A NEW PHYSIOLOGICALLY BASED, SEGREGATED-FLOW MODEL TO EXPLAIN ROUTE-DEPENDENT INTESTINAL METABOLISM

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ABSTRACT:

Processes of intestinal absorption, metabolism, and secretion must be considered simultaneously in viewing oral drug bioavailability. Existing models often fail to predict route-dependent intestinal metabolism, namely, little metabolism occurs after systemic dosing but notable metabolism exists after oral dosing. A physiologically based, Segregated-Flow Model (SFM) was developed to examine the influence of intestinal transport (absorption and exsorption), metabolism, flow, tissue-partitioning characteristics, and elimination in other organs on intestinal clearance, intestinal availability, and systemic bioavailability. For the SFM, blood flow to intestine was effectively segregated for the perfusion of two regions, with 10% reaching an absorptive layer—the enterocytes at the villus tips of the mucosa where metabolic enzymes and the P-glycoprotein reside, and the remaining 90% supplying the rest of the intestine (serosa and submucosa), a nonabsorptive layer. The traditional, physiologically-based model, which regards the intestine as a single, homogeneous compartment with all of the intestinal blood flow perfusing the tissue, was also examined for comparison. The analytical solutions under first order conditions were essentially identical for the SFM and traditional model, differing only in the flow rate to the absorptive/removal region. The presence of other elimination organs did not affect the intestinal clearance and bioavailability estimates, but reduced the percentage of dose metabolized by the intestine. For both models, intestinal availability was inversely related to the intrinsic clearances for intestinal metabolism and exsorption, and was additionally affected by both the rate constant for absorption and that denoting luminal loss when drug was exsorbed. However, the effect of secretion by P-glycoprotein became attenuated with rapid absorption. The difference in flow between models imparted a substantial influence on the intestinal clearance of flow-limited substrates, and the SFM predicted markedly higher extents of intestinal metabolism for oral over i.v. dosing. Thus, the SFM provides a physiological view of the intestine and explains the observation of route-dependent, intestinal metabolism.

Drugs administered orally must first be absorbed, either passively or via facilitated transport, across the intestinal luminal membrane to reach the systemic circulation. Much is known about the various intestinal transport proteins that participate in the uptake of drugs (Tsujii and Tamai, 1996; Lin et al., 1999). Additionally, the intestine possesses metabolic enzymes, notably the conjugating enzymes, UDP-glucuronosyltransferases, glutathione S-transferases (Dubey and Singh, 1988; Ilett et al., 1990; Koster et al., 1995), and cytochrome P450 3A (Watkins et al., 1987; Peters and Kremers, 1989; Kolars et al., 1987; Hsing et al., 1992; Saitoh and Aungst, 1995; Lown et al., 1997). In some instances, metabolism by the intestine was noted only during absorption and not on subsequent circulation through the intestinal tissue. That intestinal metabolism is “route dependent”, being greater with oral than with i.v. dosing, was observed for acetaminophen (Pang et al., 1986), enalapril (Pang et al., 1985), and morphine (Doherty and Pang, 2000), and for the conversion of the prodrug (−)-aminocarbovir to (−)-carbovir (Wen et al., 1999) in the perfused rat small intestine preparation. The observation was repeated for the oxidation of midazolam in man (Paine et al., 1996, 1997). Furthermore, a 170-kDa protein, the P-glycoprotein (Pgp),2 has been identified to be responsible for drug efflux into the intestinal lumen (Thiebault et al., 1987; Hunter et al., 1990; Hsing et al., 1992; Saitoh and Aungst, 1995; Smit et al., 1998). Intestinal metabolism and exsorption effectively reduce the bioavailability of orally administered agents (Gibaldi et al., 1971; Leu and Huang, 1995; Doherty and Pang, 1997; Lown et al.,

2 Abbreviations: Pgp, P-glycoprotein; AUC, area under the concentration-time curve; CLint, influx intrinsic clearance from blood compartment to enterocyte compartment; CLvec, efflux intrinsic clearance from enterocyte compartment to blood compartment; CLbact, influx intrinsic clearance from blood compartment to serosal compartment; CLvec, efflux intrinsic clearance from serosal compartment to blood compartment; CLe, intestinal clearance; Clec, clearance by other parallel organs; CLint, metabolic intrinsic clearance of intestine; CLsec, secretory intrinsic clearance of intestine; CLt, total body or systemic clearance; Fabs, fraction absorbed; Fint, intestinal availability; Fsys, systemic bioavailability; kD, absorption rate constant; kL, luminal degradation constant; Qtot, flow to the enterocyte layer of the mucosa; Qtot, total flow to the intestine; SFM, segregated-flow model; TM, traditional model; M, morphine; M3G, morphine-3-glucuronide.

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Despite the large body of information on intestinal exsorption and metabolism, only a few models exist to correlate these physiological processes with the overall drug absorption or bioavailability (Barr and Riegelman, 1970; Crouthamel et al., 1975; Stigsby and Krag, 1983; Nakashima et al., 1984; Choi et al., 1995; Yu and Amidon, 1998; Ito et al., 1999). Although the models would account for multiple-site/regional absorption, metabolism, secretion, or even diffusion within the tissue, few would forecast route-dependent intestinal metabolism. An exception is the model proposed by Klippert and Noordhoek (1985) that suggests shunting of intestinal blood for prediction of route-dependent metabolism.

In this communication, a physiologically based Segregated-Flow Model (SFM) was developed to explain route-dependent intestinal metabolism; the model encompassed differential blood perfusions to distinct tissue layers of the intestine. The properties of the model were investigated upon engendering intestinal blood flow, the intestinal metabolic, secretory, and intrinsic clearances, tissue-partitioning characteristics (diffusion-limited versus flow-limited distribution) of substrate, and presence of eliminatory pathways in parallel organs to predict the intestinal clearance and systemic availability. The segregated flows could be rationalized because distinct blood flow patterns have been noted for various tissue layers of the intestine—the mucosa, submucosa, and muscularis—with each contributing to one of three functions of the small intestine, absorption, secretion, and motility (Granger et al., 1980), and the serosa that lies inferior to the muscularis. The large surface area for absorption is attributed to the villi and microvilli of the mucosa, and metabolizing enzymes are located within enterocytes at the villus tip (Kolars et al., 1992; Lown et al., 1997). It has been noted that the majority of “resting” intestinal blood flow, some 60 to 70% of the intestinal flow, is distributed to the mucosa-submucosa because of greater metabolic demand (Schurgers and de Blaey, 1984), with approximately 18% (MacFerran and Mailman, 1977), 5 to 7% (Mailman, 1978; Granger et al., 1980), or 10 to 30% (Svanvik, 1973; Micflikier et al., 1976) of the intestinal blood flow perfusing the enterocyte layer of the villus tips where the majority of the absorptive, metabolic, and Pgp activities reside. Because flow perfusing the site of elimination can influence the disposal of drugs and because there are differing blood flow distributions to various tissue layers of the small intestine, it becomes important to view intestinal drug metabolism beyond what is ordinarily considered in traditional, compartmental, or physiological models, in which the absorptive layer is assumed to receive 100% of the total intestinal blood flow.

Two physiological models for the intestine were examined: the Traditional Model (TM) (Fig. 1A) and the SFM (Fig. 1B). Removal by other parallel eliminating organs exists, and the effective clearance is described by $\text{CL}_{\text{others}}$. Common features of the models include the interconnection of the blood compartment (central or reservoir compartment in this instance) to the intestinal tissue via the circulation. Only first order transport and removal processes are considered, and for the sake of simplicity, the drug is assumed to be completely unbound.

Traditional Model. The intestine is subdivided into the vascular (intestinal blood), cellular (tissue), and luminal subcompartments (Fig. 1A). The tissue is supplied with blood from the superior mesenteric artery with the flow rate, $Q_I$; venous blood returns through the portal vein to the reservoir. The exchange of substrate between the cellular and vascular compartments is described by the intrinsic transport clearance terms $\text{CL}_{\text{int},1}$ and $\text{CL}_{\text{int},2}$ that characterize, respectively, transport from intestinal blood into intestinal tissue and vice versa.
The rate constant for absorption of the substrate across the luminal membrane is denoted by \( k_a \), whereas luminal removal of the drug, either by metabolism, fecal excretion, and/or gastrointestinal transit, is represented by rate constant \( k_e \). Once in the intestinal tissue, the drug undergoes biotransformation, and is transported out to blood or effluxed into lumen—processes that are described by intrinsic clearance terms \( CL_{int} \), \( CL_{int,c} \), and \( CL_{int,s} \) respectively (Doherty and Pang, 2000).

**Segregated-Flow Model.** This model is an expansion of the physiological model normally developed for the intestine, but it further recognizes the subtle demarcation of tissue layers and distributions in blood supply. The notion of flow-bypass of tissue regions of the intestine was also recognized by Klippert and Noordhoek (1985). Drug in the serosal blood compartment equilibrates with tissue with the transfer clearances \( CL_{s\|d} \) and \( CL_{s\|c} \), whereas drug in the mucosal-blood/enterocyte-blood compartment equilibrates with tissue with the transfer clearances \( CL_{d\|i} \) and \( CL_{d\|e} \). The absorptive, metabolic, and efflux activities within the villus tips of the enterocyte compartment are denoted by the rate constant, \( k_e \), and the intrinsic clearances, \( CL_{int} \) and \( CL_{int,c} \) respectively (Fig. 1B).

### Experimental Procedures

Mass-balanced equations were written for the TM and the SFM. For emphasis of intestinal metabolism, secretion, and absorption, the system described was similar to that for the recirculating system of the perfused intestine preparation (Doherty and Pang, 2000).

**Traditional Model.** For the rate of change of drug in the reservoir (compartment “R”):

\[
\frac{dA_R}{dt} = Q_R \frac{A_{int}}{V_{int}} - (Q_t + CL_{other}\frac{A_R}{V_R})
\]

For the rate of change of drug in the intestinal blood (compartment “int,b”):

\[
\frac{dA_{int,b}}{dt} = Q_{int} \frac{A_{int,b}}{V_{int,b}} - (CL_{d\|i} + Q_t)\frac{A_{int,b}}{V_{int,b}} + CL_{int}\frac{A_{int}}{V_{int}}
\]

For the rate of change of drug and formation of metabolite \([m]\) in the intestinal tissue (compartment “int”):

\[
\frac{dA_{int}}{dt} = k_A A_{lumen} - (CL_{d\|i} + CL_{int,c} + CL_{int}\frac{A_{int}}{V_{int}} + CL_{d\|e}\frac{A_{int,b}}{V_{int,b}})
\]

\[
\frac{dA_{int}[m]}{dt} = CL_{int}\frac{A_{int}}{V_{int}}
\]

For the rate of change of drug in the intestinal lumen (compartment “lumen”):

\[
\frac{dA_{lumen}}{dt} = CL_{int}\frac{A_{lumen}}{V_{lumen}} - (k_e + k_i)A_{lumen}
\]

**Segregated-Flow Model.** For the rate of change of drug in the reservoir (compartment “R”):

\[
\frac{dA_R}{dt} = Q_R \frac{A_{int}}{V_{int}} - (Q_t + CL_{other}\frac{A_R}{V_R})
\]

For the rate of change of drug and rate of formation of metabolite \([m]\) in enterocyte layer of mucosa (compartment “en”):

\[
\frac{dA_{en}}{dt} = k_A A_{lumen} - (CL_{d\|i} + CL_{int,c} + CL_{d} A_{en}\frac{A_{en}}{V_{en}} + CL_{d\|e}\frac{A_{en,b}}{V_{en,b}})
\]

\[
\frac{dA_{en}[m]}{dt} = CL_{int}\frac{A_{en}}{V_{en}}
\]

### Table 1

<table>
<thead>
<tr>
<th>Description</th>
<th>Symbol</th>
<th>TM</th>
<th>SFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral dose</td>
<td>(Dose_{i.v.})</td>
<td>100(^\circ)</td>
<td>100(^\circ)</td>
</tr>
<tr>
<td>i.v. dose</td>
<td>(Dose_{i.v.})</td>
<td>100(^\circ)</td>
<td>100(^\circ)</td>
</tr>
<tr>
<td>Reservoir</td>
<td>(V_R)</td>
<td>200(^\circ)</td>
<td>200(^\circ)</td>
</tr>
<tr>
<td>Intestinal tissue</td>
<td>(V_{int})</td>
<td>3(^\circ)</td>
<td>3(^\circ)</td>
</tr>
<tr>
<td>Enterocyte layer</td>
<td>(V_{en})</td>
<td>0.3(^\circ)</td>
<td>0.3(^\circ)</td>
</tr>
<tr>
<td>Serosa and other tissues</td>
<td>(V_s)</td>
<td>2.7(^\circ)</td>
<td>2.7(^\circ)</td>
</tr>
<tr>
<td>Intestinal blood volume</td>
<td>(V_{int,b})</td>
<td>1.62(^\circ)</td>
<td>1.62(^\circ)</td>
</tr>
<tr>
<td>Enterocyte blood</td>
<td>(V_{en,b})</td>
<td>0.162(^\circ)</td>
<td>0.162(^\circ)</td>
</tr>
<tr>
<td>Serosal blood</td>
<td>(V_{s,b})</td>
<td>1.458(^\circ)</td>
<td>1.458(^\circ)</td>
</tr>
</tbody>
</table>

**Flow rate (ml/min)**

| Intestinal blood | \(Q_t\) | 8\(^\circ\) | 8\(^\circ\) |
| Mucosa blood to enterocyte layer | \(Q_m\) | 0.8\(^\circ\) | 0.8\(^\circ\) |
| Serosa and other tissue blood | \(Q_s\) | 7.2\(^\circ\) | 7.2\(^\circ\) |

**Clearances (ml/min)**

| Drug transport clearance | \(CL_{d}\) | 0.5 or 50 | 0.5 or 50 |
| Metabolic intrinsic clearance | \(CL_{int}\) | 0.1 to 50\(^\circ\) | 0.1 to 50\(^\circ\) |
| Secretory intrinsic clearance | \(CL_{int,c}\) | 0 to 50\(^\circ\) | 0 to 50\(^\circ\) |
| Absorption rate constant (min\(^{-1}\)) | \(k_a\) | 0.01 to 10\(^\circ\) | 0.01 to 10\(^\circ\) |
| Luminal degradation rate constant (min\(^{-1}\)) | \(k_i\) | 0.5\(^\circ\) | 0.5\(^\circ\) |

\(*\)Assigned parameters.

\(\text{\(a\) Value estimated based on Harrison and Gibaldi (1977) where 10 ml/min was used for a 360-g rat (including cecum and stomach) and the average intestinal weight = 3 g (ref. Doherty and Pang, 2000).}

\(\text{\(b\) Value associated with the designated flow to the enterocytes (0.1 \times Q_t).}

\(\text{\(c\) Value associated with the designated flow to the serosal and other tissue layer (0.9 \times Q_t).}

\(\text{\(d\) Parameters varied during simulations.)}

It is noteworthy that when \(Q_{en}\) equals \(Q_t\), the SFM simplifies to the TM. The coefficients in the mass-balanced rate equations for drug with the TM (Eqs. 1 to 4) and SFM (Eqs. 5 to 10) were represented as elements in a \(4 \times 4\) and \(6 \times 6\) matrices, respectively. Inversion of these matrices with the software Mathematica (Power Macintosh 9500/120) provided the analytical solutions for areas under the amount-time curves per unit i.v. or p.o. dose. Multiplication of these by the ratios of administered doses to reservoir volumes furnished areas under the concentration-time curves (AUC). With the assumption that clearance is constant under first order conditions, the dose-corrected areas under the curves were used to estimate model-independent parameters: 1) the total body or systemic clearance (\(CL_d\)) from Dose/s/AUC, 2) the intestinal clearance (\(CL_{int}\) or \(CL_{lumen} - CL_{other}\)), and 3) the systemic bioavailability (\(F_{sys}\)) or \(AUC_{R,i.v.}/AUC_{R,10^\circ}\). The fraction of drug that ultimately reaches the systemic circulation, \(F_{sys}\), is a product of the fraction of drug that is absorbed across the intestinal membrane (\(F_ab\)) and that portion that escapes intestinal metabolism and exsorption (\(F_E\)). Based on the calculated \(F_{sys}\) and the definition of the fraction absorbed \(F_ab\), the ratio of the absorption rate
constant to the sum of the absorption and luminal degradation rate constants or \( k_a (k_g + k_y) \), intestinal availability \( (F_I) \) was calculated as \( F_{sys} \times F_I \).

**Simulation.** Values of the intestinal clearance and the systemic and intestinal availabilities were either simulated with the equations (eqs. 1 to 10, with the program, Scientist, Micromath, Salt Lake City, UT) or calculated using the solutions obtained for both the TM and the SFM. Various values for the volume, flow, and transport and intrinsic clearances (Table 1) were placed into rows and columns of the worksheet in Excel (Version 5.0 for Macintosh, Microsoft, Seattle, WA) and substituted into the solved equations (see Table 2) for estimation of the various parameters. The overall intestinal flow rate was set as 8 ml/min. Because literature values for the blood flow to the absorptive enterocyte layer of the mucosa vary greatly, ranging from 5 to 30% (Svanvik, 1973; MacFerran and Mailman, 1977; Mailman, 1978; Granger et al., 1980), the average flow to this compartment was assigned 10% of intestinal flow for the sake of simplicity, and the remaining compartment—the serosa and other intestinal structures—received the other 90% of flow; the volumes were partitioned in the same fashion. Furthermore, simulation was performed with transport clearances between blood and tissue compartments being identical for the TM \( (CL_a = CL_d = CL_g) \) and for SFM \( (CL_g = CL_d = CL_{d1} = CL_{d2} = CL_{d3} = CL_{d4}) \). The value of \( CL_g \) was set either as 0.5 or 50 ml/min, because these represented conditions of drugs of low (diffusion-limited distribution) and high (flow-limited distribution) permeability, respectively. The intestinal metabolic intrinsic clearance \( (CL_{m}) \), ranging from 0.1 to 50 ml/min, the exsorption or secretory intrinsic clearance \( (CL_{Isec}) \), ranging from 0 to 50 ml/min, and values of the absorption rate constant \( (k_a) \), ranging from 0.01 to 10 \( \text{min}^{-1} \) were varied under a nonchanging \( k_y \) \( (0.5 \text{ min}^{-1}) \) to study the influence of these factors on the area under the curve, clearance, and bioavailability estimates.

To assess the importance of intestinal exsorption by PgP on drug bioavailability, the metabolic component was set to zero \( (CL_m=0) \). The secretory intrinsic clearance \( (CL_{Isec}) \), the absorption rate constant \( (k_a) \), and the rate constant for gastrointestinal transit/loss \( (k_y) \), were varied for a substrate with \( CL_a = 0.5 \) and 50 ml/min. Lastly, the extents of intestinal drug metabolism after i.v. and p.o. dosing were compared between the models. In these simulations, \( CL_{Isec} \) and \( k_y \) were set as zero whereas \( CL_g, CL_{Iexcess}, \) and \( CL_m \) were varied.

**Fitting of Morphine Data to the TM and SFM.** The utility of the SFM versus the TM was appraised with the recent data of Doherty and Pang (2000) in which morphine (M), a substrate which is absorbed, glucuronidated, and secreted, was given both systemically and intraduodenally to the recirculating, vascularly perfused rat small intestine preparation. The models (Fig. 1) were extended to describe not only the disposition of M but also for the formation of the metabolite, morphine-3β-glucuronide (M3G), by the rat intestine preparation; in this instance, \( CI_{Iexcess} \) was set to zero (Fig. 2). For TM, influx/efflux of M into the intestinal tissue from the blood is characterized by the transport clearance parameter, \( CL_1 \) and \( CL_2 \), respectively (Fig. 2A). Once M enters the intestinal tissue, it undergoes biotransformation to M3G with the intestinal metabolic clearance, \( CL_{I1} \), or is exsorbed across the luminal (denoted by the secretory intrinsic clearance \( CL_3 \)). The absorption intrinsic clearance of M from the intestinal lumen is denoted by \( CL_A \), and the luminal degradation clearance, \( CL_{12} \). M3G, once formed in the intestinal tissue, can either efflux out to the perfuse blood (CL10) or be excreted into the lumen (CL7), where there exists deconjugation of the glucuronide metabolite (with CL5) and glucuronidation of M (with CL6). The influx and efflux clearances for M3G across the basolateral membrane are denoted by CL9 and CL10, respectively. The data had been fitted to mass balance relationships developed previously (see Appendix of Doherty and Pang, 2000) to describe events occurring during the traverse of M and M3G across the intestine. The intrinsic clearances for drug and metabolite absorption and luminal degradation, \( CL_A \), CL8, and CL12, respectively, become the corresponding rate constants upon division by the volume of the lumen, \( Q_{lumen} \).

The SFM was used for the simultaneous fitting of the data (Fig. 2B). The distinction of this model from the TM lies in that only a fraction \( (j_1) \) of the intestinal flow \( (Q_i) \) perfuses the enterocyte layer of the mucosa where both CYP3A and PgP reside. The remaining flow of the intestine or \( (1 - j_0)Q_i \) perfuses the serosa and other structures. If \( j_0 = 1 \), the SFM simplifies to the TM. In the SFM, substrate in the serosal blood \((s,b)\) and mucosal blood to the enterocyte layer \((en,b)\) equilibrates with that in tissue; these are described by transport clearances for M \( (CL_{d1} \) and \( CL_{d2} \)) and M3G \( (CL_{d1M3G} \) and
matrices, were used to calculate the total and intestinal clearances, and of i.v. and p.o. administrations, obtained from inversion of the square on data after the administration of trace doses of $[3\text{H}]M$ alone (systemic and obtained with the Simplex method, then least square optimization was performed
differential equations for the SFM with Scientist. Initial estimates were ob-
dilation to M3G in lumen in our systemic studies, CL5 and CL6 for the TM
and flows in Table 1). Due to published accounts on the lack of deglucuronida-
tribution to changes. Equivalent total values of volume and flows were assigned,
were neglected because binding was linear and constant and would not con-
Doherty and Pang (2000). The effects of binding of M at tracer concentration
were the same for the TM and SFM although the AUC R,i.v. differed
AUC R,p.o. and $F_{\text{sys}}$ for SFM, as did CL1, $F_{\text{sys}}$, and $F_1$. Interestingly, the transport clearances of drug across the
were absent in the solutions of the SFM. This is due to the role of the
the serosa serving only as a noneliminating, drug-distribution compart-
ment (Fig. 1B). Because of exsorption of drug and readsorption, the
absorption rate constant, $k_a$, and the luminal degradation rate constant,
were present in the solutions of CL1, CL1, $F_{\text{sys}}$, and $F_1$. In the
absence of secretion by Pgp, the constants $k_a$ and $k_p$ are absent in the
equations for CL1, CL1, and $F_1$, except for AUC R,p.o., and $F_{\text{sys}}$, which are influenced by $F_{\text{abs}}$ (Table 2).
Simulations. Effects of intestinal metabolism and secretion on $CL_\text{d}$
Membrane transport clearance (CL<sub>tr</sub>) was fixed at 0.5 ml/min and at 50 ml/min for illustration of drugs of the low and high permeability, respectively.

Effects of CL<sub>sec</sub>, k<sub>a</sub>, and k<sub>g</sub> on F<sub>sys</sub> when CL<sub>tr</sub> = 0. In absence of metabolism, secretion and absorption represented the processes effecting the cycling of drug between lumen and intestine. However, the overall bioavailability depended not only on the values of CL<sub>sec</sub> and k<sub>g</sub> but also on k<sub>a</sub>, the “luminal degradation” constant associated with gastrointestinal transit time or loss. When k<sub>a</sub> was set to zero, CL<sub>I</sub> became zero regardless of the value of CL<sub>sec</sub> because of drug reabsorption and total lack of loss in the system (CL<sub>tr</sub> and k<sub>g</sub> = 0). High secretion tended to be offset with rapid absorption (high k<sub>a</sub>) when minimal loss existed in the lumen (k<sub>g</sub> = 0.01 min<sup>-1</sup>), and the systemic availability tended to remain close to unity (data not shown). At increasing values of k<sub>g</sub> (0.5 min<sup>-1</sup>), however, F<sub>sys</sub> became attenuated (Fig. 6), and the trend persisted with even higher k<sub>g</sub> (10 min<sup>-1</sup>) (data not shown).

Effects of CL<sub>tr</sub> and k<sub>a</sub> on F<sub>sys</sub> when CL<sub>sec</sub> = 0 and k<sub>g</sub> = 0.5 min<sup>-1</sup>. In the absence of secretion (CL<sub>sec</sub> = 0), increasing the values of k<sub>a</sub> failed to alter AUC<sub>R,i.v.</sub>, CR<sub>R,i.v.</sub>, or CL<sub>I</sub> (see Table 2) but increased values of F<sub>sys</sub>, the simple parameter changing with k<sub>a</sub>. The greatest changes existed for drugs with low CL<sub>tr</sub>, whereas changes were more gradual for the high-permeability drugs (Fig. 7). Similar trends were observed at CL<sub>tr</sub> = 5 ml/min, albeit the values for F<sub>sys</sub> were attenuated (data not shown). F<sub>sys</sub> bore an inverse relation to CL<sub>tr</sub>. It was noted that values of F<sub>sys</sub> for the SFM were consistently smaller than those for the TM, and the ratios of the values were always less than one.

Effects of CL<sub>other</sub>, CL<sub>tr</sub> and CL<sub>I</sub> on metabolism with constant k<sub>a</sub> (0.05 min<sup>-1</sup>). The simulation with Scientist according to the differential equations revealed different extents in intestinal metabolism
between i.v. and p.o. doses for the SFM and TM when values of \( CL_{\text{others}} \), \( CL_{\text{rec}} \), and \( CL_d \) were varied in the absence of secretion and luminal loss (\( CL_{\text{sec}} \) and \( k_g \)). When \( CL_{\text{others}} = 0 \), intestinal metabolism accounted for 100% of the administered i.v. and p.o. doses regardless of the value of \( CL_d \) for drug because metabolism was the only route of removal (data not shown). With degradation or loss occurring within the lumen (\( k_g > 0 \)), however, the percentage of dose metabolized by intestine could become greater for the i.v. over the p.o. dose due to incomplete absorption (\( F_{\text{abs}} < 1 \)).

In the presence of alternate, parallel pathways (\( CL_{\text{others}} > 0 \)), both models displayed route-dependent metabolism, with a greater extent of intestinal metabolism occurring with p.o. than with i.v. dosing.
However, the difference was much greater with the SFM. The SFM predicted that because there was slower intestinal flow rate (10% flow rate) to the enterocyte layer, the absorbed drug tended to remain longer in the intestinal tissue due to the sluggish flow, thereby allowing a greater extent of intestinal metabolism. The difference in flow for the models led to a smaller intestinal clearance for the SFM, leading to much reduced intestinal metabolism after i.v. dosing. Hence discrepancy in intestinal metabolism between the p.o. and i.v. doses was greater with the SFM, and this trend was augmented at low CL_d (Fig. 8, A versus B). The same reasoning may be used to explain the intestinal metabolism for the TM. The greater intestinal flow rate to the site of absorption would effect the dispersal of the orally absorbed drug rapidly into the systemic circulation, thereby reducing the extent of intestinal metabolism. Moreover, due to the greater flow rate to the absorptive and metabolic region of the intestine, CL and intestinal metabolism would be high with i.v. dosing. For this reason, there was less discrepancy in intestinal metabolism between the p.o. and i.v. doses with the TM. There was no change in extent of intestinal metabolism with increasing values of k_a, but the time course was shifted to the left.

**Application of SFM: Fitting of Morphine Data.** The optimized parameters obtained from simultaneous fitting of the systemic and oral data of M and M3G to the TM and SFM are summarized in Table 3. Parameter estimation for M was more reliable because the S.D. values of the estimates were less than the values of the estimates. Expectedly, those for M3G were much less reliable due to the very high S.D. values of the estimates. This situation was not unique because the metabolite was not given, and there were too many fitted parameters. Nonetheless, least-square fitting was best with a weighting scheme of unity, and the resultant fits generally yielded good correlation with the data (Table 3, Fig. 9). The quality of the fits was, however, better for the SFM. Although an adequate fit of the TM was observed for intraduodenal data (Fig. 9B), a systematic trend existed for the fit to the i.v. data of M; M3G formation, though not detected in the system, was over-predicted (Fig. 9A). The SFM furnished, in comparison, superior fits, as shown by the higher value for the MSC (Model Selection Criterion), the slightly improved correlation coefficient, the lower RSS or residual sum of square of residuals (Table 3), and increased randomness in the residual plots (Fig. 10). An improved fit was observed with the i.v. data since the serosal compartment effectively provided a distribution space for M (Fig. 9A). The fitted value for the fraction of the intestinal flow perfusing the enterocyte layer (f_Q) was very low, representing only 2.4% of the total intestinal flow, and was different from zero or unity. If f_Q were unity, the SFM would simplify to the TM.

**Discussion**

The overall systemic availability of an orally administered substrate depends on the outcome between intestinal absorption and elimination by first-pass organs such as the intestine, liver, and lungs. Indeed, the importance of the intestine as an ingress organ in regulating the net absorption of drugs into the portal circulation is well recognized (Rowland 1972; Doherty and Pang, 1997). However, unlike the attention given to the examination of physiological variables influencing liver drug clearance (for review, see Pang et al., 1998), removal processes such as metabolism and secretion (or exsorption) and the physiological variables such as intestinal flow and gastrointestinal transit time on intestinal clearance and availability have not been fully investigated.

Until now, modeling and computer fitting of drug absorption have...
been based on a simplistic view of the intestine, where the tissue is considered as a homogeneous compartment separated from the lumen compartment by an apical membrane and from the organ blood by a basolateral membrane. Although these compartmental models have been applied to describe the intestinal absorption of various agents, the models lack consideration of one or more of the processes that are critical in determining reliably the overall clearance of the intestine. More specifically, the model assumed by Barr and Riegelman (1970) allowed for efflux and intracellular metabolism of orally administered drugs but did not include the transfer constant from the blood compartment to the tissue. Crouthamel et al. (1975), on the other hand, included the reversible transfer of drugs between the tissue and blood compartments, but both intestinal secretion and metabolism were ignored in modeling of the pharmacokinetics of sulfaethidole. Transport processes, such as the exchange from blood to tissue or the efflux from tissue to lumen, and intestinal metabolic activities were absent in the kinetic models proposed by Choi et al. (1995) and Nakashima et al. (1984). Recently, Ito et al. (1999) introduced a theoretical pharmacokinetic model to relate the influence of intestinal CYP3A4 metabolism, Pgp efflux, and intracellular diffusion on drug absorption. Not unlike both of our TM and SFM, Ito’s model was able to predict the inverse relationship between bioavailability and metabolism and/or efflux. However, the transport clearance term that describes the partitioning of drug from the circulation to the epithelial cells was absent, precluding the intestinal accumulation or exsorption of i.v. administered drugs, and transfer processes between the gut lumen and epithelial cells were omitted in their definition of absorption clearance. The extended compartmental absorption and transit model developed by Yu and Amidon (1998) had simultaneously considered passive absorption, saturable absorption, degradation, and
transit kinetics in the small intestine. But processes such as luminal and intracellular metabolism and exsorption were excluded. The present model is developed to comprehensively illustrate the interaction between the effective flow to the intestine, the absorption rate constant, intestinal enzymatic and secretory activities, and the influence of other clearances on systemic bioavailability. The SFM, based on the view that the absorptive site of the intestine receives only a portion of the overall organ blood flow, is in theory not dissimilar to the bypass phenomenon proposed by Klippert and Noordhoek (1985), with the exception that the flow rate to the intestinal tissue is conserved and drug distributes into the nonabsorptive and noneliminatory layer of the serosa and submucosa.

A close scrutiny of the SFM and TM reveals notable differences because of the different effective perfusion of the absorptive/metabolic/secretory layer. Theoretical solutions for both the TM and SFM differ only in the flow terms (Q<sub>1</sub> versus Q<sub>en</sub>) (see Table 2). Elimination within other parallel (non first pass) organs fails to affect the intestinal clearance, as expected of the additivity of organ clearances among parallel elimination pathways, and does not impact on bioavailability. The present communication also uncovers that, for both the SFM and TM, CL<sub>d1</sub> and F<sub>1</sub> are directly/inversely related to the intestinal metabolic and exsorption intrinsic clearances (CL<sub>d1</sub> and CL<sub>sec</sub>) and blood flow to the absorptive layer (Figs. 3 and 4); the parameters are additionally affected by k<sub>d</sub> and k<sub>g</sub> when there is drug exsorption (Table 2). Values for the SFM are, however, consistently lower than those for the TM (Fig. 5).

The frequent question addressed on whether the role of Pgp on secretion is overemphasized (Lin et al., 1999) can now be answered. The exsorption of substrate from the intestinal tissue to the lumen (CL<sub>sec</sub> > 0) exerts a direct influence on F<sub>syst</sub>; the larger the exsorption clearance, the less the systemic availability. Drug secretion by Pgp, viewed best in absence of metabolism and loss from lumen, reveals that secretion may be obliterated when drug absorption is rapid (Fig. 6). However, the concurrent absence of secretion and metabolism (CL<sub>sec</sub> = 0; CL<sub>d1</sub> = 0) will result in a dramatic increase in the systemic (or intestinal) availability.

The difference in flow between the models also affects the extents of intestinal metabolism. The condition was best shown when CL<sub>sec</sub> and k<sub>d</sub> = 0; a greater difference in the extent of intestinal metabolism is found between the p.o. and i.v. doses with the SFM (see Fig. 8). According to the SFM, the lowered flow rate perfusing the enterocyte layer renders lower values of intestinal clearance, because there is reduced drug delivery to intestinal enzymes or secretory sites. However, during oral absorption, the entire orally administered dose must traverse the enterocyte layer before the substrate enters the circulation. The consequence of the partial flow to the enterocyte compartment leads to sluggish dispersal of drug into the circulation and a longer transit time within the intestinal tissue. The differential exposure with the site of administration results in different extents of metabolism by intestinal enzymes and exsorption, and contributes to the observation of route-dependent metabolism (Klippert and Noordhoek, 1985; Pang et al., 1985, 1986; Wen et al., 1999).

Intestinal metabolism may then be viewed effectively as a single preabsorptive event, occurring predominantly during the absorption of the substrate across the luminal membrane and is substantially lower upon recirculation of the drug. It
has been noted that flow can also be a limiting factor of intestinal absorption because it affects the net substrate flux from the lumen into the circulation and vice versa (Crouthamel et al., 1975; Winne, 1978; Schurgers and de Blaey, 1984). However, the flow rate to the enterocyte layer is now recognized as critical to intestinal clearance and bioavailability. Although the nature of the change remains largely untested, the magnitude of this flow is expected to be of paramount importance to the initial absorptive flux and drug extraction as well as on subsequent recirculation of the substrate.

Finally, the confirmatory evidence that the SFM is the better explanation of intestinal metabolism is substantiated by the fit to the experimental data of M. Statistically, the fits of the SFM to data on route-dependent glucuronidation of M in the vascularly perfused intestine preparation (data of Doherty and Pang, 2000) are improved over those afforded by the TM (Table 3, Fig. 9). In particular, the fit of the SFM to the i.v. data of M was superior because the distribution phase was better described by the SFM due to the presence of the serosal compartment acting as the storage/distribution compartment (Fig. 2B). The mass transfer equations that describe the rates of changes of M and M3G in the reservoir (R), the tissue were low (5 to 6% dose). Although there were notable levels of M3G accumulated in the reservoir after the intraduodenal dose, the tissue partitioning ratio (value of 8) for M for the SFM was more reasonable than the much higher value of 22 predicted for the TM (CL2/CL1 or CLa2/CLa1), when levels of total radioactivity in the tissue were low (5 to 6% dose). Although there were notable levels of M3G accumulated in the reservoir after the intraduodenal dose, M3G was not detected after i.v. administration. The total level of M3G predicted by the SFM was lower for the SFM (6.6% for TM and 2% for the SFM).

Currently, the intestine is regarded as a single compartment. The SFM is physiologically sound and affords a plausible explanation of route-dependent metabolism. Due to the many examples of route-dependent metabolism of the intestine, it is anticipated that the proposed intestinal SFM may be important in future endeavors to accurately relate in vitro parameters with in vivo physiological events on absorption and bioavailability. Moreover, this model may be readily expanded to describe the physiological segmental divisions of the intestine—duodenum, jejunum, and ileum—and transport and metabolic or secretory heterogeneity within these segments (Dubey and Singh, 1988; Fei et al., 1994; Saitoh and Aungst, 1995; Aldini et al., 1996; Paine et al., 1997). With the development of these kinds of models, predictions on the first pass removal/metabolism and drug-drug interactions within the intestinal tissue would then be made accurately.

**Appendix**

The equations for the TM were presented earlier (see Appendix, Doherty and Pang, 2000), and the equations for the SFM are presented below. There were segregated flows to the enterocyte layer of the mucosa [which comprised of a fraction ($f_Q$) of the total intestinal flow, $Q_I$] and to the serosa and other remaining intestinal tissues [or $(1-f_Q)Q_I$]. The enzymatic and Pgp activities are present in the enterocytes of the mucosa (Fig. 2B). The mass transfer equations that describe the rates of changes of M and M3G in the reservoir (R), the serosa (s), the enterocytes of the mucosal layer (en), and blood in serosal compartment (s,b) and enterocyte layer of the mucosal compartment (en,b), and lumen are:

For M and M3G in reservoir (R) compartment,

$$\frac{dM_I}{dt} = f_Q Q_I \frac{M_{en,b}}{V_{en,b}} + (1-f_Q)Q_I \frac{M_{s,b}}{V_{s,b}} - \frac{M_I}{V_I} \quad (B1)$$

$$\frac{dM3G_R}{dt} = f_Q Q_I \frac{M3G_{en,b}}{V_{en,b}} + (1-f_Q)Q_I \frac{M3G_{s,b}}{V_{s,b}} - \frac{M3G_R}{V_R} \quad (B2)$$

For M and M3G in serosa and other nonmucosal tissue(s) compartment

$$\frac{dM_s}{dt} = CL_{d1} M_{s,b} \frac{V_{s,b}}{V_s} - CL_{d1} M_s \frac{V_s}{V_s} \quad (B3)$$

$$\frac{dM3G_s}{dt} = CL_{d2,M3G} M3G_{s,b} \frac{V_{s,b}}{V_s} - CL_{d2,M3G} M3G_s \frac{V_s}{V_s} \quad (B4)$$

For M and M3G in enterocyte layer (en) in mucosal compartment

$$\frac{dM_{en}}{dt} = CL_{d1} M_{lumen} \frac{M_{en,b}}{V_{en,b}} - CL_{d1} V_{en,b} M_{en} \quad (B5)$$

$$\frac{dM3G_{en}}{dt} = CL_{d2,M3G} M_{en} \frac{M3G_{lumen}}{V_{en,b}} - CL_{d2,M3G} M3G_{en} \frac{V_{en,b}}{V_{en,b}} \quad (B6)$$

For M and M3G in serosal blood (s,b) compartment

$$\frac{dM_{s,b}}{dt} = (1-f_Q)Q_I \frac{M_I}{V_I} + CL_{d1} M_s \frac{V_s}{V_s} - [CL_{d1} (1-f_Q)Q_I] \frac{M_{s,b}}{V_{s,b}} \quad (B7)$$
For M and M3G in blood to enterocyte layer (en,b) in mucosal compartment

\[
\frac{dM_{3G,\text{en,b}}}{dt} = \left(1 - f_g \right) \frac{M_{3G_{\text{en}}}}{V_{\text{en}}} + CL_{\text{d,\text{en}} \cdot \text{M3G}} \frac{M_{\text{3G,b}}}{V_{\text{en}}} - \left[CL_{\text{d,\text{en}} \cdot \text{M3G}} + f_g \right] \frac{M_{\text{3G,\text{en,b}}}}{V_{\text{en,b}}}
\]

For M and M3G in lumen (lumen) compartment

\[
\frac{dM_{\text{lumen}}}{dt} = CL_{\text{sec}} \frac{M_{\text{lumen}}}{V_{\text{en}}} - \left(CL_{\text{e}} + CL_{\text{G1T}}\right) \frac{M_{\text{lumen}}}{V_{\text{en}}} + CL_{\text{G1T}} \frac{M_{\text{3G,lumen}}}{V_{\text{en}}} + CL_{\text{d,\text{en}} \cdot \text{M3G}} \frac{M_{\text{3G,\text{lumen}}}}{V_{\text{en}}}
\]

The amounts of M in exudate and lumen were summed to provide the total amount collected in the sampling tube at 120 min. The same was done for M3G.

References


