Effects of Intestinal CYP3A4 and P-Glycoprotein on Oral Drug Absorption—Theoretical Approach

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Purpose. To evaluate the effects of gut metabolism and efflux on drug absorption by simulation studies using a pharmacokinetic model involving diffusion in epithelial cells.

Methods. A pharmacokinetic model for drug absorption was constructed including metabolism by CYP3A4 inside the epithelial cells, P-gp-mediated efflux into the lumen, intracellular diffusion from the luminal side to the basal side, and subsequent permeation through the basal membrane. Partial differential equations were solved to yield an equation for the fraction absorbed from gut to the blood. Effects of inhibition of CYP3A4 and/or P-gp on the fraction absorbed were simulated for a hypothetical substrate for both CYP3A4 and P-gp.

Results. The fraction absorbed after oral administration was shown to increase following inhibition of P-gp. This increase was more marked when the efflux clearance of the drug was greater than the sum of the metabolic and absorption clearances and when the intracellular diffusion constant was small. Furthermore, it was demonstrated that the fraction absorbed was synergistically elevated by simultaneous inhibition of both CYP3A4 and P-gp.

Conclusions. The analysis using our present diffusion model is expected to allow the prediction of in vivo intestinal drug absorption and related drug interactions from in vitro studies using human intestinal microsomes, gut epithelial cells, CYP3A4-expressed Caco-2 cells, etc.

KEY WORDS: oral absorption; CYP3A4; P-glycoprotein; diffusion model; drug interaction.

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ABBREVIATIONS: P-gp, P-glycoprotein; PSinf, influx clearance from the lumen into the epithelial cells [ml/min]; C, drug concentration in the gut lumen [mol/ml]; Ce, drug concentration in the epithelial cells [mol/ml]; x, distance in the direction of luminal flow [cm]; y, distance in the direction of drug absorption [cm]; Q, luminal flow rate [ml/min]; $A_{r,L}$, sectional area of the gut lumen [cm^2]; V_L, volume of the gut lumen [ml]; V_e, volume of the epithelial cells [ml]; D, diffusion constant in the epithelial cells [cm^2/min]; PSeff, efflux clearance from the epithelial cells to the lumen [ml/min/g]; CLm, metabolic clearance in the epithelial cells [ml/min/g]; CLab, absorption clearance from the epithelial cells to the blood [ml/min/g]; Eab, fraction of drug absorbed into blood at steady-state; $A_{r,e}$, sectional area of each epithelial cell [cm^2]; M, length of each epithelial cell in the direction of drug absorption [cm]; F, drug availability in the gut lumen; L, length of the gut lumen [cm]; V_m, velocity of intracellular metabolism [mol/min]; V_{ab}, velocity of drug absorption [mol/min]; Z, length of each epithelial cell in the direction perpendicular to both drug absorption and luminal flow [cm].

INTRODUCTION

CYP3A4, an enzyme involved in the metabolism of many drugs including cyclosporin, is reported to be present not only in the liver but also in the gut and it plays an important role in the first-pass metabolism of drugs after their oral administration (1–3). De Waziers *et al.* (4) have shown using Western blot analysis that CYP3A4 is highly expressed in duodenum and jejunum, second only to liver, in humans.

Oral bioavailability of cyclosporin is reduced by co-administration of rifampicin, an inducer of CYP3A4, and increased by co-administration of ketoconazole or erythromycin which are inhibitors of CYP3A4 (5–7). Wu *et al.* (8) attempted to differentiate between the first-pass gut and hepatic metabolism of cyclosporin in humans by a kinetic analysis of rifampicin-, ketoconazole-, or erythromycin-induced changes in bioavailability. The calculated extraction ratio in the gut was larger than that in the liver and the effect of the interacting drug in the gut was about the same or greater than that in the liver.

It is also known that P-glycoprotein (P-gp) is present in the luminal membrane of gut epithelial cells where it acts as an efflux transporter (9,10). Recent studies have revealed the overlapping substrate specificity of CYP3A4 and P-gp and many substrates of CYP3A4 are also substrates or inhibitors of P-gp (11). Using a cell line derived from a human colon adenocarcinoma, many of the P-gp inducers were shown to also induce CYP3A4, suggesting the possibility of common regulatory factors for these proteins (12). Benet *et al.* have proposed that the synergistic effects of CYP3A4-mediated metabolism and P-gp-mediated efflux in the gut epithelium may result in an unexpectedly high first-pass effect in the gut after oral administration (13,14). Thus, the first-pass effect in the gut may be affected by the inhibition or induction of CYP3A4 and/or P-gp caused by drug-drug interactions.

In this study, the effects of gut metabolism and efflux on the absorption of a drug (a hypothetical substrate for both CYP3A4 and P-gp) were evaluated by simulation studies using a pharmacokinetic model with a diffusion process in epithelial cells.

METHODS

Model and Assumptions

Figure 1 shows the model used in the analysis. A tube model was assumed for the gut lumen and any diffusion and/or dispersion of the drug along the flow was ignored. The apparent influx clearance from the lumen into the epithelial cells (PSinf) was defined as a hybrid parameter including both membrane permeability and diffusion through the unstirred water layer (15).

It was assumed that a drug taken up into epithelial cells is metabolised during the diffusion process from the luminal to the basal side and that the metabolising enzyme is distributed homogeneously in the cells in the direction of both luminal flow and drug absorption. Efflux by P-gp was assumed to take place at the luminal membrane of the epithelial cells and was incorporated in the boundary condition, as well as the absorption process into blood at the basal membrane.

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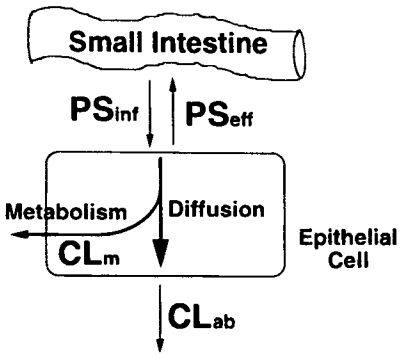

Fig. 1. The kinetic model for drug absorption used in the analysis. PSinf; the apparent influx clearance from the lumen into epithelial cells, PSeff; the efflux clearance from cells to the lumen, CLm; the metabolic clearance in the cells, and CLab; the absorption clearance from the cells to the blood.

Equations

According to the above model, mass-balance equations for drug concentrations in the gut lumen (C) and epithelial cells (Ce) can be described as follows:

$$\frac{\partial C(x,t)}{\partial t} = -\frac{Q}{A_{r,L}}\frac{\partial C(x,t)}{\partial x} - \frac{PS_{inf}}{V_L}C(x,t) + PS_{eff}\cdot\frac{V_e}{V_L}\cdot Ce(x,0,t) \quad (1)$$

$$\frac{\partial Ce(x,y,t)}{\partial t} = D\frac{\partial^2 Ce(x,y,t)}{\partial y^2} - CLm\cdot Ce(x,y,t) \quad (2)$$

where x and y represent the distance in the direction of luminal flow and drug absorption, respectively; Q is the luminal flow rate; $A_{r,L}$ is the sectional area of the lumen; V_L and V_e are the volume of the lumen and epithelial cells, respectively; D is the diffusion constant in the cells. The efflux clearance from cells to the lumen (PSeff), the metabolic clearance in cells (CLm), and the absorption clearance from cells to the blood (CLab; see below) were defined as clearances per unit volume of the epithelial cells.

These partial differential equations were solved by Laplace transformation to give the fraction of drug absorbed into blood at steady-state (Eab). The initial conditions (Eqs. 3 and 4) and the boundary conditions at the apical and basal membranes of cells (Eqs. 5 and 6, respectively) were as follows:

$$C(x,0) = 0 \quad (3)$$

$$Ce(x,y,0) = 0 \quad (4)$$

$$PS_{inf}\cdot C(x,t) = PS_{eff}\cdot V_e\cdot Ce(x,0,t) - D\cdot A_{r,e}\frac{\partial Ce(x,0,t)}{\partial y} \quad (5)$$

$$CLab\cdot V_e\cdot Ce(x,M,t) = -D\cdot A_{r,e}\frac{\partial Ce(x,M,t)}{\partial y} \quad (6)$$

where $A_{r,e}$ is the sectional area of each epithelial cell and M is the length of each cell in the direction of drug absorption.

First, the availability in the lumen (F) was calculated by dividing the steady-state drug concentration at the exit of the lumen by that at the entrance (Eq. 7; see APPENDIX):

$$F = \lim_{s\to 0}\frac{C(L,s)}{Co/s} = \exp\left\{\frac{\alpha_1\sqrt{D\cdot CLm}\,(1+YY)}{PS_{eff}\cdot M\cdot(1-YY) - \sqrt{D\cdot CLm}\,(1+YY)}\right\} \quad (7)$$

where

$$YY = \frac{M\cdot CLab + \sqrt{D\cdot CLm}}{M\cdot CLab - \sqrt{D\cdot CLm}}\exp\left(2M\sqrt{\frac{CLm}{D}}\right)$$

and L represents the length of the lumen. α_1 was defined as follows as a dimensionless parameter, dividing the influx clearance from the lumen to the cells (PSinf) by Q:

$$\alpha_1 = \frac{PS_{inf}}{Q}$$

Eab was calculated from the extraction ratio, obtained by subtracting F from 1, taking the contribution of metabolism into consideration (Eq. 8; see APPENDIX):

$$Eab = (1-F)\frac{V_{ab}}{V_{ab}+V_m} = \frac{2M\cdot CLab\,(1-F)}{\left\{(M\cdot CLab - \sqrt{D\cdot CLm})\exp\left(-M\sqrt{\frac{CLm}{D}}\right) + (M\cdot CLab + \sqrt{D\cdot CLm})\exp\left(M\sqrt{\frac{CLm}{D}}\right)\right\}} \quad (8)$$

where V_{ab} and V_m represent the velocity of absorption and metabolism, respectively.

Assuming that D is sufficiently larger than CLm ($4M^2\cdot CLm \ll D$) in Eq. 8, the following equation (Eq. 9) can be obtained, which is also derived by assuming a well-stirred model inside the cells:

$$Eab = \frac{CLab}{CLab+CLm}\times\left\{1-\exp\left(-\alpha_1\cdot\frac{CLab+CLm}{PS_{eff}+CLab+CLm}\right)\right\} \quad (9)$$

Simulations

The effects of inhibiting the efflux and/or metabolism on Eab were simulated according to Eq. 8, changing the values of PSeff, CLm, CLab, etc. The value of D is reported to be about 2×10^{-5} cm²/min for a drug with a molecular weight of about 300 (16). Therefore, 2×10^{-5}, 2×10^{-6}, and 2×10^{-7} cm²/min were used for the value of D; M was assumed to be 0.003 cm (17) and 0.1, 1, and 10 were used as values for α_1.

RESULTS

Figure 2 shows how Eab is affected when the efflux clearance is reduced due to inhibition of P-gp under various condi-

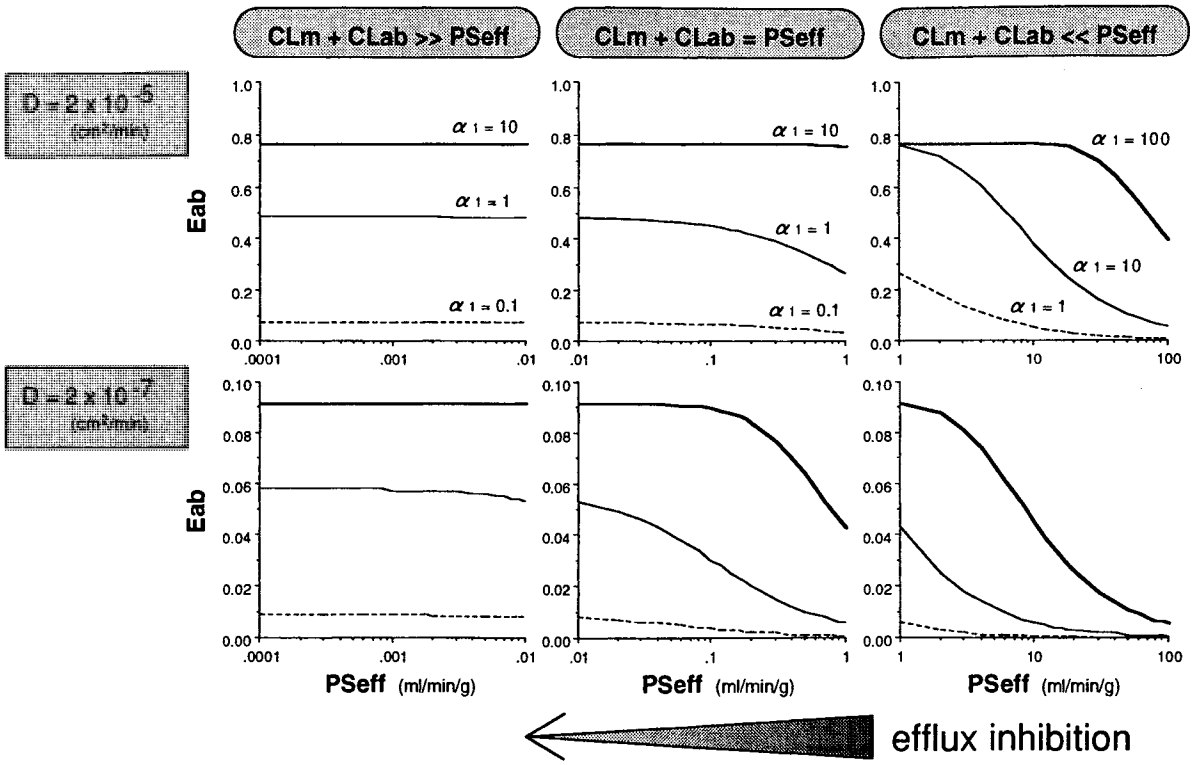

Fig. 2. The simulated effects of efflux inhibition on Eab. CLm = 0.2 ml/min/g, CLab = 0.8 ml/min/g. See text for the abbreviations.

tions. When the intracellular diffusion constant is relatively large (upper figures), the results obtained were the same as those obtained assuming a well-stirred model inside the cells (Eq. 9), where the degree of increase in Eab caused by efflux inhibition tended to be greater for a drug with a higher efflux clearance. This tendency was more marked when the diffusion constant was relatively small (lower figures).

In Fig. 3, the effect of efflux clearance on Eab was investigated by changing the values of CLm and CLab by a factor of 10. When the intracellular diffusion constant is relatively large, Eab is affected by efflux inhibition if PSeff is greater than the sum of CLm and CLab. On the other hand, when the diffusion constant is relatively small, there is a clear effect of efflux inhibition even if PSeff is not very large.

The simulated effects of simultaneous inhibition of both intracellular metabolism and efflux into the lumen are shown in Fig. 4. It can be seen that inhibition of metabolism has a greater effect on Eab than inhibition of efflux when PSeff is small, whereas inhibition of efflux has a greater effect when PSeff is large. Furthermore, Eab is synergistically increased by simultaneous inhibition of both metabolism and efflux, and this is also clearer when the intracellular diffusion constant is small.

DISCUSSION

Attempts have been made to rigorously predict in vivo pharmacokinetics and drug interactions from in vitro data (18–20). Although in vitro intrinsic clearances of many drugs obtained in metabolic studies using human liver microsomes agreed well with in vivo intrinsic clearances calculated based on physiologically-based pharmacokinetics, the intrinsic clearances of some drugs were underestimated in the in vitro studies (19). Furthermore, some of the in vivo drug-drug interactions were also underestimated from in vitro metabolic inhibition studies, taking only hepatic metabolism into consideration (21,22). In these cases, the predictability is expected to be improved by taking into account drug metabolism or drug interactions in extrahepatic tissues such as the small intestine.

It is reasonable to expect that, in gut epithelial cells, metabolism by CYP3A4 and efflux by P-gp into the lumen limits the oral bioavailability of substrates for these proteins (8,9). Benet has proposed the concept that a substrate of P-gp repeatedly circulates between the gut lumen and epithelial cells, leading to increased metabolism due to prolonged exposure to the metabolic enzyme, resulting in reduced absorption of the drug into blood (Fig. 5) (13,14). However, no one has previously attempted to carry out a model analysis to investigate this concept in quantitative terms. Therefore, in the present study, the effects of inhibiting intracellular metabolism and/or efflux into the lumen on drug absorption into the blood were investigated by a model analysis which took into consideration intracellular drug diffusion.

The diffusion constant was assumed to be 2×10^{-5} cm^2/min, which is the reported value for a compound with a molecular weight of about 300 (16), or smaller than that which might apply to drugs with substantial binding to intracellular organella (16). Studies using perfused rat liver have revealed that the diffusion of drug molecules in cells is greatly limited compared with that in water because of intracellular structures such as microfilaments and microtubles (23). Because diffusion of the intracellular organella itself is negligible, only drugs in the cytosol diffuse in cells (24). Furthermore, intracellular diffusion of drugs bound to cytosolic protein is also limited due to their

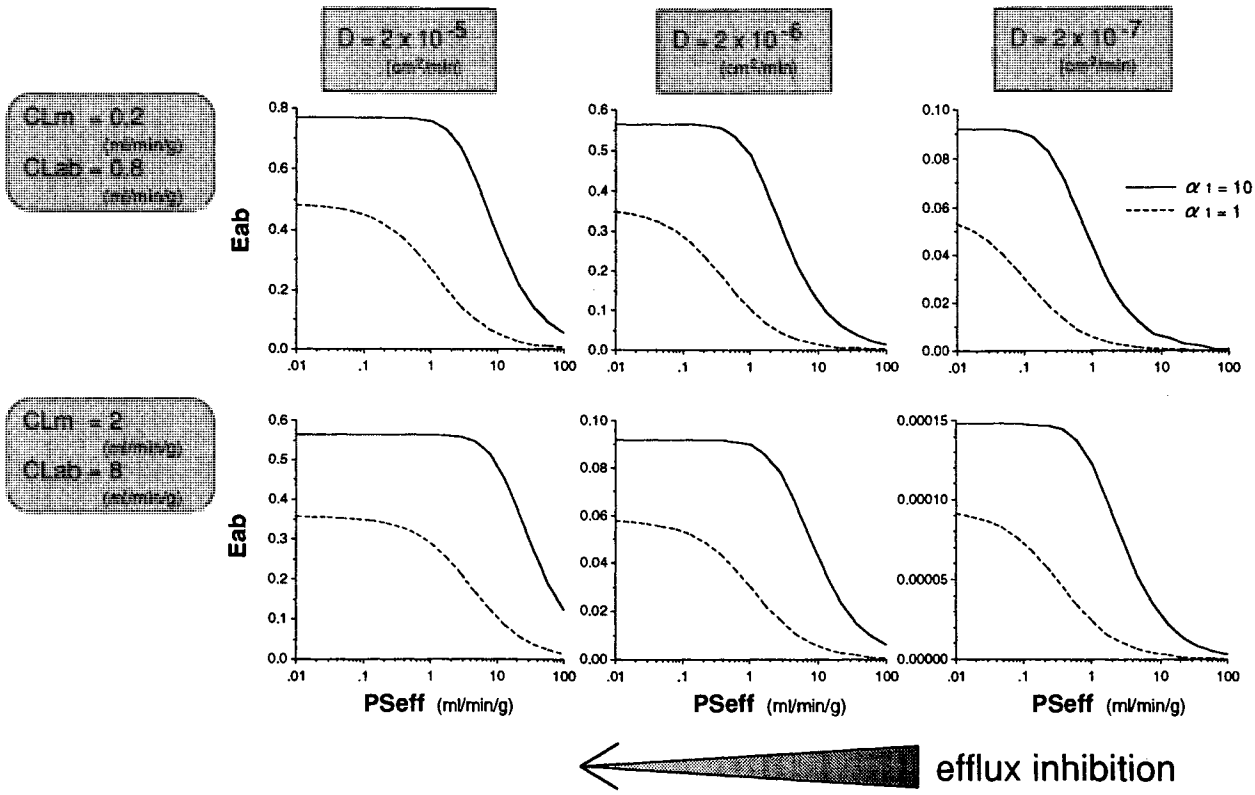

Fig. 3. The simulated effects of efflux inhibition on Eab. Values of CLm and CLab were changed as indicated. See text for the abbreviations.

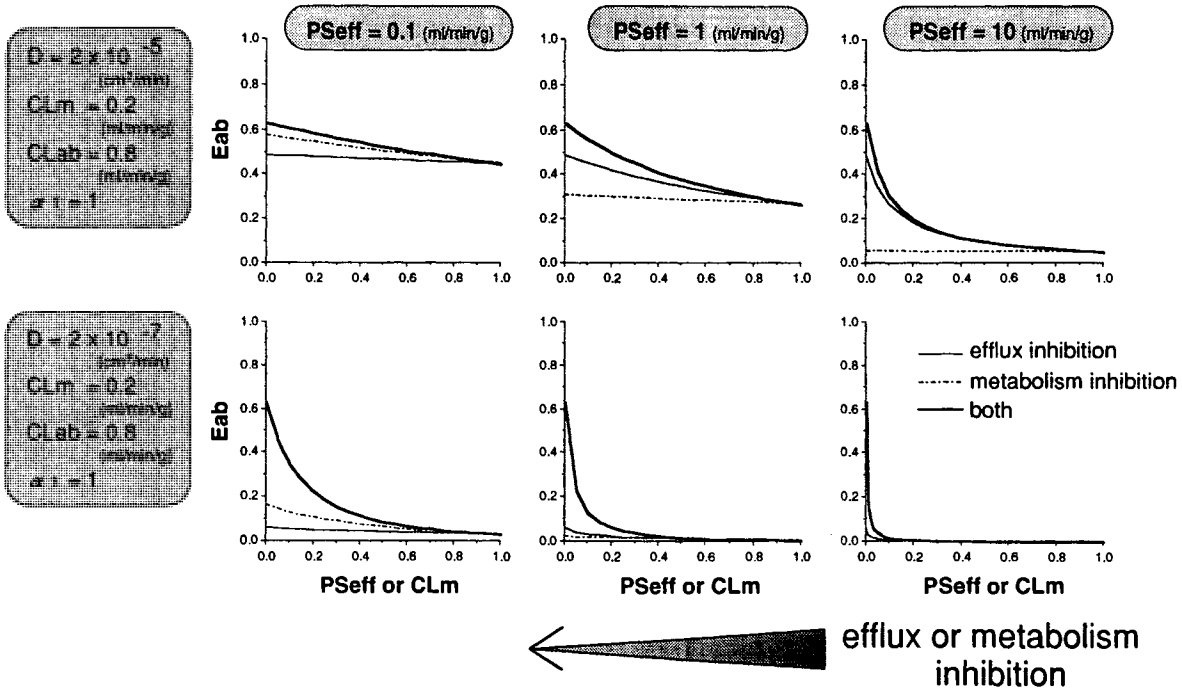

Fig. 4. The simulated effects of inhibiting metabolism and/or efflux on Eab. CLm = 0.2 ml/min/g, CLab = 0.8 ml/min/g, $\alpha_1 = 1$. See text for the abbreviations.

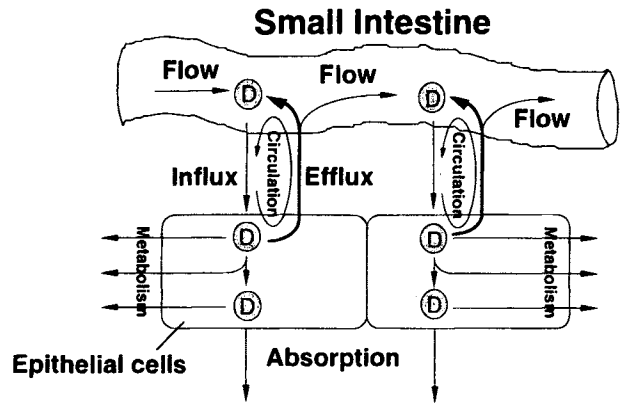

Fig. 5. The synergistic effect of intracellular metabolism and efflux into the lumen on the absorption of a substrate of both P-gp and CYP3A4.

large molecular weight. Therefore, the intracellular diffusion constant defined in terms of total drug, including the fraction bound to intracellular organella or cytosolic protein, may be much smaller than the intrinsic diffusion constant defined in terms of free drug in the cytosol. For this reason, the value of D, defined as an apparent diffusion constant for total drug in cells, was reduced in some cases to 1/10 or 1/100 of the control value in the present analysis.

Simulation studies for a hypothetical drug which undergoes both metabolism and efflux demonstrated that the fraction absorbed is increased following inhibition of efflux into the lumen. The greater the PSeff compared with the sum of CLm and CLab, and the smaller the intracellular diffusion constant, the greater the increase in the fraction absorbed following efflux inhibition (Figs. 2 and 3). Furthermore, it was shown that the fraction absorbed is synergistically increased by simultaneous inhibition of both intracellular metabolism and efflux into the lumen (Fig. 4). This provides theoretical support for the qualitative hypothesis proposed by Benet.

The finding that the fraction absorbed and the effect of metabolic and/or efflux inhibition depend on the intracellular diffusion of the drug can be explained as follows: the drug molecules taken up into epithelial cells from the gut lumen diffuse within the cells until they reach the basal side. If the diffusion constant is small (i.e., slow diffusion), the intracellular residence time of the drug molecules is long, leading to extensive metabolism due to prolonged exposure to the enzyme. In such cases, therefore, the amount of the drug absorbed into the blood is less than that in the case of rapid intracellular diffusion. In addition, the effect of efflux into the lumen is clearer in the case of slower intracellular diffusion because of the longer residence time of the drug molecules around the luminal border. The fraction absorbed is increased when either metabolism or efflux is inhibited and, in the case of slower diffusion, the effects of both metabolism and efflux are greater, which may cause the synergistic increase in the fraction absorbed to be more appearent when both processes are inhibited simultaneously.

In future, analyses based on the present diffusion model may be applied to predicting in vivo intestinal absorption and related drug interactions from in vitro studies in which parameters such as the metabolic and efflux clearance of various drugs are measured using intestinal microsomes (25,26), Caco-2 cells (27,28), or the CYP3A4-expressed or induced Caco-2 cells (29,30).

APPENDIX

Laplace transformation of Eqs. 1 and 2 gives Eqs. 10 and 11, respectively:

$$\frac{\partial C(x, s)}{\partial x} = -\frac{A_{r,L}(s + PS_{inf}/V_L)}{Q} C(x, s) + \frac{A_{r,L} PS_{eff}\, V_e/V_L}{Q} Ce(x, y, s) \quad (10)$$

$$\frac{\partial^2 Ce(x, y, s)}{\partial y^2} = \frac{s + CLm}{D} Ce\,(x, y, s) \quad (11)$$

When Ce can be expressed as $Ce = A \exp(\alpha y)$,

$$A\alpha^2 \exp(\alpha y) = \frac{s + CLm}{D} A \exp(\alpha y) \quad (12)$$

Therefore,

$$Ce(x, y, s) = A\,(x, s) \exp(\alpha y) + B\,(x, s) \exp(-\alpha y) \quad (13)$$

where

$$\alpha = \pm\sqrt{\frac{s + CLm}{D}}$$

Rearrangement of Eqs. 6 and 13 and introducing the parameter Y gives Eq. 14:

$$B = -\left\{\frac{M\,CLab + \alpha D}{M\,CLab - \alpha D} \exp(2\alpha M)\right\} A = -Y\,A \quad (14)$$

Combining Eqs. 13 and 14,

$$Ce = A\{\exp(\alpha y) - Y \exp(-\alpha y)\} \quad (15)$$

Rearrangement of Eqs. 5 and 15 and introducing the parameter X gives Eq. 16:

$$A = \frac{PS_{inf}}{PS_{eff}\, V_e(1 - Y) - \alpha D A_{r,e}(1 + Y)} C(x) = X\,C(x) \quad (16)$$

Combining Eqs. 15 and 16,

$$Ce = X\,C(x)\,\{\exp(\alpha y) - Y \exp(-\alpha y)\} \quad (17)$$

When $C(0) = 0$ and $y = 0$, Eqs. 10 and 17 can be rearranged as follows:

$$C(x) = \frac{Co}{s} \times \exp\left\{-\frac{s + PS_{inf}/V_L - PS_{eff}\, V_e/V_L\, X\,(1 - Y)}{Q/A_{r,L}} x\right\} \quad (18)$$

When $s \to 0$, introducing the parameter YY,

$$\alpha \to \sqrt{CLm/D}$$

$$Y \to \frac{M\,CLab + \sqrt{D\,CLm}}{M\,CLab - \sqrt{D\,CLm}} \exp(2M\sqrt{CLm/D}) = YY$$

$$X \to \frac{PS_{inf}}{PS_{eff} V_e(1 - YY) - \sqrt{D\,CLm}\, A_{r,e}(1 + YY)}$$

Therefore, the availability in the lumen (F) can be obtained as follows:

$$F = \lim_{s\to 0} \frac{C(L)}{Co/s}$$

$$= \lim_{s\to 0} \exp\left\{-\frac{s + PS_{inf}/V_L - PS_{eff}\, V_e/V_L \,X\, (1 - Y)}{Q/A_{r,L}} L\right\} \quad (19)$$

Rearrangement of Eq. 19 gives Eq. 7.

The velocities of intracellular metabolism and absorption (V_m and V_{ab}, respectively) can be expressed as follows:

$$V_m = CLm\, M\, L\, Z \frac{dx}{L}\frac{dy}{M}\int_0^L \int_0^M Ce(x, y, ss) \quad (20)$$

$$V_{ab} = CLab\, M\, L\, Z \int_0^L Ce(x, M, ss)\frac{dx}{L} \quad (21)$$

where Z is the length of each epithelial cell in the direction perpendicular to both drug absorption and luminal flow; and

$$Ce(x, y, ss) = \lim_{s\to 0} s\, Ce(s)$$

$$= \lim_{s\to 0} s\, C(s)\, X\, \{\exp(\alpha y) - Y \exp(-\alpha y)\}$$

$$= Co \exp\left\{-\frac{s + PS_{inf}/V_L - PS_{eff}\, V_e/V_L \,X\, (1 - Y)}{Q/A_{r,L}} s\right\}$$

$$\times X\, \{\exp(\alpha y) - Y \exp(-\alpha y)\} \quad (22)$$

Eq. 8 can be derived by combining Eqs. 20, 21, and 22.

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