articles

molecular pharmaceutics

Applications of Physiologically Based Absorption Models in Drug Discovery and Development

Neil Parrott* and Thierry Lave

F. Hoffmann-La Roche Ltd., Pharmaceuticals Division, Pharma Research Non-Clinical Development, Non-Clinical Drug Safety, Basel Switzerland

Received January 28, 2008; Revised Manuscript Received April 30, 2008; Accepted May 11, 2008

Abstract: This article describes the use of physiologically based models of intestinal drug absorption to guide the research and development of new drugs. Applications range from lead optimization in the drug discovery phase through clinical candidate selection and extrapolation to human to phase 2 formulation development. Early simulations in preclinical species integrate multiple screening data and add value by transforming these individual properties into a prediction of in vivo absorption. Comparison of simulations to plasma levels measured after oral dosing in animals highlights unexpected behavior, and parameter sensitivity analysis can explore the impact of uncertainties in key properties, point toward factors which are limiting absorption and contribute to assessment of compound developability. Physiological models provide reliable prediction of human absorption and with refinement based on phase 1 data are useful guides to further market formulation development. Improvements in the accuracy of simulations are expected as better in vitro methods generate more in vivo relevant solubility and permeability data, and modeling will play a central role in the development of more predictive methods for transporter-related effects on drug absorption.

Keywords: Modeling and simulation; pharmacokinetics; physiologically based pharmacokinetic (PBPK); absorption model; advanced compartmental and transit (ACAT); GastroPlus

Introduction

Drug delivery via the oral route is often preferable because of safety, convenience and cost, and in many cases a new therapeutic entity can only become profitable if it is able to be given orally. To aid the development of viable oral drugs there have been many attempts to model drug absorption. A simple early model combined the key compound properties of permeability, ionization and solubility in an empirical mathematical formula to calculate a number related to the absorption potential of a drug.[1] In the next years numerous more complex models were developed, one of which provided the theoretical basis for the Biopharmaceutical Classification System,[2] now widely used to aid decision making in pharmaceutical formulation development and the basis for regulatory decisions on biowaivers.[3,4] A good review of many of the more useful early models is provided by Yu.[5]

Recently there has been much activity in the development of dynamic models based on representations of gastrointestinal tract physiology which track drug transit, dissolution

* To whom correspondence should be addressed. Mailing address: Drug Metabolism and Pharmacokinetics Department, Modeling and Simulation Group, F. Hoffmann-La Roche Ltd, Basel, Switzerland. Tel: (+41)-61-688-0813. E-mail: neil_john.parrott@roche.com. Fax: (+41)-61-688-2066.

(1) Dressman, J. B.; Amidon, G. L.; Fleisher, D. Absorption Potential: Estimating the Fraction Absorbed for Orally Administered Compounds. *J. Pharm. Sci.* **1985**, *74* (5), 588–589.

(2) Amidon, G. L.; et al. A theoretical basis for a biopharmaceutic drug classification: The correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res.* **1995**, *12* (3), 413–420.

(3) FDA, Guidance for Industry—Dissolution Testing of Immediate Release Solid Oral Dosage Forms. 1997.

(4) FDA, Guidance for Industry—Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System. 2000.

(5) Yu, L. X.; et al. Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption. *Adv. Drug Delivery Rev.* **1996**, *19*, 359–376.

10.1021/mp8000155 CCC: $40.75
Published on Web 06/12/2008

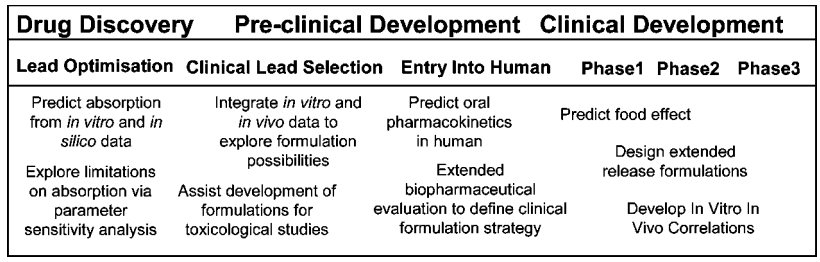

Figure 1. Applications of absorption modeling in drug discovery and development.

and absorption. Several of these models have been released as commercial software packages suitable for use by scientists working in industry, and some have published their experiences.[6–12] While workers in academia often custom-build physiologically based absorption models using general purpose modeling software,[13–16] for scientists in industry the convenience of a supported, user-friendly software package usually outweighs the cost and flexibility considerations that favor custom model building. To meet this need several commercial absorption software tools are marketed. GastroPlus, based on Yu and Amidon's compartmental absorption and transit (CAT) model,[5] was the first such commercial software. Simulations Plus (www.simulationsplus.com) extended the CAT model and produced a user-friendly software tool, adding features such as pH dependent solubility and permeability. They renamed the new model the advanced CAT or ACAT model.[17] Two other software tools largely based upon the CAT model are provided by Cyprotex (www.cyprotex.com) and SimCYP (www.simcyp.com) while an alternative to the ACAT model is implemented in the PK-Sim software.[18] PK-Sim first appeared as a whole-body physiologically based disposition model but later added a novel absorption model where the gastrointestinal tract is modeled as a continuous tube with spatially varying properties. This model has been described for rat,[19] human[20] and more recently for monkey.[21] The performance of GastroPlus to predict oral absorption and comparison with a competitor software iDEA (no longer available) has been reported previously.[11]

Modeling of drug absorption can be useful from the lead optimization of drug discovery through to phase 2 studies in clinical development as illustrated in Figure 1.

During lead optimization numerous compounds are synthesized and characterized for physicochemical and pharmacokinetic properties in screening assays. Simulations of oral plasma profiles based on such screening data have been assessed by us before.[22] We found that the reliability of the predictions for very poorly soluble lipophilic compounds is often limited by the poor relevance of aqueous solubility for the in vivo situation. However, higher throughput biorelevant solubility measurements are now possible (see Materials and Methods) and the use of such data for early simulations can be reassessed.

(6) Dannenfelser, R.-M.; et al. Development of Clinical Dosage Forms for a Poorly Water Soluble Drug I: Application of Polyethylene Glycol-Polysorbate 80 Solid Dispersion Carrier System. *J. Pharm. Sci.* **2004**, *93* (5), 1165–1175.

(7) Kuentz, M.; et al. A strategy for preclinical formulation development using GastroPlusTM as pharmacokinetic simulation tool and a statistical screening design applied to a dog study. *Eur. J. Pharm. Sci.* **2006**, *27*, 91–99.

(8) Tubic, M.; et al. In Silico Modeling of Non-Linear Drug Absorption for the P-gp Substrate Talinolol and of Consequences for the Resulting Pharmacodynamic Effect. *Pharm. Res.* **2006**, *23* (8), 1712–1720.

(9) Jones, H.; et al. Predicting Pharmacokinetic Food Effects Using Biorelevant Solubility Media and Physiologically Based Modelling. *Clin. Pharmacokinet.* **2006**, *45* (12), 1213–1226.

(10) De Buck, S. S.; et al. The Prediction of Drug Metabolism, Tissue Distribution, and Bioavailability of 50 Structurally Diverse Compounds in Rat Using Mechanism-Based Absorption, Distribution, and Metabolism Prediction Tools. *Drug Metab. Dispos.* **2007**, *35* (4), 649–659.

(11) Parrott, N.; Lave, T. Prediction of intestinal absorption: comparative assessment of GASTROPLUS and IDEA. *Eur. J. Pharm. Sci.* **2002**, *17*, 51–61.

(12) Lindahl, A.; et al. Regional Intestinal Absorption and Biliary Excretion of Fluvastatin in the Rat: Possible Involvement of mrp2. *Mol. Pharmaceutics* **2004**, *1* (5), 347–356.

(13) Grass, G. M. Simulation Models To Predict Oral Drug Absorption From In Vitro Data. *Adv. Drug Delivery Rev.* **1997**, *23*, 190–219.

(14) Nicolaides, E.; et al. Biorelevant Dissolution Testing to Predict the Plasma Profile of Lipophilic Drugs After Oral Administration. *Pharm. Res.* **2001**, *18* (3), 380–388.

(15) Fotaki, N.; Symillides, M.; Reppas, C. Canine versus in vitro data for predicting input profiles of l-sulpiride after oral administration. *Eur. J. Pharm. Sci.* **2005**, *26* (3−4), 324–333.

(16) Rausl, D. Intestinal permeability and excretion into bile control the arrival of amlodipine into the systemic circulation after oral administration. *J. Pharm. Pharmacol.* **2006**, *58*, 827–836.

(17) Agoram, B.; Woltosz, W. S.; Bolger, M. B. Predicting the impact of physiological and biochemical processes on oral drug bioavailability. *Adv. Drug Delivery Rev.* **2001**, *50* (Suppl. 1), S41–S67.

(18) Willmann, S.; et al. PK-Sim: a physiologically based pharmacokinetic "whole-body" model. *BioSilico* **2003**, *1*, 121–124.

(19) Willmann, S.; et al. A Physiologic Model for Simulating Gastrointestinal Flow and Drug Absorption in Rats. *Pharm. Res.* **2003**, *20* (11), 1766–1771.

(20) Willmann, S.; et al. A Physiological Model for the Estimation of the Fraction Dose Absorbed in Humans. *J. Med. Chem.* **2004**, *47* (16), 4022–4031.

(21) Willmann, S.; Edginton, A. N.; Dressman, J. B. Development and Validation of a Physiology-based Model for the Prediction of Oral Absorption in Monkeys. *Pharm. Res.* **2007**, *24* (7), 1275–1282.

(22) Parrott, N.; et al. An Evaluation of the Utility of Physiologically Based Models of Pharmacokinetics in Early Drug Discovery. *J. Pharm. Sci.* **2005**, *94* (10), 2327–2343.

At the clinical lead selection phase only a few compounds are still under consideration for progression to clinical development, and these compounds need to be more fully characterized. A key question is whether the desired exposure for therapeutic effect in human is achievable, and an estimation of the maximum absorbable dose (MAD) is useful in an early assessment of "developability". The MAD concept was first outlined by Johnson,[23] who developed an equation to estimate an upper limit of absorbed drug for neutral molecules which had no dissolution limitation. Various modifications to the MAD calculation have been proposed (see review by Sun et al.[24]), but we believe that a more powerful way to explore absorption across a range of doses is to perform a parameter sensitivity analysis (PSA) using a physiological absorption model. With PSA, a series of simulations is run at a range of different doses to examine how different parameters such as maximal plasma concentration (Cmax) or area under the plasma concentration versus time curve (AUC) are affected. Use of PSA to aid decision on a clinical formulation approach has been published previously.[7]

A task of preclinical research is to understand the pharmacokinetics of compounds in animals and use this knowledge to make a reliable prediction in human. Physiologically based models can incorporate the known differences across species and when combined with in vitro data on biochemical differences can provide a rational basis for such prediction. A strategy based upon such an approach has been developed within our group and was shown to provide more reliable predictions than empirical approaches.[25] This strategy relies on absorption models describing the different gastrointestinal physiologies for each of the common preclinical species (mouse, rat, dog and monkey) and on in vitro assays characterizing biochemical differences. We have applied these models to simulate literature data as well as for our own in-house projects and verified that they capture appropriately the species differences in absorption and allow reliable prediction of human oral pharmacokinetics.

An advantage of model based drug research and development is that the models act as a repository for the knowledge gained.[26] Thus, a model developed with preclinical data and applied to predict human oral pharmacokinetics can be further refined during clinical development. The human absorption model allows more mechanistic interpretation of clinical data and thus can be useful to explore hypotheses and to help guide clinical formulation development. For example, the effect of food on absorption can be simulated, leading to better understanding and guiding formulation attempts to reduce the effect.[9] In cases where limited bioavailability is an issue, the possible impact of intestinal transporters and intestinal metabolism may be explored to aid decisions on the cost to benefit ratio of extensive formulation work. Also if the shape of the plasma concentration profile is considered suboptimal, then design of modified release formulations is aided by simulation.

This paper illustrates all of these applications with examples taken from our work. The simulations were performed with GastroPlus.

Materials and Methods

Strategy. During drug research and preclinical development a wide range of data is generated to characterize compounds including properties computed based on chemical structure, in vitro measured data and measurements in vivo in animals. Taken individually, each piece of data is of limited value since its importance for the overall therapeutic profile is dependent upon other factors. Our strategy is to use physiologically based models to integrate this data and put the individual pieces into context. If the models were complete, then a single human model incorporating all relevant data from well-validated assays would be sufficient. However, models are an approximation to the more complex reality and are based upon numerous assumptions and on inputs subject to uncertainty. Therefore, comparison of simulation to in vivo results in animals is needed to verify the assumptions and allow model refinement leading to more accurate simulations as more data is integrated. This strategy, applied for prediction of human pharmacokinetics (Figure 2), has been shown to deliver more reliable prediction than more empirical approaches[25] and underlies the overall philosophy followed in this paper.

Input Parameters. The reliability of simulation depends on the input data, and this is continually developing in both quantity and quality as a compound moves forward. The assays we use in our company are described below.

(a) Permeability. GastroPlus requires an estimate of human jejunal permeability as a key input. At the lead optimization stage compound permeability is measured in the parallel artificial membrane permeability assay (PAMPA)[27,28] and for more advanced compounds in Caco-2 cells.[29] For use in simulations we transform the in vitro permeability to an estimate of human jejunal permeability based on a correlation of 20 reference drugs where human permeability has been published[29] (Figure 3).

(23) Johnson, K. C.; Swindell, A. C. Guidance in the Setting of Drug Particle Size Specifications to Minimize Variability in Absorption. *Pharm. Res.* **1996**, *13* (12), 1795–1798.

(24) Sun, D.; et al. In vitro testing of drug absorption for drug 'developability' assessment: Forming an interface between in vitro preclinical data and clinical outcome. *Curr. Opin. Drug Discovery Dev.* **2004**, *7* (1), 75–85.

(25) Jones, H.; et al. A Novel Strategy for Physiologically Based Predictions of Human Pharmacokinetics. *Clin. Pharmacokinet.* **2006**, *45* (5), 511–542.

(26) Barrett, J. S. Model based drug development applied to oncology. *Expert Opin. Drug Discovery* **2007**, *2* (2), 185–209.

(27) Kansy, M.; Avdeef, A.; Fischer, H. Advances in screening for membrane permeability: high-resolution PAMPA for medicinal chemists. *Drug Discovery Today: Technologies* **2004**, *1* (4), 349–355.

(28) Avdeef, A. *Absorption and Drug Development—Solubility Permeability and Charge State*; Wiley: New York, 2003.

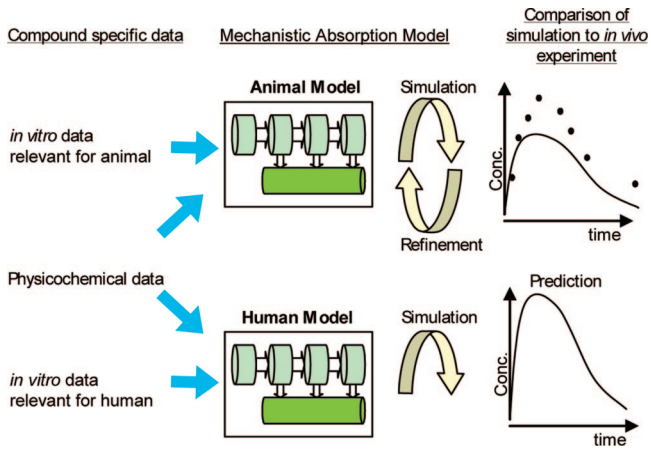

Figure 2. Illustration of the process whereby comparison of simulation to observation in animals can be used to explore hypotheses and learn about compound properties before making a prediction of behavior in human.

(b) Solubility. During lead optimization aqueous solubility is measured in a lyophilization solubility assay (LYSA),[30] and then for more advanced compounds a thermodynamic solubility assay (THESA)[30] is employed. For particularly interesting compounds a partially automated solubility screening (PASS)[31] allows determination of solubility in biorelevant media (e.g., fasted state simulating intestinal fluid (FaSSIF) at pH 6.5 and fed state simulating intestinal fluid (FeSSIF) at pH 5[32]). Using the PASS assay we often have human biorelevant solubility data for several potential candidate compounds prior to entry into human enabling studies.

(c) Lipophilicity and Ionization. Octanol/water partition coefficients at a defined pH (in general 7.4) are measured in a high throughput assay derived from the conventional "shake flask" method. Ionization constants describing the extent of ionization with respect to pH are measured in high throughput based on spectrophotometry. Both lipophilicity and ionization are key inputs for absorption modeling as they are used to determine how solubility and permeability change with pH changes in the gastrointestinal tract.

(d) In Vitro Metabolism Data. At the lead optimization stage intrinsic metabolic clearance is measured in rat/mouse and human liver microsomes and plasma protein binding and blood/plasma ratio are also measured in rat/mouse and human. Together with physicochemical data this is sufficient for an early simulation with a physiologically based disposition model.[22] For more interesting compounds additional data will be generated in rat/mouse and human microsomes and hepatocytes at a range of concentrations. Furthermore, when additional species are used for in vivo studies the associated in vitro data for those species will be generated. More detailed information on assays and scaling from in vitro to in vivo are provided in refs 22 and 25.

(e) In Vivo Pharmacokinetic Data. Typically the first in vivo data will be obtained in the species used in pharmacological screening assays, most often rat and/or mouse. Pharmacokinetics is measured in these species after intravenous and oral dosing with the oral dose usually given as a microsuspension. For more interesting compounds in vivo pharmacokinetics may be determined in a second and third species, most often dog and/or monkey to support pharmacological, toxicological or formulation studies. More details on the experimental protocols are provided in refs 22 and 25.

Example Compounds. The key input data for the three diverse compounds used in the examples is given in Table 1.

Theoretical Basis of the Models in GastroPlus. The theoretical basis and mathematical description of the CAT model is provided in the papers of Yu,[33–35] and further details of extensions made in the ACAT model are given in a paper from Simulations Plus[17] and in a book chapter.[36]

For simulation of plasma profiles after oral dosing the absorption versus time profile predicted with the ACAT model must be used as input to a disposition model. In GastroPlus this is possible in either of 2 ways. If plasma concentrations measured after an intravenous dose are available, then a compartmental model may be fit to these data and used to simulate disposition. This process is facilitated by a module which fits 1, 2 or 3 compartment open models, recommends the best fit and performs automatic linking of the chosen model to the ACAT model. The first pass effect due to metabolism can be estimated from in vitro data generated in microsomes or hepatocytes. If the observed total clearance is in agreement with the expected clearance based on scaling of in vitro metabolism data, then, assuming that there is no significant excretion of unchanged compound in bile, the first pass extraction is estimated as (100% × total blood clearance/liver blood flow). However, if the in vitro to in vivo scaling of clearance indicates significant extrahepatic clearance, then different assumptions

(29) Alsenz, J.; Haenel, E. Development of a 7-Day, 96-Well Caco-2 Permeability Assay with High-Throughput Direct UV Compound Analysis. *Pharm. Res.* **2003**, *20* (12), 1961–1969.
(30) Alsenz, J.; Kansy, M. High throughput solubility measurement in drug discovery and development. *Adv. Drug Delivery Rev.* **2007**, *59* (7), 546–567.
(31) Alsenz, J.; Meister, E.; Haenel, E. Development of a Partially Automated Solubility Screening (PASS) Assay for Early Drug Development. *J. Pharm. Sci.* **2007**, *96* (7), 1748–1762.
(32) Dressman, J. B.; et al. Estimating drug solubility in the gastrointestinal tract. *Adv. Drug Delivery Rev.* **2007**, *59* (7), 591–602.
(33) Yu, L. X.; Crison, J. R.; Amidon, G. L. Compartmental transit and dispersion model analysis of small intestinal transit flow in humans. *Int. J. Pharm.* **1996**, *140*, 111–118.
(34) Yu, L. X.; Amidon, G. L. A compartmental absorption and transit model for estimating oral drug absorption. *Int. J. Pharm.* **1999**, *186*, 119–125.
(35) Yu, L. X. An integrated model for determining causes of poor oral drug absorption. *Pharm. Res.* **1999**, *16* (12), 1883–1887.
(36) Bolger, M. B. Simulation of absorption, metabolism, and bioavailability. In *Drug bioavailability: estimation of solubility, permeability, absorption and bioavailability*; Methods and Principles in Medicinal Chemistry; Waterbeemd, H. v. d., Lennernäs, H., Artursson, P., Eds.; Wiley-VCH: Weinheim, 2003.

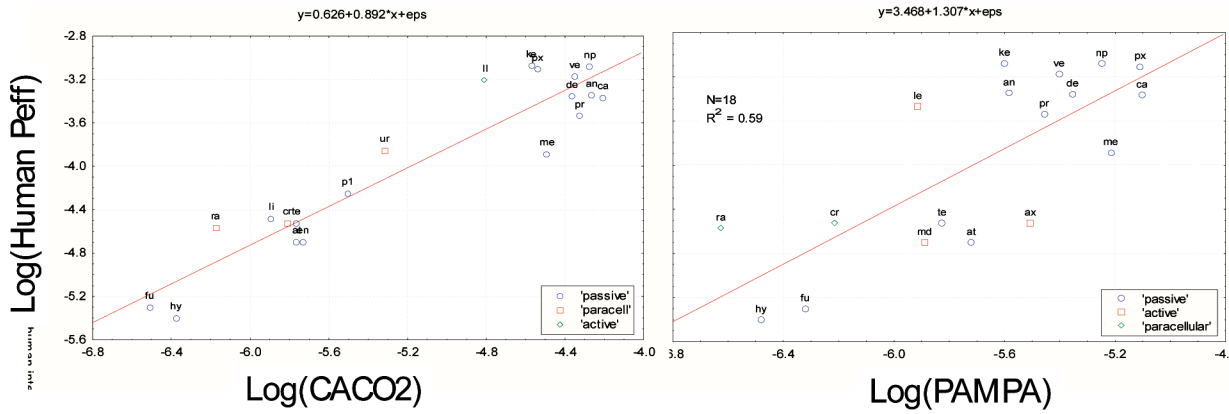

Figure 3. Log−log correlations based upon 20 reference drugs are used to transform in vitro permeability values to human jejunal permeability for use in GastroPlus. For PAMPA: Log(Hpeff) = 3.5 + 1.3 × Log(PAMPA). For Caco2: Log(Hpeff) = 0.63 + 0.89 × Log(CACO).

Table 1. Physicochemical and Pharmacokinetic Properties of Compounds **A**, **B** and **C**

compound	A	B	C
molecular weight	543	326	566
LogD pH 7.4	3.0	4.0	3.5 pH 1.1
charge	neutral	base 4.7	base 4.1
human Peff (converted from PAMPA) (×10E−4 cm/s)	1.2	1.9	1.7
human Peff (converted from Caco2) (×10E−4 cm/s)	3.5	7.5	5 (optimized)
phosphate buffer solubility pH 7 [mg/mL]	0.005	<0.001	0.013 pH 1
SGF pH 1.4 [mg/mL]	0.025	1.67	
FaSSIF pH 6.5 [mg/mL]	0.017	0.012	0.006
FeSSIF pH 5 [mg/mL]	0.063	0.135	0.038 pH 5.5
Vss rat [L/kg]	4	4	9
clearance in rat [mL/min/kg]	5	6	7
clearance in dog [mL/min/kg]	0.5		39
clearance in monkey [mL/min/kg]	4	7	18
oral clearance in human [mL/min/kg]	1.5	4.5	9 (after iv)

need to be explored. An alternative to a compartmental disposition model is a full physiologically based whole body model. This is available in GastroPlus via the physiologically based pharmacokinetic (PBPK) module which includes options for prediction of tissue distribution and metabolism scaling. For use of this model, essential inputs are lipophilicity, ionization constant, plasma protein binding and estimates of hepatic clearance (e.g., by scaling of in vitro data from microsomes or hepatocytes) and extrahepatic clearance (e.g., renal filtration).

For absorption simulations the essential inputs are lipophilicity, ionization constant, solubility and permeability. For permeability differences across species GastroPlus includes by default a simple empirical scaling between species so that dog has a 3-fold higher permeability than human while rat and monkey have similar permeability to human. However, these defaults might be questioned particularly for transported molecules,[37] and they may be overridden if measured permeability in the relevant species is available. Solubility is not changed between species except to account for differences in pH. The variation of solubility with different intestinal regions is assumed to depend only on the pH of that region and is calculated from the pK_a values of the drug according to the Henderson−Hasselbalch equation. Dissolution is calculated according to a Noyes−Whitney model.

GastroPlus also includes calculations of the maximal absorbable dose. The original Johnson MAD equation is reproduced as MAD(lit) but is modified for acids and bases by assuming that the lowest solubility in the gastrointestinal tract applies for the entire small intestine. In addition to this equation, which might be expected to lead to large underestimates for acids and bases exhibiting pH dependent solubility in the physiological range, Simulations Plus have added their own MAD equation MAD(S+). The (S+) method employs knowledge of the regional solubility of the drug within the intestinal tract. The equations for these computations are given below.

$$\mathrm{MAD(lit)} = \mathrm{lowest(Sol(pH)} \times K_{a,i}) \times \mathrm{SIVol} \times \mathrm{SITT}$$

where K_a is the absorption rate constant, SIVol is the volume of the small intestine and SITT is the total small intestinal transit time.

(37) Fagerholm, U.; Johansson, M.; Lennernas, H. Comparison between permeability coefficients in rat and human jejunum. *Pharm. Res.* **1996**, (9), 1336–1342.

$$\mathrm{MAD(S+)} = \mathrm{SUM}(Sol(\mathrm{pH}_i) \times K_{a,i} \times \mathrm{SIVol}_i \times \mathrm{TTRemaining}_i)$$

where the summation is made over all compartments in the ACAT model including the colon. $K_{a,i}$ and SIVol_i are the absorption rate constant and volume of the ith compartment and TTRemaining is the sum of transit times of all the compartments remaining to be transited before the drug is excreted.

Results

Simulation of Oral Absorption in Rat from In Vitro Screening Data. Comparison of simulated concentration versus time profiles to observed data obtained after oral dosing of a microsuspension to rats is used to gain a first impression of the factors determining drug absorption and is often performed at the lead optimization stage. Such simulations use measured data on solubility (aqueous buffer or FaSSIF data), ionization constant, lipophilicity and permeability (PAMPA or Caco2). It is assumed that permeability of the gastrointestinal wall is determined solely by passive diffusion and that dissolution rate limitations may be calculated using the Noyes−Whitney model and an assumed particle size of 10 μm. Disposition is modeled based on a compartmental model fit to data obtained after an intravenous dose. For the three diverse BCS Class 2 compounds **A**, **B** and **C** (Table 1) the clearance is low and first pass effect was assumed to be negligible. For all three compounds there is uncertainty in the estimation of permeability and solubility, but as seen in Figure 4 the simulations are most sensitive to the solubility with aqueous solubility leading to underprediction while the FeSSIF value overpredicts for compounds **A** and **B**. A parameter sensitivity analysis for compound **A** is shown in Figure 5. This is a convenient way to assess the sensitivity of the simulation to the uncertainties in key inputs. It can be seen that over a range of solubility covering the uncertainty (i.e., from the aqueous value of 5 μg/mL to the FeSSIF value of 63 μg/mL) the simulated AUC changes by 10-fold while for generous ranges covering the uncertainty in permeability (4-fold) and particle size (10-fold) the change in AUC is less than 3-fold.

Prediction of Exposures in Ascending Dose Studies in Animals and Human. Maximum absorbable dose calculations and sensitivity analyses for changes in exposure with dose were performed for compound **A** in rat, monkey and human. For all species the Caco2 permeability value was used as input and the default GastroPlus conversions of permeability were applied. For disposition, model fits to data obtained after intravenous dosing were used for rat and monkey while human disposition pharmacokinetics was predicted with PBPK modeling. This compound has a very low clearance in all species (Table 1), and so the first pass metabolism was assumed to be negligible. Formulations used were microsuspensions in rat (particles size range 1−10 μm.), an aqueous emulsion in monkey and hard gelatin capsules containing milled API in human. Rats were allowed access to food ad libitum, monkeys were in the fed state (1 banana) and humans were fasted. The calculated MAD values are

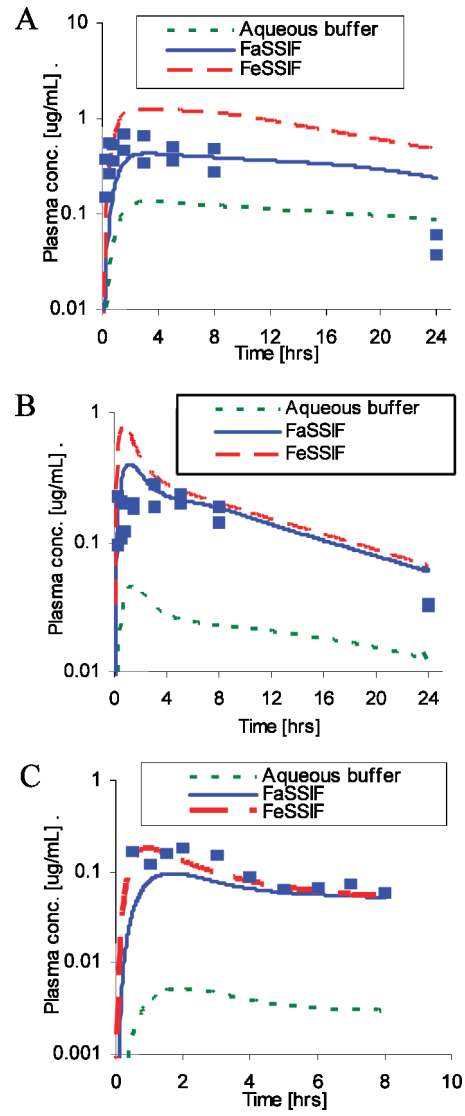

Figure 4. Simulation (line) of oral profiles in the rat using solubility measured in aqueous buffer, FaSSIF and FeSSIF. Doses were microsuspensions at (A) 10 mg/kg, (B) 2.3 mg/kg, (C) 1.3 mg/kg.

shown in Table 2 while parameter sensitivity plots (Figure 6) show the predicted and observed changes in AUC and Cmax with dose.

The parameter sensitivity analysis indicated that the dose dependent increase in exposure was best simulated using FaSSIF solubility in rat, monkey and human. The advantage of parameter sensitivity plots over the single estimated MAD is illustrated in Figure 7 which shows the simulation in human of increases in AUC and fraction absorbed over a wider range where saturation of absorption occurs. Also shown as a solid vertical line is the MAD estimated with the S+ method and corresponding to the dose at which roughly 80% is absorbed.

Simulation of Species Differences in Absorption. Refinement of a Human Absorption Model. In a preliminary evaluation of the ACAT model for rat and dog[38] fraction absorbed data were collected from the literature[39,40] for a set of 13 marketed drugs. Aqueous solubility and lipophilicity

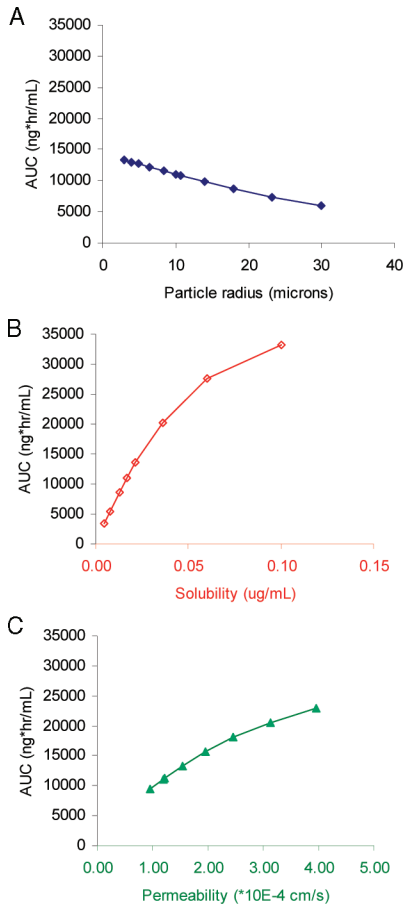

Figure 5. A parameter sensitivity analysis showing simulated AUC for compound **A** and covering ranges reflecting the uncertainty in particle radius, solubility and permeability.

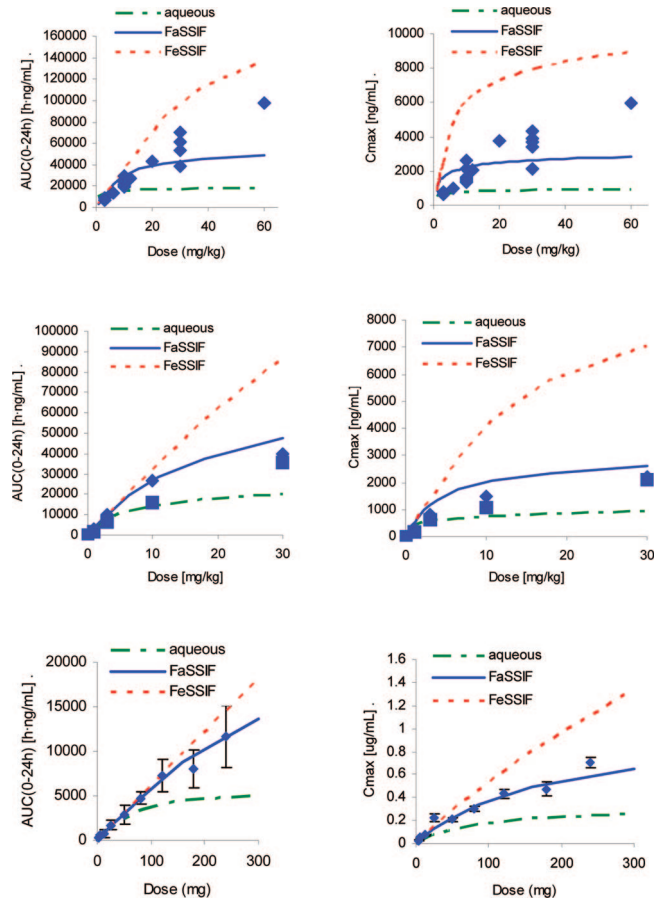

Figure 6. Parameter sensitivity plots showing the predicted and observed changes in AUC and Cmax with dose for compound **A** in rat (upper), monkey (middle) and human (lower).

Table 2. Calculated Maximum Absorbable Doses for Compound **A** in Three Species Based upon Caco2 Permeability and Different Measured Solubility Values[a]

	rat		monkey		human	
	MAD(lit) (mg/kg)	MAD(S+) (mg/kg)	MAD(lit) (mg/kg)	MAD(S+) (mg/kg)	MAD(lit) (mg/kg)	MAD(S+) (mg/kg)
aqueous	2.8	5.6	0.9	3.3	0.4	1.9
FaSSIF	9.6	18.8	3.0	11.3	1.2	6.6
FeSSIF	35.6	69.2	11.5	42.0	4.6	24.3

[a] MAD(lit) is based upon the method of Johnson[23] while MAD(S+) is a modification accounting for regional variations in intestinal solubility.

were measured in house while permeability comprised a mixture of published human data and scaled in house data. All compounds had good aqueous solubility (>0.5 mg/mL). In human 9 of the 13 drugs were correctly categorized as low (<33%), medium or high (>66%) absorption, and the mean error of the prediction was 26%. In dog an overall trend toward higher absorption was correctly predicted, and 10 of 11 drugs were correctly categorized, with a mean error of 13%. In rat 6 of 11 drugs were correctly categorized and the mean error was 22%. This verification offers encouragement that it should be possible to use the species specific models in GastroPlus to simulate absorption differences for compounds without solubility limitations. However, during early discovery work we are often faced with poorly soluble compounds which are initially dosed as simple microsuspension formulations. In such cases it can be difficult to separate limited absorption caused by slow dissolution and suboptimal formulation from more fundamental limits due to solubility or permeability. For compound **A**, an early simulation (Figure 4) based upon permeability scaled from PAMPA delivered a reasonable match to observation in the rat with a simulated fraction absorbed of 38% compared to an observed value estimated at 23%. However, simulations with the PAMPA permeability in monkey and dog tended to underestimate the initial absorption phase, which was better matched with a 3-fold higher permeability scaled from

(38) Lave, T. Interspecies scaling In *Applications of Pharmacokinetic Principles in Drug Development*; Krishna, R., Ed.; Springer: New York, 2003.

(39) Chiou, W. L.; Barve, A. Linear correlation of the fraction of oral dose absorbed of 64 drugs between humans and rats. *Pharm. Res.* **1998**, *15* (11), 1792–1795.

(40) Chiou, W. L.; et al. Evaluation of using dog as an animal model to study the fraction of oral dose absorbed of 43 drugs in humans. *Pharm. Res.* **2000**, *17* (2), 135–140.

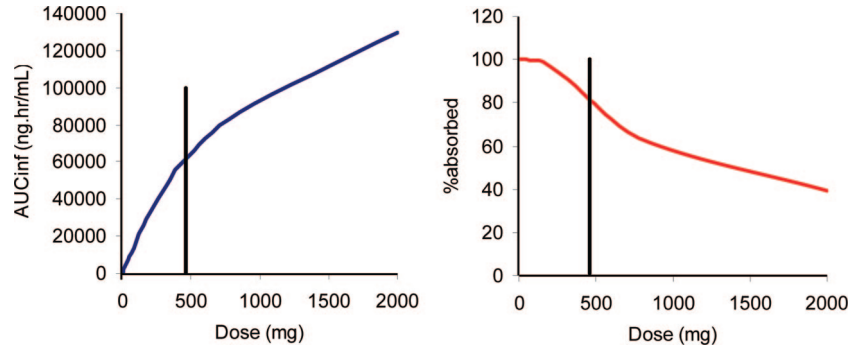

Figure 7. Simulation of changes in AUC and fraction absorbed for compound **A** for a wide range of doses. The vertical line indicates the calculated MAD of 460 mg.

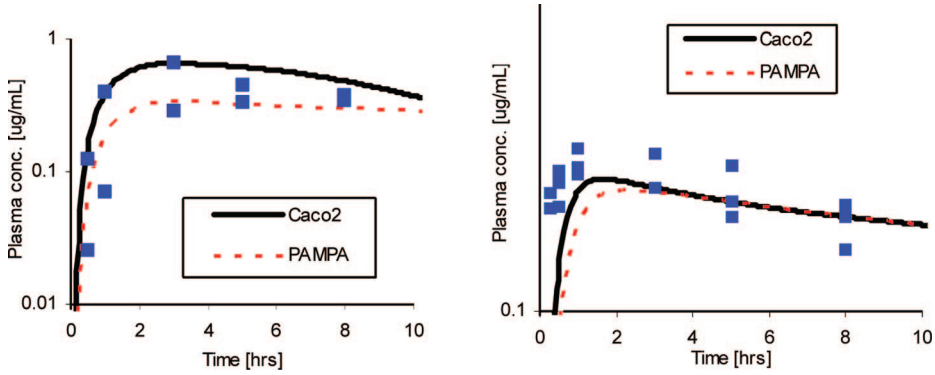

Figure 8. Simulations of oral profiles for compound **A** in monkey (left) and dog (right). The symbols are observed data obtained with microsuspensions at 3 mg/kg in monkey and 1 mg/kg in dog. In both simulations the FaSSIF solubility is used.

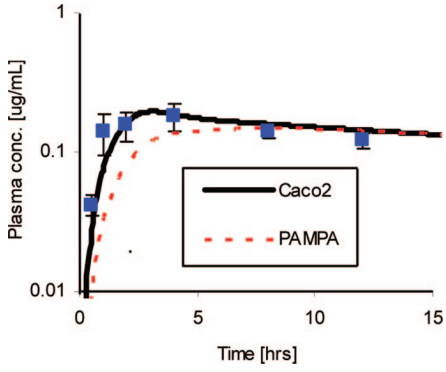

Figure 9. Simulations of oral profiles in human for compound **A**. The symbols are observed mean ± SE data in healthy volunteers ($N = 6$).

data obtained in Caco2 cells (Figure 8). The initial good match in the rat based upon PAMPA was later explained as a dissolution limitation when an experiment with an emulsion formulation showed significantly higher exposures which were matched by simulations with the Caco2 permeability.

Subsequently, when the first human data were obtained it was discovered that simulation based upon PAMPA permeability led to a clear underestimation of the absorption rate which was better matched using the Caco2 value (Figure 9).

Prediction of Oral Pharmacokinetics in Humans. For prediction of human oral pharmacokinetics we have developed a strategy based on PBPK modeling and verified this retrospectively using data from Roche databases.[25] In recent years we have applied this strategy for prospective predictions of human pharmacokinetics and have obtained clinical data for 10 compounds. Figure 10 shows the correlations of predicted AUC and Cmax for these compounds in single ascending dose studies. 80% of AUC predictions and 60% of Cmax predictions are within 2-fold of the observed mean values.

These predicted exposures include all aspects of the pharmacokinetics, not just absorption but distribution and elimination as well.

Once clinical data become available the absorption model used for predictive simulation may be refined and further used for mechanism based modeling to address clinical questions. For example with compound **B**, the prospective PBPK simulation for this compound was made using the biorelevant solubility values and a human permeability estimated by scaling from a PAMPA value (Caco2 data was not available at the time the prediction was made). The prospective prediction and observed data are shown in Figure 11.

As seen from the figure the prediction was reasonably close to observed data with Cmax well predicted. However Tmax was too late (3 h vs 1 h), AUC was overpredicted by 2-fold and the peak−trough ratio in the first 24 h was underpredicted. Subsequently the match of simulation to observed data could be improved by just 2 changes to the model. The Caco2 permeability value scaled to a human permeability

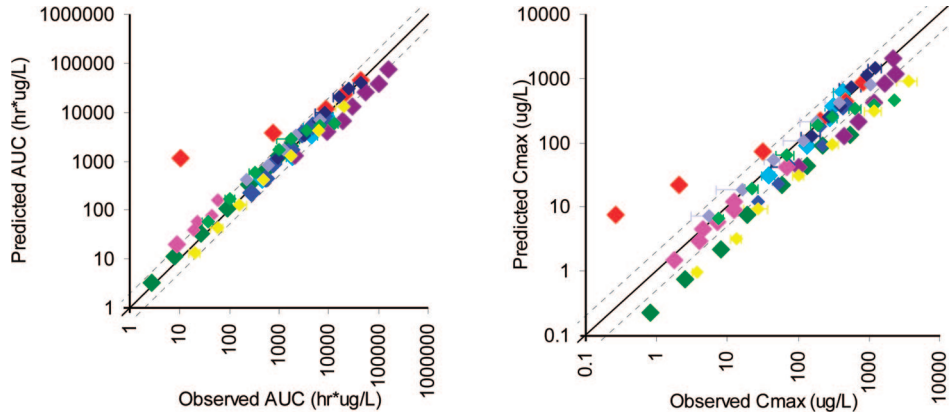

Figure 10. Predicted and observed AUC and Cmax in human for 10 diverse compounds. Each compound is represented by a single color and appears multiple times to represent different dose levels.

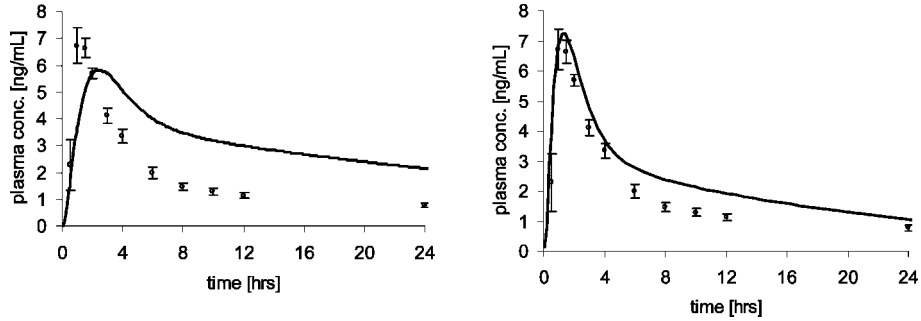

Figure 11. Predicted plasma concentrations (solid line) compared to observed data (symbols ± SD) for a human dose of 1 mg. The prospective simulation (left), a refined simulation (right) after increasing permeability and clearance.

about 3-fold higher than the PAMPA scaled value, and this improved the match of simulated and observed Tmax. In addition, if the clearance was increased by a factor of 2, the simulations delivered a very good match to observed data at all dose levels, and this refined model was used for further mechanistic modeling. Data generated in vitro with recombinant enzymes indicated that both CYP3A4 and CYP1A1 were responsible for compound turnover, and as both are known to be present extrahepatically, this may lend support to an underprediction of clearance based on the hepatocyte data.

Mechanistic Explorations of the Effect of Intestinal Metabolism and Transporters. GastroPlus was used to investigate nonlinear liver and gut metabolism in human for Compound **C**, a highly lipophilic weak base with very low solubility in water. The clinical formulation development for the compound was very challenging, and modeling was performed as an attempt to help understanding of these effects and to aid in the development of improved formulations.

First, a GastroPlus absorption model based on preclinical data was taken and linked to a model of the disposition in human. The disposition model was constructed by fitting a classical compartmental model to observed plasma levels obtained after intravenous dosing to 10 subjects. A 3 compartment model was found to provide the best fit. The total plasma clearance obtained from the fit was 0.34 L/h/kg, which was in reasonable agreement with the hepatic metabolic clearance obtained by scaling of in vitro clearance measured in human hepatocytes. Since the liver blood flow

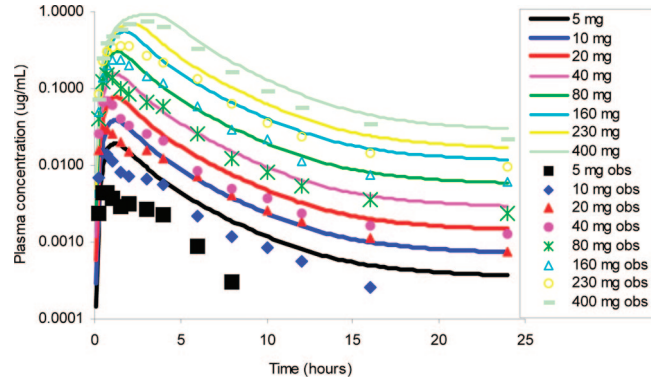

Figure 12. Initial simulations (lines) and observed data (symbols) for single doses of compound **C** at 5 to 400 mg in healthy volunteers.

in human is 1.2 L/h/kg and the blood/plasma ratio of RO0675930 is 0.65, the first pass effect is given by ((0.34/0.65)/1.2) = 44%. The next step was to use the initial model to simulate data obtained when a drinking emulsion was administered to 8 groups of 10 fed subjects at doses of 5, 10, 20, 40, 80, 160, 230 and 400 mg. Noncompartmental analysis of this data showed that bioavailability was increasing with dose from 10% at 5 mg up to 36% at 400 mg. Initial simulations showed that Tmax was well matched at all doses but plasma levels were overpredicted with the overestimation of Cmax being more pronounced for the lower doses (Figure 12).

As CYP450 3A4A was known to be the major enzyme responsible for turnover of compound **C**, the hypothesis of nonlinear 3A4 metabolism in liver and gut was investigated. In vitro clearance in microsomes showed a Vmax of 1.12 ± 0.07 nmol/min/mg protein and an apparent Km of 1.8 ± 0.5 μM. For use in GastroPlus this Vmax must be scaled to the in vivo situation and using a total liver weight of 1800 g a value of 52.5 mg of prot/g of liver and the compound molecular weight of 566 gave 1.00 mg/s as the in vivo Vmax value. For the Km parameter the value for unbound drug is required, and so an estimate of the degree of binding in the microsomal in vitro incubation was needed. Since this value was not available, the unbound Km was optimized to provide a best fit for the observed plasma concentration profile after intravenous dosing. The resulting optimized Km was 0.07 μg/mL. As the unbound Km is related to the apparent Km by Km unbound = Km apparent × fraction unbound in microsomes the optimized value would imply a fraction unbound in microsomes of 0.07/(0.0018 × 565) = 0.069. Based upon a QSAR equation for prediction of the binding in microsomes published by Austin,[41] the predicted fraction unbound in microsomes would be 0.029, roughly a factor of 2 different from the optimized value. Bearing in mind the possible error in the QSAR equation and the experimental error in the apparent Km this difference is not too big. A simulation of the highest oral dose of 400 mg showed that the highest concentration reached in the liver was 1.5 μg/mL. As the fraction unbound in plasma is 0.09%, this corresponds to an unbound concentration of 0.00135 μg/mL, indicating that the concentration in the liver, even at the highest doses in this study, are likely to be well below the Km and therefore the hepatic clearance should be linear in the dose range studied. For gut metabolism the same unbound Km value was used but the Vmax was scaled to the relative amounts of enzyme in the different regions of the intestine.[42] Again the relevant unbound fraction within the enterocyte is not known, and so the unbound fraction was optimized so that the simulated plasma levels were better matched to observation over the full dose range (Figure 13). After optimization, the simulated bioavailability increased with dose comparably to the observed values (Table 3). The optimized fraction unbound in the enterocyte was 0.05.

The inclusion of gut metabolism provided an improved simulation of the change in bioavailability with dose, but some features were still not well simulated, for example at the lower doses of 5, 10 and 20 mg a pronounced hump in the profiles beginning at around 3 h. Also, although the simulated Cmax for the lower doses matched well the observed data, Cmax at the doses 40, 80, 160 and 230 mg was slightly overpredicted. There was in vitro evidence that

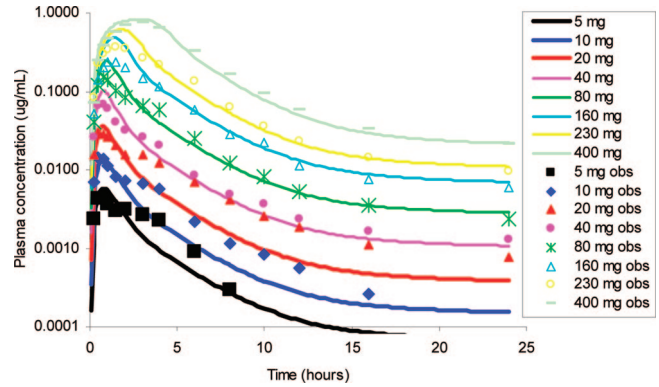

Figure 13. Simulations (lines) and observed data (symbols) for single doses of compound **C** at 5 to 400 mg in healthy volunteers using nonlinear metabolism.

Table 3. Observed Bioavailability and Values Simulated after Including Gut Metabolism

	F%	
dose (mg)	observed	simulated
5	10	16
10	15	17
20	21	19
40	18	24
80	22	30
160	22	36
230	28	39
400	36	45

compound **C** is a substrate for p-glycoprotein (P-gp) with low to intermediate affinity. However, quantitative kinetic data were not available, and even if this were the case, the regional distribution of P-gp in the gastrointestinal tract is uncertain, although it has been reported that the relative amount of P-gp in the gastrointestinal tract increases distally.[43–45] To investigate the possible influence of P-gp an assumption about the P-gp distribution was made and a scaling factor increasing linearly from 0.2 in the duodenum to 1.6 in the ileum was added. Then the Vmax, Km parameters for efflux were optimized to fit the observed data. The Vmax and Km parameters for the optimized model were 0.0042 mg/s and 1.2 μg/mL.

It is interesting to note that the model is now a good match to the observed data and that the second hump in the profiles beginning at around 3 h was captured by the simulation

(41) Austin, R. P.; et al. The Influence Of Nonspecific Microsomal Binding On Apparent Intrinsic Clearance And Its Prediction From Physicochemical Properties. *Drug Metab. Dispos.* **2002**, *30* (12), 1497–1503.

(42) Paine, M. F.; et al. Characterisation of interintestinal and intraintestinal variations in human CYP3A-Dependent metabolism. *J. Pharmacol. Exp. Ther.* **1997**, *283* (3), 1552–1562.

(43) Makhey, V. D.; Guo, A.; et al. Characterization of the regional intestinal kinetics of drug efflux in rat and human intestine and in caco2 cells. *Pharm. Res.* **1998**, *15* (8), 1160–1167.

(44) Englund, G. Regional levels of drug transporters along the human intestinal tract: Co-expression of ABC and SLC transporters and comparison with Caco-2 cells. *Eur. J. Pharm. Sci.* (Advances in Understanding Oral Absorption and Delivery of Problem Compounds—Selected Papers from the 3rd World Conference on Drug Absorption, Transport and Delivery) **2006**, *29* (3–4), 269–277.

(45) Mouly, S. P.; Paine, M. F. P-Glycoprotein Increases from Proximal to Distal Regions of Human Small Intestine. *Pharm. Res.* **2003**, *20* (10), 1595–1599.

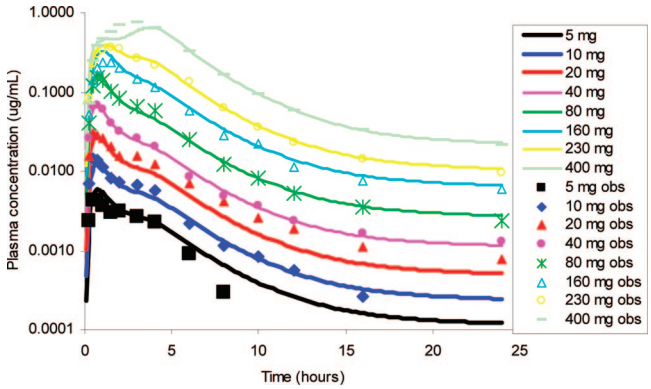

Figure 14. Simulations (lines) and observed data (symbols) for single doses of compound **C** at 5 to 400 mg in healthy volunteers using nonlinear metabolism and P-gp efflux.

(Figure 14). The explanation for the simulation of this hump can be found by looking at the simulated profiles and the regional absorption with and without P-gp efflux (Figure 15).

The efflux of drug by P-gp in the upper gastrointestinal tract is delaying absorption, so the Cmax is lower and the fraction of the dose absorbed in the duodenum, jejunum and upper ileum is lower. This results in more drug reaching the colon, which occurs at approximately 3.3 h since this is the small intestine transit time used in the model. It has been shown in the rat[46] that hydrophobic drugs (logD at pH 7.4 greater than 0) show a significant increase in permeability aborally. The default model for scaling regional permeability in GastroPlus accounts for this observation and adjusts permeability in each compartment according to the logD of the molecule being simulated. For a very lipophilic drug like compound **C** this model predicts that the colonic permeability is some 7 times higher than that in the ileum. Thus, when drug reaches the colon there can be an increase in absorption leading to the hump in the simulated profile.

Prediction of Food Effects. A prediction of food effect was made for the lipophilic neutral compound **A**. Figure 16 shows this prediction compared to observed data after an 80 mg dose was administered to 12 subjects as capsules with 200 mL of water either after an overnight fast or 30 min after a high fat, high calorie breakfast. The prediction is in agreement with the observed food effect which showed little change in AUC but an increase in Cmax of 1.4-fold with food.

The prediction of food effect is further illustrated with the example of compound **B** a lipophilic weak base showing low solubility and high permeability (Table 1) for which the refinement of the GastroPlus human model during phase 1 has already been described. Prior to the clinical food effect study the food effect was investigated in cynomolgus monkey at 0.3 mg/kg, where a marked 3-fold reduction in AUC was seen when the compound was dosed after a banana. However, simulations with GastroPlus in human predicted no change in AUC with food, a delayed Tmax and only a slight reduction in Cmax (to 80%). Due to the food effect in monkey as well as concerns over a narrow therapeutic window, a food effect study in human was performed early in phase 1, and the data obtained in 9 healthy volunteers after a standard meal were reasonably close to the predicted simulations (Figure 17). This supported the hypothesis that the food effect in human at this dose is largely due to delayed gastric emptying with solubility and pH changes less important.

Development of Modified Release Formulations. If phase 1 studies show that human pharmacokinetics are suboptimal, then it may be possible to change the oral profile via modification of drug release rate. For compound **B** it was noticed that occurrence of unwanted side effects was correlated with maximal plasma concentrations achieved 1 or 2 h after dosing. It was hypothesized that slowing drug release from the formulation could help by reducing Cmax and the initial absorption rate. However, 24 h trough levels had to be maintained in order to sustain therapeutic effects with once daily dosing. The success of a modified release formulation requires that absorption from the distal gastrointestinal tract is adequate to maintain the extent of absorption even while the initial rate is slowed. To assist with this question the refined human absorption model developed based upon phase 1 single ascending dose data was applied. It was known that formulations exhibiting zero order release in vitro at different rates were achievable via an eroding matrix technology. In addition, an enteric coating made it possible to restrict release in the stomach. Simulation was then used to explore different intestinal release rates and two variants were chosen which released the complete dose over 5 and 12 h respectively (Figure 18).

These simulations indicated that significantly slowed release might be achieved without a drastic reduction in total exposure since, even for the 12 h release profile, the model predicted that AUC up to 24 h was only reduced about 10% compared to the immediate release formulation. However, such simulations did indicate that a significant fraction of drug was being absorbed in the large intestine (30% for the 5 h release and >60% for 12 h release). To give more support to this assumption and to test the in vivo release characteristics, it was decided to perform a study with the 2 modified release formulations in the monkey. Data from the monkey study (Figure 19) showed that, in spite of the shorter intestinal transit time in monkey, the 5 h and even the 12 h modified release formulations were achieving plasma levels in reasonable agreement with the model simulations. Therefore, based upon simulations and the monkey data it was decided to proceed with the 2 modified release formulations in the clinic. Clinical data are not currently available.

Predicting In Vivo Dissolution. Modeling of the effect on in vivo exposures of different particle size distributions for compound **A** was used to aid decision on the degree of milling needed to be confident that slight differences would have no impact on the AUC. In a first model verification

(46) Ungell, A.-L.; Bergstrand, S.N., S.; Sjöberg, Å.; Lennernäs, H. Membrane transport of drugs in different regions of the intestinal tract of the rat. *J. Pharm. Sci.* **1998**, *87* (3), 360–366.

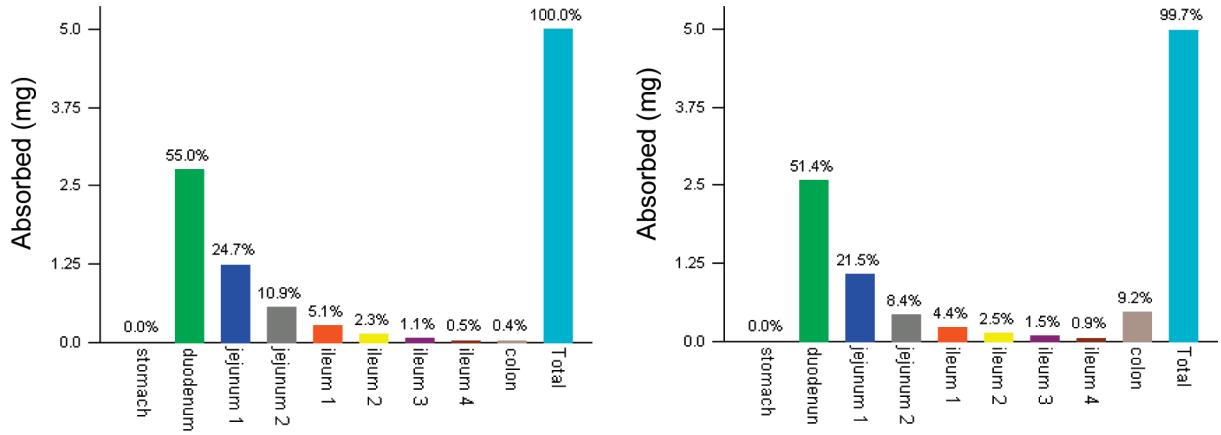

Figure 15. Regional absorption plots for model simulations without P-gp efflux (left) and with P-gp efflux (right).

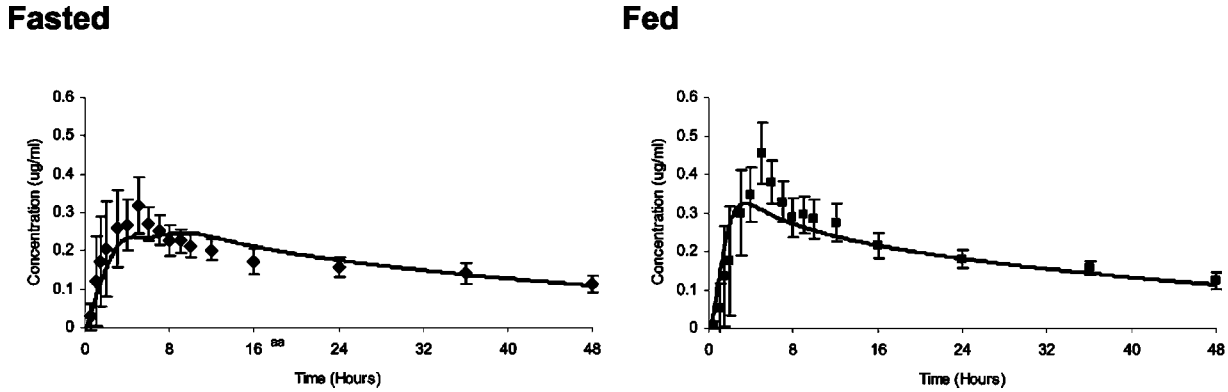

Figure 16. Simulated (line) and observed (symbol ± SE) in human for compound **A** in fasted (left) and fed (right) states

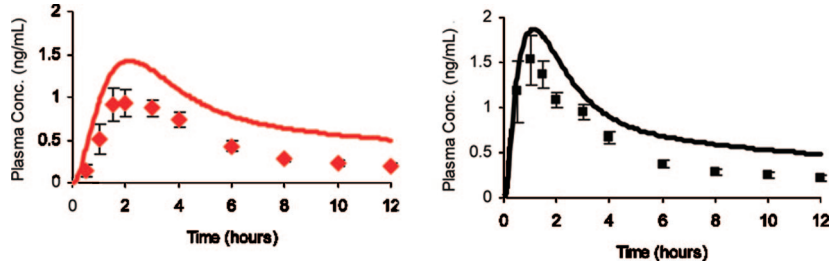

Figure 17. Simulated (line) and observed (symbols) plasma concentrations in human for 0.25 mg dose in the fed (left) and fasted (right) states.

stage, tablets containing milled and unmilled material were characterized in terms of their particle size distributions and this data was used as input to a GastroPlus monkey model. Then simulations were compared to data obtained after dosing of these tablets to three cynomolgus monkeys (Figure 20).

The simulations captured well the effect of changing particle size in the monkey, and so the effect of changes in particle size on AUC and Cmax was explored with a sensitivity analysis using the human GastroPlus model. Both changes in the mean particle size and the standard deviation of particle sizes were explored (Figure 21).

This analysis showed that the predicted effect of particle size on Cmax was more pronounced than that on AUC with a 20% reduction in Cmax expected for particle diameters above 24 μm while a 20% reduction in AUC occurred for particle diameters above 44 μm.

Discussion

The 3 compounds described here exhibit low solubility and high permeability and are typical of molecules currently studied in preclinical and early clinical development. According to recent analyses[47] such molecules make up a large percentage of the novel molecular entities currently being worked on in the pharmaceutical industry. The examples presented range from early drug research through clinical

(47) Leeson, P. D.; Springthorpe, B. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat. Rev. Drug Discovery* **2007**, *6* (11), 881–890.

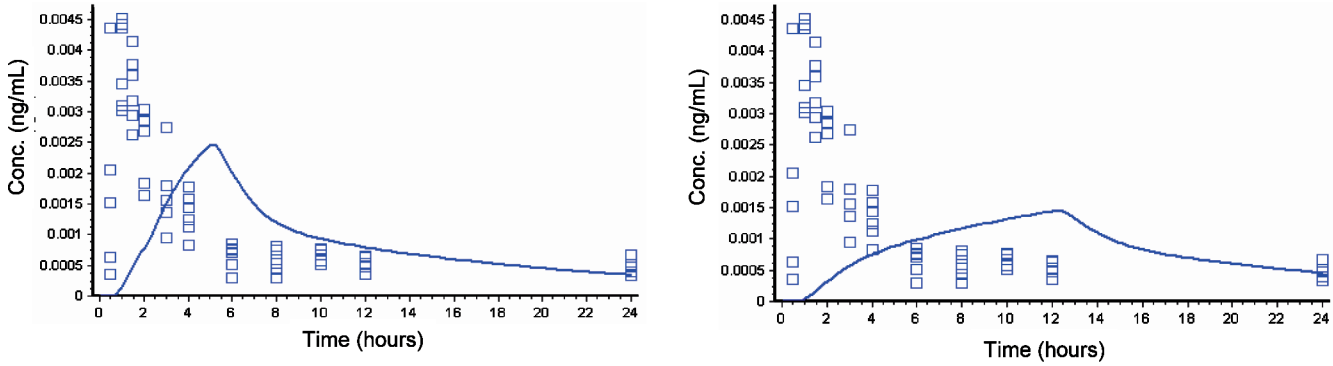

Figure 18. Simulated profile (solid line) in human for a 5 h (left) and 12 h (right) zero order slow release. Symbols show observed plasma concentrations for the immediate release formulation at the same dose of 0.5 mg.

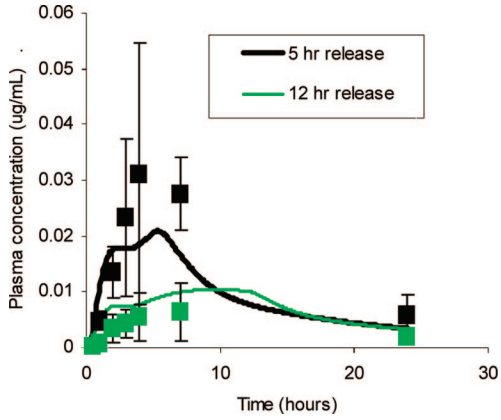

Figure 19. Observed plasma levels (symbols ± SD) and simulations (lines) for 2 modified release formulations in the monkey.

phase 2 and illustrate how an early model can develop and become more precise by incorporating new data and knowledge gained during the process. Early simulations make many assumptions and are based on screening data associated with a significant degree of uncertainty. For example, PAMPA as a measure of passive permeability is generated in higher throughput and can be a useful starting point for early absorption simulations. However, the standard error in the correlation of PAMPA to human Peff is about 0.3 log unit and this error range needs to be taken into account. The uncertainty associated with early solubility measurements can have an even more significant impact on simulations for BCS Class 2 compounds as was shown in Figures 2 and 3. Given these and other uncertainties such as in first pass effect and the impact of formulation on dissolution, it is clear that early simulations should be used rather to explore possibilities than to make definite predictions.[48] However, the benefit of simulations at this stage should not be underestimated. A parameter sensitivity analysis can incorporate the known experimental uncertainties and allow us to assess the likely causes of mismatch between simulation and observation. A significant mismatch may be indicative that processes not considered in the model are important in vivo, and simulations exploring different hypotheses may suggest useful experiments to fill in knowledge gaps. By incorporating new data and integrating in vitro simulations with in vivo results in animals the models can be refined and eventually lead to more reliable prediction. For compound **A**, the early simulation of the oral profile in rat was very uncertain and a reasonable match to observed data could be obtained via a number of different hypotheses such as uncertainty in solubility, permeability or dissolution. However, simulations in dog and monkey and later simulations with different formulations in rat could all be used to gain better understanding of the key factors determining in vivo behavior. This was then applied in the human model and led to a good prediction of absorption in human. We believe that this process of comparison of simulations to in vivo data in preclinical species (Figure 2) is of key importance as a learning process which leads to more reliable prediction.

The benefit of this PBPK modeling approach for prediction of human pharmacokinetics has been demonstrated previously for 19 Roche compounds, [25] and these findings are confirmed by our recent experiences (Figure 10) where good predictions of AUC and Cmax for 10 entries into human candidates have been demonstrated. A further benefit of the early use of such modeling is that the model built with preclinical data can be used to help interpretation of the first clinical data, refined based on these data and then further applied for simulation and prediction. This is illustrated here for all three compounds where the refined absorption models were used to simulate food effect, to develop modified release formulations and to explore the importance of intestinal metabolism and transport.

The simulated exposures for compound **A** in rat and monkey were in reasonable agreement with observation and predicted the observed saturation at high dose when FaSSIF solubility and Caco2 permeability were used as inputs (Figure 6). However, the human doses were not really high enough to show a clear flattening of the exposure versus dose relationship due to solubility. Our attempts within our company to find further examples of clinical single ascending dose studies exhibiting solubility limitation show that this

(48) Rostami-Hodjegan, A.; Tucker, G. 'In silico' simulations to assess the 'in vivo' consequences of 'in vitro' metabolic drug-drug interactions. *Drug Discovery Today: Technologies* **2004**, *1* (4), 441–448.

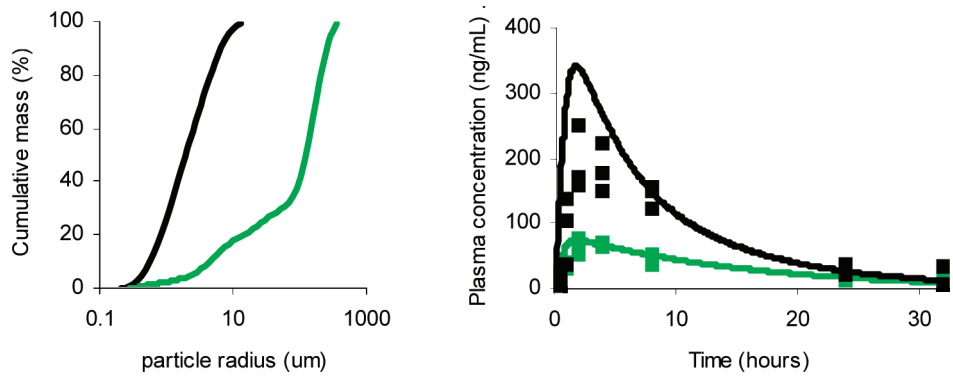

Figure 20. Left: Measured particle size distribution of milled (black) and unmilled (light green) compound **A**. Right: Simulated (lines) and observed (symbols) plasma levels after dosing monkeys with tablets containing milled (black) and unmilled (light green) material.

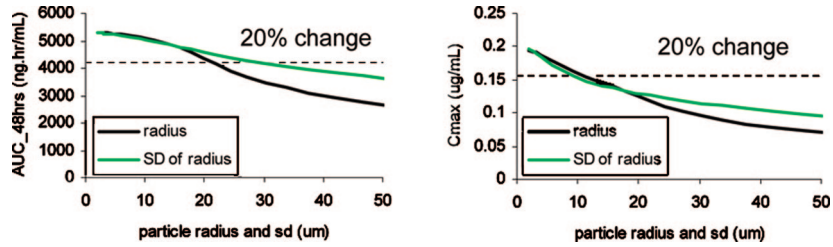

Figure 21. Simulations showing the effect of increases in particle size on the AUC and Cmax for a 50 mg dose of compound **A** in humans.

is very often the case, and others have found the same.[49] Animal toxicological studies often extend to higher doses, but again the validity of predictions based on solubility limitation is difficult to assess because it is likely that other nonlinearities in pharmacokinetics appear such as saturation of metabolism, nonlinear protein binding or changes in elimination. In these circumstances it becomes impossible to assess from the oral exposure data which part of the effect is due to solubility limitation. When used to assist decision making for estimation of maximum human dose, the parameter sensitivity analysis offers advantages over the simple MAD calculation, not only because the plot of predicted exposures versus dose conveys more information than a single number (Figure 7) but also because preclinical discussions and decisions should usually be centered around exposure and exposure-based safety margins rather than dose.

Quantitative prediction of the effects of both intestinal metabolism and transporters on absorption of drugs still remains a challenge. Particularly for uptake and efflux transporters, there is limited knowledge of the expression patterns and protein abundances along the gastrointestinal tract and the in vitro to in vivo scaling techniques remain to be validated. Also the prediction of effects in human from animal data is complicated because of large species differences in first pass metabolism and transport.[50,51] The overall importance of these effects in drug absorption remains uncertain with some evidence that transporter−enzyme interplay leads to clinically significant effects (e.g., low bioavailability and drug−drug interactions)[52] while other studies have stated that the clinical importance of P-gp influence on bioavailabilty is often not substantial.[53] However, progress is being made and there are several recent examples where intestinal metabolism has been accurately simulated for CYP3A substrates. Agoram et al.[17] used GastroPlus to simulate midazolam oral profiles, and more recently Yang et al.[54] showed good predictions of fraction metabolized in the gut for 16 CYP3A substrates using a Qgut model accounting for interplay between permeability and metabolism. The simulations described here for compound **C** implied that intestinal metabolism plays a very significant role in limiting the bioavailability of this CYP3A4 substrate, particularly at lower doses where there is less saturation of the gut enzymes. However, at present these simulations cannot be used in a predictive manner and remain explor-

(49) Ding, X.; Rose, J. P. Absorbable Dose Prediction of Select Post-Phase I Trial Internal Compounds. AAPS Annual Meeting and Exposition, 2007.

(50) Cao, X.; et al. Why is it challenging to predict intestinal drug absorption and oral bioavailability in human using rat model. *Pharmacol. Res.* **2006**, *23* (8), 1675–1686.

(51) Nishimura, T.; et al. Asymmetric intestinal first-pass metabolism causes minimal oral bioavailability of midazolam in cynomolgus monkey. *Drug Metab. Dispos.* **2007**, *35* (8), 1275–1284.

(52) Wu, C.-Y.; Benet, L. Z. Predicting Drug Disposition via Application of BCS: Transport/Absorption/Elimination Interplay and Development of a Biopharmaceutics Drug Disposition Classification System. *Pharm. Res.* **2005**, *22* (1), 11–23.

(53) Kwon, H.; Lionberger, R. A.; Yu, L. X. Impact of P-Glycoprotein-Mediated Intestinal Efflux Kinetics on Oral Bioavailability of P-Glycoprotein Substrates. *Mol. Pharmaceutics* **2004**, (6), 455–465.

(54) Yang, J.; et al. Prediction of Intestinal First-Pass Drug Metabolism. *Curr. Drug Metab.* **2007**, *8*, 676–684.

atory. This is due to the uncertainty in the relevant value of the unbound fraction of drug in the enterocytes to be used in the model. The value found to best match the observed data was 0.05, which seems reasonable in view of the values estimated as free within the microsomal incubations which were 0.07 via optimization and 0.03 using a QSAR equation. However, GastroPlus modeling done for midazolam used a value of 100% free in the enterocyte, and the predictions for 16 CYP3A substrates with the Qgut model[54] were also best when assuming no binding, although this did produce some outliers.

Compound **C** is also a P-gp substrate, and the GastroPlus model was also used to explore the possible impact of this factor. These simulations were very speculative since only semiquantitative in vitro data indicating efflux were available and the regional distribution profile of P-gp was assumed. The increase of 8-fold which was assumed from the duodenum to ileum is supported by data on levels of mRNA expression reported by Englund[44] although other data obtained via Western blot analysis indicate an increase of less than 2-fold.[45] In addition the stepwise approach followed where first the intestinal metabolism was optimized and then efflux in a second step may not allow capturing the interplay of these two processes appropriately.[55] Other workers using GastroPlus modeled the influence of P-glycoprotein's involvement in the pharmacokinetics of talinolol.[8] They used in vitro Vmax and Km values as well as experimental data on the distribution in human intestinal tissues[56] for quantitative scaling of in vitro data but were unable to accurately simulate the in vivo profiles. Thus the prediction from in vitro data to the in vivo situation for intestinal metabolism and efflux is still challenging, but physiologically based model simulations can already be used for hypothesis exploration, and there is hope that continued efforts will result in better quantitative predictions.

A strategy for prediction of food effects based upon GastroPlus models describing physiology in fed and fasted states together with biorelevant solubility and degradation data has previously been illustrated for six compounds.[9] The assumption of this approach is that the effect of food on pharmacokinetics is due to physiological changes in gastric pH and volume, gastric transit time and liver blood flow combined with changes in intestinal solubility due to food. Our experiences to date indicate that these assumptions are very often adequate to explain food effects and that this strategy adds value over the use of animal models alone to predict food effect. The benefits of such predictions include early recognition of potential issues due to high food effect, assistance in planning of clinical food effect studies including study design and timing, simulating expected food effect as a function of dose and mechanistic understanding to assist formulation work to reduce food effect. The example for compound **B** illustrates some of these benefits since the modeling was able to explain well the observed clinical food effect. However, this example also illustrates some limitations in the current strategy. Simulations in the monkey were unable to match the observed food effect in this species, and so we could not be confident that the human prediction was reliable. The current modeling approach strategy covers some of the major causes of food effects but does not cover all mechanisms. Therefore it builds confidence in the human prediction if simulations in animals are in line with observed food effect. This is analogous to the strategy defined for human PK predictions[25] where comparison of simulated to observed in vivo data in animals is used to check the completeness of the model before making the final prediction. Currently the models capture many of the known differences in gastrointestinal physiology between the animals and human, but the strategy could be improved by a better understanding of species specific solubility and how this changes with food. Some work in the dog[57] has shown significant differences in the effect of food on intestinal solubility compared to human, and the strategy would benefit from development of simulated dog intestinal fluids and use of these for generation of input data for simulations.

Modified release formulations are often considered as a way to reduce dosing frequency, reduce fluctuations in drug concentrations or control the site of drug delivery in the gastrointestinal tract. In the example presented here GastroPlus modeling was used to relate the rate of drug release to the resulting plasma concentration profiles and aid the decision of which formulation to test in the clinic. In addition the combination of modeling and a specifically designed monkey study was able to build sufficient confidence to proceed into the clinic with the designed formulations. The example where measured particle size information was used to simulate observed changes in exposures after oral dosing illustrates how absorption modeling can be used to guide decision making during the development of a market formulation. Again, specially designed preclinical in vivo studies were used to verify the reliability of the model prior to the prediction to human. Successful integration of in vitro dissolution data into GastroPlus[58,59] and development of a level A in vitro to in vivo correlation for Glyburide[60] have been reported by other groups.

Finally, some developments leading to improved accuracy of simulations are considered. In general the reliability of

(55) Benet, L. Z.; Cummins, C. L. The drug efflux-metabolism alliance: biochemical aspects. *Adv. Drug Delivery Rev.* **2001**, *50*, S3–S11.
(56) Mouly, S.; Paine, M. F. P-glycoprotein increases from proximal to distal regions of human small intestine. *Pharm. Res.* **2003**, *20*, 1595–1599.
(57) Kalantzi, L.; et al. Canine Intestinal Contents vs Simulated Media for the Assessment of Solubility of Two Weak Bases in the Human Small Intestinal Contents. *Pharm. Res.* **2006**, *23* (6), 1373–1381.
(58) Kesisoglou, F. Assessment of effect of API properties on in vivo dissolution and bioavailability through absorption modeling. AAPS Annual Meeting and Exposition, 2007.
(59) Kesisoglou, F. Assessment of bioequivalence of formulations through absorption simulations—a case for biowaivers for BCS Class II weak acids. AAPS Annual Meeting and Exposition, 2007.
(60) Wei, H.; Lobenberg, R. Biorelevant dissolution media as a predictive tool for glyburide a class II drug. *Eur. J. Pharm. Sci.* **2006**, *29* (1), 45–52.

simulations is critically dependent on the quality of the input data and on a good understanding of the conversion from in vitro measurements to in vivo relevant values. We expect that integration of work to better understand species specific differences and better simulate the in vivo situation in permeability[61,62] and solubility[32,57] will allow better understanding and bring about better prospective predictions when integrated into physiologically based models.[15] In addition to the expected improvements in measurement of relevant input data for modeling there is also scope for improvements in the models. The human ACAT models already include compartments appropriately sized according to the physiology of the corresponding intestinal regions and with realistic transit times. However, the animal models are not yet at the same level and mostly use an equal transit time for each compartment. This limitation has been due to the lack of literature data on the physiology in animals, but this is improving,[63] and custom ACAT models are easily created when the data are available.

(61) He, Y.-L.; et al. Species Differences in Size Discrimination in the Paracellular Pathway Reflected by Oral Bioavailability of Poly(ethylene glycol) and D-Peptides. *J. Pharm. Sci.* **1998**, *87* (5), 626–633.

(62) Ingels, F.; et al. Effect of simulated intestinal fluid on drug permeability estimation across Caco-2 monolayers. *Int. J. Pharm.* **2004**, *274* (1−2), 221–232.

(63) McConnell, E. L.; et al. Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. *J. Pharm. Pharmacol.* **2008**, *60* (1), 63–70.

Conclusion

This paper has outlined a strategy for deployment of physiologically based absorption models in the research and development of new drugs. Initial models integrate data generated during routine screening and compound characterization and add value by simulation of the in vivo situation. Comparison of simulation to the results of the first in vivo experiments can point toward factors which may be limiting absorption, and a model parameter sensitivity analysis can allow the uncertainties in early screening data to be accounted for. As a compound advances, experiments are performed at different doses and with different formulations in several preclinical species, and further comparison of simulation to experimental in vivo data helps to refine the model and build up a consistent picture of the absorption behavior. When simulation and observed data cannot be reconciled by accounting for the uncertainties, simulation may suggest additional experiments to characterize factors not yet included in the model. For compounds approaching the clinic, a parameter sensitivity analysis shows how exposure changes with dose and can assist design of animal toxicological studies and contribute to an assessment of developability. Eventually, a refined preclinical absorption model is a solid basis for a prediction of oral absorption in human and with further refinement based upon phase 1 data provides mechanistic insights to assist market formulation development.

MP8000155