A New Physiologically-Based Pharmacokinetic Model for the Prediction of Gastrointestinal Drug Absorption: Translocation Model

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Abbreviations: F, bioavailability; FG, intestinal availability; FA, fraction absorbed; FH, hepatic availability; FAFG, product of FA and FG; PBPK, physiologically-based pharmacokinetic; GI, gastrointestinal; ACAT, advanced compartmental absorption and transit; Q, blood flow rate; L, length; MRT, mean residence time; VRT, variance of residence time; z, location; DTPA, diethylenetriamine pentaacetic acid; AIC, Akaike’s information criterion; kGE, gastric emptying rate constant; PE, plicate expansion; VE, villi expansion; ME, microvilli expansion; V, volume; HLw, half-life for disappearance of water; T„enter„, thickness of enterocyte; Hvilli, height of villi; FaSSIF, fasted state simulated intestinal fluid; CYP3A, cytochrome P450 3A; A, protein amount; P-gp, P-glycoprotein; Ht, hematocrit; SMA, superior mesenteric artery; Papp, apparent permeability; Km, Michaelis constant; Vmax, maximum rate; P, permeability; DDI, drug-drug interaction; f, free fraction; CLint,H, hepatic intrinsic clearance; AFE, average fold error; RMSE, root mean square prediction error.
Abstract

This study aimed to construct a new local pharmacokinetic model of gastrointestinal absorption, the translocation model (TLM), using an anatomically relevant, minimally segmented structure to explain linear and nonlinear intestinal absorption, metabolism, and transport. The TLM was based on the concept of a single absorption site that flexibly moves, expands, and shrinks along with the length of the gastrointestinal tract after the intake of an oral dose. The structure of the small intestine is continuous, and various time- and location-dependent issues are freely incorporated in the analysis. Since the model has only one absorption site, understanding and modification of factors affecting the absorption are simple. The absorption site is composed of four compartments: solid drug in the lumen, solution drug in the lumen, concentration in the enterocytes, and concentration in the lamina propria. The lamina propria includes the blood capillaries. Blood flow in the absorption site of the lamina propria appropriately accounts for the absorption. In the TLM, the permeability of the apical membrane and that of the basolateral membrane are distinct. By considering plicate, villi, and microvilli expansions of the surface area, the apparent permeability ($P_{app}$) measured in Caco-2 experiments was converted to the effective permeability ($P_{eff}$) in vivo. The intestinal availability, bioavailability, and dose-product

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of intestinal availability and absorption rate ($F_A F_G$) relationship of model drugs were well explained using the TLM. The TLM would be a useful tool for the consideration of local pharmacokinetics in the GI tract in various situations.
Introduction

In drug discovery, the prediction and understanding of the absorption profile of oral drug candidates are required because they are key information to determine the exposure of the drug to therapeutic targets in the body. Accordingly, the development of a logical physiologically based mathematical model that can predict the absorption process of drugs in the gastrointestinal (GI) tract is important. Several models for the prediction of local pharmacokinetics in the GI tract have been reported so far, including the advanced compartmental absorption and transit (ACAT) model (Yu and Amidon, 1999; Agoram et al., 2001), segregated-flow model (SFM) (Cong et al., 2000), $Q_{\text{Gut}}$ model (Yang et al., 2007), advanced dissolution, absorption and metabolism (ADAM) model (Jamei et al., 2009), and GI Transit Time (GITT) model (Bergstrand et al., 2009; Bergstrand et al., 2012; Hénin et al., 2012). Among these, the $Q_{\text{Gut}}$ model is the simplest one in model description that predicts intestinal availability ($F_{\text{G}}$) by using $Q$, which is a virtual hybrid parameter of the membrane permeability clearance and blood flow rate in the intestinal villi (Gertz et al., 2010). Although the fundamental principal of $Q_{\text{Gut}}$ model are similar with SFM (Pang and Chow, 2012), the $Q_{\text{Gut}}$ model is not capable of analyzing nonlinear pharmacokinetics because the model does not
consider drug concentration in the enterocytes, where nonlinear processes such as metabolism and transport actually occur (Hisaka et al., 2010).

The ACAT, and ADAM models assume a compartmentalized structure of the intestine, and first order kinetics is applied to the movement of drugs within it. These models can consider location dependent longitudinal changes in the physiological conditions. It has been reported that the ACAT model successfully predicts the oral pharmacokinetics of various drugs (Heikkinen et al., 2012). However, even by incorporating the ACAT model into a sophisticated whole body physiologically based pharmacokinetic (PBPK) model, the oral pharmacokinetics of only 23% of drugs are accurately predicted (Poulin et al., 2011).

Furthermore, it should be noted that diagonal (moves from mucosa to submucosa) changes are taken into consideration less than longitudinal (moves along with the absorption site) changes in these models. The concentration of drugs in the enterocytes is sometimes vague since the necessary distinction of permeability between the apical and basolateral membranes is lacking or only implicitly defined. Furthermore, heterogeneous flow that is caused by the differences in villi abundance in different regions should be adequately considered (Kalampokis et al., 1999).
In most of the past physiological absorption models, the blood flow rate to the whole small intestine is used for the analysis of absorption. In addition, these models often assume that the basolateral transfer is mostly unidirectional, thus the effect of the blood stream on the absorption rate is ignored. The consequence of changes in the blood flow on the absorption rate becomes important when the basolateral transfer of drugs from the enterocytes to the blood stream is assumed as reversible. The importance of the blood flow is discussed more appropriately in several articles (Winne, 1978; Shulz and Winne, 1987; Chen and Pang, 1997; Pang and Chow, 2012); a physiological model was reported by Cong (Cong et al., 2001) in which branching of the blood flow to the mucosa and submucosa was considered. Pang mentioned the importance of intestinal blood flow branching and proposed the adequate mucosal to intestinal blood flow ratio ($f_Q$) (Pang and Chow, 2012). When the effect of the blood stream on the absorption of drugs is considered based on the physiological structure of the intestine, branching in both the diagonal and longitudinal directions should be considered. It is well known that blood flow to the duodenum and the jejunum is greater than that to the ileum, which is in accord with the physiological function of the intestine. To date, few absorption models appropriately take into consideration both the diagonal and longitudinal blood flow
branching, except the segregated-flow model, which can factor for the longitudinal blood flow in animals by dividing the lumen and enterocytes into three compartments (Tam et al., 2003).

In this study, a new model is developed to solve these issues and to describe drug absorption in the GI tract in a more physiological manner. Although complicated factors needed to be considered in the new model, we hoped to keep the whole structure as simple as possible. This is because the model should be comprehensive and completely open to the users. Considering all of these issues, we aimed to construct a new model that could accurately predict various absorption events with only one absorption site.
Materials and Methods

Theory of location and distribution functions

In the translocation model (TLM), the location and length of the absorption site in the small intestine were calculated using $\lambda(t)$, the location function, and $\sigma^2(t)$, the variance function, at time, $t$. Those functions were obtained by using a fitting analysis of the observed location and its variance for a non-absorbable index drug in the intestinal tract. We assume that a drug is being dissolved completely here. The dissolution process will be considered later. When a total drug concentration (density per length) at a distance $z$ from an exit of the stomach is expressed as $C_{\text{lumen,total}}(z)$, $n$-th order moment of distribution of a drug in the intestinal tract is defined by Equation 1 where $L_{si}$ is the length of the small intestine from the duodenum to the ileum:

$$M_{\text{location,n}} = \int_0^{L_{si}} z^n \cdot C_{\text{lumen,total}}(z)dz$$

The mean and variance of location of the index drug in the intestine are given by $\lambda$ and $\sigma^2$, respectively.
\[ \lambda = \frac{M_{\text{location},1}}{M_{\text{location},0}} \]  

(2)

\[ \sigma^2 = \frac{M_{\text{location},2}}{M_{\text{location},0}} - \lambda^2 \]  

(3)

Equations 2 and 3 correspond to the mean residence time (MRT) and variance of residence time (VRT) in the moment analysis of blood drug concentration-time profiles. In the TLM, it was assumed that \( C_{\text{lumen}}(z) \) at the observation time is constant within the absorption site, and it is otherwise zero, for simplification. Based on this hypothesis, the length of the absorption site \( L_{\text{abs}} \) is given by Equation 4, which was derived from Equations 2 and 3. In the following, subscript abs denotes the absorption site:

\[ L_{\text{abs}} = 2\sqrt{3\sigma} \]  

(4)

In theory, the equations for \( \lambda \) and \( \sigma \) can be arbitrarily selected as capable of explaining the observed movements of a drug in the GI tract. In this study, Equations 5 and 6 were obtained using a fitting analysis of the reported data (Kimura et al., 2002) that showed the time-course of % dose of radioactivity in the jejunum, ileum, and colon after administration of non-
absorbable $^{99m}$Tc-DTPA in humans (Figure 5A):

$$\lambda_{\text{bolus}}(t) = \frac{m_1}{m_2} \frac{t}{m_2 + t}$$  \hspace{1cm} (5)

$$\sigma_{\text{bolus}}(t) = s_1 \left( e^{-s_2 t} - e^{-s_3 t} \right) + s_4$$  \hspace{1cm} (6)

Subscript bolus means that the functions correspond to bolus inputs of a drug into the system, and $t$ is the observation time. As a result of fitting analysis, $m_1$, $m_2$, $s_1$, $s_2$, $s_3$, and $s_4$ were obtained as $415$ (1.35%), $1.50$ (4.71%), $92.4$ (4.68%), $0.605$ (4.35%), $45.9$ (36.8%), and $3.72$ (5.03%), respectively (values in the parenthesis are CV values calculated from the fitting analysis). Equations 5 and 6 were used to describe the time dependent change of the drug absorption site, and were shown in Scheme 1.

When a drug enters the GI tract with an input function $I(t)$ but not as a bolus, such as continuous or intermittent administration, the location function should be given with a convolution of $I(t)$ and Equation 5.
\[
\lambda(t) = (\lambda_{\text{bolus}} \ast I)(t) = \int \lambda_{\text{bolus}}(\nu)I(t-\nu)d\nu
\]  

(7)

To simplify the convolution analysis, another moment analysis is applied to drugs in the intestine to calculate mean residence time, \( \tau \).

\[
M_{\text{time},n} = \int_0^t s^n \cdot X_{\text{lumen, total}}(s)ds
\]  

(8)

\[
\tau = \frac{M_{\text{time},1}}{M_{\text{time},0}}
\]  

(9)

where \( X_{\text{lumen, total}}(t) \) is the total amount of drug in the lumen at time \( t \). Calculation of differentials of \( M_{\text{time},n} \) is described in the Supplementary Data (Appendix) and applied for calculation of \( M_{\text{time},1} \) in Scheme 1. When the velocity of a drug in the absorption site is constant even in the absence or presence of continuous drug input/output, a location of the average of drug distribution moves in accordance with \( \tau \). Therefore, the convolution of Equation 7 can be approximated as follows.

\[
\lambda(t) = (\lambda_{\text{bolus}} \ast I)(t) \approx \lambda_{\text{bolus}}(\tau)
\]  

(10)
Equation 10 means that if we calculate $\tau$ from the time-courses of a drug input/output appropriately, the drug movement is described apparently with $\lambda_{bolus}(\tau)$. Equation 10 generates errors when velocity of the drug movement is variable in the absorption site. Theoretically, it is possible to cover these errors by calculating higher moments. However, the simplest model was adopted in this study because it showed sufficient performance (Figure 10). Similarly, $\sigma$ was also approximated using $\tau$ for simplification.

$$\delta(t) \cong \delta_{bolus}(\tau)$$  \hspace{1cm} (11)

In the TLM, parameters such as permeability and clearance are determined depending on the location of the absorption site (Table 1). Strictly speaking, these parameters are possibly variable even within the absorption site. However, for simplification, it was assumed that representative values at the average location could be applied monotonously for the absorption site in the TLM. In the present model, the stomach and large intestine were modeled separately as well-stirred compartments. It was assumed that a drug in the stomach traverses to the
entrance of duodenum with a first order rate constant of $k_{GE}$. The value for the $k_{GE}$ for a solution was assumed as 5.46 h$^{-1}$ from the fitting analysis using Equations 5 and 6. Input into the large intestine is determined by movements of a drug in the small intestine. In the present study, it was assumed that absorption does not occur from the stomach and large intestine, considering the characteristics of the drugs used for the analysis.

**Radius and adjusted location of the intestinal tracts**

The function for the radius ($r$) of the lumina of the small intestine, consisting of the duodenum, jejunum, and ileum, was described by linear function in accordance with a report by Willmann (Figure 4a; Willmann et al., 2003, 2004).

$$r = r_{ini} - r_{grad} \cdot z_c$$ (cm) (12)

where $z_c$ (cm) was the center of the absorption site. It needs to be noted that the intestine should be filled with the contents adequately to agree its true radius to the value calculated by Equation 12. In the process of absorption, this is not always realized. For this reason, the
internal volume of intestine will be calculated based on different assumption later. The values of $r_{ini}$ and $r_{grad}$ (1.56 and 0.00265, respectively with $r = -1.00$, and AIC = -48.7) were obtained from a regression analysis to the radii that were used in GastroPlus™.

**Surface area and volume at the absorption site**

The surface areas of the absorption sites of the basolateral and apical membranes of the small intestine ($S_{abs,bas}$ and $S_{abs,api}$, respectively) were calculated using Equations 13 and 14:

$$S_{abs,bas} = \pi \left( r_{in} + r_{out} \right) \sqrt{ \left( r_{in} - r_{out} \right)^2 + L_{abs}^2 } \times PE \times VE_{abs} \times ME$$  \hspace{1cm} \text{(cm}^2) \hspace{1cm} \text{(13)}

$$S_{abs,api} = S_{abs,bas} \times ME$$  \hspace{1cm} \text{(cm}^2) \hspace{1cm} \text{(14)}

where $r_{in}$ and $r_{out}$ are the radii at the proximal and distal end of the absorption site, and PE is plicate expansion, $VE_{abs}$ is villi expansion, and ME is microvilli expansion (Figure 3) at the absorption site. Equation 13 was derived from the formula of surface area of frustum. $VE_{abs}$ was calculated using Equation 15 because of a gradual decrease in the size of villi depending on the location in the small intestine.
where $VE_{ini}$ is villi expansion at the entrance of the duodenum. We assumed that PE and ME are constant in the small intestine. For PE, $VE_{ini}$, and ME, 3, 10 and 20, respectively, were used for the small intestine (DeSesso and Jacobson, 2001).

The surface area of the whole length of the small intestine ($S_{si}$) was calculated using Equation 16 as a solution of integration of cross-sectional area of intestinal lumen from 0 to $L_{si}$:

$$S_{si} = 2\pi \cdot PE \cdot ME \left[ \frac{(1 + VE_{ini}) r_{int} \cdot L_{si}}{2} - \left( \frac{1}{3} + \frac{V_{ini}}{6} \right) r_{grad} \cdot L_{si}^2 \right] \quad \text{(cm}^2\text{)} \quad (16)$$

$S_{si}$ will be used for calculation of the blood flow (Equation 20). The volume of the absorption site for the intestinal lumen, $V_{lumen,abs}$, was not calculated from the sizes of the absorption site described by Equations 4 and 12 because these equations assume that the lumen is adequately filled with contents. Instead, it was calculated using Equation 17 as the total of water volume taken with drug and physiological intestinal water volume in absorption site:
where $V_{\text{water,ini}}$ is the volume of water that is taken with an orally administered drug (200 mL) and $V_{\text{cont}}$ represents volume of contents in the intestine under normal fasted conditions (105 mL, Schiller et al., 2005). HLw is the half-life for disappearance of water taken with the drug. In this study, we assumed that HLw is 1 hr. Several fold changes of HLw did not affect on the absorption profile of drugs in preliminary simulations (data not shown).

The volume of the enterocytes at the absorption site, $V_{\text{ent,abs}}$, was calculated as the product of the thickness of enterocytes ($T_{\text{ent}}$, 0.0018 cm) (Sugano, 2009) and the $S_{\text{abs,bas}}$:

$$V_{\text{ent,abs}} = S_{\text{abs,bas}} \cdot T_{\text{ent}}$$  \hspace{1cm} (18)

where volume of the lamina propria, $V_{\text{propria,abs}}$ at the absorption site was calculated, based on the assumption of almost complete coverage of the intestinal lumen by villi, as the product of the villi height ($H_{\text{villi}}$, 0.07 cm) (Sugano, 2009) and the surface area of the intestinal lumen.
Plasma flow at the absorption site

The plasma flow rate to enterocytes at the absorption site, $Q_{\text{plasma,abs}}$, was calculated using Equation 20 by multiplying the blood flow rate to all enterocytes, $Q_{\text{blood,enterocyte}}$ and the ratio of the surface area of the absorption site ($S_{\text{abs,api}}$) to the whole small intestine ($S_{\text{si}}$), and then converting to plasma flow considering hematocrit, $H_t$ (0.45):

$$Q_{\text{plasma,abs}} = (1 - H_t) \frac{Q_{\text{blood,enterocyte}} S_{\text{abs,api}}}{S_{\text{si}}} (\text{mL/h}) \quad (20)$$

$Q_{\text{blood,enterocyte}}$ was calculated to be 18,000 mL/h by assuming that the blood flow into the superior mesenteric artery (SMA) was 37,200 mL/h, which accounts for 10% of the cardiac output; 80% of the blood flow of the SMA flows into the mucosa, and then 60% of the mucosal blood flows into the epithelium cells of villi (Jamei et al., 2009).
Dissolution of a solid formulation

Solubility in fasted-state simulated intestinal fluid (FaSSIF; \( S_{\text{FaSSIF}} \)) was calculated using Equation 21 as reported by Poulin et al., (Poulin et al., 2011):

\[
S_{\text{FaSSIF}} = S_{\text{Water}} + S_{\text{Water}} \frac{M_{\text{Water}}}{M_{\text{Drug}}} \left(10^{0.75\log P - 2.27}\right) \cdot M_{\text{Drug}} \cdot C_{\text{BS}} \quad (\mu g/mL) \quad (21)
\]

where \( S_{\text{Water}} \), \( M_{\text{Water}} \), \( M_{\text{Drug}} \), \( \log P \), and \( C_{\text{BS}} \) represent water solubility, molecular weight of water and drug, n-octanol:buffer partition coefficient of the drug, and the bile salt concentration of sodium taurocholate (4 nM), respectively. The dissolution constants for the small intestine were defined using Equations 22 and 23:

\[
k_{j\text{dis}} = 3 \gamma \frac{S_{\text{Sol}} - C}{\rho \ r_{j} \ T} \quad (h^{-1}) \quad (22)
\]

\[
\gamma = 9.9 \times 10^{-5} \cdot M_{W}^{-0.413} \quad (cm^2/s) \quad (23)
\]
where Sol, $\gamma$, $\rho$, $r_j$, T, and $C_i$ represent solubility, diffusion coefficient, density (1.2 mg/mL), particle radius (0.0025 cm), diffusion layer thickness (0.003 cm), and the concentration in the stomach or lumen, respectively (Agoram et al., 2001).

**Expression level of metabolizing enzymes and transporters**

It has been reported that cytochrome P450 (CYP)3A is most abundantly expressed in the duodenum, and less expressed in the distal end of the ileum (Paine et al., 1997). Therefore, a linear gradient was assumed for the expression of CYP3A, decreasing from the proximal end of the duodenum to the distal end of the ileum. The expression of CYP3A at the absorption site, $A_{\text{CYP3A,abs}}$, was calculated as a product of $L_{\text{abs}}$ and the density of CYP3A expression (Equation 24, Table 1) for the small intestine ($z < L_{\text{si}}$, $L_{\text{si}} = 306$ cm):

$$A_{\text{CYP3A,abs}} = 2A_{\text{CYP3A,total}} \left[ 1 - \frac{z_c}{L_{\text{si}}} \right] \frac{L_{\text{abs}}}{L_{\text{si}}} \quad \text{(pmol)} \quad (24)$$

where $A_{\text{CYP3A,total}}$ is the total amount of CYP3A expressed in the small intestine (70500 pmol).

The factor 2 in Equation 24 indicated that the density is double when $z_c$ is zero. Conversely,
$A_{\text{CYP3A}_\text{abs}}$ decreases to zero as the location moves to the large intestine since no expression of CYP3A was reported in the colon (Bruyere et al., 2010). Metabolic clearance of substrates of CYP3A was calculated from $A_{\text{CYP3A}_\text{abs}}$ and Michaelis constants for each substrate (Scheme 1).

It has been reported that the expression level of P-glycoprotein (P-gp) relatively increases from proximal to distal end of small intestine (Stephens et al., 2001; Englund et al., 2006; Bruyere et al., 2010), however, the absolute expression level is rarely reported. Therefore, we assumed that the relative expression level of P-gp in interest ($A_{\text{rel,Pgp}_\text{abs}}$) increases linearly in the lower part of intestine. For the lower site below the $A_{\text{rel,Pgp}_\text{abs}}$ was calculated using Equation 25:

$$A_{\text{rel,Pgp}_\text{abs}} = \left( A_{\text{init,Pgp}} + A_{\text{grad,Pgp}} \frac{z}{L_{s}} \right) \frac{L_{\text{abs}}}{L_{s}} \text{ (pmol)}$$ (25)

where $A_{\text{init,Pgp}}$ and $A_{\text{grad,Pgp}}$ represent the amount of P-gp in the entrance of duodenum, and the slope for increase in P-gp expression amount, respectively. In this study, 0 and 1 were used for $A_{\text{init,Pgp}}$ and $A_{\text{grad,Pgp}}$, respectively. To calculate the actual expression level of P-gp, a scaling factor was estimated from in vivo observations as will be described later.
Membrane permeability

The components of the permeability constants of the enterocytes, i.e. passive intake of apical, passive efflux of apical, active efflux of apical by P-gp, passive output from basal, and passive reverse intake from basal, were designated as $P_1$, $P_2$, $P_{P-gp}$, $P_3$, and $P_4$, respectively (Figure 3). Based on the structural symmetry and similarity of membranes, values for the passive permeability ($P_1$, $P_2$, $P_3$, and $P_4$) were assumed to be the same to the passive permeability of a single membrane which is denoted as $P_s$ (when pH of the environments are the same for both side of the membrane). $P_s$ for Caco-2 system was calculated from the apparent permeability ($P_{app}$) determined in Caco-2 assays (Gertz et al., 2010) using Equation 26:

\[
P_{s,Caco-2} = \frac{1 + M_{E_{Caco-2}}}{M_{E_{Caco-2}}} P_{app,Caco-2}
\]  

where $M_{E_{Caco-2}}$ is the apical/basolateral relative surface area ratio for Caco-2 cells. Equation 26 was derived from the equation reported by Tachibana and co-authors (Tachibana et al., 2010).

For the equation, $P_{S1} = P_{1,Caco-2} S \cdot M_{E_{Caco-2}}$, $P_{S2} = P_{2,Caco-2} S \cdot M_{E_{Caco-2}}$, $P_{S3} = P_{3,Caco-2} S$, and $P_{S4} = P_{4,Caco-2} S$ were assumed. The terms of $K_m$ and $V_{max}$ were removed because we used the equation
to calculate passive diffusion.

In the present study, an $ME_{Caco-2}$ value of 4 was adopted, as the average of values determined for propranolol and naproxen, as non-substrates of P-gp (Ohura et al., 2011). The values of $P_{app,Caco-2}$ for the substrates of P-gp (quinidine and verapamil) were calculated as the average values of $P_{app,Caco-2}$ at the three highest concentrations (approximately 10-100 μM), because the activity of P-gp was saturated at the three highest concentrations in the cited experiments (Tachibana et al., 2010).

$P_5$ for the in vivo system was determined by Equation 27:

$$P_{invivo} = P_2 = P_3 = P_4 = P_{Caco-2} \times psf_{passive}$$

(27)

where $psf_{passive}$ is the scaling factor, which is obtained from approximation of the simulated and reported $F_A$ for nine drugs (acyclovir, atenolol, cimetidine, ciprofloxacin, enalaprilat, gabapentin, methotrexate, ranitidine, and sulpiride). These drugs were selected based on the following criteria; (1) $F_A < 0.9$, (2) Caco-2 permeability is available in the literature (Thomas et al, 2008), (3) $F_H > 0.7$, and (4) $CL_H/CL_{tot} < 0.3$. First, each value of psf for the individual drug was
estimated by fitting analysis to agree the reported $F_A$ and predicted $F_A$ as possible using TLM (Supplemental Table 1). And then the $\text{psf}_{\text{passive}}$ was determined as the average of $\text{psf}$ for nine drugs. Calculation of $P_1$ will be described later.

For the drugs that are substrates of P-gp, the active permeability by P-gp \textit{in vitro} (Equation 28) and \textit{in vivo} (Equation 29) were described as follows:

$$P_{\text{Pgp,Caco-2}} S_{\text{Caco-2}} = \frac{V_{\text{max,Pgp,Caco-2}}}{K_{m,Pgp} + C_{\text{cell}}}$$  \hspace{1cm} (28)

$$P_{\text{Pgp,abs,api}} = \frac{\text{psf}_{\text{Pgp}} \cdot V_{\text{max,Pgp}} \cdot A_{\text{rel,Pgp}}}{K_{m,Pgp} + C_{\text{Enterocyte,u}}}$$  \hspace{1cm} (29)

where $\text{psf}_{\text{Pgp}}$, $C_{\text{cell}}$ and $C_{\text{Enterocyte,u}}$ represent the scaling factor for P-gp transport, the concentrations in Caco-2 cell and enterocyte, respectively. Michaelis constants for the transport by P-gp ($K_{m,Pgp}$ and $V_{\text{max,Pgp,Caco-2}}$) were calculated using a fitting analysis with data from Caco-2 cell assays (Tachibana et al., 2010), as previously described with a slight modification using Equation 30:
where, $C_a$ represents the drug concentration in the apical chamber. Respective $K_{m,Pgp}$ values were 0.113 and 0.283 $\mu$g/mL for quinidine and verapamil. For $P_{s,Caco-2}$ values, it was confirmed that the values obtained by fitting analysis with Equation 30 were the practically the same as those obtained from Equation 26.

For apical uptake permeability, $P_1$ was calculated using Equation 31 considering differences of pH between the Caco-2 assay and intestinal lumen. Caco-2 assays are generally carried out at pH 7.4, but it has been reported that the surface of intestinal lumen is somewhat more acidic (Table 1, Bolger et al., 2009).

$$P_1 = P_{s,\text{in vivo}} \times \frac{1 + 10^{pH_{\text{Caco-2}} - pK_a_{\text{acid}}} + 10^{pK_a - pH_{\text{Caco-2}}}}{1 + 10^{pH_{\text{in vivo}} - pK_a_{\text{acid}}} + 10^{pK_a_{\text{base}} - pH_{\text{in vivo}}}}$$

(31)

where $pH_{\text{Caco-2}}$, $pK_a_{\text{acid}}$, and $pK_a_{\text{base}}$ represent the pH for the Caco-2 assay (i.e. 7.4), acidic pK$a$, and basic pK$a$, respectively. A value for $pH_{\text{lumen}}$ represents the pH of the intestinal lumen of the small intestine that is described by the following equation:
The values for $\text{pH}_{\text{ini}}$ and $\text{pH}_{\text{grad}}$ were 5.85 and 0.00455, respectively with $r = 0.979$ and AIC = -13.7). The values for a product of $\text{psf}_{\text{Pgp}}$ and $V_{\text{max, Pgp}}$ in Equation 26 were obtained simultaneously by a fitting analysis using the TLM; the values were estimated to explain the dose-response of $F_A F_G$ appropriately for verapamil and quinidine, which is a substrate of P-gp but a weak substrate of CYP3A (Supplemental Figure 2). Based on the above theory, fundamental simultaneous ordinary differential equations for the TLM were constructed as shown in Scheme 1.

Data collection for drug related parameters

The drug-related parameters for 20 drugs (alfentanil, alprazolam, buspirone, cisapride, cyclosporin, felodipine, lovastatin, midazolam, nifedipine, nisoldipine, repaglinide, rifabutin, saquinavir, sildenafil, simvastatin, trazodone, triazolam, zolpidem, quinidine, verapamil) used in this model are summarized in Table 3. $K_m$ values for CYP3A were taken from the literature
(Lavrijsen et al., 1988; Ekins et al., 1999, Heikkinen et al., 2012; Gertz et al., 2011; Kajosaari et al., 2005; Ku et al., 2008; Rotzinger et al., 1998; Polasek et al., 2010). When no information was available for the $K_m$ values of CYP3A, the $K_m$ values of CYP3A4 were used. Metabolic intrinsic clearance, membrane permeability, and blood free fraction were taken from the literature (Gertz et al, 2011). Observed $F_G$ was estimated using the DDI method reported by Hisaka and coworkers (Hisaka et al., 2014) and taken from the other sources (Varma et al., 2010). The other observed or reported values required for analysis in this study are shown in Table 3 and Table 4. Unbounded fractions in the intestinal lumen ($f_{lumen}$), in the enterocyte ($f_{ent}$), and in the lamina propria ($f_{propria}$) were assumed to be 1.

**Calculation of pharmacokinetics parameters ($F_G$ and bioavailability $[F]$)**

$F_AF_G$ values were calculated by TLM as a ratio of the cumulative drug amount flown into the portal vein to the administered dose (Equation 33). $F_G$ values were calculated by TLM as a ratio of $F_AF_G$ to $F_A$ the later was calculated by subtracting the ratio of the cumulative drug amount remained in the colon and rectum to the administered dose from one.
By using in vitro hepatic intrinsic clearance (CL$_{int,HLM}$) estimated using human liver microsomes (Gertz et al., 2011), the blood free fraction ($f_B$), hepatic blood flow rate ($Q_H$), and the hepatic availability were estimated with in vitro information using the following equation ($F_{H,in vitro}$):

$$F_{H,in vitro} = \frac{Q_H}{Q_H + f_B CL_{int,HLM,in vitro}}$$

(35)

By multiplying the predicted $F_A F_G$ with $F_{H,in vitro}$, $F$ was predicted. The observed $F$ values were collected from two reports, (Varma et al., 2010; Appendix of Goodman & Gilman’s The Pharmacological Basis of Therapeutics 11th edition).

**Sensitivity analysis**

A sensitivity analysis of TLM was performed to estimate the effect of the surface area of the apical membrane of enterocytes by using verapamil as a model drug. Absorptions of forty-mg
verapamil was simulated by TLM with the ME values of 1, 5, and 20. Other parameters for TLM were not changed in this sensitivity analysis. Then changes in the intestinal lumen concentration, enterocyte concentration, lamina propria concentration in the absorption site, and $F_A F_G$ were investigated by comparing the time-course of these concentrations.

**Evaluation of precision and accuracy of the prediction**

Precision and accuracy of the prediction for $F_G$ and $F$ were evaluated by using the within 2-fold error, within $\pm 0.3$ error, average fold error (AFE, equation 36), and root mean square prediction error (RMSE, equation 37):

$$AFE = 10 \frac{\sum \log_{predicted}^{\text{observed}}}{N}$$  \hspace{1cm} (36)

$$RMSE = \sqrt{\frac{\sum (predicted - observed)^2}{N}}$$  \hspace{1cm} (37)

where $N$ represents the number of the drugs that used for the calculation.
Prediction of nonlinear pharmacokinetics

A prediction of nonlinear pharmacokinetics was performed by simulating the $F_{\text{AFG}}$-dose relationship for midazolam. Average $F_{\text{AFG}}$ values in the range from 0.01 to 10000 mg administered dose were plotted followed by confirmation of the similarity of the simulated value and observed values, which were calculated by using reported clinical data based on the assumption that the major site to induce the non-linear systemic exposure after oral administration is the intestine. (Bornemann et al., 1985; Misaka et al., 2010). For the calculation, body weight, $R_B$, and hepatic blood flow rate were assumed to be 70 kg, 1, and 25.5 mL/min/kg, respectively. Total clearance and fraction excreted in urine were cited from the following source (Appendix of Goodman & Gilman’s The Pharmacological Basis of Therapeutics 11th edition). “

Systems and application

Calculation of the ordinary differential equations constructed in this study was performed using Napp (http://square.umin.ac.jp/todaiyak/download.htm) with the Runge-Kutta–Fehlberg method (Hisaka and Sugiyama, 1998).
Results

Construction of the TLM

The TLM was constructed based on the concept that a drug is transferred from the lumen to the blood stream across the enterocytes and lamina propria at an absorption site, which is relocated, expanded, and decreased in size along the length of the GI tract in a time-dependent manner (Figure 1). The locatable absorption site consisted of four compartments: solid formulation in the lumen, dissolved drug in the intestinal lumen, concentration in the enterocytes, and concentration in the lamina propria including the capillaries. The absorption site continuously traversed from the duodenum to the ileum. In accordance with the physiological structure, the stomach and the colon were defined as a separate compartment.

Definition of location and distribution functions

The location and distribution functions that determine the movement of a drug absorption site in the TLM were obtained using a fitting analysis of the results of the gamma scintigraphy reported by Kimura (Kimura and Higaki, 2002). The movement of a non-absorbable index drug in the gastrointestinal tract was analyzed by using the location and distribution functions, and
then agreement was confirmed between the fitted and reported values (Figure 5A). By applying
a simplified convolution with the MRT, these functions were extended to cover any drug input
situation in the intestine, such as reduction of the gastric emptying rate, multiple inputs, and so
forth (Figure 10).

**Definition of location dependent parameters**

All location dependent parameters defined for the absorption site of the TLM were described by
z, the location in the intestine. The equations that defined the location dependent parameters are
represented in Table 1. Changes of the parameters defined by z are shown in Figure 4. The
calculated total surface area and the total volume of lumen, enterocyte, and lamina propria were
751000 cm$^2$, 311 mL, 67.6 mL, and 378 mL, respectively.

**Approximation of psf, and $V_{max,Pgp}$**

To determine the correspondence of passive permeability between *in vitro* and *in vivo* systems, a
fitting analysis was performed for the $F_A$ values of nine drugs (Supplemental Table 1). The
value of $psf_{passive}$ was determined as 2.23 from the analysis. This result suggests that the passive
membrane permeability of enterocytes in vivo is apparently 2.23-fold higher than that in vitro.

By using this $psf_{\text{passive}}$, $F_A$ was estimated within $\pm 0.3$ of observed value for all compounds.

(Supplemental Figure 1).

There were few reports on the in vivo $V_{\text{max}}$ value for P-gp. Therefore, the product of $psf_{\text{Pgp}}$ and $V_{\text{max,Pgp}}$ was obtained by simultaneous fitting analysis to the human $F_AF_G$ values for quinidine (0.1, 1, 10, and 100 mg) and verapamil (0.1, 3, 16, and 80 mg) (Supplemental Figure 2). The value was estimated as 1.66 g/h ($CV = 11.1\%$) assuming that $V_{\text{max}}$ is the same for quinidine and verapamil (Table 3).

Sensitivity analysis for ME

In the TLM, the model description of the surface area of the enterocytes is different for the apical and basolateral membranes. The ratio of the surface area of apical to basolateral membrane was defined as ME; ME was assumed to be 20 in this study. This means the surface area of the apical membrane is 20-fold larger than that of the basolateral membrane. The effect of ME on drug exposure in the lumen, enterocytes, lamina propria, and $F_AF_G$ were confirmed by
sensitivity analysis using 40 mg verapamil (Figure 6). Increasing ME reduced the concentration in the intestinal lumen, whereas $F_A F_G$ was increased by an increase in ME.

**Simulation of nonlinear pharmacokinetics and plasma time profile of midazolam**

$F_A F_G$-dose relationships were simulated using TLM for midazolam (Figure 7A). The predicted dose-dependent increase of $F_A F_G$ was well agreed with observed $F_A F_G$. The plasma time profile was predicted for oral midazolam after the administration with therapeutic dose or microdose by using pharmacokinetic parameters after intravenous administration that were obtained from the same experiment with the oral administration (Figure 7B). As a result, although plasma concentration after microdose was slightly under-estimated, overall time-course of drug concentration after therapeutic dose and microdose was well predicted.

**Prediction of intestinal availability and bioavailability**

To validate the further utility of the TLM after confirming the validity of the model’s construction, $F_G$ was calculated for 18 drugs and $F$ was calculated for 15 drugs that the information about their affinity for CYP3A and P-gp are available (Table 3). The reported $F_G$
and F values, the predicted $F_G$ and F values were shown in Table 4 and Figure 8. Number of drugs within 2-fold errors for $F_G$ and F were 50% and 53%, respectively, of drugs analyzed in this study. The ratio within ±0.3 errors for $F_G$ and F were 72% and 93%, respectively. The AFE and RMSE for $F_G$ prediction were 1.10 and 1.48, while AFE and RMSE for F prediction were 0.347 and 0.230, respectively (Table 5).

**Simulation of dose-dependent absorption**

The time course of drug amount in the stomach, intestinal lumen, enterocyte, and lamina propria, the cumulative drug amount in the portal vein and feces, and the cumulative amount of metabolized drug were simulated with input conditions at 40 mg (clinically effective dose) and 0.1 mg (microdose) of oral verapamil using the TLM (Figure 9). At 40 mg, most of the unchanged drug was rapidly absorbed and then 32.7% of the dose traversed into the portal vein while 57.9% was metabolized and 9.4% reached to the colon. On the other hand, the extent of drug absorbed into the portal vein was lower at 0.1mg; only 16.6% of the dose traversed into the portal vein while 32.2% was metabolized and 51.2% reached to the colon.
Application to multiple administration

The time course of drug location (Figure 10A), and drug concentration in the intestinal lumen, enterocyte, and lamina propria (Figure 10B) were simulated when the location and distribution functions were applied to multiple inputs of a low permeability drug. The simulation revealed that the drug absorption site could move both back and forth as needed. A tendency for drug concentration to decrease from the intestinal lumen to the lamina propria was observed appropriately.
Discussion

The TLM was developed to construct a physiologically precise and relatively simple absorption model which is easy to modify the structure and parameters if needed. The model structure was simplified by introducing single absorption site which is moved in accord with the location and variance functions. Previous models represent location-dependent changes of physiological parameters with the fragmented intestinal compartments (Yu and Amidon, 1999; Agoram et al., 2001; Jamei et al., 2009). Conversely, the TLM enabled them by describing each parameter with a location dependent function under various input/administration conditions.

The location and distribution functions set in this study reproduced the delay in gastric emptying under the fed state and the corresponding changes in the distribution of a non-absorbable index drug, reported by Kimura (Kimura and Higaki, 2002). Moreover, the profile of distribution in Figure 5B was similar to the results that were reported by Willman and co-workers (Willmann et al., 2003). From these results, it was indicated that the location and variance functions used in this study could represent drug movement in the intestinal lumen.

Most of reported models use the effective permeability (P_{eff}) as the parameter for the passive permeability of the enterocytes (Yang et al., 2007; Badhan et al., 2009; Gertz et al.,
2011). $P_{\text{eff}}$ is the parameter that is calculated for the human jejunum permeability and can be estimated by $P_{\text{app}}$ in Caco-2 cells by using the result of the regression analysis between $P_{\text{app}}$ and $P_{\text{eff}}$ (Sun et al., 2002). In the TLM, in vivo passive permeability was integrated using the in vitro $P_{\text{app}}$ of Caco-2 cells and multiplying the constants of surface area expansion (PE for plicate, VE for villi, and ME for micro-villi). This direct integration of the passive permeability enables the user to take the intestinal physiological factor and the effect of the changes in the intestinal pH into consideration compared to the empirical approach of the extrapolation from $P_{\text{app}}$ to $P_{\text{eff}}$. To confirm the utility of the direct integration from single membrane permeability, $P_{\text{eff}}$ values from the TLM were simulated by modifying the TLM that mimicked the reported experimental conditions of Loc-I-Gut (Petri et al., 2003); the location and the size of absorption site (10 cm) was placed in the jejunum by TLM in exactly the same way. Finally, the reported and simulated $P_{\text{eff}}$ values were compared (Supplemental Figure 3). Our results indicate that the passive permeability could be calculated adequately from the single membrane permeability.

To confirm the effect of ME on drug absorption, a sensitivity analysis was performed for verapamil after the oral administration of a therapeutic dose (40 mg). Results showed that the increase of ME reduced verapamil exposure to the lumen, and increased the maximum
concentration in the enterocytes in the early time point that might be important for the first-pass metabolism due to the higher expression of CYP3A in the proximal part of small intestine.

FAFG, was also increased depending on the extent of the ME increase (Figure 6). These results indicate that discrimination of the apical and basolateral membranes by using adequate ME would be advantageous to avoid potential underestimation of the systemic exposure.

The obtained parameters were used for prediction of the dose-FAFG relationship of midazolam. As shown in Figure 7A, predicted and observed FAFG for midazolam were in close agreement, suggesting appropriateness of parameters obtained from the fitting analyses of FA values for nine drugs. In addition to the nonlinearity in FAFG, the plasma time profile of midazolam was also predicted after oral administration of therapeutic dose and microdose (Figure 7B). These results suggest that the time-course of plasma concentration could be well predicted as well as nonlinear absorption kinetics by TLM.

In this research, the FG for 18 drugs and F for 15 drugs were simulated, as they are substrates of CYP3A and P-gp (Figure 8). As a result, the FG and F of these drugs were reasonably predicted using the TLM from in vitro parameters. As shown in Table 5, AFE and RMSE in all predictions were acceptable by which it was demonstrated the potential of TLM for
prediction of clinical pharmacokinetics after oral administration of various drug candidates from 
in vitro data from Caco-2 cell assays and microsomal metabolic kinetics studies during the drug
discovery stage.

By using the TLM, the simulation of the local pharmacokinetics of the intestine was
performed at the clinical effective dose (40 mg) and a micro-dose (0.1 mg) of verapamil. Lower
availability was predicted with a microdose than that with 40 mg which corresponds to the
clinical observation (Maeda et al., 2011). The simulated maximum concentration in the
enterocytes was approximately 6.7 μM, which was smaller than the Kₘ value of CYP3A4 (49.0
μM) and higher than the Kₘ for P-gp (0.622 μM). This would indicate larger contribution of P-
gp to the nonlinear drug absorption after 40 mg of oral verapamil.

The recent commercially available applications for prediction of drug absorption are
generally expensive and not easy to modify because of the complex and veiled structure. The
TLM was developed by using Napp, a program for the analysis of pharmacokinetics, which is
available for free. This program would assist researchers to perform detailed analyses and
predictions of drug absorption. Moreover, an advantage of the TLM is that the model structure
is completely transparent to the users.
In conclusion, we successfully developed the TLM in order to describe physiology of
drug absorption precisely as possible; i.e. the movement of a drug in the intestine, permeation
through the apical and basolateral membranes, and the contributing blood flow. The structure of
TLM is relatively simple compared with the previous available models. It would be useful for
the prediction of drug absorption during new drug development in the future. Moreover,
various events during the absorption process would be analyzed more accurately with TLM by
adjusting its structure and parameters if necessary.
Authorship Contributions

*Participated in research design:* Hisaka, Ando, and Suzuki

*Conducted experiments:* Ando and Hisaka.

*Contributed new reagents or analytic tools:* Ando and Hisaka.

*Performed data analysis:* Ando, Hisaka, and Suzuki.

*Wrote or contributed to the writing of the manuscript:* Ando, Hisaka, and Suzuki.
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Bornemann LD, Min BH, Crews T, Rees MM, Blumenthal HP, Colburn WA, and Patel IH


Hisaka A, Nakamura M, Tsukihashi A, Koh S, and Suzuki H (2014) Assessment of intestinal availability (FG) of substrate drugs of cytochrome p450s by analyzing changes in


Footnotes

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Legends for Schemes

Scheme 1. Differential equations of translocation model.
Legends for Figures

Figure 1. The concept of the translocation model. A) The anatomical structure of the intestinal villus reported by Lin (Lin et al., 1999). B) The anatomical structure of the gastrointestinal tract included in the translocation model. C) State of the compartments that were created for the translocation model (translocation compartments) immediately after a solid drug reached the duodenum. D) State of the translocation compartments after a dissolved drug reached the colon.

Figure 2. The structure of the translocation model. The translocation model includes four movable compartments; solid and solution in lumen, enterocyte, and lamina propria containing the capillaries.

Figure 3. Definitions of apical and basolateral permeability in the translocation model.

In this model, different surface areas were defined for apical and basolateral membrane. \( P_1, P_2, P_3, \) and \( P_4 \) represent the passive permeability from lumen to enterocyte, enterocyte to lumen, enterocyte to lamina propria, and lamina propria to enterocyte, respectively. \( P_{\text{P-gp}} \) represents the active permeability by P-gp (Equation 29).
Figure 4. Location dependent parameters defined for the translocation model. Location dependent expression of A) radius of the intestinal lumen, B) plasma flow rate, C) cytochrome P450 (CYP)3A amount, D) P-glycoprotein level, E) apical surface area, F) volume of the lumen, G) volume of the enterocytes, and H) volume of the lamina propria.

Figure 5. Time-dependent parameters defined for the translocation model. A) Time course of $^{99m}$Tc-DTPA amount in each site of the gastrointestinal tract reported by Kimura et al (Kimura and Higaki, 2002). Open circle, closed triangle, open square, and closed circle represent the drug in the stomach, jejunum, ileum, and colon, respectively. Time course of B) location (solid line) with proximal and distal ends of the absorption site (dotted line), C) drug in the lumen, and D) Mean residence time (MRT) are simulated using a non-absorbable condition after a single input.

Figure 6. Effect of microvilli expansion (ME) on lumen concentration (A), enterocyte concentration (B), lamina propria concentration (C), and $F_A F_G$ (D) after 40 mg oral verapamil.
Dotted, dashed, and solid lines represent the simulation curve under 1, 5, or 20 of the ME condition.

Figure 7. The prediction results of A) the dose versus $F_A F_G$ relationship of midazolam and B) plasma time course of midazolam after oral administration of therapeutic dose and microdose. 

A) The dashed line represents the predicted dose-$F_A F_G$ and closed circles represent the reported $F_A F_G$. B) The closed and open circles represent the observed plasma concentration after oral administration of the therapeutic dose (TD) and microdose (MD), respectively. The solid line and dotted line represent the predicted plasma concentration after TD and MD.

Figure 8. Prediction result of bioavailability for 15 drugs by using TLM. Closed circles represent the predicted F. The black dotted lines represent within ±0.3 error lines. The solid line represents the unity.

Figure 9. Simulation results of the amount of drug in the stomach (black solid line), lumen (black dashed spaced line), enterocytes (gray solid line), lamina propria (black dashed line), and
cumulative amounts in the portal vein (gray dashed line), metabolite (gray dotted line), and rectum (black dotted line) after oral (A) and (B) 40 mg, and (C) and (D) 0.1 mg verapamil. Normal plots are shown in (A) and (C), and logarithm scale plots are shown in (B) and (D), respectively.

Figure 10. Time course of (A) average location (solid line) and proximal and distal ends of the absorption site (dotted lines), and (B) concentration in the lumen (dotted line), enterocytes (dashed line), and lamina propria (solid line). Simulations were performed with multiple input conditions for an absorbable drug with low permeability (apparent permeability $[P_{app}] = 0.0007$ cm/h).
### Table 1. Properties of intestine in the translocation model

<table>
<thead>
<tr>
<th>Category</th>
<th>Parameters</th>
<th>Equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical</td>
<td>pH&lt;sub&gt;lumen&lt;/sub&gt;</td>
<td>( pH_{lumen} = 5.85 + 1.39 \frac{z_e}{L_{sl}} )</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>( r = -0.00265 \cdot z_e + 1.56 )</td>
</tr>
<tr>
<td></td>
<td>( S_{abs,api} )</td>
<td>( S_{abs,api} = \pi \left( r_{in} + r_{out} \right) \left( r_{in} - r_{out} \right)^2 + \frac{L_{abs}}{2} \times PE \times VE_{abs} \times ME )</td>
</tr>
<tr>
<td></td>
<td>( V_{lumen} )</td>
<td>( V_{lumen,abs} = \frac{L_{abs}}{L_{all}} \cdot V_{cont} + V_{water,put} \cdot e^{-\ln(2)t/HLW} )</td>
</tr>
<tr>
<td></td>
<td>( V_{ent} )</td>
<td>( V_{ent,abs} = S_{abs,abs} \times T_{ent} )</td>
</tr>
<tr>
<td></td>
<td>( V_{propria} )</td>
<td>( V_{propria,abs} = \frac{S_{abs,abs}}{VE_{abs}} \times V_{ent,abs} )</td>
</tr>
<tr>
<td></td>
<td>Q</td>
<td>( Q_{plasma,abs} = (1 - H)Q_{blood,enterocyte} \cdot \frac{S_{abs}}{S_{sl}} )</td>
</tr>
<tr>
<td></td>
<td>( A_{CYP3A} )</td>
<td>( A_{CYP3A,abs} = 2 \cdot A_{CYP3A,total} \left( 1 - \frac{z_e}{L_{sl}} \right) \frac{L_{abs}}{L_{sl}} )</td>
</tr>
<tr>
<td>Protein expression</td>
<td>( A_{Pgp} )</td>
<td>( A_{vol,Pgp,abs} = \left( A_{abs,Pgp} + A_{grad,Pgp} \frac{z_e}{L_{sl}} \right) \frac{L_{abs}}{L_{sl}} )</td>
</tr>
<tr>
<td>Permeability</td>
<td>( P_{1} )</td>
<td>( P_{i} = P_{s,invivo} \times \frac{1 + 10^{\frac{pH_{lumen} - pK_a,acid}{10}}} {1 + 10^{\frac{pH_{lumen} - pK_a,acid}{10}}} + \frac{10^{pK_a,basic - pH_{lumen}}}{1 + 10^{\frac{pK_a,basic - pH_{lumen}}{10}}} )</td>
</tr>
<tr>
<td></td>
<td>( P_{2} \sim P_{4} )</td>
<td>( P_{2} = P_{3} = P_{4} = P_{s,invivo} )</td>
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Table 2. Size of the gastrointestinal tract used for the translocation model

<table>
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<tr>
<th></th>
<th>Radius&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Length&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Volume&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cumulative length</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>9.67 cm</td>
<td>28.3 cm</td>
<td>47 mL</td>
<td>- cm</td>
<td>Stomach end</td>
</tr>
<tr>
<td>Duodenum</td>
<td>1.53 cm</td>
<td>14.1 cm</td>
<td>42 mL</td>
<td>14.1 cm</td>
<td>Duodenum end</td>
</tr>
<tr>
<td>Jejunum1</td>
<td>1.45 cm</td>
<td>58.4 cm</td>
<td>154 mL</td>
<td>72.5 cm</td>
<td></td>
</tr>
<tr>
<td>Jejunum2</td>
<td>1.29 cm</td>
<td>58.4 cm</td>
<td>122 mL</td>
<td>130.9 cm</td>
<td>Jejunum end</td>
</tr>
<tr>
<td>Ileum1</td>
<td>1.13 cm</td>
<td>58.4 cm</td>
<td>94 mL</td>
<td>189.3 cm</td>
<td></td>
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<tr>
<td>Ileum2</td>
<td>0.980 cm</td>
<td>58.4 cm</td>
<td>71 mL</td>
<td>247.7 cm</td>
<td></td>
</tr>
<tr>
<td>Ileum3</td>
<td>0.820 cm</td>
<td>58.4 cm</td>
<td>49 mL</td>
<td>306.1 cm</td>
<td>Ileum end</td>
</tr>
</tbody>
</table>

<sup>a</sup> Used in the GastroPlus™ (Bolger et al., 2009, Heikkinen et al., 2012).

<sup>b</sup> Length of stomach was not incorporated into translocation model.
Table 3. Drug specific parameters used for the prediction of absorption

<table>
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<tr>
<th>Drugs</th>
<th>Dose</th>
<th>$K_{\text{m,u}}$</th>
<th>$V_{\text{max}}$</th>
<th>$P_{\text{app,caco-2}}$</th>
<th>$f_{\beta}$</th>
<th>MW</th>
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<tr>
<td></td>
<td>µmol</td>
<td>µmol/h/pmol</td>
<td>µmol/h/pmol</td>
<td>µmol/h/pmol</td>
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<tr>
<td></td>
<td>µmol</td>
<td>CYP3A P-gp</td>
<td>CYP3A P-gp</td>
<td>CYP3A P-gp</td>
<td>CYP3A P-gp</td>
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<tr>
<td>Alfentanil</td>
<td>7220</td>
<td>22.8 NA</td>
<td>1.30 0</td>
<td>0.105 0.137</td>
<td>417</td>
<td></td>
</tr>
<tr>
<td>Alprazolam</td>
<td>1290</td>
<td>265 NA</td>
<td>0.238 0</td>
<td>0.0918 0.341</td>
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<tr>
<td>Buspirone</td>
<td>4150</td>
<td>7.98 NA</td>
<td>1.04 0</td>
<td>0.0914 0.062</td>
<td>386</td>
<td></td>
</tr>
<tr>
<td>Cisapride</td>
<td>16100</td>
<td>3.20 NA</td>
<td>0.526 0</td>
<td>0.108 0.0200</td>
<td>466</td>
<td></td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>474000</td>
<td>1.40 0.0567</td>
<td>0.0333 1.38 x 10^6</td>
<td>0.0286 0.0140</td>
<td>1203</td>
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<tr>
<td>Felodipine</td>
<td>26000</td>
<td>5.31 NA</td>
<td>7.45 0</td>
<td>0.0151 0.068</td>
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<tr>
<td>Lovastatin</td>
<td>49400</td>
<td>7.80 NA</td>
<td>22.8 0</td>
<td>0.0522 0.03</td>
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<td>Midazolam</td>
<td>9200</td>
<td>3.31 NA</td>
<td>1.35 0</td>
<td>0.117 0.056</td>
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<tr>
<td>Nifedipine</td>
<td>28900</td>
<td>11.0 NA</td>
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<td>Nisoldipine</td>
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<td>0.0720 0.003</td>
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<td>Repaglinide</td>
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<td>0.0868 0.025</td>
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<td>Rifabutin</td>
<td>177000</td>
<td>10.9 NA</td>
<td>0.338 0</td>
<td>0.0342 0.483</td>
<td>847</td>
<td></td>
</tr>
<tr>
<td>Drug</td>
<td>IC50</td>
<td>PDE5</td>
<td>IC25</td>
<td>IC10</td>
<td>IC0.05</td>
<td>IC0.025</td>
</tr>
<tr>
<td>------------</td>
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<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>--------</td>
<td>---------</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>1490000</td>
<td>0.300</td>
<td>0.863</td>
<td>1.09</td>
<td>2.47×10^6</td>
<td>0.0497</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>52600</td>
<td>15.0</td>
<td>NA</td>
<td>1.01</td>
<td>0</td>
<td>0.0922</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>95500</td>
<td>3.39</td>
<td>NA</td>
<td>14.2</td>
<td>0</td>
<td>0.0245</td>
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<tr>
<td>Trazodone</td>
<td>202000</td>
<td>312</td>
<td>NA</td>
<td>4.41</td>
<td>0</td>
<td>0.0871</td>
</tr>
<tr>
<td>Triazolam</td>
<td>729</td>
<td>85.1</td>
<td>NA</td>
<td>0.261</td>
<td>0</td>
<td>0.101</td>
</tr>
<tr>
<td>Zolpidem</td>
<td>16300</td>
<td>140</td>
<td>NA</td>
<td>0.185</td>
<td>0</td>
<td>0.115</td>
</tr>
<tr>
<td>Quinidine</td>
<td>615000</td>
<td>4.00</td>
<td>0.348</td>
<td>0.00766</td>
<td>5.11×10^6</td>
<td>0.0491</td>
</tr>
<tr>
<td>Verapamil</td>
<td>87900</td>
<td>49.0</td>
<td>0.622</td>
<td>6.92</td>
<td>3.65×10^6</td>
<td>0.0516</td>
</tr>
</tbody>
</table>
Table 4. Reported and prediction results of $F_G$, and observed and prediction results of $F$

<table>
<thead>
<tr>
<th>Drugs</th>
<th>$F_G$</th>
<th>$\text{CL}_{\text{int,HLM}}$</th>
<th>$F_{\text{H,in vitro}}$</th>
<th>$F$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predicted</td>
<td>Reported</td>
<td>μL/min/pmol</td>
<td>Predicted</td>
</tr>
<tr>
<td>Alfentanil</td>
<td>0.72</td>
<td>0.60</td>
<td>0.890</td>
<td>0.613</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>0.99</td>
<td>0.99</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Buspirone</td>
<td>0.51</td>
<td>0.22</td>
<td>1.84</td>
<td>0.629</td>
</tr>
<tr>
<td>Cisapride</td>
<td>0.56</td>
<td>0.55</td>
<td>2.13</td>
<td>0.819</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>0.99</td>
<td>0.35</td>
<td>0.474</td>
<td>0.957</td>
</tr>
<tr>
<td>Felodipine</td>
<td>0.02</td>
<td>0.53</td>
<td>15.5</td>
<td>0.155</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>0.04</td>
<td>0.09</td>
<td>35.4</td>
<td>0.154</td>
</tr>
<tr>
<td>Midazolam</td>
<td>0.36</td>
<td>0.48</td>
<td>3.75</td>
<td>0.476</td>
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<tr>
<td>Nifedipine</td>
<td>0.60</td>
<td>0.74</td>
<td>2.00</td>
<td>0.594</td>
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<tr>
<td>Nisoldipine</td>
<td>0.03</td>
<td>0.11</td>
<td>53.1</td>
<td>0.547</td>
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<tr>
<td>Repaglinide</td>
<td>0.84</td>
<td>0.89</td>
<td>0.737</td>
<td>0.913</td>
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<tr>
<td>Rifabutin</td>
<td>0.85</td>
<td>0.21</td>
<td>0.514</td>
<td>0.436</td>
</tr>
<tr>
<td>Drug</td>
<td>Emax</td>
<td>EC50</td>
<td>AUC0-24h</td>
<td>FL-app</td>
</tr>
<tr>
<td>--------------</td>
<td>------</td>
<td>------</td>
<td>----------</td>
<td>--------</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>0.92</td>
<td>0.18</td>
<td>51.3</td>
<td>0.090</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>0.77</td>
<td>0.54</td>
<td>1.07</td>
<td>0.742</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>0.02</td>
<td>0.19</td>
<td>51.7</td>
<td>0.131</td>
</tr>
<tr>
<td>Trazodone</td>
<td>0.90</td>
<td>0.83</td>
<td>0.370</td>
<td>0.881</td>
</tr>
<tr>
<td>Triazolam</td>
<td>0.98</td>
<td>0.45</td>
<td>NA</td>
<td>NC</td>
</tr>
<tr>
<td>Zolpidem</td>
<td>0.99</td>
<td>0.81</td>
<td>0.0850</td>
<td>0.956</td>
</tr>
</tbody>
</table>

a: Calculated using Equation 34.
b: Hisaka et al., 2014.
c: Gertz et al., 2011.
d: Calculated using Equation 35.
e: Calculated as the product of $F_AF_G$ (equation 33) and $F_{IL,in vitro}$.
f: Appendix of Goodman & Gilman’s The Pharmacological Basis of Therapeutics 11th edition.
Table 5. Prediction results of F_G and F, and statistics for the prediction results.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>F_G^a</th>
<th>F^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within 2-fold (%)</td>
<td>50</td>
<td>53</td>
</tr>
<tr>
<td>Within ±0.3 (%)</td>
<td>72</td>
<td>93</td>
</tr>
<tr>
<td>AFE</td>
<td>1.10</td>
<td>1.48</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.347</td>
<td>0.230</td>
</tr>
</tbody>
</table>

AFE: Average fold error.
RMSE: Root mean square prediction error.
Schemes

Scheme 1

\[
\frac{dX_{\text{solid, stomach}}}{dt} = Dose_{\text{solid}} - (k_{GE,\text{Solid}} + k_{\text{dis, stomach}}) X_{\text{solid, stomach}}
\]

\[
\frac{dX_{\text{stomach}}}{dt} = Dose_{\text{solution}} + k_{\text{dis, stomach}} X_{\text{solid, stomach}} - k_{GE} X_{\text{stomach}}
\]

\[
\frac{dX_{\text{solid, lumen}}}{dt} = k_{GE} X_{\text{stomach}} - (k_{\text{dis, lumen}} + k_{\text{exc, lumen}}) X_{\text{solid, lumen}}
\]

\[
\frac{dX_{\text{lumen}}}{dt} = k_{GE} X_{\text{stomach}} + k_{\text{dis, lumen}} X_{\text{solid, lumen}} - FL_{\text{lumen}}
\]

\[
\frac{dX_{\text{ent}}}{dt} = \frac{f_{\text{ent}} P_S \text{abs, ap} V_{\text{lumen, abs}}}{V_{\text{lumen, abs}}} X_{\text{lumen}} - \frac{CL_{\text{out}} + CL_{\text{met}} + f_{\text{ent}} P_S \text{abs, bas} V_{\text{ent, abs}}}{V_{\text{ent, abs}}} X_{\text{ent}} + \frac{f_{\text{propria}} P_S \text{abs, bas} V_{\text{propria, abs}}}{V_{\text{propria, abs}}} X_{\text{propria}}
\]

\[
\frac{dX_{\text{propria}}}{dt} = f_{\text{ent}} P_S \text{abs, bas} V_{\text{ent, abs}} X_{\text{ent}} - \frac{f_{\text{propria}} P_S \text{abs, bas} + Q_{\text{plasma, bas}} / f_B}{V_{\text{propria, abs}}} X_{\text{propria}}
\]

\[
\frac{dM_{\text{time, l}}}{dt} = X_{\text{lumen}} + X_{\text{solid, lumen}} - FL_{\text{lumen}} + k_{\text{exc, lumen}} X_{\text{solid, lumen}} + M_{\text{time, l}}
\]

\[
FL_{\text{lumen}} = \frac{f_{\text{lumen}} P_S \text{abs, ap} V_{\text{lumen, abs}}}{V_{\text{lumen, abs}}} X_{\text{lumen}} + k_{\text{exc, lumen}} X_{\text{lumen}} - \frac{CL_{\text{out}} V_{\text{ent, abs}}}{V_{\text{ent, abs}}} X_{\text{ent}}
\]

\[
CL_{\text{out}} = f_{\text{ent}} P_S \text{abs, ap} + \frac{psf_{\text{PS}} V_{\text{max, PS}} A_{\text{rel, PS, abs}}}{K_{m, PS} / f_{\text{ent, bas}} + X_{\text{ent}} / V_{\text{ent, abs}}}
\]

\[
CL_{\text{met}} = \frac{V_{\text{max, CYP3A}} A_{\text{CYP3A, abs}}}{K_{m, CYP3A} / f_{\text{ent, bas}} + X_{\text{ent}} / V_{\text{ent, abs}}}
\]

\[
k_{\text{exc, lumen}} = \frac{d\lambda(\tau)}{d\tau} L_{\text{abs}} \quad \text{(when the absorption site reaches the distal end of small intestine)}
\]

\[
\tau = M_{\text{time, l}} / (X_{\text{lumen}} + X_{\text{solid, lumen}})
\]

\[
z_{\text{abs}} = \lambda(\tau)
\]

\[
L_{\text{abs}} = 2\sqrt{3} \times \sigma(\tau)
\]
\(X_{\text{solid, stomach}}\), \(X_{\text{stomach}}\), \(X_{\text{solid, lumen}}\), \(X_{\text{lumen}}\), \(X_{\text{ents}}\), \(X_{\text{propria}}\): amount of drug in stomach (as solid), stomach (as solution), lumen (as solid), lumen (as solution), enterocytes and lamina propria, respectively.

\(M_{\text{time,1}}\): first order moments for time of drug as solid and solution in lumen

\(F_{\text{lumen}}\): flux for disappearance from lumen

\(C_{\text{out}}\): output clearance from enterocytes

\(C_{\text{met}}\): metabolic clearance in enterocytes

\(k_{\text{excr}}\): rate constant for excretion from lumen

\(k_{\text{GE, Solid}}\): gastric emptying rate of solid form drug (Jamei et al., 2009)

\(\tau\): MRT in lumen

\(z_{\text{abs}}\): location of absorption site

\(L_{\text{abs}}\): length of absorption site
Figure 2

Portal vein \( V_{pv} \) flows into the capillaries and lamina propria \( V_{propria} \) and then into the enterocyte \( V_{ent} \). The enterocyte exchanges with the stomach lumen \( V_{stomach} \) and the lumen of the small intestine \( V_{lumen} \) through the locatable compartments. The colon \( V_{col,ent} \) and rectum \( V_{rec} \) are also depicted, with the colon exchanging with the lumen of the small intestine and the rectum exchanging with the colon.

Transport rates include:
- \( k_{GE} \) from the enterocyte to the stomach lumen
- \( k_{excr} \) from the enterocyte to the colon
- \( k_{rec} \) from the colon to the rectum

Metabolism rate is denoted as \( CL_{met} \).
undissociated + dissociated

undissociated

apical

P₂

P₄

P₋gp

S₂₁₃₄

S₂₁₃₄

basolateral

P₁

P₃

P₄

S₂₁₃₄

S₂₁₃₄

enterocyte
Figure 4

A) Radius (cm) vs. Location (cm)

B) Plasma flow rate (mL/h/cm) vs. Location (cm)

C) CYP3A (pmol/cm) vs. Location (cm)

D) Relative P-gp (pmol/cm) vs. Location (cm)

E) Surface area (cm²/cm) vs. Location (cm)

F) Lumen volume (mL/cm) vs. Location (cm)

G) Enterocyte volume (mL/cm) vs. Location (cm)

H) Lamina propria volume (mL/cm) vs. Location (cm)
Figure 5
Figure 6

A) Lumen Conc. (μM) vs Time (h)

B) Enterocyte Conc. (μM) vs Time (h)

C) Lamina propria Conc. (μM) vs Time (h)

D) $F_A F_G$ vs Time (h)
Figure 8
Figure 9

A) Drug amount versus Time (h)

B) Drug amount versus Time (h)

C) Drug amount versus Time (h)

D) Drug amount versus Time (h)