SIMDOT-AbMe: Microphysiologically Based Simulation Tool for Quantitative Prediction of Systemic and Local Bioavailability of Targeted Oral Delivery Formulations

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ABSTRACT:
The purpose of this study was to develop a physiologically based simulation tool that is able to predict local as well as systemic bioavailability of 5-aminosalicylic acid (5-ASA)-targeted delivery formulations using the existing understanding of the transport and metabolism mechanisms of 5-ASA. The model accounts for active and passive transcellular transport (absorptive and efflux), passive paracellular transport, intestinal biotransformation, and systemic metabolism and clearance. The intestinal physiology was represented by transverse segments for ileum and proximal colon and longitudinal compartments for the microphysiology of the intestinal tissue. The tool, equipped with an optimization routine that enables tuning model parameters, was developed in Matlab and uses a user-friendly graphical interface for data input and output. Physiologic and kinetic model parameters were estimated either from literature monolayer transport studies using nonlinear curve fitting or obtained directly from the literature. 5-ASA clinical pharmacokinetic profiles of a once-daily (one 4-g/day dose) and twice-daily (two 2-g/day doses) dosing regimen were used to partially calibrate and validate the model, respectively. Simulation results showed that drug C\text{max} in the gut mucosal layers reached a higher level and was achieved sooner than in the systemic blood level. The computed relative local bioavailability with respect to the systemic bioavailability was 0.063. With use of the model, the relative local bioavailability of different formulations can be established for fast performance verification of new preparations based on measured systemic bioavailability. These types of models play a critical role in designing such preparations and rapidly assessing their effectiveness and will foster efficient experimental designs, saving time and resources.

5-Aminosalicylic acid (5-ASA) is one of the well known anti-inflammatory agents that is currently being used in the treatment of ulcerative colitis (UC) and Crohn’s disease (CD), two major idiopathic inflammatory bowel diseases (IBDs). In fact, the first-line therapies for induction and maintenance of remission in patients with these diseases are the oral mesalazine formulations (Asacol, Pentasa, Salofalk, Mesasal, and Cleversal) and prodrug (sulfasalazine, olsalazine, and balsalazide) and rectal formulations (Sandborn and Hanauer, 2003). After oral or rectal administration, 5-ASA acts locally at the inflammation site, often the colon, and is absorbed by intestinal epithelial cells. The effectiveness of the drug is related to its mucosal concentration and the fact that systemic dosages remain low after oral and rectal administration (Christensen et al., 1990; Frieri et al., 2000). Despite the drug’s long history and numerous experimental and clinical studies on this group of medicines, the basic mechanism of action of 5-ASA is still the subject of extensive discussion (Gandia et al., 2007). The putative anti-inflammatory actions of 5-ASA include modulation of inflammatory cytokine production (Kaiser et al., 1999), decreased transcriptional activity of nuclear factor-κB by modulating RelA/p65 phosphorylation (Egan et al., 1999), inhibition of the biosynthesis of prostaglandins and leukotrienes (Sharon et al., 1978; Lauritsen et al., 1985), inhibition of intestinal epithelial cell injury, and apoptosis induced by oxidative stress (Dallegrì et al., 1990; Sandoval et al., 1997). Desreumaux and Ghosh (2006) recently argued that even though these mechanisms are not mutually exclusive, the integrated mechanism of action of 5-ASA can be described through its effect on peroxisome proliferator-activated receptor-γ, a class of receptors involved in the control of inflammation, cell proliferation, apoptosis, and metabolic function.

Regardless of the mechanism of action, the desired effect of 5-ASA could be attributed to a local effect of the medicine, which could be directly related to the local bioavailability (effective mucosal drug concentration) that is achieved using targeted delivery. In contrast, the systemic bioavailability may contribute to adverse effects (Klotz and Schwab, 2005). Several in vitro, ex vivo, and clinical comparative experiments have been performed in an attempt to study the mechanism of action of 5-ASA. Furthermore, these experiments have shown that 5-ASA can affect the peroxisome proliferator-activated receptor-γ (PPAR-γ) and other targets by modulating the expression of proinflammatory mediators.

ABBREVIATIONS: 5-ASA, 5-aminosalicylic acid; UC, ulcerative colitis; CD, Crohn’s disease; IBD, inflammatory bowel disease; PK, pharmacokinetics; PD, pharmacodynamics; GIT, gastrointestinal tract; GUI, graphical user interface; WRSS, weighted residual sum of squares; RMSE, root mean square error; AUC, area under the curve; RHS, right-hand side.
studies have reported the pharmacokinetic (PK) parameters of this group of medicines and 5-ASA in particular (Bondesen et al., 1991; Swift et al., 1992; Klotz and Stracciari, 1993; Yu et al., 1995; Sandborn and Hanauer, 2003; Sandborn et al., 2004). There are a few studies that investigated intestinal absorption and metabolism of 5-ASA (e.g., Yokoe et al., 2003). However, these studies focused on drug plasma concentration, while giving little emphasis to the drug mucosal concentration. In fact, studies that assess the concentration of 5-ASA in the intestinal mucosa could provide direct evidence regarding adequate tissue concentrations of 5-ASA at the site of action. A lack of requirements by regulatory authorities and the absence of standard methodology for site and timing of tissue acquisition and issues regarding methods of gut preparation can significantly affect the result of local drug concentration measurements (Gandia et al., 2007). In addition, the use of existing general PK/PD mathematical models has only offered the standard PK parameters. Therefore, there is a clear need to develop a simulation model to estimate the local bioavailability of the oral targeted delivery formulation such as 5-ASA in various parts of the gastrointestinal tract.

Local bioavailability of the targeted delivery formulation can be

Fig. 1. Mechanisms of absorption and elimination pathways of 5-ASA in healthy and compromised states. The transport mechanism depends on the concentration of the drug. At high concentrations, the transcellular transport reaches saturation and paracellular transport becomes dominant. D, drug; M, metabolite.

Fig. 2. Transversal (horizontal) and longitudinal (vertical) physiologically based compartmental model structure for 5-ASA absorption. $q_{in}$ and $q_{out}$, in and out bolus flow, respectively; $i$, longitudinal compartment index; $j$, transversal compartment index; $r_{M}$, drug biotransformation; $C_{D}$, drug concentration; $C_{M}$, metabolite concentration; $q_{bin}$ and $q_{bout}$, blood flow in and out of the intestinal tissue, respectively; $q_{cl}$, clearance.
affected by many physiological factors such as gastrointestinal tract (GIT) pH and transit time and pathologic conditions. In our drug release model (Haddish-Berhane et al., 2006), we incorporated many of these biological factors to predict drug concentration in the various part of GIT after administration of targeted delivery formulations such as controlled-release 5-ASA. Predictions from our model were comparable with clinical observations (Haddish-Berhane et al., 2007). Using the model, we were able to predict the amount of drug released in the different parts of the distal intestine. The next logical step is to develop a drug absorption, intestinal biotransformation, and systemic metabolism model that focuses on the prediction of local (GIT mucosal) concentration based on the results of the drug release model.

In the present work, a physiologically based model that considers the characteristics of the intestinal luminal conditions such as the transit time and pH as well as the physiological properties of the mucosal layer of bowel (subtle microlayers of the intestinal tissue) was developed. The model development is based on the transport and metabolism mechanism proposed by Zhou et al. (1999) for 5-ASA and its metabolite (Fig. 1). The model has been validated using literature data. This model will be integrated with the previously developed drug release model for enhanced prediction of local drug bioavailability.

Materials and Methods

The SIMDOT-AbMe Theoretical Model. To develop SIMDOT-AbMe, the mechanism proposed by Zhou et al. (1999) for absorption, biotransformation, and metabolism of 5-ASA and the exosorption of metabolite in health and disease was adopted as shown in Fig. 1. The mathematical description considered the intestinal mucosa as longitudinally (vertical) organized compartments and the various sites of the bowel as transversely (horizontal) organized segments. Targeted oral drug delivery formulations in the treatment of IBD mainly focus on drug release at the distal part of the intestine and colon. Hence, the window of drug release and, thus, the transversal segmentation was limited to the distal part of the small intestine and the colon. A schematic representation of the SIMDOT-AbMe model is depicted in Fig. 2. The generic model accounts for active and passive transcellular transport (absorptive and efflux), passive paracellular transport, and biotransformation. The assumptions considered in the building of the model were the following: 1) transport between two adjacent compartments in the segments is ignored except in the luminal compartment where the transit occurs; 2) blood flow to the intestinal tissue is divided into the flow to the lamina propria, which equilibrates with the epithelial tissue and the flow to serosal/submucosal compartment; 3) only passive absorptive and efflux transport of the compound is considered in all vertical compartments except at the apical and basolateral sides of the epithelial compartment; and 4) active transport is assumed to obey the Michaelis-Menten saturable kinetics.

For a given segment, $j$, if $C$ is the concentration of the drug/metabolite in a given compartment, $i$, the mass balance around the compartment can generally be represented by

$$V_i \frac{dC_i}{dt} = K_{TPi} + K_{TAi} + Q_i + r_i \quad (1)$$

where $V$ is the volume of the compartment, $t$ is time; $K_{TPi}$ is the passive transport term, $K_{TAi}$ is the active transport term, $Q$ is the flow term, and $r$ is the metabolism or elimination term. These terms can be described mathematically as (the subscript $j$ is omitted for clarity)

$$K_{TPi} = k^{TPi}_{i-1}C_{i-1} - (k^{TPi}_{0j} + k^{TPi}_{ej})C_i + k^{TPi}_{i+1}C_{i+1} \quad (2)$$

$$K_{TAi} = k^{TAi}_{i-1}C_{i-1} \frac{k^{Ap}_{0j} + k^{Ap}_{ej}}{K_{Mei0} + C_i} + k^{Ap}_{i+1}C_{i+1} + C_i + k^{Ap}_{Mei1}C_{i+1} + C_i + 1 \quad (3)$$

$$Q_i = q_{i0}C_i - q_{i1}C_i \quad (4)$$

$$r_i = -\frac{k^{Ap}_{0j}}{K_{Mei0} + C_i} \quad (5)$$

where $k^{TP}$ is AP, which is the area multiplied by the permeability coefficient, $k^{Ap}$ is the maximum rate of transport constant, $K_{TP}$ is the Michaelis-Menten constant, $C_{TP}$ is the concentration of drug entering from the adjacent compartment from the $(j-1)$th compartment, and $q_{i0}$ is the flow rate. The subscripts $a$, $e$, and $m$ denote absorption, efflux, and metabolism/biotransformation, respectively, and the superscripts $P$ and $A$ represent passive and active transport, respectively.

All of the terms may or may not be applicable to a given compartment. The reader is referred to the Appendix (eqs. A1–A6) for the detailed model equations for a given segment representing the ileum or colon and consisting of five compartments representing the lumen, mucus layer, epithelium, lamina propria, and submucosa/serosa.

Model Parameters. The model (eqs. 1–6) requires information about the compartmental volumes, absorptive surface areas, intestinal transit, intestinal blood flow, passive and active transport, biotransformation, and metabolic kinetic parameters. The compartmental volumes, absorptive surface areas, intestinal transit, and blood flow data were obtained from the literature (Rouge et al., 1996; Chawla et al., 2003; Tam et al., 2003). The passive transport permeability coefficients were assigned for the active drug (5-ASA) and its metabolite (Ac-5-ASA). The permeability coefficients and the active transport kinetic parameter data for the epithelial layer were obtained from literature (Zhou et al., 1999; Xin et al., 2006). In these studies the apparent permeability, $P_{app}$ was calculated according to the following formula:
where \( \frac{dM}{dt} \) is the apparent appearance rate of drug in the receiver side determined from the receiver amount versus time profile, \( A \) is surface area of the cell line, and \( C \) is the donor concentration. When both active and passive transport routes are involved, the term “apparent appearance rate” is the sum of both transport routes, namely,

\[
\frac{dM}{dt} = P_{app}
\]

(6)

where \( P \) is the permeation coefficient and \( V_m \) and \( K_m \) are the Michaelis-Menten parameters describing the active transport. In a normal gut, it is believed that there is no secretion of the drug (5-ASA) (Xin et al., 2006). Therefore, only the absorption term is represented (Michaelis-Menten term) in the mechanism shown in Fig. 1. The kinetic and transport parameters for the epithelial compartment were determined by fitting the data provided by Xin et al. (2006) (at low donor concentration) and Zhou et al. (1999) (at high donor concentration). The permeability coefficients for the other compartments were assigned, given that the model assumed that active transport only occurred at the epithelial compartment. Coefficients were assigned on the basis of the difficulty obtaining experimental data for each microscopic compartment. An optimization routine (see Appendix) was implemented to train the model and reestimate the assigned parameters using systemic concentration and other available data. The type and number of parameters to be optimized were based on average values provided in the literature for healthy subjects and patients with UC and CD. For example, Haddish-Berhane et al. (2007) compiled more than 200 data sets from the literature to determine the average transit times for the compartments. All parameters used in the present model are summarized in Table 1.

**Implementation.** The model and a user-friendly graphical user interface (GUI) were developed using Matlab (The MathWorks, Inc., Natick, MA). The GUI was designed for users such as pharmaceutical and clinical scientists with a variety of backgrounds. Owing to the dependence of absorption and biotransformation processes based on the state of the gut (diseased or healthy), the structure of the input data were designed as such to incorporate the status of disease remission or flare for both UC and CD as was proposed by DiPiro et al. (2005) using sulfasalazine or mesalamine. The output of the simulation is presented graphically and the important PK parameters are displayed. An optimization tool was incorporated for tuning and calibration of the model. The optimization results are also presented graphically and the statistical parameters that indicate the information content of the input experimental data and the accuracy of the estimates are displayed.

**Validation.** The model was calibrated and validated using experimental data presented by Gandia et al. (2007) for the systemic concentration of both the drug and metabolite. The clinical data (randomized, two-way, crossover study) compared the pharmacokinetic profile of a new once-daily dosing regimen of mesalazine (one 4-g/day dose) with the current twice-daily dosage (two 2-g/day doses) used in many European countries. In the present study, the drug and metabolite concentration data for the once-daily dosing regimen were used for calibration and the twice-daily dosage regimen was used for validation.

**Results**

**Kinetic Model Parameters.** The kinetic model parameters of the epithelial layer were estimated using literature data from Zhou et al.
who studied the transport of 5-ASA at high donor concentrations using in vitro Caco-2 cells. Using eq. 7, the data were analyzed and displayed in Fig. 3A. The continuous line represents the original data and the dotted lines with square and triangle symbols represent the nonsaturable (permeation) and Michaelis-Menten (saturable) kinetics. The permeability coefficient, the maximum rate $V_m$, and the kinetic constant $K_m$ values, estimated using a nonlinear fitting procedure, were $0.98 \pm 0.29 \times 10^{-6}$ cm min$^{-1}$, $4.9 \times 10^{-3} \pm 1.10 \times 10^{-2}$ $\mu$g min$^{-1}$, and $922 \pm 2989$ $\mu$g ml$^{-1}$, respectively. Further analyses of the data were performed by plotting the weighted residual sum of squares (WRSS) (Fig. 3B). [See Appendix for definition (eq. A7).] It was observed that minimum values do not exist for the given set of parameters, which implies that the information content of the data from the literature is not sufficient to accurately and uniquely estimate the kinetic parameters. The large confidence intervals also support this observation. Using the biotransformation data, the maximum conversion rate, $V_m$, and the Michaelis-Menten constant, $K_m$, were determined to be $6.8 \times 10^{-4} \pm 2.4 \times 10^{-5}$ $\mu$g min$^{-1}$ and $13.19 \pm 2.72$ $\mu$g ml$^{-1}$ (Fig. 4A). The WRSS plot (Fig. 4B) shows that the kinetic parameters were uniquely and accurately identified. A similar analysis was not possible for the metabolite (Ac-5-ASA) kinetic parameters because of the small number of data points reported.

Implementation. The graphical user interface for the software under development is shown in Fig. 5. One of the main attributes of the model is its flexibility. The number of transverse segments and longitudinal compartments (Fig. 2) used to model the lumen is selected by the user depending upon the level of detail desired and the calibration and parameter data available. Model inputs include the drug type, dose, and frequency and the disease type and stage. The pertinent parameter data are loaded automatically for the simulation on the basis of data provided by the user. The concentration profiles for each compartment are displayed graphically. Local and systemic PK/PD parameters are also computed and displayed upon selection. Because the model requires an extensive set of parameters and because some of them are assigned arbitrarily, it is essential that the data are refined through an iterative process as absorption data become available. Therefore, an optimization routine has been developed to readjust the parameters using an iterative process. On the basis of a priori sensitivity analyses, the parameters are ranked according to their sensitivity. The user selects the parameters to be optimized on the basis of the model type and experimental data available. The output of the optimization routine includes the predicted profile with the confidence interval, the number of parameters optimized, the correlation coefficient, and the root mean square error to quantify the quality of fit between the experimental and simulated profiles.

Simulation and Validation Results. Clinical literature data from Gandia et al. (2007) were used to tune and validate the proposed model. A model consisting of one segment and five longitudinal compartments and a dosage regiment of 4 g of mesalamine, once daily, was used for tuning and validation. Figure 6 shows the simulated drug (Fig. 6A) and metabolite (Fig. 6B) concentration profiles.
for each compartment and the experimental systemic plasma concentrations (circles). Although the simulated systemic concentrations of the drug and metabolite predict the experimental data trends; relatively high root mean square errors (RMSE) between the predicted and measured concentrations were calculated (1.6 and 2.7 for the drug and metabolite, respectively). A sensitivity analysis performed (see Appendix for details) before calibration of the model highlighted the fact that the model response was sensitive to the parameters for systemic metabolism and clearance (CL). Therefore, these parameters were reestimated using the optimization tool. The optimized values were $K_m = 13.2 \text{ mg} \text{ l}^{-1}$, $V_m = 35.7 \text{ mg} \text{ min}^{-1}$, $CL_d = 38 \text{ ml} \text{ min}^{-1}$; and $CL_m = 28 \text{ ml} \text{ min}^{-1}$. Repeating the simulation using the optimized parameters for the 4-g once-daily dose resulted in an improved correlation between the experimental and predicted data. The correlation coefficients for this simulation were 0.98 and 0.99 and the RMSEs were 0.38 and 0.37 for drug and metabolite, respectively (Fig. 7). The confidence interval for the predicted systemic concentration profile (dotted line in Fig. 7) is within the error range of the clinical data.

The optimized model was validated using systemic data for a twice-daily, 2-g dose of mesalamine. The results are shown in Fig. 8. The model accurately predicted the experimental data; the RMSEs were 0.6 and 0.3 for the drug and the metabolite, respectively. These values were significantly less than the S.D.s of the measured data, which were 0.9 and 0.95 for the drug and metabolite, respectively. The model also accurately predicted the residual plasma concentration of the drug. Table 1 shows a comparison between the calculated and measured PK parameters. It is also noteworthy that although the computed and experimental $t_{\text{max}}$ and the area under the curve (AUC) values agreed, the $C_{\text{max}}$ values differed. Comparing the two peaks for the 2-g, twice-daily administration, the AUC of the second peak (12–24 h) was observed to be substantially higher than that for the first peak. The PK parameters for the epithelial layer were computed to be $12.4 \text{ mg} \text{ l}^{-1}$, 15 min, and 23.3 h for the $C_{\text{max}}$, $t_{\text{max}}$, and AUC, respectively. The relative local bioavailability with respect to the systemic bioavailability defined as the ratio of systemic to local AUC was 0.063. This result implies that when systemic bioavailability is known, the local bioavailability can be predicted using the model.

**Discussion**

**Kinetic Model Parameters.** Among other factors such as the underlying assumptions, the accuracy of the model prediction depends on the accuracy of the model parameters. Because the epithelial layer has a pivotal role in drug absorption and biotransformation, the accuracy of the parameters pertaining to this compartment significantly influences the overall model prediction especially with orally administered targeted delivery formulations. In their study, Liang et al. (2000) considered the different transport routes for a slightly different molecule (sulfasalazine) and reported an apparent permeability value of $1.14 \times 10^{-6} \text{ cm} \text{ min}^{-1}$, which is similar to ours. However, the saturable kinetic parameters reported in the same study were 1 order of magnitude lower for the Michaelis-Menten constant and 2 orders of magnitude higher for the maximum rate than our
estimated parameters. It is evident that the experimental data used in our analysis did not contain enough information to estimate the saturable and nonsaturable parameters simultaneously, resulting in a wide confidence interval especially for the saturable kinetic parameters. Liang et al. (2000) did not report the confidence interval for their estimates. Therefore, a conclusive comparison with our model could not be made. Furthermore, in their study Zhou et al. (1999) reported the concentration at which passive transport becomes dominant to be $100 \mu g/m^2$ l, which is approximately 1 order of magnitude higher than the value calculated in this study (Fig. 3). In the absence of reliable estimates for the kinetic parameters, this value gives an idea to the value of $V_{max}$. To investigate the reason for this discrepancy, low donor drug concentration (0.8–80 $\mu g/m^2$ l) data presented by Xin et al. (2006) were used to perform parameter estimation. Even though the low concentration data cannot be used reliably to estimate the kinetic parameters, especially $V_{max}$, the crude estimation of this parameter would give an estimate as to how far the rate at 80 $\mu g/ml$ is from the saturation. At a donor concentration of 80 $\mu g/ml$, the active transport was far from saturation, and we estimated a maximum rate of $2.9 \times 10^{-3}$ $\mu g/min$, which was on the same order of magnitude as our previous estimate. This result implies that the concentration at which the passive transport becomes dominant is close to that estimated in the present study.

The estimation of the biotransformation kinetic parameters and the information content analysis demonstrated that the estimated parameters are unique and accurate. It should be noted that the conversion of 5-ASA into its metabolite was assumed to be the rate-determining step because the enzymatic process is slower than binding and transport. However, Zhou et al. (1999) reported a significant influence of the saturable efflux transport on the rate of appearance of the metabolite on the apical side, which would mean that the model used for estimating the parameters (eq. 7) is not adequate. In this scenario, a model that differentiates between the two processes (biotransformation and saturable efflux transport) would be needed to estimate the biotransformation and the efflux transport parameters. Overall, the analysis showed that the estimated parameters should be used with caution because of the large confidence intervals calculated for the transport data and the significant influence of the saturable efflux transport of the metabolite on the biotransformation data. It also highlights the fact that optimal in vitro and in vivo experiments need to be designed to obtain informative data to ascertain the accuracy and uniqueness of the parameter estimates for high-fidelity predictive models. To further tune the parameters with experimental data, the simulation tool is equipped with an optimization tool that enables reestimation of parameters that are sensitive to the model prediction. In the present study, only the systemic response was used to calibrate the parameters. Thus, the optimized parameters should also be treated with caution.

Simulation and Validation. Successful development of targeted delivery systems for existing potent drugs such as 5-ASA requires an in-depth understanding of the multiple biochemical and physiological processes and mechanisms involved in GIT epithelial transport (absorption and exsorbtion), gastrointestinal transit, the first-pass effect, and local and systemic exposure time profiles of the drugs. Therefore, it is critical to develop in silico models, based upon mechanism-dependent and physiologically sound principles, to accurately predict local bioavailability of 5-ASA. In the present work, a physiologically relevant simulation tool was developed to model the absorption, biotransformation, metabolism, and clearance of 5-ASA.
In the model presented, passive absorption, saturable absorption, biotransformation, and transit kinetics were simultaneously considered, whereas general purpose absorption and transit models (Ito et al., 1999; Agoram et al., 2001; Kimura and Higaki, 2002) exclude extracellular metabolism and exsorption (efflux transport). On the other hand, not unlike our model, the traditional and segregated-flow models developed by Cong et al. (2000) comprehensively illustrate the interactions among the effective flow to the intestine, absorption rate, and intestinal epithelial cell activities including enzymatic, secretory, and metabolism activities. Furthermore, for interaction in the present model we considered the above-mentioned factors with other variables, such as the blood flow of the intestinal tissue, that influence distribution of the drug to the nonabsorptive and noneliminatory layers of the submucosa. In addition, prior models excluded mechanism-dependent transport explicitly and focused on predicting the relationship between systemic bioavailability and metabolism and efflux. By considering mechanism-dependent transfer processes be-

Fig. 8. A, top, simulated drug concentration profiles in the lumen (---) and epithelial layer (——) compartments. Bottom, simulated systemic compartment drug concentration (——) and clinical data (●). B, simulated lumen (---), epithelial (——), and systemic (—— with symbols) compartment metabolite concentration profiles and clinical data (●). All simulations and data were at 2-g twice-daily dose. The vertical lines at each data point represent the S.E.

Fig. 9. Rate of drug leaving the small intestine (SI) versus time.
tween gut lumen and epithelial cells, and luminal biotransformation in our model, the relationships among local bioavailability, metabolism, efflux, and systemic bioavailability were established. Thus, our computation explicitly takes into account the role of local metabolic enzymes and efflux transporters (P-glycoprotein and multidrug-resistant protein 2) on absorption and local bioavailability, which has been the point of discussion in the past few years. Thus, one can use the present model to evaluate modulation of the different kinetic and clearance parameters pertaining to these processes.

Simulation of an in vivo clinical situation was demonstrated by modeling the effects of once- and twice-daily administration of 5-ASA. Very few reports that investigated the relationship between mucosal concentration profile and local bioavailability of 5-ASA exist because of a lack of availability of standard methods to measure these concentrations. With use of the relative local bioavailability predicted by the present model, the local bioavailability of new preparations can quickly be calculated to verify their effectiveness on the basis of measured systemic bioavailability. Hence, this model will be a beneficial tool for researchers and clinicians because it will save time and resources associated with the experimental stages during the design of new preparations.

Moreover, the effect of the residual systemic concentration, which is an important factor for therapeutic effect of 5-ASA products in patients with IBD who need to take high doses of the compound many times each day can now be estimated.

In our previous drug release model, we established that approximately 48% of the 5-ASA dose is released in the small bowel from the targeted drug delivery dosage form Asacol (Haddish-Berhane et al., 2006). Given the equivalence of performance of Eudragit L (Asacol), the coated targeted delivery formulation used in our drug release simulation, and ethyl cellulose-coated formulations used in the calibration and validation of the current model (Gibson et al., 2006), computations can now be made to indirectly validate the predicted luminal compartment drug concentration. With use of the 4-g once-daily dose, the area under the drug rate versus time curve (Fig. 9) gives the amount of drug that entered the colon, which was 963 mg. Likewise, the amount of drug biotransformed and exsorbed in the luminal compartments was calculated to be 62 mg. Now, by combining the findings of the previous model (Haddish-Berhane et al., 2006) with this result, approximately 22% of the drug was absorbed in the small bowel. If we assume that the majority of the drug that entered the systemic circulation was a result of the small bowel absorption, this percentage can be compared with the total (5-ASA and its metabolite) in vivo recovery. After compiling several clinical results for Asacol, Nugent et al. (2001) reported that total urinary recovery ranges between 13 and 36%. It can be observed that the predicted value falls within this range. Hence, one can have confidence in the predicted luminal compartment concentration profiles.

In the twice-daily dosage simulation, the systemic profile showed an offset from the x-axis after 12 h due to the residual systemic drug/metabolite concentration, which resulted in a higher value of $C_{\text{max}}$ in the second peak (Fig. 8). This was reflected in the observed significantly higher AUC value of the second peak (Table 2) and demonstrates that the model can be used to study alterations in drug plasma pharmacokinetics as a result of repetitive administration. The current model can predict simple additive accumulation of residual drug from repetitive treatments; however, the model can be enhanced to address additional effects related to vascular barrier properties and efflux of drug from tissue.

It appears that our present model underpredicts the concentrations between $C_{\text{max}}$ and the residual. This could mainly be due to the first-order drug clearance assumption and also to the fact that the drug metabolism in the liver and possibly other organs such as the kidney was represented by one rate equation. Nonetheless, it is anticipated that the tool will allow researchers to accurately relate in vitro parameters with in vivo physiological events on absorption and local bioavailability of 5-ASA.

The limitation of development of any physiological base model is that it requires a substantial number of physiological and kinetic parameters for calibration and validation. Examples of these parameters are blood flow rate, tissue volumes, partition coefficients, and solubility values. However, the scarcity of data for local intestinal concentration profiles has resulted in only partial calibration and validation of the model. Future studies with close collaboration between modeling experts and biological scientists are required to fully validate the model. The next step in model development will include designing in vitro and clinical experiments that will generate the necessary data for better characterization, calibration, and validation of the simulation model especially with respect to local drug bioavailability. In addition, one cannot ignore the biological variability when the bioavailability of the formulations is simulated. The current model is easily expandable to include stochastic simulations for the physiological parameters, which will provide realistic predictions that are readily usable by clinical pharmacologists.

Finally, we hope that the development of such computational models will provide a guideline and a roadmap for optimal design of ex vivo and clinical experiments that will assess the clinically relevant intestinal tissue concentrations of targeted delivery products such as 5-ASA. Development of standard methodologies regarding the optimal region of the intestine to biopsy and the appropriate time interval for sample collection after oral administration of the product will enable regulatory agencies to include these aspects in the evaluation of the effectiveness of targeted delivery formulations such as 5-ASA for the treatment of IBD.

### Appendix

#### Model Equations

The detailed model equations for two segments representing ileum and colon each with five compartments, namely lumen, mucus layer, epithelial, lumina propria, and submucosa/serosal, is given below for the drug concentration. Similar equations were also implemented for the metabolite concentration. When the numbers

<table>
<thead>
<tr>
<th>Drug</th>
<th>Clinical data</th>
<th>Simulation</th>
<th>Simulation</th>
<th>AUC_{0–12h} (h μg ml$^{-1}$)</th>
<th>AUC_{12–24h} (h μg ml$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (μg ml$^{-1}$)</td>
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<td>3.3</td>
<td>5.5</td>
<td>15.4</td>
<td>26.8</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>2*</td>
<td>1.8</td>
<td>2</td>
<td>12.1</td>
<td>16.1</td>
</tr>
</tbody>
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*Median with minimum and maximum of 1–6 h.
of segments and compartments are selected by the user, the model equations are created automatically for the selected options.

**Lumen (L)**

\[ V_L \frac{dD_L}{dt} = k_a L D_L - k_e M L D_M L - \frac{v_m L D_L}{k_m a + D_L} - q_L D_L \]  \hspace{1cm} (A1)

The first term on the RHS represents the passive efflux from the compartment below (mucous layer), the second term represents the passive absorption from the lumen to the compartment below, the third term represents the biotransformation of the drug in the lumen, and the last term stands for the elimination of the drug from the intestinal segment. The dose that is released in the ileal segment is input to the model as an initial value for the drug concentration.

**Mucus Layer (ML)**

\[ V_{ML} \frac{dD_{ML}}{dt} = k_a M L D_{ML} - k_e M L D_{ML} - \frac{v_{ML} D_{ML}}{k_m a + D_{ML}} - k_e M L D_{ML} \]  \hspace{1cm} (A2)

The first term on the RHS represents the passive absorption from the compartment below to the mucous layer compartment, the second term is passive and active efflux from the current (ML) to the epithelial compartments, and the fifth term is passive efflux from the ML compartment to the one above.

**Epithelial (E)**

The first two terms on the RHS represent the passive and active absorption to the epithelial compartment, and the third term is for the passive and active efflux terms from the LP compartment below, the fifth and sixth terms stand for the passive and active absorption from the epithelial layer, and the last term is for epithelial biotransformation.

**Lumina Propria (LP)**

\[ V_{LP} \frac{dD_{LP}}{dt} = k_a E D_{LP} + \frac{v_{LP} D_{LP}}{k_e E + D_{LP}} - k_a E D_{LP} - \frac{v_{E} D_{LP}}{k_e E + D_{LP}} + f E (D_e - D_{LP}) \]  \hspace{1cm} (A4)

The first and second terms on the RHS are for passive and active absorption to the current compartment (LP), the third term is for the absorption from the current compartment, the fourth and fifth terms are passive and active efflux from the current compartment to the epithelial compartment, and the last term is the exchange with the systemic circulation through the blood flow with a partition coefficient \( f \) to the LP compartment.

**Submucosa (SM)**

\[ V_{SM} \frac{dD_{SM}}{dt} = k_a L D_{LP} - k_a S M D_{SM} + (1 - f) q_S (D_e - D_{SM}) \]  \hspace{1cm} (A5)

The first term on the RHS is the passive absorption to the current (SM) compartment, the second term represents the passive absorption from the current compartment, and the last term is the exchange with the systemic circulation through the blood flow to the SM. The term \( 1 - f \) represents the blood flow partition coefficient. Here it is assumed that no passive efflux is present from this compartment to the one above.
The absolute change, $J$, of the model prediction due to an increase or decrease of the parameter value by $\Delta p$ was analyzed using (A8).

\[
J = \left| \frac{\partial y}{\partial p} \right| \frac{y_{p+\Delta p} - y_{p-\Delta p}}{2\Delta p}
\]

### References


