INFLUENCE OF P-GLYCOPROTEIN, TRANSFER CLEARANCES, AND DRUG BINDING ON INTESTINAL METABOLISM IN CACO-2 CELL MONOLAYERS OR MEMBRANE PREPARATIONS: A THEORETICAL ANALYSIS

DEBBIE TAM, HUADONG SUN, AND K. SANDY PANG

Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada

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ABSTRACT:

Studies on the Caco-2 cell monolayer system that contained cytochrome P450 and P-glycoprotein activities have advanced the theory that increased intestinal metabolism resulted with increased drug efflux due to an increase in mean residence time (MRT) in the system. To confirm or refute the claim, we developed compartmental models to study the effects of intestinal secretion on the MRT and rates of metabolism under first-order and nonlinear conditions. The theoretical examinations showed that under first-order conditions, intestinal secretion increased the MRT of drug in all compartments but failed to increase the rate of metabolite formation or the total amount of metabolite formed. Instead, reduced metabolic rates arose with increased efflux from cell, either into the apical or the basolateral compartment. By contrast, under saturable metabolic conditions, there were some conditions found whereby rates of metabolism increased with intestinal secretion and rapid reabsorption, albeit the total amount of metabolite formed eventually equaled the administered dose. Intestinal secretion failed to induce higher rates of metabolism for other conditions (saturable cellular binding, cellular efflux, or cell entry). With saturation of metabolic enzymes, drug efflux brought about desaturation, and, upon rapid recovery of drug into the cellular compartment, higher rates of metabolite formation were attained. The simulation study showed that, under first-order conditions, intestinal secretion reduced the rate of metabolism even though the MRT was prolonged within the cell preparation. With nonlinear metabolism, however, instances may exist whereby higher rates of metabolism would result with secretion.

The intestine is the first physical barrier to which drug is presented following oral administration. In addition to transporters for uptake (Tsuij and Tama, 1996), drug-metabolizing enzymes for oxidation and conjugation (Dubey and Singh, 1988; Ilett et al., 1990) and efflux transporters for excretion are present (Lin et al., 1999; Suzuki and Sugiyama, 2000). An important efflux transporter is P-glycoprotein (Pgp), a multidrug resistance (MDR1) gene product that is present at the villous tips of the enterocytes (Thiebaut et al., 1987). Drugs that are substrates of Pgp include verapamil (Saitoh and Aungst, 1995; Sandström et al., 1998; Johnson et al., 2001); the anticancer drugs vincristine, etoposide, daunorubicin, and paclitaxel (Leu and Huang, 1995; Sonnichsen et al., 1995; Nakayama et al., 2000; Chico et al., 2001; Wacher et al., 2001; Abraham et al., 2002); digoxin (Cavet et al., 1996; Greiner et al., 1999); the human immunodeficiency virus protease inhibitor indinavir (Hochman et al., 2000; Li et al., 2002); and immunosuppressive agents cyclosporin (Gan et al., 1996; Lown et al., 1997), tacrolimus (Lampen et al., 1996; Hashimoto et al., 1998; Hashida et al., 2001), and sirolimus (Paine et al., 2002).

Because of the significance of the intestine as an important first-pass organ, in vitro systems have been developed to assess the importance of intestinal uptake, metabolism, and excretion for the prediction of permeability and overall absorption. Among these are intestinal membrane segments/preparations (Johnson et al., 2001), everted sacs (Carreno-Gomez and Duncan, 2000), and the Ussing chamber (Fiddian-Green and Silen, 1975). In these systems, a donor compartment, a receiving compartment, and a receiving compartment for sampling allow the estimation of drug absorption, metabolism, and efflux. Mucosal administration allows investigation of drug flux in the mucosal to serosal direction, whereas drug given at the serosal side permits the flux in the opposite direction, that is, from the serosal (basolateral) to mucosal compartment to be estimated. Another system is the cultured Caco-2 cell monolayer derived from human colon carcinoma cells. When differentiated Caco-2 cells were employed to study drug efflux by Pgp (Saitoh and Aungst, 1995), the involvement of Pgp was inferred when the basolateral to apical flux (B to A) exceeded that of A to B. It was further found that, upon culture in 1α,25-dihydroxy vitamin D3 for 2 weeks postconfluence, cytochrome P450 3A activity was up-regulated (Schmiedlin-Ren et al., 1997;
described by the first-order, metabolic intrinsic clearance, CL_{int,met} (Scheme A), or by a saturable system characterized by the Michaelis-Menten constant, K_m, and the maximum velocity, V_{max} (Scheme B). In Scheme B, nonlinear conditions for absorption, efflux, or cellular binding were readily accommodated. The mass balance equations for the apical (denoted by subscript, ap), cell (denoted by subscript, cell) and the basolateral (denoted by subscript, baso) compartments are shown in the Appendix.

Scheme A: Linear conditions. Permeation of drug (D) from the apical compartment into the cell layer, whether mediated by uptake transporters or passive diffusion, is associated with the absorption rate constant, k_a, whereas secretion from the cell back into the apical compartment occurs with the intrinsic clearance, CL_{int,sec} . Drug partitioning between the cell and the basolateral compartment is mediated by influx and efflux clearances, CL_{int,ap} and CL_{int,baso}, respectively, as shown. Drug binding to proteins present in the apical compartment due to sloughed off mucosal cells (unbound fraction f_{ap}), within the cell (unbound fraction f_{cell}), and in the basolateral compartment (unbound fraction f_{baso}) affects the transfer and metabolic rates based on unbound drug concentrations. Metabolite formation is assumed to occur from the cellular compartment. At any time, the rate of total metabolite formed under first-order conditions is given by \( f_{cell}D_{cell}CL_{int,met}/V_{cell} \), where \( D_{cell} \) is the amount of drug within the cellular compartment of volume, \( V_{cell} \). Estimation of metabolic formation under first-order conditions was simplified by setting the efflux clearances of metabolite from the cellular compartment as zero. The total amount of metabolite formed within the cellular compartment may be obtained by integration of the metabolite formation rates with respect to time, and this amount was further normalized to the dose.

Under first order conditions, the area under the curve for drug (AUC) and area under the moment curve for drug (AUMC) were solved by inversion of the square matrix (shown in the Appendix for Scheme A) with the program, Theorist, as described previously (Pang, 1995). The MRT for the drug for first-order conditions was obtained as the ratio of AUMC/AUC (Eq. 1). The MRT_{cell} within the cell or AUMC_{cell}/AUC_{cell} provides a pertinent parameter of the duration of drug in the eliminating tissue.

\[
\text{MRT}_{cell} = \frac{\text{AUMC}_{cell}}{\text{AUC}_{cell}} = \int_0^t C_{cell}(t) \, dt \div \int_0^t C(t) \, dt
\]

Scheme B: Nonlinear metabolism. Scheme B further considers nonlinear drug metabolism (characterized by \( V_{max} \) and \( K_m \)) to form the metabolite, M (Fig 1B), and nonlinearity in absorption, cellular efflux, or tissue binding. The formed metabolite may, in some instances, affect the transport processes or the binding of drug and, in turn, the rate of drug metabolism. Binding of metabolite is assumed to be unity, and transport processes of the metabolite may be described in Scheme B since the formed metabolite may be an inhibitor or inducer for drug transport or metabolism, and these may be modeled, in future studies. Metabolite within the cell (M_{cell}) may be effluxed out with clearances, CL_{int,ap}[mi] and CL_{int,baso}[mi], respectively, into the apical and basolateral compartments. The metabolite from the apical compartment (M_{ap}) is transported into the cell monolayer with the rate constant, \( k_{ap} \), whereas that from the cellular compartment as zero. The total amount of metabolite formed within the cellular compartment may be obtained by integration of the metabolite formation rates with respect to time, and this amount was further normalized to the dose.

Materials and Methods

Models and Solutions. The schematic depictions of the Caco-2 cell monolayer and membrane preparation are similar, and are shown in Fig. 1. It was assumed that drug metabolism is confined to the cellular monolayer and is described by the first-order, metabolic intrinsic clearance, CL_{int,met} (Scheme A), or by a saturable system characterized by the Michaelis-Menten constant, K_m, and the maximum velocity, V_{max} (Scheme B). In Scheme B, nonlinear conditions for absorption, efflux, or cellular binding were readily accommodated. The mass balance equations for the apical (denoted by subscript, ap), cell (denoted by subscript, cell) and the basolateral (denoted by subscript, baso) compartments are shown in the Appendix.
The total amount of metabolite formed was provided by the sum of the amounts of metabolite in all compartments.

**Simulations.** Data were simulated for apical (denoted by subscript, A) and basolateral (denoted by subscript, B) dosings. Recovery of the dose was tested and was complete in all simulations. Values of $V_{ap}$ (1.5 ml) and $V_{baso}$ (2.5 ml) were based on the data of Cummins et al. (2002). The value of $V_{cell}$ (0.1 ml) was arbitrarily chosen, since the volume of the cell layer was unimportant for both linear and nonlinear simulations (data not shown).

**Linear cases.** The circumstances for simulations of the first-order conditions are summarized in Table 1 (Scheme A). An initial drug concentration of 1 μM was used. The solutions (shown in Table 2) were used to estimate the AUC, AUMC, and MRT with apical (mucosal) or basolateral administration with the assigned values of $V_{ap}$, $V_{baso}$, and $V_{cell}$. Values of $k$, $k_{[m]}$, $f_{ap}$, $f_{cell}$, $f_{int}$, $CL_{d1}$, $CL_{d2}$, $CL_{int,sec}$, $CL_{int,mi}$, and $CL_{int,sec,mi}$ were assumed to be independent of concentration and time for simulations of the linear cases. Initially, values of the intrinsic clearances and the absorption constants were varied to provide suitable profiles, and these served as the basis for simulations (Table 1). The intrinsic clearance for secretion ($CL_{int,sec}$) was varied from 0 to 10 ml/min whereas the intrinsic clearance for metabolism ($CL_{int,met}$) was varied from 0 to 1 ml/min for cases 1 to 4. The value of $k_{f}$ was set as 0.01 min⁻¹ since this provided a reasonable time course for transport and metabolism (see later simulations). $CL_{d1}$ and $CL_{d2}$ values of 50, 5, 0.01 to 50 ml/min, spanning from low to high transmembrane permeability, and unbound fractions of unity for $f_{ap}$, $f_{cell}$, and $f_{baso}$, were used (Cases 1–4, Table 1).

**Nonlinear cases.** In the second set of simulations, saturable metabolism/absorption/binding/efflux was included (Scheme B, Fig. 1). The chosen values for the metabolite: $k_{f}[m] = 0.05$ min⁻¹, $CL_{int,mi} = 0.05$ ml/min, $CL_{int,mi}[m] = 0.1$ ml/min, $CL_{int,sec,mi} = 1$ ml/min, for simulation were inconstant in the present simulation since it was assumed that the metabolite failed to alter the kinetics of drug.

As for the saturable metabolism, the $V_{cell}$ (10 to 50 nmol/min) and $K_{m}$ (10 to 50 μM) were varied for the metabolic pathway. Initial drug concentrations of 100 and 300 μM were used, and simulations were performed with $f_{ap} = f_{cell} = f_{baso} = 1$, $CL_{d1}$ and $CL_{d2}$ were varied from 0.02, 0.5, 1, and 5 ml/min, spanning from low to high transmembrane permeability. Values of $k_{f}$ (1 and 30 min⁻¹) and $CL_{int,sec}$ (1, 5, and 10 ml/min) were used.

Saturable absorption ($k_{f}$ pathway) was examined when the metabolic intrinsic clearance was linear and constant (first-order; $CL_{int,met} = 0.5$ ml/min). $CL_{d1}$ and $CL_{d2}$ were assigned the value of 5 ml/min such that drug partitioning was rapid and not an issue. Various $V_{cell}$ (10 and 50 nmol/min) and $K_{m}$ (10, 

### TABLE 1

**Simulations performed for Scheme A**

<table>
<thead>
<tr>
<th>Case</th>
<th>$CL_{int,met}$</th>
<th>$CL_{int,sec}$</th>
<th>$CL_{d1}$</th>
<th>$CL_{d2}$</th>
<th>$f_{ap}$</th>
<th>$f_{cell}$</th>
<th>$f_{baso}$</th>
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<tbody>
<tr>
<td>1</td>
<td>0 to 1</td>
<td>0 to 10</td>
<td>50</td>
<td>50</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0 to 1</td>
<td>0 to 10</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0 to 1</td>
<td>0 to 10</td>
<td>0.01</td>
<td>0.01</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>0 to 1</td>
<td>0 to 10</td>
<td>5</td>
<td>0.01</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>0.01 to 0.5</td>
<td>1</td>
<td>5</td>
<td>50</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>0.01</td>
<td>0.1 to 1</td>
<td>5</td>
<td>50</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>0.01</td>
<td>1</td>
<td>0 to 10</td>
<td>50</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>0.01</td>
<td>1</td>
<td>5</td>
<td>50</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>0.01</td>
<td>1</td>
<td>5</td>
<td>0.01 to 50</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>10–13</td>
<td>0.01</td>
<td>1</td>
<td>5</td>
<td>50</td>
<td>0.01 to 1</td>
<td>1</td>
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</tr>
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### TABLE 2

**Solutions to Scheme A**

<table>
<thead>
<tr>
<th>Administration to Apical Side</th>
<th>$AUC_{ap}$</th>
<th>$AUC_{cell}$</th>
<th>$AUC_{baso}$</th>
<th>$AUC_{ap}/AUC_{baso}$</th>
<th>$MRT_{ap}$</th>
<th>$MRT_{cell}$</th>
<th>$MRT_{baso}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>($CL_{int,sec} + CL_{int,mi} f_{dose})$</td>
<td>$CL_{int,sec} V_{cell} f_{ap}$</td>
<td>$CL_{int,sec} V_{baso} f_{dose}$</td>
<td>($CL_{int,sec} + CL_{int,mi}) CL_{int,sec}$</td>
<td>$CL_{int,sec} + CL_{int,mi} f_{dose}$</td>
<td>$f_{dose}(CL_{int,sec} V_{cell} f_{dose} + CL_{int,sec} V_{baso} f_{dose}) + CL_{int,sec} CL_{int,sec} f_{dose}$</td>
<td>$f_{dose}(CL_{int,sec} V_{cell} f_{dose} + CL_{int,sec} V_{baso} f_{dose}) + CL_{int,sec} CL_{int,sec} f_{dose}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Administration to Basolateral Side</th>
<th>$AUC_{ap}$</th>
<th>$AUC_{cell}$</th>
<th>$AUC_{baso}$</th>
<th>$AUC_{ap}/AUC_{baso}$</th>
<th>$MRT_{ap}$</th>
<th>$MRT_{cell}$</th>
<th>$MRT_{baso}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>($CL_{int,sec} + CL_{int,mi} f_{dose})$</td>
<td>$CL_{int,sec} V_{cell} f_{dose}$</td>
<td>$CL_{int,sec} V_{baso} f_{dose}$</td>
<td>($CL_{int,sec} + CL_{int,mi}) CL_{int,sec}$</td>
<td>$CL_{int,sec} + CL_{int,mi} f_{dose}$</td>
<td>$f_{dose}(CL_{int,sec} V_{cell} f_{dose} + CL_{int,sec} V_{baso} f_{dose}) + CL_{int,sec} CL_{int,sec} f_{dose}$</td>
<td>$f_{dose}(CL_{int,sec} V_{cell} f_{dose} + CL_{int,sec} V_{baso} f_{dose}) + CL_{int,sec} CL_{int,sec} f_{dose}$</td>
</tr>
</tbody>
</table>
The binding association constant \( K_A \) was varied from \( 10^4 \) to \( 10^8 \) \( \text{M}^{-1} \), and the classes and number of binding sites were set as unity. The cellular protein concentration \( [P_t] \) was assumed to be 4,000 \( \mu\text{M} \) (assuming 16 g of cytosolic protein/100 ml of intracellular fluid and an average molecular weight of 40,000 for the cytosolic proteins). The absorption rate constant \( (k_a) \) was varied from 1 to 30 \( \text{min}^{-1} \), and \( CL_{d1} \) and \( CL_{d2} \) were varied from 0.05 to 5 \( \text{ml/min} \); the metabolic intrinsic clearance, \( CL_{int,met} \), was kept constant at 0.5 \( \text{ml/min} \). The input concentration was varied from 100 to 5,000 \( \mu\text{M} \).

\[
C_{cell,u} = -\frac{(1+nK_A[P_t]-K_A C_{cell}) + \sqrt{(1+nK_A[P_t]-K_A C_{cell})^2 + 4K_A C_{cell}}}{2K_A}
\]

The patterns of the MRTs were similar for both routes of drug administration, since \( CL_{d1} \) and \( CL_{d2} \) were high.

**Results**

**Scheme A: Linear Conditions.** Effect of \( CL_{ap} \) and tissue partitioning on \( AUC \) and MRT. The simpler model (Scheme A) revealed that the solution for \( AUC \) in both the apical and basolateral compartments (\( AUC_{ap} \) and \( AUC_{baso} \)) differed according to the site of drug application (Table 2). All of the \( AUCs \) were inversely related to the unbound fraction for the compartment and were route-dependent except for \( AUC_{cell} \), which remained identical for apical and basolateral administrations. \( AUC_{cell} \) was independent of \( CL_{int,sec} \) and was equal to the quotient of the dose and the metabolic intrinsic clearance, \( CL_{int,met} \), multiplied to the unbound fraction in cell. The total metabolite formation (\( CL_{int,met} [C_{cell} AUC_{cell} - Met_{cell}] \)) was independent of secretion and equaled the administered dose when time reached infinity. The ratio of \( AUC_{ap,B}/AUC_{baso,B} \) after apical dosing was \( (CL_{int,met} + CL_{int,sec} V_{ap, ap} V_{baso, baso} / CL_{d1} V_{ap, ap} k_a) \), and that of \( AUC_{baso,B} / AUC_{ap,B} \) after basolateral dosing was \( (CL_{d2} + CL_{int,met} V_{ap, ap} V_{baso, baso}) / (CL_{d1} CL_{int,sec} / CL_{d2}) \). It could readily be deciphered that the metabolic and

**Fig. 2. Simulations of MRTs for apical (A) and basolateral (B) administrations for Cases 1 and 2, Table 1 (Scheme A).**
secretory intrinsic clearances, the transfer or partitioning clearances, the volumes, the unbound fractions, and the absorption rate constant all influence these ratios.

The MRTs in the apical, cell, and basolateral compartments were all increased with $CL_{int,sec}$, since the term was present in the numerators of the solutions (Table 2). At high $CL_{d1}$ and $CL_{d2}$ (5 or 50 ml/min, Cases 1 and 2, Table 1), an increase in $CL_{int,sec}$ alone increased the MRT$_{ap}$, MRT$_{cell}$, and MRT$_{baso}$ and prolonged the mean residence times of substrate in all compartments. The trends were similar for both apical and basolateral administrations, reflecting the absence of a permeation barrier for cases 1 and 2 (Fig. 2). Reducing $CL_{d1}$ (value reduced to 0.01 ml/min at $CL_{d2}$ of 5 ml/min; Case 3, Table 1) failed to alter the $CL_{int,sec}$-induced changes of the MRTs among the apical, cell, and basolateral compartments after apical dosing and for the basolateral compartment after dosing into that compartment, MRT$_{baso,B}$. In all cases, the MRTs increased with increasing $CL_{int,sec}$ (Fig. 3); the trends persisted even when $CL_{d2}$ was reduced from 5 to 0.5 ml/min, at $CL_{d1} = 0.01$ ml/min (data not shown). A reduction of $CL_{d2}$ only (value reduced to 0.01 ml/min at $CL_{d1}$ of 5 ml/min; Case 4, Table 1) failed to perturb the trends for the MRTs in apical, cell, and basolateral compartments following apical administration. But distinct patterns were observed, especially for MRT$_{ap,B}$ following basolateral dosing. The MRT$_{cell,B}$, MRT$_{ap,B}$, and MRT$_{baso,B}$ arising from basolateral dosing were considerably lower than comparable ones after apical administration (Fig. 4). These scenarios persisted when $CL_{d2}$ was reduced from 5 to 0.5 ml/min.

The above simulations (Figs. 2–4) showed that the permeation barrier was absent at 5 ml/min, whereas at lower $CL_{d1}$ and $CL_{d2}$, the transmembrane barriers started to affect the MRTs for both routes of administration. Nevertheless, the trend of increasing values of the MRT with increasing $CL_{int,sec}$ persisted for cases 1 to 4.

**Effect of $k_a$.** The effects of $k_a$ on the AUC and the MRT are shown in Table 2. AUC$_{ap}$ bore an inverse relationship with $k_a$ regardless of the route of drug dosing. Moreover, $k_a$ bore an inverse relationship with all of the MRTs. With increasing $k_a$, all of the MRTs decreased for all values of $CL_{int,met}$ (data not shown). Increasing the $k_a$ reduced

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**Fig. 3. Simulations of MRTs for apical (A) and basolateral (B) administrations for Case 3, Table 1 (Scheme A).**

A transmembrane barrier existed, being more severe for transport from the basolateral compartment into the cellular compartment. The patterns of the MRTs were different for both routes of drug administration.
the MRTs within the compartments and this persisted at different values of CL_{d1} and CL_{d2} (data not shown).

Factors affecting time course of metabolite formation. Although the AUC_{cell} and the total amount of metabolite formed ultimately equaled the dose for both apical and basolateral dosing, the rate of accrual of metabolite was dependent on the incubation time. The time courses differed for the various cases. Metabolite accrual was strongly affected by $k_a$, CL_{int,sec}, CL_{int,met}, CL_{d1}, CL_{d2}, the unbound fractions, and the route of administration. Increasing the $k_a$, CL_{d1}, or CL_{int,met} increased the rate of metabolite accrual for both apical and basolateral administrations (Fig. 5). By contrast, increasing the CL_{int,sec} or CL_{d2} (right panel) decreased the accrual rate of metabolite. At infinite time, the entire dose would ultimately be all metabolized.

Effect of binding on the time courses of metabolite formation. To investigate the effects of protein binding on the time course of the drug concentrations in the apical (C_{ap}), cell (C_{cell}) and basolateral (C_{baso}) compartments, high values (50 ml/min) were chosen for CL_{d1} and CL_{d2} to eliminate any barrier effect on drug permeation at the basolateral membrane. Binding and changes in binding in the apical, cellular, and basolateral compartments failed to alter the concentration-time profiles of substrate in the cell compartment (data not shown) for both routes of dosing, although the concentration-time courses of C_{ap} and C_{baso} and metabolite formation were affected by the unbound fractions $f_{ap}$ (Fig. 6) and $f_{baso}$ (Fig. 7). Binding also reduced the rates of metabolite accrual with either apical or basolateral administration (Figs. 6 and 7, lower panels). Similar effects of $f_{baso}$ on metabolite formation were observed for midazolam metabolism in the Caco-2 system (Fisher et al., 1999).

Scheme B: Nonlinear Cases. Values of $V_{max}$ and $K_m$ for metabolism, and values of $k_a$, CL_{d1}, and CL_{d2} were altered by trial and error to identify a condition whereby the rate of metabolite accrual and the MRT_{cell} would increase upon increasing the CL_{int,sec}. It was observed that increasing the $K_m$ (compare Tables 3–5) and decreasing the $V_{max}$ (compare Tables 5 and 6) for metabolism decreased the MRT_{cell} at
given values of $k_a$ (1 and 30 min$^{-1}$) and designated transmembrane clearances of drug (CL$_{d1}$ and CL$_{d2}$ at 0.02–5 ml/min). Increasing the absorption rate constant, $k_a$ (cf. values at 1 and 30 min$^{-1}$) and the permeability (CL$_{d1}$ and CL$_{d2}$) reduced the MRT$_{cell}$ (compare Tables 3–6). Increasing the applied concentration to the apical or basolateral compartment usually prolonged MRT$_{cell}$ due to a greater saturation of the metabolic enzymes, thereby prolonging the residence of drug within the cell. Generally, increasing the CL$_{int,sec}$ brought about a prolongation in the MRT$_{cell}$ with drug administration into the apical compartment, except some cases of low CL$_{d1}$ and CL$_{d2}$ (0.02 and 0.5 ml/min, starred examples; Tables 3–6). For basolateral application, however, a prolongation in the MRT$_{cell,B}$ occurred with increasing CL$_{int,sec}$ (Tables 3 and 6).

Under conditions of saturable metabolism, the rates of metabolite formation (Met) were increased with increasing secretion for several simulated cases where the drug permeability was low. Upon apical administration and rapid absorption (high $k_a$ of 30 min$^{-1}$) at $K_m$ of 50 μM and $V_{max}$ of 10 nmol/min, increasing secretion resulted in increased rates of metabolism (Figs. 8C and 9C). The pattern occurred at both 100 μM (Fig. 8) and 300 μM (Fig. 9). At the higher $V_{max}$ (50 nmol/min), metabolite accrual rates were faster. This trend of increased rates of cellular metabolism with increased secretion persisted at the $k_a$ of 30 min$^{-1}$ and low drug permeability (data not shown). Under saturable conditions for apical absorption, cellular efflux (CL$_{d2}$ pathway), or cellular binding, however, metabolite accrual failed to increase with secretion (data not shown).

**Discussion**

The competing interactions of absorption, metabolism, and exsorption/efflux in the intestine have been the subject of recent investigations (Lin et al., 1999; Suzuki and Sugiyama, 2000; Wacher et al., 2001). The interplay is recognized to be of paramount importance in the determination of drug bioavailability since intestinal efflux is inferred to increase the mean residence time of drugs and to increase intestinal metabolism (Johnson et al., 2001, 2003; Cummins et al., 2002). The role of Pgp on intestinal drug metabolism has been much

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**FIG. 5. Simulations of the rate of metabolite accrual with apical administration (Met$_{cell,A}$) for Cases 5 to 9, Table 1 (Scheme A) with increasing values of $k_a$, CL$_{d1}$, and CL$_{int,sec}$.**

These resulted in increased rates of metabolite produced (left panel). By contrast, upon increasing the values of CL$_{int,sec}$ and CL$_{d2}$ (right panel), the rates of metabolite produced decreased. The same patterns were observed for Met$_{cell,B}$ after basolateral administration.
debated. The consideration is important since Caco-2 systems and similar techniques are the mainstay of high-throughput, in vitro systems, and perturbations are readily achieved with addition of putative inhibitors of Pgp or cytochrome P450 to the system. Lin et al. (1999) had questioned whether the role of Pgp was overemphasized. The role of Pgp is de-emphasized when saturation of the secretory pathway exists (Dr. Jiunn Lin, personal communication) and, as shown in this simulation study, when reabsorption (high $k_a$) is facile in recovery of the drug. Several reports had asserted that increased intestinal metabolism was associated with the actions of Pgp, and the total amount of metabolite accumulated in all compartments increased with increased mean residence time (Johnson et al., 2001, 2003; Cummins et al.,
Roles. A faster absorption rate constant, $k$ for all compartments (apical, cell, and basolateral). But there are open, cellular compartment. The consequence is an increase in the compartments due to efflux and reabsorption presents a closed loop is not unexpected since the cycling between drug in the cell and apical Indeed, under first-order conditions, there is increase in the mean linear treatise and competition reactions (Sirianni and Pang, 1997). However, the above conjecture is against theory based on contrast to previous speculation, there was a lack of increase in virtual, peripheral compartment and reduces the MRT. However, in sampling is conducted at infinite time, no apparent difference should be observed for total metabolism. Thus, in contrast to popular thinking, Pgp would not increase the ultimate production of metabolite.

TABLE 3

Scheme B: $MRT_{cell}$ as functions of $CL_{int,sec}$, $CL_{d1}$, $CL_{d2}$, $k_a$, and nonlinear metabolism ($K_m$ of 10 $\mu$M and $V_{max}$ of 10 nmol/min).

<table>
<thead>
<tr>
<th>$C_{in}$</th>
<th>$CL_{int,sec}$</th>
<th>$MRT_{cell}$ (min) at $k_a = 1$ min$^{-1}$</th>
<th>$MRT_{cell}$ (min) at $k_a = 30$ min$^{-1}$</th>
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</thead>
<tbody>
<tr>
<td>$\mu$M</td>
<td>ml/min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Apical Administration
- 100 1 24.3* 12.7 12.1 11.8 72.6* 12.4* 11.4 10.7
- 100 5 19.0* 16.3 16.1 15.9 49.6* 12.4* 11.4 10.8
- 100 10 22.7* 21.3 21.2 21.1 40.5* 12.4* 11.5 10.9
- 300 1 53.7* 27.4 27.1 26.9 101* 26.6 26.0 25.4
- 300 5 38.6* 31.1 30.9 30.8 84.6* 26.7 26.2 25.6
- 300 10 39.7* 35.9 35.7 35.6 77.1* 26.8 26.3 25.8

Basolateral Administration
- 100 1 132 17.9 17.0 16.4 131 16.9 16.0 15.6
- 100 5 136 22.5 21.4 20.7 131 17.1 16.2 15.7
- 100 10 142 28.0 26.6 25.9 131 17.2 16.3 15.9
- 300 1 133 41.1 40.5 40.1 132 39.8 38.8 37.8
- 300 5 139 46.3 45.7 45.4 132 39.9 39.0 38.1
- 300 10 145 52.2 51.5 51.1 132 40.2 39.3 38.5

* Deviating trend: increasing values of $CL_{int,sec}$ failed to increase the MRT.

TABLE 4

Scheme B: $MRT_{cell}$ as functions of $CL_{int,sec}$, $CL_{d1}$, $CL_{d2}$, $k_a$, and nonlinear metabolism ($K_m$ of 20 $\mu$M and $V_{max}$ of 10 nmol/min).

<table>
<thead>
<tr>
<th>$C_{in}$</th>
<th>$CL_{int,sec}$</th>
<th>$MRT_{cell}$ (min) at $k_a = 1$ min$^{-1}$</th>
<th>$MRT_{cell}$ (min) at $k_a = 30$ min$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$M</td>
<td>ml/min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Apical Administration
- 100 1 27.9* 16.5 15.8 15.4 75.1* 15.5* 14.2 13.3
- 100 5 26.8* 24.1 23.9 23.7 53.0* 15.5* 14.4 13.5
- 100 10 35.5* 34.0 33.9 33.8 43.3* 15.6* 14.6 13.8
- 300 1 57.3* 31.1 30.7 30.5 104* 29.6 29.1 28.7
- 300 5 45.9* 38.8 38.6 38.3 87.9* 29.8 29.3 29.0
- 300 10 52.5* 48.9 48.7 48.6 78.5* 30.0 29.5 29.3

Basolateral Administration
- 100 1 136 22.2 21.0 20.2 134 20.1 18.9 18.0
- 100 5 145 30.8 29.3 28.4 134 20.8 19.2 18.3
- 100 10 156 41.3 39.6 38.6 134 20.8 19.5 18.7
- 300 1 138 46.0 45.4 44.9 136 43.7 43.1 42.7
- 300 5 149 55.0 54.2 53.7 136 44.0 43.4 43.0
- 300 10 162 64.9 63.8 63.2 137 44.5 43.8 43.4

* Deviating trend: increasing values of $CL_{int,sec}$ failed to increase the MRT.

From results of the present theoretical examination, the question of secretion increasing the MRT and metabolism has become clarified. Indeed, under first-order conditions, there is increase in the mean residence time of drug within all of the compartments. The outcome is not unexpected since the cycling between drug in the cell and apical compartments due to efflux and reabsorption presents a closed loop that is akin to the addition of a peripheral (apical) compartment to the open, cellular compartment. The consequence is an increase in the MRT for all compartments (apical, cell, and basolateral). But there are differences in the extents of the increase in MRTs, since the diffusional constants, $CL_{d1}$ and $CL_{d2}$, and $k_a$ play additional modulating roles. A faster absorption rate constant, $k_a$ counters the depth of the virtual, peripheral compartment and reduces the MRT. However, in contrast to previous speculation, there was a lack of increase in metabolite formation with increasing $CL_{int,sec}$. If sampling is conducted at infinite time, no apparent difference should be observed for
increasing drug efflux and is time-dependent, additional questions may be asked on the rationale of the sampling times for the in vitro experiments.

The present investigation also explored the effects of nonlinear cellular binding, absorption, or metabolism, and whether increasing secretion could bring about increased rates of metabolite accrual. Since increased efflux would desaturate the enzymes, there were instances in which increasing secretion could bring about increased rates of intestinal metabolism, especially when rapid recovery into the cell existed. The extent of intestine metabolism in this in vitro system, however, remained unchanged and eventually equaled the dose. Nonlinear protein binding inside the cell showed lower free fractions at declining drug concentrations that ensued with time, and this would slow down drug metabolism. With nonlinear absorption, increased efflux to the apical compartment would saturate drug absorption into the cell, and under extreme conditions, absorption may even become zero-order, further reducing the intracellular drug concentration and slowing down drug metabolism.

Although our investigation was of limited scope, the findings revealed that increased secretion brought about increased metabolite accrual only with saturable metabolism, rapid apical absorption, and low drug partitioning at the basolateral compartment upon apical dosing (Figs. 8C and 9C). Secretion brought about desaturation of the enzymatic system. At high dosing (Figs. 8C and 9C), secretory drug could bring about the rapid reabsorption replenished the substrate for cellular metabolism that was occurring more optimally under desaturated conditions. The total amount of metabolite ultimately formed, however, remained equal to the dose. However, there was no apparent correlation between MRT cell and metabolite accrual (Tables 3–6; Figs. 8 and 9).

To properly address the type of changes expected of Pgp and cytochrome P450 and the presence of the inhibitors (Johnson et al., 2003), the experimenter needs to be cognizant that many of the described variables are capable of affecting drug disappearance and metabolite accrual. A better approach, in our minds, is to describe the absorption and efflux as separate events instead of “net absorption” (Johnson et al., 2003), so as to segregate the effect of entry versus metabolism after the drug has gained entry into the cell.

### TABLE 5

<table>
<thead>
<tr>
<th>C10</th>
<th>MRT cell (min) at k = 1 min⁻¹</th>
<th>MRT cell (min) at k = 30 min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>μM</td>
<td>m/l/min</td>
<td>m/l/min</td>
</tr>
<tr>
<td>100</td>
<td>50.0</td>
<td>24.1</td>
</tr>
<tr>
<td>100</td>
<td>74.0</td>
<td>24.3</td>
</tr>
<tr>
<td>100</td>
<td>68.8</td>
<td>2.4</td>
</tr>
<tr>
<td>100</td>
<td>90.8</td>
<td>3.9</td>
</tr>
</tbody>
</table>

* Deviating trend: increasing values of CL int,sec failed to increase the MRT.

### TABLE 6

<table>
<thead>
<tr>
<th>C10</th>
<th>MRT cell (min) at k = 1 min⁻¹</th>
<th>MRT cell (min) at k = 30 min⁻¹</th>
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<td>90.8</td>
<td>3.9</td>
</tr>
</tbody>
</table>

* Deviating trend: increasing values of CL int,sec failed to increase the MRT.
f_{ap} \text{ and } f_{baso} \text{ increased, whereas increasing values of } CL_{int,sec} \text{ and } CL_{d2} \text{ decreased the rate of metabolite formation (Figs. 5–7). Alternately, when drug metabolism occurs under saturating conditions, there are instances where an increase in } CL_{int,sec} \text{ may evoke higher rates of metabolite accrual (Figs. 8C and 9C). The complete understanding of the interaction between drug partitioning, metabolic enzymes, and Pgp secretion lends to the better prediction of intestinal drug absorption and bioavailability.}

**Appendix**

**Scheme A: Linear case.** The mass balance rate-equations for Scheme A (eqs. A1–A3) are presented below, where D and M are the amounts of drug and metabolite, respectively, and subscripts ap, cell, and baso denote the apical, cellular, and basolateral compartments, respectively.

\[
\frac{dD_{ap}}{dt} = -k_{ap}D_{ap} + CL_{int,sec} \frac{f_{cell}D_{cell}}{V_{cell}} \quad (A1)
\]

\[
\frac{dD_{baso}}{dt} = -CL_{d1} \frac{f_{baso}D_{baso}}{V_{baso}} + CL_{d2} \frac{f_{cell}D_{cell}}{V_{cell}} \quad (A2)
\]

\[
\frac{dD_{cell}}{dt} = k_{ap}D_{ap} + CL_{d1} \frac{f_{baso}D_{baso}}{V_{baso}} - (CL_{int,sec} + CL_{int,met} + CL_{d2}) \frac{f_{cell}D_{cell}}{V_{cell}} \quad (A3)
\]

\[
\frac{dM_{cell}}{dt} = CL_{int,met} \frac{f_{cell}}{V_{cell}} \quad (A4)
\]

The following square matrix resulted for Scheme A,

\[
\begin{pmatrix}
-f_{ap}k_{a} & f_{cell}CL_{int,sec} & 0 \\
-f_{ap}k_{a} & f_{cell}(CL_{int,sec} + CL_{int,met} + CL_{d2}) & -f_{baso}CL_{d1} \\
0 & -f_{cell}CL_{d2} & f_{baso}CL_{d1}
\end{pmatrix}
\]

**Scheme B: Nonlinear Case.** The rate equations for the apical and basolateral compartments were identical to those for Scheme A (eqs. A1 and A2). However, the rate equation for drug in the cellular compartment differed.

\[
\frac{dD_{cell}}{dt} = k_{ap}D_{ap} + CL_{d1} \frac{f_{baso}D_{baso}}{V_{baso}} - (CL_{int,sec} + CL_{int,met} + CL_{d2}) \frac{f_{cell}D_{cell}}{V_{cell}} \quad (A5)
\]

Similarly, the rate equations for the rate of change of metabolite (M) in the apical, basolateral, and cellular compartments are shown below.
EFFECT OF SECRETION ON INTESTINAL METABOLISM

Fig. 9. Scheme B: nonlinear metabolism and metabolite accrual at \( k_a = 1 \text{ min}^{-1} \) [(A) and (B)] or at \( k_a = 30 \text{ min}^{-1} \) [(C) and (D)] for the initial concentration of 300 \( \mu M \).

Simulations were conducted for \( V_{m_{max}} = 10 \text{ mmol/min} \) and \( K_{m} = 50 \mu M \), when drug permeability was low (CL_{int,sec} = CL_{baso} = 5 \text{ mmol/min} ) [(B) and (D)]. The CL_{int,sec} varied from 1 \( (\square, \bigcirc) \) to 10 \( (\triangle, \triangle) \) mmol/min; solid symbols are associated with apical administration and open symbols represent basolateral administration. Usually, increasing values of CL_{int,sec} decreased the rates of metabolite accrual [see (A), (B), and (D)], except when reabsorption was rapid and permeability of drug was low [see (C)].

The dose-corrected amount of metabolite formed at any time is given by the sum of the amounts of \( M \) in the compartments.

\[
\text{Met} = \left( M_{ap} + M_{baso} + M_{cell} \right) / \text{dose}
\]  

\[
\frac{dM_{ap}}{dt} = -k_{ap}[m_{ap}]M_{ap} + CL_{int,sec}[m_{ap}]M_{cell}/V_{cell}
\]

\[
\frac{dM_{baso}}{dt} = -CL_{baso}[m_{baso}]M_{baso} + CL_{int,sec}[m_{baso}]M_{cell}/V_{cell}
\]

\[
\frac{dM_{cell}}{dt} = k_{cell}[m_{cell}]M_{ap} + CL_{int,sec}[m_{cell}]M_{cell}/V_{cell}
\]

\[
V_{m_{max}} \left( f_{cell}D_{cell} \right) / V_{cell} + K_{m} + f_{cell}D_{cell} = \left( CL_{int,sec}[m_{cell}] + CL_{baso}[m_{baso}] \right)M_{cell}/V_{cell}
\]

References


