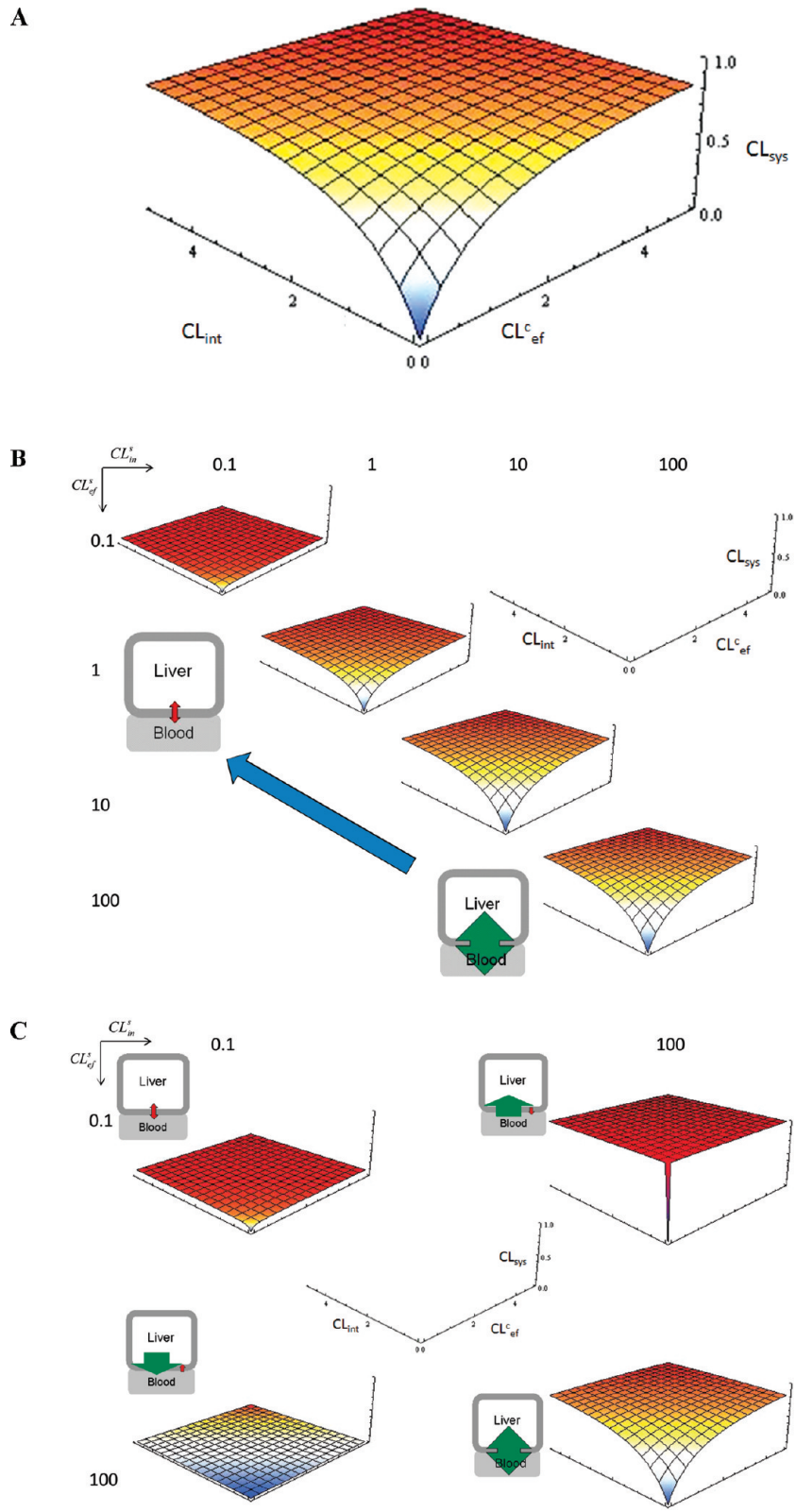

Figure 3. Systemic clearance (CL_{sys}, mL/min per g) of a drug is a function of intrinsic metabolic (CL_{int}) and canalicular efflux (CL^{c}_{ef}) clearance. (A) When sinusoidal influx and efflux are substantially greater than CL^{s}_{in} and CL^{s}_{ef} (100 mL/min per g), CL_{sys} will approach hepatic blood flow (Q_L, 1.0 mL/min per g) as CL_{int} or CL^{c}_{ef} or both exceed Q_L. (B) In contrast, when sinusoidal hepatic distribution (mL/min per g) becomes permeability rate-limited, CL_{sys} will not exceed CL^{s}_{in} even when CL_{int} or CL^{c}_{ef} or both are larger than Q_L. (C) A summary of the behavior of CL_{sys} at the extremes of CL^{s}_{in} and CL^{s}_{ef}. The fraction unbound in the plasma (f_p) was fixed at 1, and CL_{other} was fixed at 0 mL/min per g. The scale (mL/min per g) and axis labels of the inset graphs are equivalent to the reference graph.

"net uptake" at the hepatocyte sinusoidal membrane (i.e., $CL^{s}_{in} > CL^{s}_{ef}$), the shape of the curved portion of the surface is much steeper (Figure 3C, upper right). Because of this, when considering the magnitude of the change in systemic clearance that may occur from a change in either metabolic or biliary clearance, the steepness of this surface plot and the relative magnitude of both the metabolic and biliary intrinsic clearance determine the magnitude of that change. Finally, it is important to note that depending on the relative value of CL^{s}_{in}, CL^{s}_{ef}, CL_{int} and CL^{c}_{ef}, there are clearly conditions where a change in intrinsic metabolic or biliary efflux clearance will or will not result in a change in total systemic clearance. For example, when the intrinsic clearance of the elimination pathways (either metabolic or biliary efflux) is sufficiently large (e.g., 4 mL/min/g) relative to hepatic blood flow, the total hepatic clearance is blood-flow limited, and a change in one elimination pathway is insufficient to change the total hepatic clearance.

When the apparent metabolic ($CL^{app}_{metabolic}$) and biliary ($CL^{app}_{biliary}$) clearance are calculated using the reservoir AUC as the reference (see Supporting Information), these clearances become dependent not only on the pathway involved but also on a number of other model parameters. For example, the apparent biliary clearance was simulated, and plotted as a function of intrinsic metabolic and biliary efflux clearance when sinusoidal influx and efflux clearances are equal and large when compared to hepatic blood flow (Figure 4A). The upward curve in the surface plot indicates that, for a fixed value of canalicular efflux clearance (i.e., ~1.5 mL/min per g), a decrease in intrinsic metabolic clearance results in an increase in apparent biliary clearance. Additionally, the magnitude of this increase depends on the magnitude of the difference between CL^{s}_{in} and CL^{s}_{ef}, and the relative magnitude and decrease in CL^{c}_{ef} or CL_{int}. For example, when the sinusoidal influx and efflux become small and rate limiting with respect to hepatic blood flow, the steepness of this upward curve in the surface plot decreases (Figure 4B). Additionally, when CL^{s}_{in} is equal to or substantially greater than CL^{s}_{ef} (i.e., net sinusoidal influx, Figure 4C), the same decrease in intrinsic metabolic clearance results in substantially larger apparent increase in biliary clearance. For example, when CL^{s}_{in} and CL^{s}_{ef} are 10 and 0.1 mL/min per g, a decrease in CL_{int} from 2 to 0.5 mL/min per g results in a substantial increase (2.5-fold, from 0.18 to 0.45 mL/min per g) in apparent biliary clearance when CL^{c}_{ef} is low (i.e., 0.5 mL/min per g), but a relatively minor increase (1.3-fold, from 0.61 to 0.81 mL/min per g) in apparent biliary clearance when CL^{c}_{ef} is high (i.e., 4 mL/min per g). This is because when CL_{int} is large (and CL^{c}_{ef} is small), it is the dominant mechanism for clearance of the drug from the hepatocytes. When this clearance pathway is inhibited, the hepatocyte concentration of the drug increases (Figure 5A). This increase in hepatocyte concentration is not significantly modulated by redistribution to the reservoir because of the active influx of the drug. Consequently, the higher hepatocyte concentration of the drug results in an increase rate of biliary efflux ($f_L \times CL^{c}_{ef} \times C_L$). Since such inhibition does not result in a significant change in reservoir concentration (as viewed from the reservoir), such inhibition appears as an increase in hepatic clearance of the drug (rate of biliary elimination/C_R). The magnitude of change in hepatic concentration will be the largest when CL_{int} (without inhibition) is a dominant clearance mechanism (and therefore CL^{c}_{ef} is a minor contributor). For this reason, it is not surprising that the apparent increase in biliary clearance will be the largest when CL^{c}_{ef} is small. The same argument can also be presented for the reverse situation when CL^{c}_{ef} is the dominant player and CL_{int} is a minor contributor to the hepatic clearance of the drug. In this event, inhibition of CL^{c}_{ef} will result in an increase in apparent metabolic clearance and this increase will be the largest when CL_{int} is small.

Conversely, when CL^{s}_{ef} is greater than CL^{s}_{in} (Figure 4C), the same decrease in CL^{c}_{ef} would result in a relatively minor change in the apparent metabolic clearance. This is because, upon inhibition, the systemic concentration increases by a similar magnitude as the hepatic drug concentration (Figure 5B). For example, when CL^{s}_{in} and CL^{s}_{ef} are 0.1 and 10 mL/min per g, a decrease in CL_{int} from 2 to 0.5 mL/min per g results in a minor increase (1.1-fold) in apparent biliary clearance when CL^{c}_{ef} is low (i.e., 0.5 mL/min per g) and approximately the same minor increase (1.1-fold) in apparent metabolic clearance when CL^{c}_{ef} is high (i.e., 4 mL/min per g). This is because, when CL^{s}_{ef} is greater than CL^{s}_{in} (i.e., net sinusoidal efflux), changes in the hepatic concentration result in changes in the reservoir concentration. The same principles apply for the effect of inhibition of biliary efflux clearance on the apparent metabolic clearance.

This phenomenon of an "apparent" increase in metabolic clearance when biliary clearance is inhibited (and vice versa) occurs due to the drug concentration at the site of measurement (plasma/blood) not representing the drug concentration at the site of elimination (hepatocytes). Consequently, this phenomenon should disappear when the drug concentration at the site of measurement (hepatocytes) is used as a reference. Indeed it does. When the metabolic or biliary clearance were calculated using the hepatic AUC as the reference (Figure 6), these "true" clearances were dependent only on the mechanistically important clearance (i.e., CL_{int} for metabolic clearance and CL^{c}_{ef} for biliary clearance) and the free fraction in the liver (f_L). On the other hand, in the presence of a nonhepatic elimination pathway, the hepatic AUC becomes dependent on the magnitude of that pathway (CL_{other}) as well as the transport (CL^{s}_{in}, CL^{s}_{ef} and CL^{c}_{ef}) and metabolism (CL_{int}) in the liver.

Discussion

With discovery and burgeoning knowledge of transporters, there is now increased recognition that they play a vital role in the hepatic distribution and biliary elimination of drugs (Figure 1). Thus, the widely accepted hepatic clearance models (e.g., well-stirred) need to be modified to take into consideration the role of transporters in hepatic clearance of drugs. The simulations presented above delineate drug interaction conditions where hepatic transporters impact

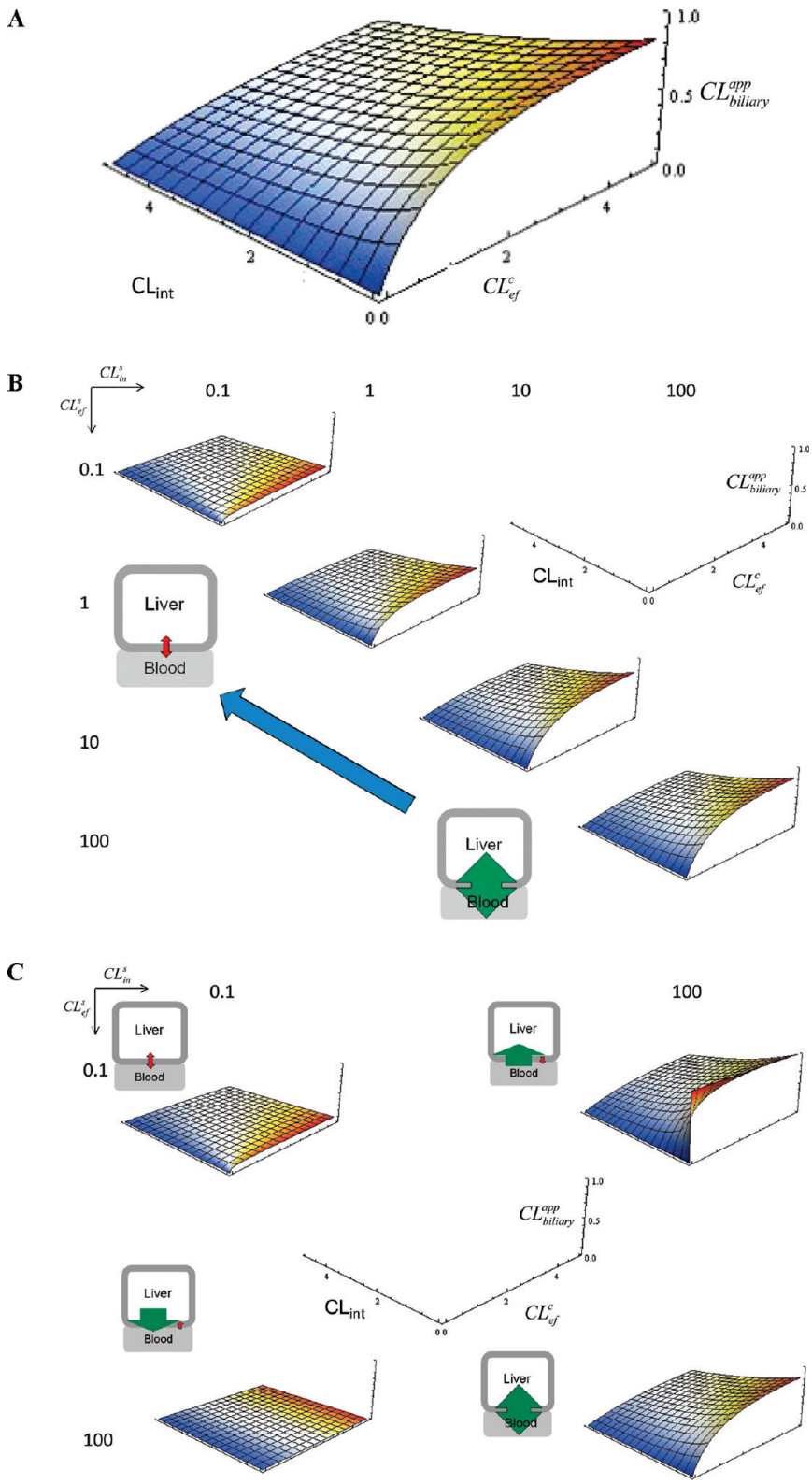

Figure 4. The apparent biliary clearance ($CL^{app}_{biliary}$) is a function of the intrinsic metabolic (CL_{int}) and canalicular efflux (CL^{c}_{ef}) clearances. (A) When sinusoidal influx and efflux are substantially greater (CL^{s}_{in} = CL^{s}_{ef} = 100 mL/min per g) than hepatic blood flow (Q_L, 1.0 mL/min per g), a decrease in CL_{int} results in an "apparent" increase in $CL^{app}_{biliary.}$ (B) This effect persists until sinusoidal hepatic distribution (mL/min per g) becomes permeability rate-limited (upper left-hand). (C) This effect is most prominent when CL^{s}_{in} is much greater than CL^{s}_{ef} and when CL_{int} is substantially inhibited (upper right-hand). When CL^{s}_{ef} is much greater than CL^{s}_{in}, the effect is absent (lower left-hand). The fraction unbound in the plasma (f_p) was fixed at 1, and CL_{other} was fixed at 0 mL/min per g. The scale (mL/min per g) and axis labels of the inset graphs are equivalent to the reference graph.

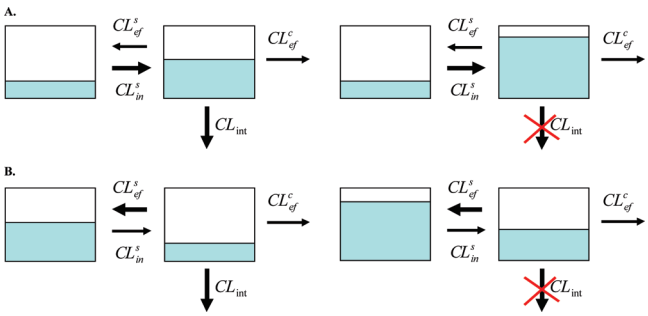

Figure 5. Illustration of interplay phenomenon when systemic concentration is used as a reference point. The effect of inhibition of metabolic intrinisic clearance is illustrated when intrinsic metabolic clearance is the dominant elimination pathway. In the case where sinusoidal influx clearance is substantially greater than sinusoidal efflux clearance (A) inhibition of hepatic metabolism results in an increase in the hepatic drug concentration. This is not reflected by an increase in systemic drug concentration. For this reason, the rate of biliary elimination (concentration × intrinsic biliary clearance) is increased resulting in an "apparent" increase in biliary clearance. In the case where sinusoidal influx clearance is substantially less than sinusoidal efflux clearance (B), a similar inhibition of metabolism results in an increase in both hepatic and systemic drug concentration. This results in an increase in the rate of biliary elimination, but the apparent biliary clearance does not change because of the systemic concentration increases by a similar magnitude.

clearance of drugs and where their inhibition could be erroneously interpreted as an "apparent" increase in the metabolic clearance of a drug or vice versa.

Liu and Pang have previously described how, in the absence of nonhepatic elimination, changes in CL^s_{in} and CL^s_{ef} can modulate the shape of the hepatic concentration−time profile without changing the hepatic AUC.[9] The solution for the hepatic clearance (using hepatic concentration as the point of reference, eq 11) clearly illustrates that, in the absence of nonhepatic clearance (CL_{other}), the hepatic clearance is independent of both CL^s_{in} and CL^s_{ef}. Because of this, while the hepatic concentration−time profile is dependent on these parameters, the hepatic clearance is independent of the distribution of drug across the sinusoidal membrane. This is because, in the absence of nonhepatic clearance, all drug must eventually be present in the liver to be eliminated. This conclusion is consistent with the observations of previous derivations of AUC_L based on similar models.[9,10]

In contrast, when biliary or metabolic clearance is computed using the plasma or blood concentration as the point of reference, an "apparent" interplay in the values of these parameters is observed. This "interplay" is best observed when biliary clearance is determined in the presence of inhibition of metabolic clearance or vice versa (Figure 4). In this event, the biliary (or metabolic) clearance is found to be increased especially when the drug is transported into the hepatocytes ($CL^s_{in} > CL^s_{ef}$). This apparent increase in

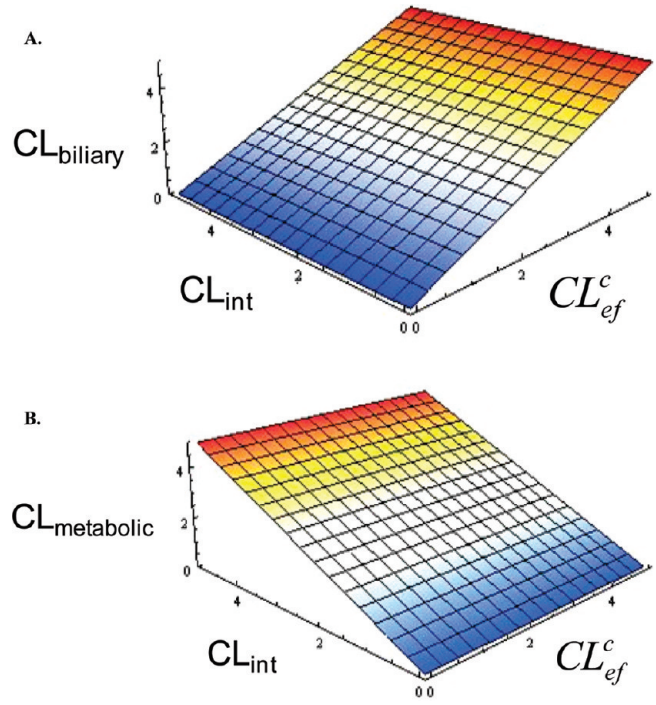

Figure 6. Hepatic and metabolic clearance when hepatic drug concentration is used as a reference point. In contrast to Figure 5, when the hepatic drug concentration is used as a reference point to compute the true metabolic ($CL_{metabolic}$) or biliary clearance ($CL_{biliary}$), the apparent interplay between the intrinsic metabolic (CL_{int}) and canalicular efflux (CL^c_{ef}) clearances disappears. The units are (mL/min per g), and the value of the fraction unbound in the liver (f_L) was fixed to 1 and that of nonhepatic clearance (CL_{other}) was fixed to 0 mL/min per g.

hepatic metabolic and biliary clearance that results from inhibition of the alternate pathway is due to the choice of the point of reference (plasma or blood concentrations). In reality, these clearances are independent as is apparent when hepatic concentrations are used as a point of reference to calculate these values (Figure 6). Furthermore, this phenomenon also occurs in the presence of enterohepatic recirculation (EHR), which, for simplicity, we have not included in this model. In the absence of EHR, the biliary clearance pathway is a true elimination pathway, as described in the model presented here. Conversely, in the presence of complete EHR (i.e., all drug excreted into the bile is reabsorbed), the biliary efflux is a purely distributional phenomenon that would affect the plasma concentrations, but not the plasma AUC. Because of this, inhibition of biliary efflux with complete EHR will result in an increase in hepatic drug concentration and therefore the rate of hepatic metabolic elimination. Consequently, in the absence of a change in plasma AUC this will manifest as an "apparent" increase in metabolic clearance. Thus, even in the presence of complete EHR, the phenomenon of transporter−metabolism interplay manifests.

While the above simulations are theoretical, the phenomenon of an apparent increase in metabolic clearance when

biliary clearance is inhibited (or vice versa) has been observed *in vivo*. For example, Lam et al. studied the effect of GG918 (GF120918, an inhibitor of P-gp) on the pharmacokinetics of erythromycin (a substrate of P-gp and CYP3A) administered intravenously (10 mg/kg) in bile-duct cannulated rats.[19] In this study, GG918 (0.25 mg/kg) significantly increased the plasma $AUC_{0-\infty}$ (~2.2-fold), and decreased the total (systemic) blood clearance (~1.9-fold) and the biliary clearance (~3.2-fold) of erythromycin, which is consistent with GG918 inhibition of erythromycin biliary clearance by P-gp. Furthermore, in the presence of GG918, the "metabolism" of erythromycin was enhanced. Specifically, 6 h after administration, the hepatic concentration of *N*-desmethyl-erythromycine (metabolite) of erythromycin (parent) was significantly increased in the presence of GG918. The authors state that this increase indicates "erythromycin metabolism increased when P-gp was inhibited." These observations are likely due to an "apparent" change in metabolic clearance. Similar results were observed in bile-duct cannulated rates, whereby ketoconazole "increased" the biliary clearance of erythromycin.[20] This "interplay" phenomenon has also been observed in humans with erythromycin.[21] In that study, the apparent metabolic clearance of CYP3A4 was assessed using the erythromycin breath test in the absence and presence of rifampin (an inhibitor of erythromycin sinusoidal influx clearance) and lansoprazole (an inhibitor of erythromycin canalicular efflux clearance). Rifampin decreased the apparent metabolic clearance of erythromycin, whereas lansoprazole increased the apparent metabolic clearance of erythromycin. These results are consistent with the predictions of the model presented here, and the *in vivo* observations in which rifampin and GG918 respectively decreased and increased the hepatic concentration of erythromycin in rats.

Based on the solutions and simulations predicted by this model, we propose a clarification of the concept that there is "interplay" between hepatic efflux and metabolic clearance. The term "interplay" suggests that these processes are mechanistically linked, while the classical clearance paradigm defines parallel clearance processes as being independent of each other. One would never suggest that, in the instance of parallel metabolic pathways, inhibition of one pathway results in an increase in the clearance of the other pathway. In the case of parallel metabolism and biliary efflux, the apparent interplay results only from the use of the plasma AUC as the reference in determining metabolic or biliary clearance. It is noted that this interpretation is often necessary, primarily because hepatic drug concentrations and AUC are unknown, but perhaps more importantly because it is considered most clinically relevant to use the plasma or, more robustly, blood concentrations as a reference. However, we caution that, mechanistically (and intuitively), metabolic and biliary efflux intrinsic clearances are independent. This is an important distinction to make, especially if successful *in vitro* to *in vivo* predictions of pharmacokinetic parameters are to be based on mechanistic principles. This does not rule out the possibility of concordant regulation of mechanistically distinct drug elimination pathways. For example, there is strong evidence of coordinately regulated drug metabolizing enzyme or drug transporter gene expression and activity, the classical example being PXR or CAR regulation of CYP3A4 and P-gp.[22−24]

In summary, we have presented a comprehensive model of hepatic clearance that incorporates the contribution of drug transporters to hepatic distribution as well as elimination by biliary excretion and metabolism. The classical and universally accepted well-stirred model is a subcondition of the model we have presented here, specifically when there is instantaneous distribution of drug between the plasma and the hepatocytes (i.e., perfusion rate limited), and when there is no biliary excretion. By incorporating both the sinusoidal and canalicular transport into this model, we have highlighted the importance of these to the total, hepatic, metabolic and biliary clearance of a drug. In addition, we have described how inhibition of biliary or metabolic clearance can result in an "apparent" interplay between these pathways. The implications of this "apparent" interplay phenomenon are directly relevant to the interpretation of experimental observations of drug−drug interaction studies where these somewhat paradoxical results are obtained. For example, inhibition of biliary efflux of a drug that is substantially cleared by this pathway will result in an apparent increase in the metabolic clearance of the drug (or vice versa). If the study is a single-dose drug-interaction study, an erroneous conclusion of activation of the metabolism of the drug may be drawn. If the study is a multiple-dose study, an erroneous conclusion of induction of metabolism may be drawn, triggering a host of additional *in vitro* and *in vivo* studies. The latter may be particularly resource intensive if a NDA application to market the drug is being submitted to the FDA.

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In addition, this interplay phenomenon also has relevance to the hepatic concentration of a drug. When biliary clearance is inhibited, hepatic drug concentrations will increase especially when $CL^{s}_{in} > CL^{s}_{ef}$. Such an increase, could, for a narrow therapeutic window drug where the liver is a target, result in increased toxicity or efficacy of the drug.

Abbreviations Used

PBPK, physiologically based pharmacokinetic; ER, extraction ratio; CL, clearance; AUC, area under the concentration−time curve; CL_{other}, nonhepatic clearance; CL_{int}, intrinsic metabolic clearance; CL^{s}_{ef}, intrinsic sinusoidal efflux clearance; CL^{s}_{in}, intrinsic sinusoidal influx clearance; CL^{c}_{ef}, intrinsic canalicular efflux clearance; CL_{dif}, diffusional clearance; CL_R, reservoir (systemic) clearance; CL_H, hepatic clearance; $CL^{app}_{metabolic}$, apparent metabolic clearance; $CL_{metabolic}$, true metabolic clearance; $CL^{app}_{biliary}$, apparent biliary clearance; $CL_{biliary}$, true biliary clearance; f_p, plasma free-fraction; f_L, hepatic free-fraction; C_R, reservoir (systemic) concentration; C_{PL}, plasma concentration; C_L, hepatic concentration; Q_L, hepatic blood flow; F_H, hepatic first-pass availability; V, volume of distribution; V_R, volume of distribution of the reservoir (central compartment); V_{PL}, volume of distribution of the hepatic plasma compartment; V_L, volume of distribution of the liver; V_M, volume of distribution of the metabolite; V_{bile}, volume of distribution of the bile compartment; CL_M, metabolite clearance; Q_{bile}, bile flow; AUC_R, systemic area under the concentration−time curve; AUC_{PL}, hepatic plasma area under the concentration−time curve; AUC_L, hepatic area under the concentration−time curve; AUC_M, metabolite area under the concentration−time curve; AUC_{bile}, bile area under the concentration−time curve; V_{max}, maximum velocity of transport or metabolism at saturation; K_M, Michaelis−Menten constant; C_{in}, compartment input concentration; C_{out}, compartment output concentration; AUC^{R}_{PV}, systemic AUC after dosing in the portal vein; AUC^{R}_{R}, systemic AUC after iv administration; A^{∞}_{M}, total amount metabolized; F_{met}, fraction metabolized; A^{∞}_{bile}, total amount excreted in the bile; F_{bile}, fraction biliary excreted; EHR, enterohepatic recirculation; PXR, pregnane X receptor; CAR, constitutive androstane receptor; CYP3A4, cytochrome P450 3A4.

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Supporting Information Available: Derivations and expressions for the dependence of F_H on the transport and metabolic intrinsic clearances. This material is available free of charge via the Internet at http://pubs.acs.org.

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