An investigation into the possibilities and limitations of *in silico* absorption modeling: GastroPlus™ simulation of nimesulide oral absorption

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Abstract

In silico modeling of oral drug absorption has received widespread attention over the past few years. These models require a number of input parameters and the accurate prediction is often limited by the lack and/or inappropriate selection of reliable input data. The objective of this study was to explore the predictive properties of GastroPlus™ simulation technology using BCS class II drug nimesulide as a model compound. Model sensitivity to the input data and accuracy to predict drug pharmacokinetic profile observed *in vivo* were evaluated by constructing drug specific absorption models by two independent analysts, using different presumptions regarding the key factors that govern nimesulide absorption from gastrointestinal tract. Model 1 was constructed assuming that nimesulide might be a substrate for influx transporters in the intestine. Therefore, the absorption scale factors (ASFs) were adjusted to best match the resultant profile to the *in vivo* observed data. Experimentally determined intrinsic solubility was used as the input value, and human jejunal permeability was *in silico* predicted. Drug particle radius was assumed to be 5 microns. The main premise in the Model 2 was that nimesulide is well absorbed after oral administration mainly due to the pH-surfactant induced increase in solubility in the gastrointestinal milieu. Therefore, the ASFs were kept on default GastroPlus™ values, and input solubility and permeability values were optimized to best match the *in vivo* data. Drug particle size was 25 microns. The results of the simulations were compared with actual clinical data in order to identify the model yielding the best estimation. The presented data indicate the potential of gastrointestinal simulation technology to predict oral drug absorption. However, in order to obtain meaningful *in silico* modeling, the necessary input data have to be carefully selected and/or experimentally verified.

Keywords

nimesulide, *in silico* modeling, absorption

Introduction

Application of *in silico* simulation technologies has infiltrated different areas of pharmaceutical sciences, and has been widely used in drug discovery and drug product development. A growing concern for the biopharmaceutical characterization of pharmaceutical products increased the interest in development and evaluation of *in silico* tools capable to identify critical factors (i.e. drug physicochemical properties, dosage form factors) influencing drug biological performance, and to forecast drug absorption based on the selected dataset(s) of input factors. Many research articles discussed the utility of such technologies, emphasizing both their advantages, and possible drawbacks (1–7). Nowadays, several commercial programs for *in silico* simulations of oral drug absorption are available (1, 8-10). So far, they have been applied in the formulation development, assessment of the influence of drug properties on oral drug absorption, prediction of food effects, prediction of drug-drug interactions, establishment of *in vitro-in vivo* correlation (IVIVC), and in certain cases, to aid justification of biowaivers.

In order to establish the relationship between drug physicochemical data and its clinical performance, a mechanistic approach to oral drug absorption based on the Biopharmaceutics Classification System (BCS) was introduced (11). According to the BCS concept, drug dose solubility and dissolution rate from pharmaceutical preparations, along with intestinal permeability, are major determinants of its absorption. Based on the theory of the BCS and prior knowledge of gastrointestinal (GI) physiology, a semiphysiological absorption model named Advanced Compartmental Absorption and Transit model (ACAT; commercially available as GastroPlus™ software package) for *in silico* prediction of oral drug absorption was developed (8). GastroPlus™ requires a number of input parameters which should adequately reflect drug biopharmaceutical properties. Default physiology parameters under fasted and fed states are population mean values obtained from published data. The other input parameters, including drug physicochemical properties and pharmacokinetic (PK) parameters, along with certain formulation characteristics, could be experimentally determined, *in silico* predicted, extracted from PK data after intravenous or oral administration, or taken from the literature. Depending on the source, values of the input parameters might vary considerably, so the accurate prediction might be limited by the lack and/or inappropriate selection of the input data.

Nimesulide (NIM) is a nonsteroidal anti-inflammatory agent with analgesic and antipyretic activity, with the recommended dosage of 100 mg twice daily. This is a BCS Class II drug, with a pK_a value within the physiological pH range (pK_a 6.4 (12), and therefore, with pH-dependent solubility in gastrointestinal environment. Literature data about nimesulide solubility are rather diverse, depending on the experimental conditions employed (e.g. media composition, temperature) and the applied methodology (12-15). Nevertheless, all the data available indicate poor solubility in acidic environment (< 30 μg/ml; dose number (D_0) > 1). The presence of naturally occurring surfactants might also impact NIM solubilization and dissolution. Reliable data on human permeability are not available, but the absorption experiments revealed that nimesulide shows good permeability through the everted rat intestinal mucosa (16). Reports from the *in vivo* studies show that, after oral administration, the drug is rapidly and extensively absorbed (17). However, no studies of nimesulide administered intravenously or in a form of a solution have been performed, and therefore, its absorption profile is difficult to decipher using conventional pharmacokinetic analysis.

The objective of this study was to explore the predictive properties of GastroPlus™ simulation technology using nimesulide as a model compound. Model sensitivity to the input data and accuracy to predict drug pharmacokinetic profile observed *in vivo* were evaluated by

constructing drug specific absorption models by two independent analysts, using different sets of the input parameters. The accuracy of the predictions was discussed in terms of the extent in which the simulated pharmacokinetic profiles matched actual clinical data.

Material and Methods

Gastrointestinal Simulation

Gastrointestinal simulation technology (GIST) based on the ACAT model (GastroPlus™ version 6.0.1004, Simulations Plus Inc., Lancaster, CA, USA) was used for mechanistic simulations. The form of the ACAT model implemented in GastroPlus™ is operated by a system of coupled linear and nonlinear rate equations used to simulate the effect of physiological conditions on drug absorption as it transits through successive GI compartments. The equations include the consideration of six states of drug substance (unreleased, undissolved, dissolved, degraded, metabolized, and absorbed), eighteen compartments (stomach, seven compartments for the small intestine, colon, and nine enterocyte compartments), three states of excreted material (unreleased, undissolved, and dissolved), and the amount of drug in up to three PK compartments (when PK parameters are available). The total amount of absorbed material is summed over the integrated amounts being absorbed/exsorbed from each absorption/transit compartment (8).

Drug specific absorption models were constructed by two independent analysts, using different presumptions regarding the key factors that govern nimesulide absorption from gastrointestinal tract. Summary of the input parameters concerning nimesulide physicochemical and PK data is given in Table I.

Table I. Summary of nimesulide input parameters employed for gastrointestinal simulation

Parameter	Model 1	Model 2
Molecular weight (g/mol)	308.31	
logD (pH 7.4)	1.8[a]	1.48[b]
pK_a	6.4[b]	
Human jejunal permeability (cm/s)	2.225×10^{-4} [c]	1.116×10^{-4} [a] 2.002×10^{-4} [d]
Dose (mg)	100	
Dose volume (ml)	200[e]	
Solubility at pH 4.5 (mg/ml)	0.007[f]	0.017[b] 0.030[d]
Mean precipitation time (s)	900[g]	
Diffusion coefficient (cm^2/s)	0.757×10^{-5} [c]	
Drug particle density (g/ml)	1.2[g]	
Effective particle radius (μm)	25[g] 5[d]	25[g]
Body weight (kg)	88[e]	
First pass extraction in liver (%)	0.1[h]	/
Blood/plasma conc. ratio	0.668[c]	1[g]
Unbound percent in plasma (%)	4.513[c]	3[a]
Clearance (L/h/kg)	0.039[h]	0.028[a]
Volume of distribution (L/kg)	0.226[h]	0.14[a]
Elimination half-life (h)	4.02	3.42
Simulation time (h)	15	
Dosage form	IR tablet	IR suspension/IR tablet

[a] literature values taken from ref (13); [b] literature values taken from ref (12); [c] *in silico* predicted (ADMETPredictor™ module); [d] optimized values; [e] literature values taken from ref (18); [f] experimental value (19); [g] default GastroPlus™ values; [h] literature values taken from ref (17)

Model 1 was constructed assuming that nimesulide might be a substrate for influx transporters in the intestine. Therefore, the absorption scale factors (ASFs), which scale the effective permeability to account for variations in absorption rate-determining effects (e.g. pH effects, the presence of influx and efflux transporters) that differ from one compartment to another (8), were adjusted to best match the resultant profile to the *in vivo* observed data. Experimentally determined intrinsic solubility was used as the input value, and human jejunal permeability was *in silico* predicted. Drug particle radius was assumed to be 5 microns. All other parameters were fixed at default values that represent human fasted physiology.

The approach used to construct and validate Model 2 was based on the comparative study of two dosage forms of nimesulide (IR suspension and IR tablet). The absorption model was initially constructed for IR suspension, and was afterwards validated for IR tablet formulation. The main premise in the Model 2 was that nimesulide is well absorbed after oral administration mainly due to the pH-surfactant induced increase in solubility in the gastrointestinal milieu. Therefore, the ASFs were kept on default GastroPlus^{TM} values, and input solubility and permeability values were optimized to best match the *in vivo* data. Parameter sensitivity analysis (PSA) was used to assess the influence of the selected input parameters (solubility and permeability) on the predicted rate and extent of drug absorption. In the Physiology tab, the Opt logD Model SA/V 6.1 was used to estimate the changes in permeability as drug transits along the GI tract.

All simulations were performed using GastroPlus^{TM} Single Simulation Mode, and the corresponding form (IR suspension or IR tablet) was specified as the dosage form.

In Vivo Data

Published data from nimesulide bioequivalence study were used to evaluate the resultant nimesulide absorption models (18).

The predictability of the generated absorption models was measured by the percent prediction error (% PE) between the simulated and *in vivo* observed data (20):

$$\%PE = \frac{observed - predicted}{observed} \times 100$$

Results and Discussion

Nimesulide PK profile obtained using the Model 1 input data set and default ASF values showed notable differences when compared to the average profile observed from the *in vivo* study (Fig.1a). The predicted C_{max} was about two times lower and the time to reach it (t_{max}) about 1.5 times longer than the *in vivo* observed values. Therefore, value for drug particle radius was decreased to 5 microns, and the ASF values were adjusted to best match the observed plasma concentration–time data. Default and adjusted ASF values are given in Table II.

The simulated NIM plasma concentration-time profile, based on the selected input parameters, along with the adjusted drug particle radius and ASF values, is presented in the Fig. 1a, together with the mean plasma profile taken from the literature. The predicted nimesulide absorption and dissolution profiles are presented in Fig. 1b. The predicted and *in vivo* observed pharmacokinetic parameters following oral administration of 100 mg NIM IR tablets are shown in Table III. The percent prediction errors for C_{max} and area-under-the-curve (AUC) values were less than 10%, indicating that the model has predicted these parameters well. The largest

deviation was observed for t_{max} (PE = 21.25%). However, this value was calculated based on the mean t_{max} estimated for a particular *in vivo* observed data set (18). Considering that reported t_{max} values after oral administration of nimesulide IR tablets varied between 1 and 4 h (18, 21), the simulated value of 3.15 h can be considered as a reasonable estimate.

Table II. ASF values employed

Compartment	Model 1	Model 2 (GastroPlus™ default)
Stomach	0	0
Duodenum	1000	2.687
Jejunum 1	500	2.668
Jejunum 2	2.600	2.633
Ileum 1	0.500	2.588
Ileum 2	0.500	2.551
Ileum 3	5.547	2.460
Caecum	6.098	1.328
Asc colon	12.240	1.995

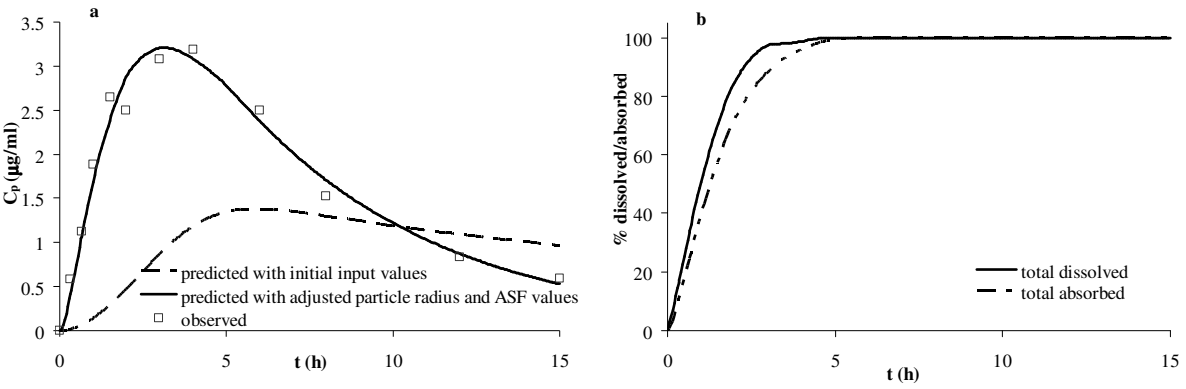

Fig. 1. GastroPlus™ Model 1 predicted and *in vivo* observed mean NIM plasma C_p–time profiles following administration of a single 100 mg nimesulide IR tablet (a); predicted dissolution and absorption profiles (b)

Table III. Comparison of pharmacokinetic parameters between Model 1 simulated and *in vivo* observed data

Parameter	Observed	Simulated	PE (%)
C_{max} (µg/ml)	3.19	3.21	-0.63
t_{max} (h)	4.00	3.15	21.25
$AUC_{0\rightarrow t}$ (μg h/mL)	25.78	25.96	-0.70
$AUC_{0\rightarrow\infty}$ (μg h/mL)	30.96	29.10	6.01

The generated regional absorption demonstrated that majority of NIM, formulated as an IR dosage form, is absorbed in duodenum and jejunum (67%), while the rest of the dose is absorbed in mid and distal GI regions (Fig. 2).

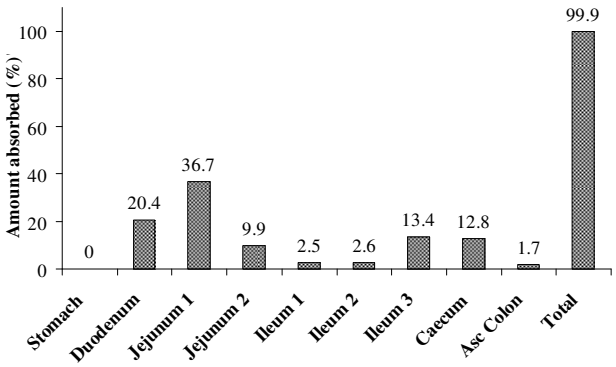

Fig. 2. Compartmental absorption of nimesulide generated using the GastroPlus™ Model 1

Gastrointestinal simulation based on the Model 2 initial input data set (Table I), using the default ASF values in GastroPlus™ Opt logD Model SA/V 6.1 (Table II), resulted in underestimation of C_{max} and overestimation of t_{max}, compared to the *in vivo* observed nimesulide absorption profile after oral administration of IR suspension (Fig.3).

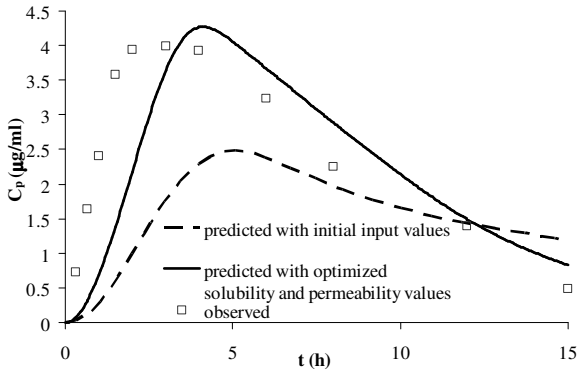

Fig. 3. GastroPlus™ Model 2 predicted and *in vivo* observed mean NIM plasma C_p–time profiles following administration of a single 100 mg nimesulide dose from IR suspension

Therefore, parameter sensitivity analysis was performed in order to evaluate the effects of solubility and effective permeability on nimesulide absorption. According to the PSA outcomes, both drug solubility and permeability were found to affect the predicted PK parameters, indicating that higher solubility and/or higher permeability values than those initially used for simulation would result with the predicted C_p-time profile similar to that observed *in vivo*. The obtained results enabled optimization of the investigated input factors: PSA showed that drug solubility increase from 0.017 mg/ml to 0.03 mg/ml and drug permeability increase from 1.116 x 10^{-4} to 2.023 x 10^{-4} cm/s would yield the PK parameter values close to those observed *in vivo*.

The simulated NIM plasma concentration-time profile after oral administration of IR suspension based on the selected, along with the parameters optimized using PSA analysis, is presented in Fig.3. The predicted and *in vivo* observed (18) PK parameters are shown in Table IV. The percent prediction errors for C_{max} and AUC values were less than 10%, while the calculated %PE value for t_{max} was relatively high (-36.67%). Similarly to Model 1 considerations, when interpreting the significance of this value, it had to be considered that it was calculated

based on the mean t_{max} for a particular *in vivo* observed data set, while the individual t_{max} values varied between 1.5 and 6 hours (18). Therefore, it could be concluded that the predicted value of 4.1 h was within the acceptable range.

Table IV. Comparison of pharmacokinetic parameters between Model 2 simulated and *in vivo* observed data

Parameter	IR Suspension			IR tablet		
	Observed	Simulated	PE (%)	Observed	Simulated	PE (%)
C_{max} (µg/ml)	3.98	4.27	-7.18	3.19	3.39	-6.16
t_{max} (h)	3.00	4.10	-36.67	4.00	3.40	15.00
$AUC_{0\rightarrow t}$ (μg h/mL)	35.23	35.68	-1.30	25.78	25.69	0.35
$AUC_{0\rightarrow\infty}$ (μg h/mL)	36.61	39.60	-8.17	30.96	27.92	9.82

In the next step, the generated absorption model was used to predict PK profile after oral administration of 100 mg nimesulide IR tablet. The obtained profile is presented in Fig. 4a, together with the mean plasma profile taken from the literature (18). The predicted nimesulide absorption and dissolution profiles from IR tablet are presented in Fig. 4b.

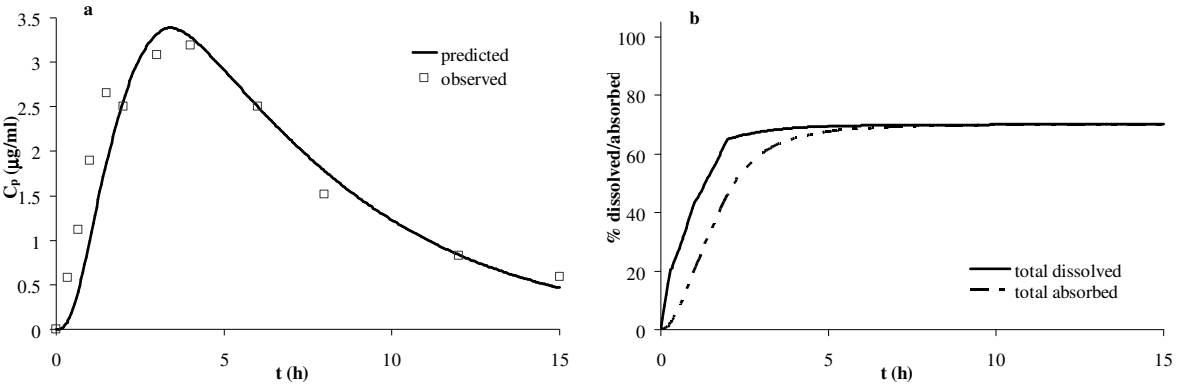

Fig. 4. GastroPlus™ Model 2 predicted and *in vivo* observed mean NIM plasma C_p–time profiles following administration of a single 100 mg nimesulide IR tablet (a); predicted dissolution and absorption profiles (b)

The simulation results indicated incomplete drug dissolution, and consequently, absorption (i.e. the extent of dissolution/absorption corresponding to approximately 70% of the administered dose). In addition, GastroPlus™ generated regional absorption of nimesulide showed that about 50% of the dose administered in a form of IR tablet (corresponding to 72% of total drug absorbed) was absorbed in duodenum and jejunum, 13% (19% of total absorbed) in ileum, while the rest of the dose was absorbed in caecum (Fig. 5).

The predicted pharmacokinetic parameters and those observed *in vivo* are presented in Table IV. The Model 2 was able to predict well the C_{max} and AUC values (PE less than 10%), and the highest degree of deviation from the mean *in vivo* estimated value was observed for t_{max} (PE = 15%). Nevertheless, the predicted value of 3.4 h was considered as reasonable estimate since it was within the reported t_{max} range (18, 21).

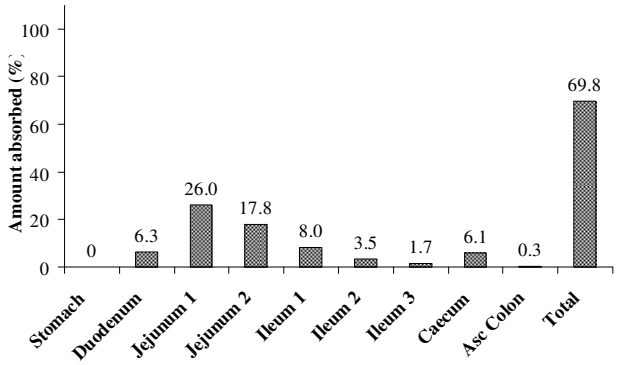

Fig. 5. Compartmental absorption of nimesulide generated using the GastroPlus Model 2

Both Models 1 and 2 gave accurate prediction of nimesulide average plasma profile after oral administration. In the Model 1, the resultant ASF values in duodenum and jejunum were much higher than the Opt logD Model SA/V 6.1 default values, reflecting fast absorption of NIM in the proximal parts of the intestine. There are two distinct interpretations: Model 1 outcomes indicate involvement of influx transporters in nimesulide absorption, while according to the Model 2 outcomes, high drug permeability is predominating factor leading to relatively rapid absorption in the proximal intestine. This later assumption is supported by the concept of Biopharmaceutics Drug Disposition Classification System (BDCCS) according to which BCS Class II drugs are not expected to be substrates for influx transporters (22). In Model 2, PSA was used to optimize parameters for which accurate data were not available (i.e. *in vivo* solubility and human jejunal permeability). Since this model was developed using the set of *in vivo* data for two dosage forms (oral suspension and IR tablet), it also revealed incomplete drug absorption from IR tablet (approximately 70% of the administered dose as compared to almost 100% drug absorbed estimated for the same set of *in vivo* data when Model 1 was applied). According to the Model 2 outcomes, nimesulide dissolution from IR tablets would be the limiting factor for the rate and extent of the drug absorption.

Conclusions

The described independent procedures to build nimesulide specific absorption model illustrate the importance of understanding complex interplay between drug physicochemical and pharmacokinetic properties, formulation factors, and human physiology characteristics in order to forecast drug PK profile *in vivo*. Although both Models 1 and 2 gave accurate prediction of nimesulide average plasma profile after oral administration, the approach applied in Model 2 might be considered as more realistic, leading to the conclusion that the related absorption model more likely reflects nimesulide *in vivo* absorption.

The presented data indicate potential of gastrointestinal simulation technology to be used for evaluation and prediction of oral drug absorption. It should be stressed out that, in order to obtain meaningful *in silico* modeling, the necessary input data have to be carefully selected and/or experimentally verified. In addition, PSA represents valuable tool for identification of critical parameters affecting the rate and extent of drug absorption, and optimization of parameters for which accurate data are not available.

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