ABSTRACT:
A simple, physiological model was used to illustrate the competing nature of transporters and metabolic enzymes in hepatic drug processing. Enalapril, a drug whose basolateral influx and canalicular efflux are mediated by rat organic anion-transporting polypeptide 1 (Oatp1) and rat multidrug resistance-associated protein 2 (Mrp2), respectively, and metabolism by the carboxylesterases, was enlisted as the example to illustrate how the transport and intrinsic clearances are inter-related in the estimation of the hepatic and metabolic, and excretion clearances. Moreover, simulations were performed to explore the effects of inhibitors or inducers of transporters/enzymes to unravel the compensatory changes of alternate pathways. Generally speaking, inhibition of one pathway led to an apparent increase in the alternate (competing) pathway and total hepatic clearance was decreased; induction would lead to an apparent decrease in the alternate pathway and an increase in total hepatic clearance. A reduction in influx clearance brought about parallel decreases in the biliary and metabolic clearances, whereas a reduction in efflux basolateral clearance evoked similar increases in biliary and metabolic clearances. However, the steady-state tissue concentration (C\text{ss}) or area under the tissue concentration-time curve (AUC\text{L}) was reliant only on the unbound fraction in liver, and the secretory and metabolic intrinsic clearances and not the influx and efflux clearances. Variations in the influx and efflux intrinsic clearances evoked temporal changes in the tissue concentration-time profile but not the AUC\text{L} or C\text{ss}.

The pharmacokinetic theory developed offers data interpretation from literature reports on P-glycoprotein and cytochrome P450 substrates with mdr1a/1b knockout versus wild-type mice, and rat liver perfusion studies, with and without the use of inhibitors. In some cases, critiques on data interpretation were made.

Kinetic principles have historically been applied to appraise kinetic constants of competing enzymes. A natural observation is that the metabolic reaction rate of a given pathway will be underscored by the presence of alternate, competing metabolic pathways (Morris and Pang, 1987; Pang and Chiba, 1994). The enzymatic activities of the enzymes will be underestimated unless all competing reaction rates are considered in unison, since competing pathways diminish the intracellular substrate concentration, thereby reducing rates of reaction (Morris and Pang, 1987). An analogy may be drawn for the competing pathways of metabolism and transport. With the recognition that transporters mediate biliary excretion and basolateral efflux, transporters compete with enzymes for drug within the cell.

However, there are some issues surrounding the role of transporters and enzymes in drug removal within eliminating organs. The controversy first appeared for the simple Caco2 cell system and suggests that intestinal apical secretion prolongs the mean residence time and increases metabolism (Benet and Cummins, 2001; Johnson et al., 2001). For these considerations, total metabolite formation in the Caco2 cell system (total amount in donor, cell, and receiver compartments) has been normalized to the amount of drug in the receiver compartment (Fisher et al., 1999) or the amounts of drug in the receiver and cell compartments, namely, the extraction ratio (Cummins et al., 2002). For the Caco2 system, in which the apical, cellular, and basolateral compartments are dynamically in equilibrium, other investigators considered that total aggregate metabolism should be normalized to dose, since drug secreted into the apical compartment is subject to reabsorption back into the cell compartment and to be available for metabolism (Tam et al., 2003). Hence, secretion removes the cellular substrate from enzymes and reduces the rates of metabolism, although the extent of metabolism ultimately will not be altered (Tam et al., 2003). This view complies with those of others who state that a competing pathway detracts from other pathways due to reduction of the substrate concentration (Morris and Pang, 1987; Sirianni and Pang, 1997; Ito et al., 1999; Schuetz and Schinkel, 1999).

The liver is known for its drug-metabolizing activities and is endowed with ATP-binding cassette proteins to mediate biliary excretion at the apical membrane. Several basolateral transporters are endowed with ATP-binding cassette proteins to mediate biliary excretion at the apical membrane. Several basolateral transporters are.
present to conduct transport of organic anions, cations, and bile acids into the liver. In the past, our laboratory has utilized pharmacokinetic principles to stress the importance of competing metabolic reactions (Morris and Pang, 1987; Pang and Chiba, 1994). The physiologically based models developed include consideration of influx/exflux transporters and enzymes in the liver and kidney (Sirianni and Pang, 1997). Although enalapril was chosen as an example because of the existence of in vitro data on transport and metabolism (Pang et al., 1998; Abu-Zahra et al., 2000), the developed concepts are pertinent to all drugs. The analytical solutions may be used to predict the biliary, metabolic, and total hepatic clearances from in vitro estimates. The theory predictions serve to explain trends in the observed data, regardless of whether the well stirred model or parallel tube model assumptions apply. The concepts developed should allow us to quantitatively explain altered changes in transport or metabolism for most drugs upon addition of inhibitors or inducers and assess their impact on the concentration in liver.

We wish to illustrate this theory with existing data sets for enalapril, a drug that is metabolized by the carboxylesterases to enalaprilat (Pang et al., 1984; de Lambey et al., 1989; Pang et al., 1998), excreted by Mrp2 (Pang et al., 2002), and transported into the liver cell at the basolateral membrane by the rat organic anion-transporting polypeptide 1 (Oatp1a1) (Pang et al., 1998) (Fig. 1). We predict that decreased biliary excretion results in an increase in the metabolic clearance but a reduction in the total hepatic clearance, whereas increases in biliary excretion would lead to reduction in metabolic clearance and increased total hepatic clearance. The corollary holds for decreased/increased metabolism. We utilize this theoretical treatment to show the usefulness of this approach to explain the possible mechanism for sets of observations. Any inconsistency between theory and data suggests that the proposed mechanism alone cannot explain the set of observed changes.

**Theory**

Sirianni and Pang (1997) had used a physiological model that describes drug removal from the liver, based on the simplified view of a recirculating liver perfusion system (Fig. 2), and had solved for the determinants of clearance. There remains, however, some confusion surrounding the definition of the (metabolic or excretion) intrinsic clearance, both in the absence or presence of basolateral influx and efflux transporters. The term intrinsic clearance (CLint) is used to define the activity pertaining to transport or metabolism; multiplication of the intrinsic clearance to the (unbound) substrate concentration [S] provides the rate. As shown in eq. 1 below, the rate also relates to the Vmax and Kst, the maximum velocity and the Michaelis-Menten constant of the saturable process for basolateral transport, excretion, or metabolism.

**TABLE 1**

<table>
<thead>
<tr>
<th>Clearance Terms</th>
<th>Solutions Depending on Whether CLinflux and CLefflux Are Rapid at Basolateral Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLinflux, CLefflux Are Comparable to QL, CLint,met, and CLint,sec</td>
<td>QL, fCLinflux, and fCLint,met</td>
</tr>
<tr>
<td>CLinflux, CLefflux Are Rapid at Basolateral Membrane</td>
<td>QL, fCLinflux, and fCLint,met</td>
</tr>
</tbody>
</table>

Figure 2. A schematic depiction of the liver in a physiological model. The liver is divided into three compartments: the reservoir (R, or blood compartment), liver plasma (PL), and tissue (L); excretion occurs with drug and metabolite appearing in the bile compartment. Q, C, and V represent flow, concentration, and volume, respectively, and are further qualified by the subscripts for the various compartments, whereas the unbound fractions in plasma and liver are denoted by subscripts P and L, respectively. The transport clearances across the sinusoidal membrane for drug from hepatic plasma to tissue and from tissue to hepatic plasma are characterized by influx (CLinflux) and efflux (CLefflux) clearances, respectively. Drug within tissue is metabolized by enzymes of intrinsic clearance, CLint,met (Vmax/Km for first-order conditions). Biliary excretion of drug is a function of the biliary intrinsic clearance, CLint,sec, across the canalicular membrane.

Solved equations on the biliary excretion clearance, metabolic clearance, and total hepatic clearance by Sirianni and Pang (1997)

These equations are reduced in complexity when CLinflux and CLefflux Are Comparable to QL, CLint,met, and CLint,sec; QL is the plasma flow rate to the liver.

![Diagram of the liver in a physiological model](image-url)
### Table 2

Estimation of the influx (CL\textsubscript{influx}), efflux (CL\textsubscript{efflux}), metabolic intrinsic clearance (CL\textsubscript{int,met}), and excretory intrinsic clearance (CL\textsubscript{int,sec}) consistent with the clearances observed for the single-pass perfused rat liver preparation.

<table>
<thead>
<tr>
<th></th>
<th>Observed</th>
<th>Predicted by Sirianni and Pang (1997)</th>
<th>New and Improved Predictions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic metabolic clearance</td>
<td>CL\textsubscript{v,met} (ml/min/g)</td>
<td>0.345&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.348</td>
</tr>
<tr>
<td>Biliary clearance</td>
<td>CL\textsubscript{v,ex} (ml/min/g)</td>
<td>0.022&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.017</td>
</tr>
<tr>
<td>Total hepatic clearance</td>
<td>CL\textsubscript{liver} (ml/min/g)</td>
<td>0.366&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.365</td>
</tr>
<tr>
<td>Observed CL\textsubscript{v,ex}/CL\textsubscript{v,met}</td>
<td>1.5 to 19&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL\textsubscript{int,met} (ml/min/g)</td>
<td>15.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>20.1</td>
<td>15.7</td>
</tr>
<tr>
<td>CL\textsubscript{int,sec} (ml/min/g)</td>
<td>2.13</td>
<td>2.13</td>
<td>1.46</td>
</tr>
<tr>
<td>CL\textsubscript{int,met} (ml/min/g)</td>
<td>2.72</td>
<td>0.61&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CL\textsubscript{int,met} (ml/min/g)</td>
<td>0.135</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td>Calculated CL\textsubscript{int,met}/CL\textsubscript{int,sec}</td>
<td>6.56&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.78&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.56&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>CL\textsubscript{influx}/CL\textsubscript{efflux}</td>
<td>0.43</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>CL\textsubscript{influx}/CL\textsubscript{int,met}</td>
<td>1.00</td>
<td>2.7</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on data of deLannoy et al. (1993).
<sup>b</sup> Observed by Pang et al. (1991).
<sup>c</sup> Based on equations.
<sup>d</sup> Did not agree with observations of Abu-Zahra et al. (2000) and Abu-Zahra and Pang (2000).
<sup>e</sup> Based on data of deLannoy et al. (1993).

From this equation, the overall removal rate normalized by the arterial concentration (overall removal rate/C\textsubscript{A}) is the total hepatic clearance, CL\textsubscript{liver,met}, which is related to all the transport and metabolic intrinsic clearance terms as well as Q\textsubscript{L} and f\textsubscript{p}.

Overall removal rate =

\[ Q\textsubscript{L}/(CL\textsubscript{influx} + CL\textsubscript{int,sec} + CL\textsubscript{int,met}) + f\textsubscript{p}CL\textsubscript{influx}/(CL\textsubscript{efflux} + CL\textsubscript{int,sec} + CL\textsubscript{int,met} + Q\textsubscript{L}) \]

(3)

### Table 3

Kinetic parameters for metabolism of enalapril by S9 enzymes derived from homogeneous hepatocytes, and the transport constants derived from initial uptake velocities by homogeneous isolated rat hepatocytes (data of Abu-Zahra et al., 2000; Abu-Zahra and Pang, 2000).

<table>
<thead>
<tr>
<th></th>
<th>( K_m )</th>
<th>( V_{max} )</th>
<th>CL\textsubscript{int} = ( V_{max}/K_m )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneous hepatocytes (S9 metabolism)</td>
<td>1308 ± 419</td>
<td>8 ± 3 nmol/min/mg S9 protein</td>
<td>0.61</td>
</tr>
<tr>
<td>Homogeneous hepatocytes (transport)</td>
<td>344 to 361</td>
<td>11.0 to 11.6 nmol/min/10&lt;sup&gt;6&lt;/sup&gt; cells</td>
<td>4.00</td>
</tr>
<tr>
<td>Transport/metabolism</td>
<td>1,425 nmol/min/g liver&lt;sup&gt;a&lt;/sup&gt;</td>
<td>800 nmol/min/g liver&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.56</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on 100 mg of protein per g of liver.
<sup>b</sup> Based on 130 × 10<sup>6</sup> cells per g of liver (Zahlten and Stratman, 1974).
perfusion experiments (A. J. Schwab and K. S. Pang, unpublished data) for influx. By assigning an equal value for efflux (CL_{influx} = 2.13 ml/min/g), they solved for the metabolic and secretory intrinsic clearances of enalapril so as to reflect the binding and liver data of de Lannoy et al. (1989, 1993), the ratio of metabolic/excretion clearance, yields the secretory intrinsic clearance. According to the observations of de Lannoy et al. (1993), the ratio of metabolic/excretion clearances should be 15.7. With 0.61 ml/min/g as CL_{int,met}, the initial uptake velocity in hepatocytes as 1.46 ml/min/g. The choices would yield consistent values: 15.7 as the ratio of CL_{int,met}/CL_{int,sec}, and 6.56 for CL_{influx}/CL_{int,met}. These outcomes are more congruent with the transport and metabolic data of Abu-Zahra et al. (2000) and Abu-Zahra and Pang (2000). By contrast, metabolism in vitro (Abu-Zahra et al., 2000) would serve as the building blocks for our improved estimation within the physiological model. Using the same strategy of Sirianni and Pang (1997), we updated the estimates of transport and metabolism (as well as secretion) intrinsic clearances in the physiologically based model. The ratio of the biliary (CL_{liver,ex}) and metabolic (CL_{liver,met}) clearances (same as CL_{int,sec}/CL_{int,met}), when multiplied to the metabolic intrinsic clearance, yields the secretory intrinsic clearance. According to the observations of de Lannoy et al. (1993), the ratio of metabolic/excretion clearances should be 15.7. With 0.61 ml/min/g as CL_{int,met}, CL_{int,sec} was estimated as 1/15.7 = 0.0388 ml/min/g. Upon assignment of 4 ml/min/g, the initial uptake velocity in hepatocytes as CL_{influx}, the CL_{efflux} that would match the observations on total, metabolic, and biliary clearances of enalapril, could be calculated from the equations shown in Table 1, with f_s = 0.55 and Q_L = 0.8 ml/min/g (de Lannoy et al., 1989, 1993). This efflux clearance was 1.46 ml/min/g. The choices would yield consistent values: 15.7 as the ratio of CL_{int,met}/CL_{int,sec}, and 6.56 for CL_{influx}/CL_{int,met}. These outcomes are more congruent with the transport and metabolic data of Abu-Zahra et al. (2000) and Abu-Zahra and Pang (2000). By contrast,

**Improved CL_{influx}, CL_{int,sec}, and CL_{int,met} Estimates for Physiological Modeling**

Since 1997, we have obtained additional in vitro estimates on transport and metabolic activities toward enalapril in isolated hepatocytes (Table 3) (Abu-Zahra et al., 2000; Abu-Zahra and Pang, 2000). With these improved parameters to render improved estimates of the transport and metabolic intrinsic clearances, we re-examined the roles of transport and metabolism on the clearances. The in vitro data showed that transport was much faster than metabolism (6.56 times); metabolism and excretion, but not transport, are rate-limiting in the overall removal of enalapril.

The values observed for homogeneous hepatocytes on transport and metabolism in vitro (Abu-Zahra et al., 2000) would serve as the building blocks for our improved estimation within the physiological model. Using the same strategy of Sirianni and Pang (1997), we updated the estimates of transport and metabolism (as well as secretion) intrinsic clearances in the physiologically based model. The ratio of the biliary (CL_{liver,ex}) and metabolic (CL_{liver,met}) clearances (same as CL_{int,sec}/CL_{int,met}), when multiplied to the metabolic intrinsic clearance, yields the secretory intrinsic clearance. According to the observations of de Lannoy et al. (1993), the ratio of metabolic/excretion clearances should be 15.7. With 0.61 ml/min/g as CL_{int,met}, CL_{int,sec} was estimated as 1/15.7 = 0.0388 ml/min/g. Upon assignment of 4 ml/min/g, the initial uptake velocity in hepatocytes as CL_{influx}, the CL_{efflux} that would match the observations on total, metabolic, and biliary clearances of enalapril, could be calculated from the equations shown in Table 1, with f_s = 0.55 and Q_L = 0.8 ml/min/g (de Lannoy et al., 1989, 1993). This efflux clearance was 1.46 ml/min/g. The choices would yield consistent values: 15.7 as the ratio of CL_{int,met}/CL_{int,sec}, and 6.56 for CL_{influx}/CL_{int,met}. These outcomes are more congruent with the transport and metabolic data of Abu-Zahra et al. (2000) and Abu-Zahra and Pang (2000). By contrast,
re-examination of the values proposed by Sirianni and Pang (1997) showed that the ratio (CL_{influx}/CL_{int,met}) was 0.78 and not 6.56, and CL_{liver,met}/CL_{liver,ex} was 20 and not 15.7, despite the fact that the set of parameters is consistent with the data.

Another parameter that may be considered is the ratio of the observed concentrations of tissue to plasma (CL_{l,ss}/CP_{l,ss}), which may be calculated from f_{p}CL_{influx}/[f_{L}(CL_{efflux} + CL_{int,sec} + CL_{int,met})] and varied from 1.5 to 19 (Pang et al., 1991). The estimated value of CL_{influx}/(CL_{efflux} + CL_{int,sec} + CL_{int,met}) was 1.9 and greater than unity. Although f_{p} was experimentally determined as 0.55, f_{L} is unknown. If f_{L} is assumed to be equal to f_{p}, then the ratio (CL_{l,ss}/CP_{l,ss}) may serve as another check for the estimated CL_{influx}/(CL_{efflux} + CL_{int,sec} + CL_{int,met}).

### Dynamics of Secretory and Metabolic Intrinsic Clearances and Basolateral Influx and Efflux Clearances on Hepatic Metabolic and Excretory Clearances

Using the improved parameters, we proceeded to examine the effect of secretory metabolism, and vice versa. Upon setting the CL_{int,sec} as zero, we mimicked the condition of the EHBR livers that lack Mrp2, the multidrug resistance-associated protein 2 that excretes enalapril (Pang et al., 2002). Only small but insignificant changes in metabolic and total clearances were observed even though the excretion clearance was dramatically reduced to almost zero (Pang et al., 2002). This was also predictable: reduction of the biliary clearance, a small component of clearance, to zero yielded an almost imperceptible increase of the metabolic clearance from 0.345 to 0.358 ml/min/g (Table 4). However, when the CL_{int,met} was set as zero, the newly predicted biliary clearance was 2 1/2 times that seen before (Table 4). It should be mentioned that, in the absence of any other removal pathway, the estimated clearance reflects the “true” basal clearance. Upon reduction of the efflux clearance by 50% while maintaining the CL_{influx}, CL_{int,sec}, and CL_{int,met} constant, the biliary, metabolic, and total clearances are all predicted to be increased. Alternately, when the influx clearance was reduced while maintaining the CL_{efflux}, CL_{int,met}, and CL_{int,sec} constant, the biliary, metabolic, and total clearances all decreased. The ratio of the metabolic/excretion or metabolic/intrinsic secretory intrinsic clearances remained constant under these conditions (Table 4).

The next questions are, what are the expectations for the metabolic and total hepatic clearances when the biliary (secretory) intrinsic clearance is increased or decreased, and, what are the associated changes of the metabolic and total hepatic clearances? Table 5 summarizes these results. A decrease in intrinsic clearance of one pathway leads to an observed increased clearance of the alternate pathway because of compensatory mechanism (a see-saw phenomenon), due to an increase in intracellular substrate concentration. However, the total clearance is decreased. Decreased secretion would bring about increased metabolic clearance but decreased total hepatic clearance (example 5, Table 5). By contrast, an increase in the intrinsic clearance of one pathway leads to a decrease in the observed clearance of the alternate pathway, but the total clearance is increased. When influx is decreased, all observed biliary, metabolic, and total clearances are decreased. When efflux is inhibited, however, all clearances: excretion, metabolic and total hepatic clearances, are increased. If secretion is inhibited and efflux is reduced only slightly, the biliary and total clearances will both be diminished and metabolic clearance is elevated, as expected (see example 5, Table 5). However, when influx is inhibited to larger extents (example 6, Table 5), the biliary and total clearances, in addition to metabolic clearance, may all show increases. We highly recommend use of an Excel Worksheet by setting various values for f_{p}, Q_{L}, CL_{int,sec}, CL_{int,met}, CL_{influx}, and CL_{efflux} into the equations shown in Table 1 to examine the resultant changes in biliary (CL_{liver,ex}), metabolic (CL_{liver,met}), and total hepatic (CL_{liver,tot}) clearances under these first-order conditions.

### Liver Tissue Concentration Profiles

With linear transport and metabolism, changes in the tissue concentration were also examined. The steady-state concentration of drug in liver tissue, CL_{l,ss}, was found as the ratio of infusion rate (R_{o}) divided by the product of the unbound fraction in liver (f_{L}) and the sum of the intrinsic clearances.

\[ \text{CL}_{l,ss} = \frac{R_{o}}{(\text{CL}_{\text{int,sec}} + \text{CL}_{\text{int,met}}) f_{L}} \]  

(4)

The value of CL_{l,ss}, corrected per unit of infusion rate (R_{o}), equals the area under the liver tissue concentration-time curve, AUC_{L}, per unit of dose.

\[ \text{AUC}_{L} = \frac{\text{dose}}{(\text{CL}_{\text{int,sec}} + \text{CL}_{\text{int,met}}) f_{L}} \]  

(5)

However, for any given AUC_{L}, the concentrations of drug in tissue changed with time. This was demonstrated in a simulation for first-
order conditions; the volume and flow parameter values were similar to those of de Lamboy et al. (1993), as summarized in Table 6. The $CL_{int,sec}$ was 0.1 ml/min/g, and the $CL_{int,max}$ was 2.5 ml/min/g; the $CL_{influx}$ and $CL_{efflux}$ were 4 and 2 ml/min/g, respectively. Only one parameter was changed at a time; the $CL_{int,sec}$ was varied to either 0.05 or 0.2 ml/min/g; and the $CL_{int,max}$ was changed to 0.125 or 0.5 ml/min/g (A). Upon decreasing the secretory and/or metabolic intrinsic clearances, $CL_{int,sec}$ and/or $CL_{int,max}$, the liver concentrations of drug were decreased with time (Fig. 3A). However, although temporal changes were observed upon changes in $CL_{influx}$ and $CL_{efflux}$ in comparison to those of the control, the AUC$_L$ was unchanged (Fig. 3B). The same relationships were obtained for averaged tissue concentration in liver or AUC$_L$ (eqs. 4 and 5) for the case when the liver is viewed as three zonal regions of equal transport and metabolic activities (data not shown).

**Effects of the Secretion, the Influx and Efflux Clearances, and Saturable Metabolism on Hepatic Clearance and Metabolite Formation**

Although the effects of transporters on metabolism under first-order conditions were well predicted by the equations shown in Table 1, modulation of the apparent activity of metabolic enzymes under nonlinear conditions was virtually unknown. For appraisal of these effects, additional simulations were performed under the nonlinear metabolic condition [the metabolic intrinsic clearance is $V_{max}/(K_m + C_m)$, where $V_{max}$ and $K_m$ were assigned values of 5 nmol/min/g liver and 20 μM, respectively, as listed in Table 6, with other parameter values] at two different input concentration levels (50 and 500 μM). The mass balance rate equations utilized for simulation are shown in the Appendix. Figure 4 summarizes the resultant concentration-time profiles at the low dose (Fig. 4, left panel) versus high dose (Fig. 4, right panel); the integral provides the area under the curves (Table 7). The summarized complementary results on metabolism and excretion (Table 7) showed trends similar to those observed under first-order conditions. It may be seen that with increases of $CL_{int,sec}$, the apparent total clearance, which bore an inverse relationship to AUC of reservoir (or blood), increased (Fig. 4, A and D) and biliary excretion increased, whereas metabolite formation decreased (Table 7). With increases in $CL_{influx}$, the apparent total hepatic clearance increased (Fig. 4, B and E) when the drug was entering the cell more readily; formation of metabolite would be reduced due to a greater degree of saturation of metabolism because of the faster rate of drug entry that results in higher cellular concentrations. The consequence is an increase in biliary excretion (Table 7). An increase in $CL_{efflux}$ reduces the hepatic clearance (Fig. 4, C and F), since drug in the intracellular compartment is readily extruded out of the cell; the lessened cellular concentration decreased the extent of excretion into bile but increased the extent of metabolism due to desaturation of the metabolic system (Table 7). Upon comparison of the extents of metabolism at the two doses (Table 7), we found that more metabolite was formed (percentage of dose) at the lower dose than that at the higher dose. On the contrary, the excreted amount (percentage of dose) was higher at the higher dose. The above trends could be deduced because of saturable metabolism at the higher dose (Table 7).

**Literature Reports**

It is well recognized that the pregnane X receptor (PXR) transcriptionally up-regulates both cytochrome P450 3A4 and Pgp of MDR1 (Goodwin et al., 2000; Geick et al., 2001), and we may find both 3A4 and Pgp being coordinately induced by PXR ligands (Wacher et al., 1998). How do these findings translate to observations on competition between transporters and enzymes in vivo? For vinblastine, a P-glycoprotein substrate (Takanaga et al., 2000), the absence of P-glycoprotein in knockout mice [KO; mdr1a(-/-)] increased the area under the curve (AUC) and decreased the total body clearance (CL) (van Asperen et al., 1996;
Spareboom et al., 1997; Kawahara et al., 1999; Yokogawa et al., 1999) (see Table 8) in single [mdr1a(/+)] and double [mdr1a/1b(/+)] KO mice in which the Cyp3a activities remained similar to that of control [mdr1a(+/+)]. However, because of reduced excretion in the KOs, the production of CO2 from erythromycin increased (Lan et al., 2000). These findings were consistent with the predictions on decreased excretion clearance (Table 5). Induction of P-glycoprotein with dexamethasone (a PXR ligand) increased the total body clearance of rhodamine, whereas cyclosporine, an inhibitor of P-glycoprotein, resulted in reduced total body clearance of rhodamine (Yumoto et al., 2001).

There have been recent studies on rat liver perfusions with the use of inhibitors of P-glycoprotein, cytochrome P450 3a, or both with FK506 (Wu and Benet, 2003). With troleandomycin (Cyp3a inhibitor) or cyclosporine (Cyp3a and P-glycoprotein inhibitor), the AUC was increased, whereas CL was decreased (Table 9), as predicted by Table 5. However, with GG918, a P-glycoprotein inhibitor, the AUC was decreased, leading to an increased CL. The result was unexpected, and the data could be explained only when efflux of FK506 was inhibited (example 6, Table 5). Yet in another recirculating liver perfusion study, there existed data on digoxin, a substrate that enters the liver via Oatp2 (or Oatp1a4) (Noé et al., 1997). Rifampin, an Oatp2 inhibitor (Fattenger et al., 2000), and quinidine, a P-glycoprotein inhibitor (Makhey et al., 1998), were used (Lau et al., 2004). With rifampin, digoxin transformation to its bis-digitoxoside was impaired, and the AUC was increased, as expected (Lau et al., 2004). However, significantly decreased liver digoxin concentrations occurred at 60 min after the commencement of the perfusion.

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**Fig. 4.** Simulations for nonlinear metabolic conditions at two different input concentration levels (50 and 500 \( \mu \)M). The CL_{influx} was varied from 0.1 to 0.5, 0.25, and 0.05 ml/min/g, whereas the CL_{efflux} ranged from 2 to 4, 1, and 0.5 ml/min/g. With increases of the CL_{influx}, the apparent total clearance, which bears an inverse relationship to AUC, increased (A and D). With increases in the CL_{efflux}, the apparent total hepatic clearance increased when the drug was entering the cell more readily (B and E). With increases in CL_{efflux}, the apparent total hepatic clearance decreased (C and F) since the drug in the intracellular compartment is readily extruded out of the cell.
of perfusion. The change in liver digoxin concentration with rifampin at one single time point would not afford sound predictions, since the liver tissue concentrations could be initially lower than those of control. But the curve eventually crossed over and concentrations became higher than those of control; the AUCL remains unchanged if changes only in transport exist (see Fig. 3B). With quinidine acting as a P-glycoprotein inhibitor, digoxin metabolism was increased, as expected. However, the quinidine-treated group resulted in significantly lower reservoir digoxin concentrations in comparison to those of controls. The trend for AUCperfusion, although not measured to time infinity, implied an increase in total hepatic clearance CLliver,tot at reduced Pgp activity and decreased CLint,sec (Fig. 3A). According to theory, the observation of increased CLliver,tot is more consistent with inhibition of efflux or an enhancement in metabolism (Table 5). The unexplained findings suggest that there may be actions exerted by inhibitors other than inhibition of the intended pathway.

**Concluding Remarks**

Pharmacokinetic analyses of this kind are quantitative tools that provide undisputed evidence on the dynamic interactions between enzyme and enzyme, and transporter and enzymes. The suggested strategy is to apply intrinsic clearance terms for basolateral transport clearances for influx and efflux, and for the metabolic (first-order and nonlinear metabolic conditions) and secretory pathways. The timely examination provides a clear picture of the compensatory trends of alternate pathways, the expected changes in tissue concentrations, and how the biliary, metabolic, and total clearances change according to inhibition (total clearance will be decreased) or induction (total clearance will be increased) of one of the elimination pathways. The
analysis further shows the scenarios when one considers changes in influx and efflux clearances, revealing that these will not affect the ratio for metabolism/excretion, although the absolute values of the clearances are changed. The above analysis clarifies that inhibitors that are used to demonstrate principles of drug-drug interactions are prone to scrutiny when the inhibitor is not specific in its intended use (Wu and Benet, 2003; Laut et al., 2004).

**Appendix**

Mass balance equations were written for the rate of change of drug in reservoir, liver plasma, liver tissue, and bile compartments (see Fig. 2 for definition of terms).

For the rate of change of drug in reservoir,

\[ V_R \frac{dC_R}{dt} = Q_L C_{pl} - Q_R C_R \]  

(A1)

For the rate of change of drug in liver plasma

\[ V_{pl} \frac{dC_{pl}}{dt} = Q_R C_R - Q_{pl} C_{pl} - f_P C_{pl} C_{influx} + f_I C_I C_{efflux} \]  

(A2)

For the rate of change of drug in liver tissue

\[ V_I \frac{dC_I}{dt} = f_P C_{pl} C_{influx} - f_I C_I C_{efflux} - \frac{V_{max}}{K_a + f_I C_I} - \frac{V_{max}}{K_{int,sec} + f_I C_I} \]  

(A3)

For the rate of change of drug in bile

\[ V_{bile} \frac{dC_{bile}}{dt} = f_I C_I C_{int,sec} - Q_{bile} C_{bile} \]  

(A4)

For cumulative amount excreted into bile

\[ A_{bile} = \int f_I C_I C_{int,sec} dt \]  

(A5)

For cumulative amount metabolized

\[ A_{net} = \int \frac{V_{max} f_I C_I}{K_a + f_I C_I} dt \]  

(A6)

**References**


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