Computer-aided biopharmaceutical characterization: gastrointestinal absorption simulation

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Abstract: This chapter introduces the concept of gastrointestinal absorption simulation using in silico methodology. Parameters used for model construction and the sensitivity predicted pharmacokinetic responses to various input parameters are described. Virtual trials for in silico modeling of drug absorption are presented. The influence of food on drug absorption, as well as correlation between the in vitro and in vivo results, are also addressed, followed by biowaiver considerations. Numerous examples are provided throughout the chapter.

1. Introduction

Biopharmaceutical assessment of drugs is of crucial importance in different phases of drug discovery and development. In early phases, pharmaceutical profiling can help to find an appropriate 'drug- like' molecule for preclinical and clinical development, and in later stages, extended biopharmaceutical evaluation can be used to guide formulation strategy or to predict the effect of food on drug absorption. A growing concern for biopharmaceutical characterization of drugs/pharmaceutical products increased the interest in development and evaluation of in silico tools capable of identifying critical factors (i.e. drug physicochemical properties, dosage form factors) influencing drug in vivo performance, and predicting drug absorption based on the selected data set(s) of input factors.

Although an in silico pharmacokinetic (PK) model can confirm different drug administration routes (Gonda and Gipps, 1990; Grass and Vee, 1993; Mahar Doan and Boje, 2000), the main focus has been on prediction of pharmacokinetics of orally administered drugs (Yu et al., 1996; Grass, 1997; Grass and Sinko, 2002; Norris et al., 2000; Agoram et al., 2001; Boobil et al., 2002). Drug absorption from the gastrointestinal (GI) tract is a complex interplay between a large number of factors (i.e. drug physicochemical properties, physiological factors, and formulation related factors), and its

correct representation in the in silico models has been a major challenge. Various qualitative/quantitative approaches have been proposed, starting from the pH-partition hypothesis (Shore et al., 1957), and later moving to the more complex models, such as the Compartmental Absorption and Transit (CAT) model (Yu and Amidon, 1999). Yu et al. gave a good review of these models, classifying them into quasi-equilibrium, steady-state, and dynamic models categories (Yu et al., 1996).

In recent years, substantial effort has been allocated to develop and promote dynamic models that represent GI tract physiology in view of drug transit, dissolution, and absorption. Among these are the Advanced Dissolution, Absorption and Metabolism (ADAM) model, the Grass model, the GI-Transit-Absorption (GITA) model, the CAT model, and the Advanced CAT (ACAT) model (Huang et al., 2009). Some of them have been integrated in commercial software packages, such as GastroPlus™, SimCYP, PK-Sim® , IDEA™ (no longer available), Cloe® PK, Cloe® HIA, and INTELLIPHARM® PKCR (Norris et al., 2000; www.Simulator.plus.com; www.Symcyp.com; Willmann et al., 2003; www.Cyprotex.com; www.Intellipharm.com PKCR. One of the first overviews of the available software intended for in silico prediction of absorption, distribution, metabolism, and excretion (ADME) properties was given in the report of Boobis et al. (2002). Cross-evaluation of the presented software packages was interpreted in terms of software purpose and function, scientific basis, nature of the software, required data to run the simulations, performance, predictive power, user friendliness, flexibility, and evolution possibilities.

Due to dynamic interpretation of the processes a drug undergoes in the GI tract, dynamic models are able to predict both the fraction of dose absorbed and the rate of drug absorption, and can be related to PK models to evaluate plasma concentration-time profiles (Yu et al., 1996). Such models can be beneficial at different stages of formulation development. For example, taking into account all the relevant biopharmaceutical properties of the compound of interest, the potential advantage of various drug properties in terms of improving oral bioavailability can be in silico assessed, before proceeding to in vivo studies. Also, by providing more mechanistic interpretation of PK data, these models can be utilized to explore mechanistic hypotheses and to help define a formulation strategy. The effect of food on drug absorption or possible impact of intestinal transporters and intestinal metabolism can be explored, leading to a better understanding of the observed pharmacokinetics, and guiding subsequent formulation attempts to reduce these effects.

The decisive advantage of in silico simulation tools is that they require less investment in resources and time in comparison to in vivo studies. Also, they offer a potential to screen virtual compounds. As a consequence, the number of experiments, and concomitant costs and time required for compound selection and development, is considerably reduced. In addition, in silico methods can be applied to predict oral drug absorption when conventional PK analysis is limited, such as when intravenous data are lacking due to poor drug solubility and/or if the drug shows nonlinear kinetics. Many research articles have discussed and explored the predictive properties of such mechanism-based models, emphasizing both their advantages and possible drawbacks (Norris et al., 2000; Parrott and Lave, 2002, Yokoe et al., 2003; Tubic et al., 2006; Kovacevic et al., 2009; Parrott et al., 2009; Jones et al., 2011; Reddy et al., 2011; Zhang et al., 2011; Abuasal et al., 2012). Several reviews on this subject have been published (Agoram et al., 2009; Grass and Sinko, 2002; Kesisoglou and Wu, 2008; Kuentz, 2008; Huang et al., 2009).

In the following, selected studies concerning the employment of GI simulation technology (GIST), in particular GastroPlus™ simulation technology, will be reviewed. Basic principles of GIST will be presented, along with the possibilities and limitations of using this mechanistic approach to predict oral drug absorption, estimate the influence of drug and/or formulation properties on the resulting

absorption profile, predict the effects of food, assess the relationship between the in vitro and in vivo data, and aid justification of biowaivers.

6.2 Theoretical background

Simulation software packages, such as GastroPlus™, are advanced technology computer programs designed to predict PK, and optionally, pharmacodynamic effects of drugs in humans and certain animals.

The underlying model in GastroPlus™ is the ACAT model (Agoram et al., 2001), an improved version of the original CAT model described by Yu and Amidon (1999). This semi-physiological absorption model is based on the concept of the Biopharmaceutics Classification System (BCS) (Amidon et al., 1995) and prior knowledge of GI physiology, and is modeled 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 ACAT model of the human GI tract (Figure 6.1) consists of nine compartments linked in series, each of them representing a different segment of the GI tract (stomach, duodenum, two jejunum compartments, three ileum compartments, caecum, and ascending colon). These compartments are further subdivided to comprise the drug that is unreleased, undissolved, dissolved, and absorbed (entered into the enterocytes). Movement of the drug between each sub-compartment is described by a series of differential equations. In general, the rate of change of dissolved drug concentration in each GI compartment depends on ten processes:

Figure 6.1 ACAT model interpretation of in vivo drug behavior (according to SimulationPlus, Inc. GastroPlus™ version 8.0 manual)

I.transit of drug into the compartment;

II.transit of drug out of the compartment;

III.release of drug from the formulation into the compartment; IV dissolution of drug particles;

V.precipitation of drug;

VI.lumenal degradation of drug;

VII.absorption of drug into the enterocytes;

VIII.exsorption of drug from the enterocytes back into the lumen; IX absorption of drug into portal

vein via paracellular pathway; and X exsorption of drug from portal vein via paracellular pathway.

The time scale associated with each of these processes is set by an adequate rate constant. Transfer rate constant (kt), associated with lumenal transit, is determined from the mean transit time within each compartment. The dissolution rate constant (kd) for each compartment at each time step is calculated based on the relevant formulation parameters and the conditions (pH, drug concentration, % fluid, and bile salt concentration) in the compartment at that time. Absorption rate constant (ka) depends on drug effective permeability multiplied by an absorption scale factor (ASF) for each compartment. The ASF corrects for changes in permeability due to changes in physiological conditions along the GI tract (e.g. surface area available for absorption, pH, expression of

transport/efflux proteins). Default ASF values are estimated on the basis of the so-called logD model, which considers the influence of logD of the drug on the effective permeability. According to this model, as the ionized fraction of a compound increases, the effective permeability decreases. Besides passive absorption, including both transcellular and paracellular routes, the ACAT model also accounts for influx and efflux transport processes, and presystemic metabolism in the gut wall. Lumenal degradation rate constant (kdegrad) is interpolated from the degradation rate (or half-iife) vs. pH, and the pH in the compartment. Finally, the rates of absorption and exsorption depend on the concentration gradients across the apical and basolateral enterocyte membranes. The total amount of absorbed drug is summed over the integrated amounts being absorbed/exsorbed from each absorption/transit compartment (Agoram et al., 2001; SimulationPlus, Inc. GastroPlus™, 2012).

Once the drug passes through the basolateral membrane of enterocytes, it reaches the portal vein and liver, where it can undergo first pass metabolism. From the liver, it goes into the systemic circulation from where the ACAT model is connected to either a conventional PK compartment model or a physiologically based PK (PBPK) disposition model. PBPK is an additional feature included in more recent versions of GastroPlus™. This model describes drug distribution in major tissues, which can be treated as either perfusion limited or permeability limited. Each tissue is represented by a single compartment, whereas different compartments are linked together by blood circulation. By integrating the key input parameters regarding drug absorption, distribution, metabolism, and excretion (e.g. partition coefficients, metabolic rate constants, elimination rate constants, permeability coefficients, diffusion coefficients, protein binding constants), we can not only estimate drug PK parameters and plasma and tissue concentration-time profiles, but also gain a more mechanistic insight into the properties of a compound. In addition, several authors reported an improved prediction accuracy of human pharmacokinetics using such an approach (Jones et al., 2006a, 2012; De Buck et al., 2007b). One of the major obstacles for the wider application of this model has been the vast number of input data required.

However, advances in the prediction of liver metabolism (Houston, 1994; Howgate et al., 2006), tissue distribution (Poulin et al., 2001; Poulin and Theil, 2002; Rodgers et al., 2005, 2006), and absorption (Agoram et al., 2001; Willmann et al., 2004) from in vitro and in silico data have made the PBPK model more attractive, leading to an increase in its use (Jones et al., 2011; 2006a, 2012; De Buck et al., 2007a; Theil et al., 2003; Lave et al., 2007).

GastroPlus™ ACAT modeling requires a number of input parameters, which should adequately reflect drug biopharmaceutical properties. Default physiology parameters under fasted and fed states (e.g. transit time, pH, volume, length, radii of the corresponding GI region) are population mean values obtained from published data. The other input parameters include drug physicochemical properties (i.e. solubility, permeability, logP, pKa, diffusion coefficient) and PK parameters (clearance (CL), volume of distribution (Yc), percentage of drug extracted in the oral cavity, gut or liver, etc.), along with certain formulation characteristics (e.g. particle size distribution and density, drug release profiles for controlled-release formulations). Given a known solubility at any single pH and drug pKa value(s), GastroPlus™ calculates regional solubility based on the fraction of drug ionized at each compartmental pH according to the Henderson-Hasselbalch relation. Recent versions of the software have the ability to account for the bile salts effect on in vivo drug solubility and dissolution (GastroPlus™, 2012). The program also includes a mean precipitation time, to model possible precipitation of poorly soluble weak bases when moving from stomach to the small intestine. Effective permeability value (Peff) refers to human jejunal permeability. However, in the absence of the measured value, an estimated value (derived from in silico prediction (ADMET Predictor), in vitro measurements (e.g. CaCo–2, PAMPA assay), or animal (rat, dog) studies) can be

used in the simulation. For this purpose, the program has provided a permeability converter that transforms the selected input value to human Peff, based on the correlation model generated on the basis of a chosen training data set.

In general, modeling and simulation start from data collection, and continue with parameter optimization (if needed) and model validation. The generated drug-specific absorption model can further be utilized to understand how formulation parameters or drug physicochemical properties affect the drug PK profile, to provide the target in vivo dissolution profile for in vitro-in vivo correlation (IVIVC) and identification of biorelevant dissolution specification for the formulation of interest, to simulate the effect of different dosing regiments, to predict food effects on drug pharmacokinetics, or to perform stochastic simulations on a group of virtual subjects (Figure 6.2).Figure 6.2 GI simulation: general modeling and simulation strategy

6.3 Model construction

Modeling and simulation start from data collection. Mechanistic absorption models require a number of input parameters, which can either be experimentally determined or in silico predicted. The common approach is to use literature reported values as initial inputs.

There is a number of examples in the literature describing the use of GastroPlus™ to predict the drug PK profile after oral administration (Tubic et al., 2006; Wei and Löbenberg, 2006; De Buck et al., 2007a; Aburub et al., 2008; Okumu et al., 2008, 2009; Tubic-Grozdanis et al., 2008; Wei et al., 2008; Kovacevic et al., 2009; Parrott et al., 2009; Grbic et al., 2011; Jones et al., 2011; Parojčić et al., 2011; Reddy et al., 2011; Zhang et al., 2011; Abuasal et al., 2012; Crison et al., 2012; Kocic et al., 2012). The reported studies involved different dosage forms, including solutions, suspensions, immediate and controlled release (CR) formulations, and all four BCS classes of drugs. Depending on the objective of the study, human or animal physiologies under fasted or fed conditions were selected for simulations. The required input parameters were taken from the literature, in silico predicted, or experimentally determined, highlighting diversity in the approaches to build a drug specific absorption model. The feasibility of using either Single Simulation or Virtual Trial mode (enables incorporation of inter-subject variability in the model) has also been explored.

A recently published study on GI simulation of nimesulide oral absorption is an interesting example on how selection of input data might influence model accuracy to predict a drug PK profile (Grbic et al., 2012). Drug specific absorption models were constructed by two independent analysts, using the same set of in vivo data, but with different presumptions regarding the key factors that govern nimesulide absorption.

A summary of the input parameters concerning nimesulide physicochemical and PK data is given in Table 6.1.Table 6.1Summary of nimesulide input parameters employed for GI simulation

A literature values taken from Rainsford, 2005; B literature values taken from Dellis et al., 2007;

C in silico predicted (ADMETPredictor™ module);

D optimized values;

E literature values taken from Jovanovic et al., 2005; F experimental value (Grbic et al., 2009);

G default GastroPlus™ values;

H literature values taken from Bernareggi, 1998.

Model 1 was constructed, assuming that nimesulide might be a substrate for influx transporters in the intestine. Therefore, the ASFs were adjusted to best match the resultant profile to the in vivo observed data (Table 6.2). 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 (immediate-release (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 Model 2 was that nimesulide is well absorbed after oral administration mainly due to the pH-surfactant induced increase in solubility in the GI milieu. Therefore, the ASFs were kept on default GastroPlus™ values (Table 6.2), and input solubility and permeability values were optimized to best match the in vivo data.

The simulation results were compared with actual clinical data (Jovanovic et al., 2005), in order to identify the model yielding the best estimation.

The simulation results (nimesulide plasma concentration-time profiles, absorption and dissolution profiles, and the predicted and in vivo observed PK parameters) obtained using the Model 1 and 2 input data sets, are presented in Figure 6.3 and Table 6.3.

According to the obtained data, both Models 1 and 2 gave accurate predictions of nimesulide average plasma profile after oral administration. In both cases, the percentage prediction errors for Cmax and area under the curve (AUC) values were less than 10%, indicating that the models have predicted these parameters well. The largest deviation was observed for tmax (PE of 21.25a/a and 15% in Model 1 and Model 2, respectively). Nevertheless, the predicted values of 3.15 h (Model 1) and 3.4 h (Model 2) were considered as reasonable estimates, since the reported tmax values after oral administration of nimesulide IR tablets varied between 1 and 4 h (Jovanovic et al., 2005; Rainsford, 2006).

However, according to Model 1, the resultant ASF values in the duodenum and jejunum were much higher than the default GastroPlus™ values, reflecting fast absorption of NIM in the proximal parts of the intestine. There were two distinct interpretations: Model 1 outcomes indicated involvement of influx transporters in nimesulide absorption, while according to the Model 2 outcomes, the pH-surfactant induced increase in drug solubility was a predominant factor leading to relatively rapid absorption in the proximal intestine. It should be noted that the Model 2 assumption was 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 (Wu and Benet, 2005). In addition, parameters for which accurate data were not available (i.e. in vivo solubility and human jejunal permeability) were optimized in Model 2. Also, Model 2 was developed using the set of in vivo data for two dosage forms (oral suspension and IR tablet), and revealed incomplete drug absorption from the IR tablet (~ 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). This finding indicated that nimesulide dissolution from IR tablets is expected to be the limiting factor for drug absorption.

Overall, the described independent procedures to build a nimesulide specific absorption model illustrated the importance of understanding complex interplay between drug physicochemical and PK properties, formulation factors, and human physiology characteristics, in order to predict drug PK profile in vivo. Interpretation of the obtained data indicated that the approach applied in Model 2 might be considered as more realistic, signifying that the related absorption model more likely reflects nimesulide in vivo absorption. It was also stressed that, in order to obtain meaningful in silico modeling, the necessary input data have to be carefully selected and/or experimentally verified.

In the next example, gliclazide (GLK) was used as the model drug to illustrate general steps of mechanistic modeling and simulation using GastroPlus™ to predict oral drug absorption. GLK is an ampholyte with pH-dependent solubility in the GI pH range (Grbic et al., 2011). According to the BCS, GLK meets the criteria of a low solubility drug. Reports from the in vivo studies show that, after oral
administration, GLK is almost completely absorbed (Delrat et al., 2002; Najib et al., 2002), although its absorption rate appears to be slow and variable (Kobayashi et al., 1981; Hong et al., 1998; Davis et al., 2000). A summary of the input parameters employed for GI simulation is given in Table 6.4.

In the initial attempt to construct a GLK-specific absorption model, Opt logD Model SA/V 6.1, considering default values for the absorption gradient coefficients C1–C4 (used to calculate the ASFs), was used to estimate changes in permeability as the drug travels along the GI tract. The resultant GLK absorption profile, based on the selected input parameters (Table 6.4) and default C1–C4 values, diverged from the mean in vivo observed Cp-time data (Najib et al., 2002) (Figure 6.4). Therefore, the absorption gradient coefficients, and consequently, the ASF values, were adjusted (using the Optimization module) to best match the resultant model to the in vivo data. Default and adjusted ASF values are given in Table 6.5.

The resultant ASF values in the small intestine, adjusted to best fit the observed plasma concentration-time data for GLK IR tablets, were lower than GastroPlus™ generated values, indicating the possible influence of efflux transporters on GLK absorption through this part of the intestine. This assumption was supported by the results of Al-Salami and associates, who revealed that GLK is a substrate of the ileal efflux drug transporters Mrp2 and Mrp3 (Al-Salami et al., 2008, 2009). The generated plasma concentration-time profile, based on the selected input parameters along with the adjusted ASF values, is presented in Figure 6.4

The predicted fraction of drug absorbed (Fa) was 99.94%, which is in accordance with the literature reporting almost 100% bioavailability of GLK after oral administration (Delrat et al., 2002; Najib et al., 2002). The predicted and in vivo observed PK parameters rendered percentage prediction errors of less than 10% for Cmax and AUC values, indicating that the model has predicted these parameters well. The largest deviation was observed for tmax (PE = 18.22%). However, considering variable GLK in vivo kinetics (reported mean tmax values after oral administration of IR tablets varied between 2.3 and 4.5 h (Kobayashi et al., 1981; Glowka et al., 1998; Najib et al., 2002)), the simulated value of 3.68 h was considered a reasonable estimate.

GastroPlus™ generated regional absorption distribution demonstrated that the majority of GLK, formulated in IR dosage form, is absorbed in the duodenum and jejunum (69.9%), while the rest of the dose is absorbed in the mid-and distal GI regions (Figure 6.5).Figure 6.5 Compartmental absorption of GLKSeveral other examples from the literature summarize values of the input parameters employed to design GI absorption models for the selected drugs. One of the most detailed descriptions of modeling and simulation strategy using GastroPlus™ was given by Zhang et al. (2011), who used carbamazepine (CBZ), a BCS class II compound, as an example to illustrate the

general steps of applying mechanistic modeling and simulation to identify important factors in formulation design and discuss important aspects of modeling and simulation. Four oral dosage forms of CBZ, namely IR suspension, IR tablet, extended-release (XR) tablet, and XR capsule, under both fasted and fed state were modeled. The required input parameters were collected from the literature, New Drug Applications (NDAs), Abbreviated NDAs (ANDAs), or in silico predicted, except the particle density for the IR tablet, which was a GastroPlus™ optimized value. A summary of the CBZ input parameters employed for ACAT model simulation is presented in Table 6.6.Table 6.6Summary of the CBZ input parameters employed for ACAT model simulation (data from Zhang et al., 2011)

The PK parameters and ASFs were obtained by two methods. The first method included deconvolution of the PK data for IR suspension under fasted conditions, to obtain systemic CL, Vc, distribution constants between central and peripheral compartments (K12, K21), and absorption rate constant (Ka), and tlag. These values were then fixed and the ASF values were optimized to obtain the physiology model. The optimized ASFs were about 10 times higher than the default Opt logD Model values, indicating rapid absorption of CBZ in the small intestine. The other approach considered fitting nine parameters in the ACAT model (Vc, CL, K12, K21, Ka, mean particle radius, drug particle density, solubility, and C1 and C2 constants used in calculation of ASFs), using the Optimization module. Coefficients C3 and C4, used to calculate the ASFs of the colon, were kept as default values. The optimized PK values revealed no significant differences in comparison to the PK parameters obtained by the first method; therefore PK parameter values obtained by fitting the conventional PK model were used for further simulations. Stomach transit times of 0.1 and 0.25 h were used for the IR suspension, and tablet and capsule under the fasted state, respectively, while a stomach transit time of 1 h was used for all dosage forms under fed conditions. A colon transit time of 36 h was used for all dosage forms under both fasted and fed conditions. All other parameters were GastroPlus™ default values. In the case of XR products, Weibull controlled-release functions were used as inputs for GI simulation (Weibull parameters were obtained by deconvoluting mean PK profiles after p.o. administration of XR tablets and capsules under fasted and fed conditions).

Predicted CBZ PK profiles were close to the observed mean PK profiles for all tested CBZ products under both fasted and fed conditions, as indicated by correlation coefficients, which ranged between 0.876 and 0.991. The model was also able to capture the absorption plateau that exists after oral administration of the investigated CBZ IR tablet under fasted conditions (the observed peak occupancy time (POT20, time span over which the concentration was within 20% of Cmax) ranged from 3.7 to 41 h under fasted conditions, while the predicted POT20 ranged from 2.9 to 40 h).

Regional absorption distribution revealed that CBZ was mainly absorbed in the small intestine for IR formulation, but in caecum and colon for XR formulation, under both fasted and fed conditions, indicating formulation may have significant impact on CBZ regional absorption (Figure 6.6). Comparing the percentage of drug absorbed in different GI regions under fasted and fed conditions revealed that food had the greatest effects on the rate of absorption from the IR suspension and tablet, and increased CBZ absorption in duodenum.

Another study of CBZ oral absorption simulation using GastroPlus™ was conducted by our group (Kovacevic et al. (2009). The prime objective of this study was to use GIST, in conjunction with IVIVC, to investigate a possible extension of biowaiver criteria to CBZ IR tablets. In this context, GIST was used to predict the fraction of CBZ dose absorbed under fasted state, and the drug disposition based on its physicochemical and PK parameters. Table 6.7 shows that some of the input parameters selected for simulation differed from the values used by Zhang et al. (2011). For example, drug

particle radius was three times larger in the study of Zhang et al. (2011), which inevitably led to slower in vivo dissolution, and consequently, drug absorption. Another notable difference referred to PK parameters employed for the simulations. Opposite to Zhang et al. (2011), who used a two-compartment model to describe CBZ pharmacokinetics following administration of an IR formulation, in our study, a one-compartment model was employed, and the corresponding PK parameters were used as inputs. Consequently, the generated absorption models differed, and the simulated PK profiles diverged, as illustrated by the predicted plasma PK parameters (Table 6.8).

However, in both studies, it was concluded that the model predicted well the average in vivo observed PK profile used as a reference. These conclusions come from the fact that different in vivo observed plasma profiles were used for model validation. The in vivo bioequivalence (BE) data used in our study indicated fast CBZ absorption (mean tmax = 7 h) in comparison to the in vivo profile rendered by Zhang et al. (2011) (characterized by a plateau absorption phase, with a mean tmax of 16 h). Although seemingly diverse, the results of both studies could be considered as reasonable estimates. Namely, considering CBZ variable pharmacokinetics after oral administration (reported tmax ranged between 2 and 24 h (Bauer et al., 2008)), it could be concluded that the PK parameters predicted with both models were within the acceptable range.

The presented examples illustrate that the form of the generated absorption model highly depends upon the PK profile used as a reference. This emphasizes the importance of considering the widest possible range of literature reported and/or experimental values of drug PK parameters, in order to fully perceive model predictability.

6.4 Parameter sensitivity analysis

The generated drug-ipecific absorption model can be used to further explore within the model, such as understanding how the formulation parameters and/or drug physicochemical properties affect the predicted PK profiles. This kind of evaluation is performed by the Parameter Sensitivity Analysis (PSA) feature in GastroPlus™. When performing PSA, one parameter is changed gradually within a predetermined range, which should be based on prior knowledge, while keeping all other parameters at baseline levels. Another option is to use three-dimensional PSA when two parameters are varied at a time, so the combined effect of these parameters is assessed. In addition, an optimized design space can be constructed as a function of the selected parameters. PSA can serve as a useful tool when the input values for some of the physicochemical properties of a compound are rough estimates (e.g. from in silico predictions), and when model predictions do not correlate well with in vivo values. In these cases, the analyst can perform PSA to define more biorelevant input value(s), and in extension, to use them to generate a drug-specific absorption model. Another useful application of this feature concerns highly variable drugs, where PSA can predict the effect of inter- individual variation in PK parameters on drug absorption. PSA can also be used to guide formulation design. For example, if a compound has a poor predicted percentage of drug absorbed, PSA can aid identification of critical parameters limiting the absorption or bioavailability of a drug. Once the limiting factors are known, it may be possible to devise methods to overcome these limitations (e.g. reduction of drug particle size, addition of solubilizers, co-solvents, permeability enhancers, use of different salt forms). In this way, researchers can save a great deal of time and effort, and minimize loss of resources in (pre)formulation processes.

In the previously described case of GLK, PSA was performed to assess the effect of the selected formulation parameters (i.e. effective particle radius, drug particle density), and certain drug physicochemical properties (i.e. solubility and permeability) on the predicted rate and extent of GLK absorption. The selected parameters were varied in the range covering one-tenth to ten-fold actual

input parameter value, except for the human effective permeability, which was varied from one-half to two-fold input value. The results are presented in Figure 6.7.

According to the PSA outcomes, the percentage of GLK absorbed (Fa) would not be significantly influenced by variations in drug particle density and effective particle radius. The PSA for solubility showed that even a 10-fold decrease in solubility would not cause bioavailability problems (Fa > 85%) (Figure 6.7a). However, it was demonstrated that larger particles, higher density and/or lower solubility values than the ones used for simulation would decrease the rate of GLK absorption (Figure 6.7c). The results also indicated that variations in the input effective permeability did not significantly affect the drug absorption profile.

Other examples describe the use of PSA to investigate the effects of different input parameters on GastroPlus™ predicted drug PK performance. In our CBZ study (Kovacevic et al., 2009), PSA was used to assess the importance of the selected input parameters (i.e. drug solubility, dose, effective particle radius, and drug particle density) in predicting the percentage of CBZ absorbed. The selected parameters were varied in the range from one-tenth to ten-fold actual input parameter value. According to the results, the extent of drug absorption was rather insensitive to the variation in the input parameters tested. PSA for drug solubility indicated that complete absorption (Fa > 85%) could be achieved with CBZ solubility 2.5 times lower than the initially used input value (0.05 mg/mL in comparison to 0.12 mg/mL), signifying that eventual CBZ transformation to less soluble polymorph would not cause bioavailability problems. PSA for particle radius revealed that high bioavailability would be achieved with CBZ particle sizes up to 90 μm (25 μm was used as the input value), and PSA for drug dose indicated that single doses up to 1200 mg would not impair the extent of CBZ absorption (Figure 6.8).

In another case where CBZ was used as the model drug (Zhang et al., 2011), PSA was performed for parameters for which accurate data were not available and the selected formulation parameters, including mean particle radius, particle radius standard deviation, drug particle density, diffusion coefficient, dose volume, drug permeability, drug solubility, precipitation time, and four Weibull parameters were used to describe release profile of the XR formulations. Four dosage forms of CBZ (IR suspension, IR tablet, XR tablet, and XR capsule), under both fasted and fed conditions, were studied. PSA results for solubility indicated that drug in vivo solubility had a significant impact on PK profiles when it was less than 0.2 mg/mL under the fasted state. However, since this border value was within the reported range of aqueous solubility of CBZ, the authors speculated that CBZ absorption is dissolution rate-limited rather than solubility-limited. This assumption coincides well with our findings (Kovacevic et al., 2009) that CBZ in vivo solubility would not cause bioavailability problems. PSA also denoted that permeability had less effect on the predicted PK parameters (Cmax, tmax, AUC0-t) when CBZ was formulated as a suspension. As for the formulation factors, it was shown that drug particle size and density had a significant effect on CBZ PK from IR formulations, being more pronounced in the case of IR tablet in comparison to the IR suspension, but having no effect on drug PK from XR formulations. However, the authors elucidated that this occurred because in XR formulations the particle size effect was integrated in the dissolution profiles, which were translated into Weibull functions for input into the ACAT model. Another phenomenon observed was that CBZ absorption profiles showed different sensitivity to the same factors, depending on whether the PSA was performed for fasted state or fed state. In general, it was shown that CBZ absorption profiles were more sensitive to variations in input parameters tested in fasted state than in fed state.

The work of Kuentz et al. (2006) is a good example of how PSA can be used as an integral part of a strategy for preclinical formulation development. In order to obtain detailed biopharmaceutical data

on the selected model drug, initially profiled to have poor solubility and high permeability, GastroPlus™ simulations, together with the statistically designed study in dogs, were conducted. In the first step, the software was used to simulate the absorption process based on pre-formulation data. Then PSA was performed where drug particle size and solubility values were varied (>100-fold range) and their impact on the oral drug bioavailability was assessed. PK experiments in beagle dogs were run according to the factorial design set-up to examine the effect of the formulation in parallel with a potential food effect in a clinically foreseen dose range. The obtained PSA results revealed that changes in particle size and reference solubility in the investigated range would not significantly affect drug bioavailability (Figure 6.9), and the beagle dogs study indicated that different dosage forms (solution and capsules filled with micronized drug) were not expected to be significantly different in terms of AUC0-inf. Based on the findings that particle size reduction and/or solubility enhancement would not lead to increased absorption, it was decided that there was no need to develop a sophisticated drug delivery system; instead, capsule formulation was selected for phase I clinical studies, leading to considerable resources being saved.

Dannenfelser et al. (2004) reported a case where PSA analysis revealed that drug solubility and particle size clearly influenced oral absorption of a poorly soluble drug. Additional PK studies in dogs revealed that solid dispersion containing water soluble polymer with a surface active agent showed comparable bioavailability with the cosolvent-surfactant solution (considered to be 100% bioavailable), both of which showed 10-fold higher bioavailability than the dry blend of micronized drug with microcrystalline cellulose. Thus, a capsule containing solid dispersion formulation was selected for clinical development.

6.5 Virtual trial

In the later stages of formulation development, it is especially valuable to anticipate inter-subject variability that may influence oral drug bioavailability. In this way, the formulator might gain a better insight on what can be achieved by means of the formulation.

In order to in silico simulate the influence of population variability and/or the combined effect of formulation variables that are not precise values, but for which distributions of values can be estimated, the Virtual Trial feature in GastroPlus™ can be used. This feature allows the user to perform stochastic simulations on a number of virtual subjects, wherein the values of the selected variables are randomly sampled from predetermined distributions (defined as means with coefficients of variation (CV%) in absolute or log space). CV% values are usually estimated on the basis of previous knowledge or analysis of literature data. The results of the simulations are expressed as means and coefficients of variation for fraction of drug absorbed, bioavailability, tmax, Cmax, and AUC values, as well as absolute minimum and maximum values for each of these parameters reached during the trials. Also, the average Cp-time curve, 90% confidence intervals, probability contours (10, 25, 50, 75, 90, 95, and 100%), and experimental data with possible BE limits (if available), are displayed.

An illustration of the use of virtual trials for in silico modeling of oral drug absorption can be seen in the paper of Tubic et al. (2006). Although the prime objective of this study was to demonstrate how an in silico approach can be used to predict nonlinear dose-dependent absorption properties of talinolol, this section will focus solely on the results of virtual trial simulations. The reason why the authors performed simulations in a virtual trial mode was to include the effects of physiological variables, such as transit times in various GI compartments, GI pH, lengths and radii, PK parameters, plasma protein binding, and renal CL on talinolol absorption. Stochastic variables were randomly selected within the range defined by the means with predetermined coefficients of variation in log

normal space, and used for the simulation. Virtual trials were performed with 12 subjects (equal to the number of subjects used in the clinical study), and the results were presented as mean Cp vs. time profile with 90% confidence intervals around the mean, along with Cp vs. time curves for 25, 75, and 100% probability of simulated patient data. The simulation results revealed that all of the observed clinical data lay within the minimal and maximal individual patient simulations, suggesting that the CV% values used for the log normal distributions of the stochastic variables produced variability that encompasses the observed clinical results. Thus, it was deduced that virtual trial simulations based on the presumed distribution of the selected variables were able to predict variability associated with the observed clinical data.

The Virtual Trial mode can also be used to conduct virtual BE studies, as demonstrated in the work of Tsume and Amidon (2010) (Section 6.8: Biowaiver Considerations) and Zhang et al. (2011). In the latter example, virtual BE studies on 25 subjects were performed for a hypothetical XR CBZ tablet under fasted and fed conditions, using a conventional 2 × 2 crossover design. Stochastic variables included physiological and PK parameters, which were randomly sampled from the predefined range in log-normal scale. Along with the reference product, two virtual test formulations were examined: Test 1 having similar dissolution profile to the reference formulation (f2 = 67.4), and Test 2 that differed in in vitro dissolution compared to the reference product (f2 = 38.2) (Figure 6.10a). Drug PK profiles were predicted from the corresponding in vitro dissolution profiles described by the Weibull function. A random sequence was assigned to the test formulations for 90% confidence intervals (CI) calculation of Cmax, AUC0-t, and AUC0-inf. The simulation results showed that, in spite of the difference in in vitro dissolution, Test 2 was bioequivalent to the reference formulation using the 80 to 125% criteria (Figures 6.10b,c), indicating that the in vitro dissolution test was more sensitive to formulation differences than an in vivo study. Also, it was perceived that the confidence intervals calculated for the test/reference ratios from virtual BE studies were narrower than the observed ones. This was attributed to the fact that physiological and PK parameters of the same subjects were equal when the subjects were administered with the test vs. reference formulations. Therefore, the authors speculated that the Test 2 formulation might not be bioequivalent to the reference formulation if intra-subject variability was included in the simulations.

6.6 Fed vs. fasted state

The presence of food may affect drug absorption via a variety of mechanisms; by impacting GI tract physiology (e.g. food-induced changes in gastric emptying time, gastric pH, intestinal fluid composition, hepatic blood flow), drug solubility and dissolution, and drug permeation (Welling, 1996). For example, lipophilic drugs often show increased systemic exposure with food, and this phenomenon is attributable to improved solubilization due to higher bile salt and lipid concentrations. Negative food effects are mostly seen for hydrophilic drugs, where food impedes permeation (Gu et al., 2007). One of the frequently used approaches to assess the effect of food on oral drug absorption involves animal studies (Humberstone et al., 1996; Paulson et al., 2001; Wu et al., 2004; Xu et al., 2012). However, due to the fact that physiological factors are species dependent, the magnitude of food effect for a given compound across species is usually different, thus complicating the prediction of food effects in humans (Jones et al., 2006b). One alternative to animal experiments is to simulate food effects in humans using physiologically based absorption models.

Considering that these models are built based on a prior knowledge of GI physiology in the fasted and fed states, they are able to describe the kinetics of drug transit, dissolution, and absorption on the basis of drug-specific features such as permeability, biorelevant solubility, ionization constant(s), dose, metabolism and distribution data, etc. GastroPlus™ default physiology parameters, which differ between fasted and fed states, are given in

Several studies have confirmed the usefulness of the in silico modeling approach to assess food effects on oral drug absorption. For example, Jones et al. (2006b) incorporated biorelevant solubility and degradation data into the GastroPlus™ absorption model to predict plasma profiles in fed, fasted, and/or high-fat conditions for six model compounds. Biorelevant solubilities were measured in different media: simulated human gastric fluid for the fasted and fed state, simulated human intestinal fluid for the fasted, fed, and high-fat state, and simulated human colonic fluid for the upper and the lower colon. The effect of food was simulated by changing physiological parameters and inserting the relevant solubility data into the appropriate ACAT compartments (stomach, intestine, and colon). The food effect for each drug was estimated by comparing AUC or Cmax between fasted, fed, and/or high-fat conditions. Predicted and observed plasma concentration-time profiles and food effects were compared for a range of doses to assess the accuracy of the simulations. The obtained results demonstrated that GI simulation using GastroPlus™ was able to correctly predict the observed plasma exposure in fasted, fed, and high-fat conditions for all six compounds. Also, the applied method was able to accurately distinguish between minor and significant food effects. Therefore, it was concluded that biorelevant solubility tests, in conjunction with physiologically based absorption modeling, can be used to predict food effects caused by solubility and dissolution rate limitations, and/or degradation. However, it was stressed that the accuracy of a generated drug-specific absorption model needs to be carefully verified before proceeding to predict the effect of food.

An important issue emphasized from different studies (Mueller et al., 1994; Schug et al., 2002a,b; Zhang et al., 2011) is related to the formulation-dependent food effects. Zhang et al. (2011) incorporated gastric emptying time and different drug in vivo solubilities under fasted and fed states into the generated CBZ absorption model and observed that co-administration of CBZ IR suspension with food resulted in decreased Cmax and prolonged tmax, probably due to a prolonged gastric emptying time, while co-administration of the IR tablet and XR capsule with food resulted in increased Cmax and earlier tmax in comparison with the PK parameters obtained under fasted state. A possible explanation of this phenomenon was that the presence of a high-fat meal induced the increase in bile salts concentration in the GI tract, thus enhancing the dissolution rate of low soluble CBZ from the IR tablet and XR capsule.

Jones et al. developed a novel strategy for predicting human pharmacokinetics in fasted and fed states, by using PBPK absorption modeling across different species (Jones et al., 2006a). The proposed strategy implies that the absorption models are first generated for the selected preclinical species (e.g. mouse, rat, dog, monkey) on the basis of data generated during drug research and preclinical development, and afterwards verified thoroughly by comparing the simulation outcomes with the results of in vivo animal studies. If the prediction was proven to be accurate, then the same in vitro absorption parameters and the same assumptions can be used to predict human pharmacokinetics. However, if the animal model was incomplete, further refinement of the model is needed in order to provide more accurate simulations in humans (Figure 6.11).

The overall concept of this strategy is illustrated in several papers published by this group (Jones et al., 2006b; Parrott and Lave, 2008; Parrott et al., 2009). For example, in one of these (Parrott et al., 2009), GastroPlus™ PBPK absorption models for dog and human for two model drugs (theophylline and aprepitant) were constructed in parallel by integrating various predictive data, including drug physicochemical properties, biorelevant solubility and dissolution, and in vivo study results. Verification of model assumptions was performed by comparing simulation results to the food effects measured in carefully designed in vivo dog studies, whereas a good match of simulated and observed plasma concentrations in the fasted and fed dogs indicated that the model has captured

well the mechanisms responsible for food effects, allowing a reliable prediction for humans. The results indicated that the strategy to predict food effects via PBPK modeling highly depended on drug biopharmaceutical properties. For theophylline, a BCS class I compound, the food effects for immediate and CR formulations could be well simulated by default GastroPlus™ models for both dog and human. However, simulations for aprepitant, a BCS II drug, required several changes to the default GastroPlus™ models (e.g. adjustment of regional solubility data, modification of the diffusion coefficient used to calculate the dissolution rate), indicating that PBPK modeling based on in vitro data for challenging drugs should be conducted in conjunction with preclinical in vivo dog studies.

6.7 In vitro dissolution and in vitro-in vivo correlation

There are two approaches enabling the GastroPlus™ generated drug-specific absorption model to be used to assess the relationship between the in vitro and in vivo data: convolution to predict the plasma concentration profile, and deconvolution to estimate the in vivo dissolution profile. Once an IVIVC is developed, an in vitro dissolution test can be used to identify changes that may affect the efficacy and safety of the drug product. In addition, biowaiver justification could be discussed in terms of whether dissolution from the dosage form is expected to be the rate-limiting factor for drug in vivo absorption.

In the convolution approach, a set of in vitro data representing different dissolution scenarios is used as the input function in GastroPlus™ software to estimate the expected drug plasma concentration- time profiles. In the next step, the obtained profiles are compared with the mean drug plasma concentration profile observed in vivo, in order to establish an IVIVC. In the deconvolution approach, the GastroPlus™ generated in vivo dissolution profile is plotted against the in vitro obtained
dissolution profiles, so that 'bioperformance' dissolution condition(s) can be identified.

In the previously described case study of GLK IR tablets (Grbic et al., 2011), a set of virtual in vitro data, based on the experimentally obtained results (in media pH 1.2, 4.0, 4.5, 6.8, 7.2, and 7.4) and literature reported data (Hong et al., 1998), was used as the input function in GastroPlus™ software to estimate the expected GLK plasma concentration profiles. The investigated in vitro profiles (presented in Figure 6.12a) were generated to reflect the situation where:

Figure 6.12 (a) Virtual GLK dissolution profiles, and (b) the corresponding simulated in vivo profiles, along with the actual in vivo data (from Najib et al., 2002) (the simulated profiles b, c, and d overlap)

I less than 85% of the drug is dissolved – incomplete dissolution (profile a); II more than 85% of the

drug is dissolved in 60 min (profile b);

III.more than 85% of the drug dissolved in 45 min (profile c);

IV.more than 85% of the drug dissolved in 30 min – 'rapid' dissolution criteria (profile d); or V more

than 85% of the drug dissolved in 15 min – 'very rapid' dissolution criteria (profile e).

The corresponding Cp–time profiles (Figure 6.12b), estimated on the basis of the generated GLK-specific absorption model, were plotted against the in vivo observed data (Najib et al., 2002), in order to develop a level A IVIVC model (Figure 6.13a). The obtained correlation coefficients and slopes of the regression lines are given in Table 6.10.

Table 6.10IVIVC statistical parameters for GLK IR tabletsa – slope of the regression line, r – coefficient of correlationFigure 6.13 IVIVC plot for GLK IR tablets: (a) convolution approach; (b) deconvolution approach

The results indicated that variations in drug input kinetics were well reflected in the simulated in vivo profiles. However, it was evident that differences observed in vitro were less pronounced in the predicted PK profiles (the simulated profiles b, c, and d overlapped). The highest degree of deviation from the in vivo observed profile was demonstrated for profile a, representing a scenario in which less than 85% of the drug is dissolved. On the other hand, values of the slope close to unity, as well as high coefficients of correlation, indicated the presence of a level A correlation for the profiles b, c, d, and e.

In the attempt to establish IVIVC for the same data set using the deconvolution approach, the hypothetical GLK in vivo absorption profile estimated by GastroPlus™ was compared with previously described in vitro dissolution profiles. Since in vitro drug dissolution was faster than the corresponding in vivo process, it was necessary to rescale the time axis when progressing from in vitro to in vivo. The IVIVC plot of the percentage dissolved in vitro vs. the percentage absorbed in vivo, is presented in Figure 6.13b. The outcomes of deconvolution revealed that the in vitro profile e (stretched by 12-fold linear rescaling of the time axis) has the same general shape (morphology) as the estimated hypothetical in vivo dissolution profile, although a good correlation was also achieved for the in vitro profiles b, c, and d (Table 6.10). These results were in accordance with those obtained by the convolution approach. Since both convolution and deconvolution approaches were successful in establishing a level A IVIVC, it was suggested that dissolution specification of more than 85% GLK dose dissolved in 60 min may be considered as biorelevant dissolution acceptance criteria for GLK IR tablets.

Other examples can also serve as a good illustration of how GIST can be used to develop IVIVC. In our previous work (Kovacevic et al., 2009), a convolution based approach was applied to simulate CBZ plasma concentration-time profiles based on different in vitro dissolution rates, with the aim to evaluate whether IVIVC for IR and CR CBZ tablets could be established. Dissolution studies of the investigated IR and CR CBZ tablets were performed in the United States Pharmacopoeia (USP) rotating paddle apparatus at 75 rpm, using 900 mL of various dissolution media. In the case of IR tablets, the employed media included water, 0.1, 0.25, 0.5, and 1% sodium lauryl sulfate (SLS) aqueous solution, 0.1 M HCl, USP acetate buffer pH 4.5, and USP phosphate buffer pH 6.8. In the case of CR tablets, drug release studies were performed in water, 1% SLS, and according to the half-change methodology (HCM). The obtained dissolution data were later used as the input function in the GastroPlus™ Single Simulation Mode, to evaluate the influence of in vitro drug dissolution rate on the predicted CBZ plasma concentration-time profiles. The dissolution profiles used as inputs, and the corresponding Cp–time profiles, are presented in Figure 6.14. PK parameters predicted on the basis of different input CBZ dissolution rates and the relevant prediction error statistics are given in Tables 6.11 and 6.12. Figures 6.14b and d illustrate that, in the case of CBZ IR tablets, the simulated in vivo profiles did not appear to be strongly affected by the differences in drug dissolution rate. The best match between the predicted and the observed Cmax and AUC values was accomplished for drug dissolution in 0.5 and 1% SLS. An interesting phenomenon concerned the deviations between the predicted Cmax and tmax values obtained for different pH dissolution media (water, media pH 1.2, 4.5, and 6.8), which were not consistent with the almost superimposable in vitro dissolution profiles in these media (Figures 6.14c and d). It was postulated that the obtained differences were caused by a simulation artifact resulting from the software approximation of the time needed to accomplish 100% drug dissolution, which was estimated as 5.5 and 15.4 h for water and pH 6.8 media, respectively. In the case of CR tablets, the simulated profiles based on CBZ dissolution in 1% SLS and HCM were in best agreement with the in vivo observed data, while the PK profile predicted on the basis of the CR tablets dissolution in water indicated slow and incomplete drug absorption. It was noted that such results were in accordance with the software calculated 39.29 h to be the time

needed for 100% drug dissolution to be accomplished, which exceeds the physiologically relevant GI transit time.

In order to develop a level A IVIVC, CBZ plasma concentration profiles simulated on the basis of drug dissolution data obtained in water and media containing 1% SLS for IR and CR tablets (Figure 6.15) were plotted against the in vivo observed data. Linear regression analysis of the pooled data for both the generic and reference IR and CR tablets indicated high level A IVIVC, especially for predictions based on the in vitro data observed in 1% SLS (Figure 6.16). According to these results, it was suggested that 1% SLS might be considered as the 'bioperformance' dissolution medium for both the IR and CR CBZ tablets. However, it was noted that the possibility to obtain a universal IVIVC model for both IR and CR products resulted from the fact that CBZ in vivo behavior is determined by its PK characteristics (i.e. long elimination half-life) rather than the dosage form properties, and that any further generalization of this concept to other compounds should be carefully evaluated.

Another example considering identification of the predictive in vitro dissolution of CBZ formulations was given by Zhang et al. (2011). The authors reviewed a set of in vitro dissolution data obtained under different conditions for different CBZ products, which were submitted to the FDA, and made a selection of the representative in vitro dissolution profiles, which were compared with the GastroPlus™ predicted CBZ in vivo dissolution profiles in the fed and fasted states. The data collected demonstrated that in vitro dissolution of CBZ from the IR suspension, conducted in 900 mL water using USP Apparatus 2 with a rotation speed of 50 rpm, was slower than the simulated in vivo dissolution in the fed state but faster than in vivo dissolution in the fasted state, indicating that the employed in vitro dissolution test conditions for CBZ IR suspension could not be considered biorelevant (Figure 6.17a). In the case of the CBZ IR tablet, in vitro dissolution profiles obtained in 900 mL media containing 0.1% SLS, using USP Apparatus 2 with paddle speed of 75 rpm, were close to the in vivo dissolution in the fed state (Figure 6.17b). For the CBZ XR tablet, the dissolution profile obtained in 900 mL buffer (pH 1.1, 4.5, and 6.8), using USP Apparatus 1 at 100 rpm, correlated well with in vivo dissolution under fed conditions (Figure 6.17c). For the XR capsule, the best relationship between in vitro and in vivo data under both fasted and fed conditions was achieved with the dissolution profile obtained in 900 mL buffer containing 0.1% SLS using USP Apparatus 2 at 50 rpm (Figure 6.17d). In addition, the repeated simulations performed for fasted state, using the same solubility as for the fed state, gave an almost identical in vivo dissolution rate to that obtained under the fed state, indicating that the differences in in vivo dissolution rates between fasted and fed states, for both IR and XR formulations, were caused by the difference in in vivo solubility under fasted and fed states.

Another example of using computer simulations to establish IVIVC referred to etoricoxib solid oral dosage forms (Okumu et al., 2008). Dissolution profiles of etoricoxib from the film-coated tablets were performed in USP Apparatus 2 at 75 rpm, using conventional dissolution media: simulated gastric fluid (SGF) and USP-simulated intestinal fluid (USP-SIF) (900 mL), and fasted state simulated intestinal fluid (FaSSIF) (500 and 900 mL) as 'biorelevant' media. The in vitro data obtained were then used as input functions in GastroPlus™ to predict the corresponding drug absorption profiles (Figure 6.18). A comparison of the simulated profiles with the in vivo observed data (Table 6.13) indicated that the profiles obtained in SGF and 900 mL FaSSIF appeared to simulate the in vivo profile better when compared with that in SIF and 500 mL FaSSIF. These results suggested that USP-SIF might not be the best choice of media, and that recommended 500 mL FaSSIF (Galia et al., 1998; Marques, 2004) may not be the right choice of volume for 'biorelevant' in vitro testing of etoricoxib tablets. However, the simulation results based on the dissolution data obtained in 900 mL FaSSIF and SGF provided a comparatively good IVIVC ($r2 = 0.899$ and 0.898, respectively).

6.8 Biowaiver considerations

The role of biowaivers in the drug approval process has been emphasized since the introduction of BCS (Amidon et al., 1995) and the release of FDA guidance on waiver of in vivo bioavailability and BE studies (US Food and Drug Adminstration, 2000). In this context, the term biowaiver refers to the situations in which in vivo BE studies can be substituted with the relevant in vitro data. The main premise, when adopting the biowaiver concept, was to reduce time and costs, and to offer benefits in terms of ethical considerations. The most common type of biowaiver adopted by the regulatory authorities includes the application of the BCS-based scheme (similar or rapid/very rapid dissolution profiles of the test and reference product in pH 1.2, 4.5, and 6.8 media) or the application of IVIVC. According to the FDA, biowaivers for IR drug products may be requested solely in the cases of highly soluble and highly permeable substances (BCS class I) when the drug product is (very) rapidly dissolving and exhibits similar dissolution profile to the reference product, while the IVIVC-based approach has been narrowed down to applications for XR products (US Food and Drug Administration, 2000, 1997). The EMA and WHO issued guidelines widened the eligibility for biowaiver to some BCS class III (eligible if very rapidly dissolving) (European Medicines Agency, 2010; WHO Expert Committee on Specifications for Pharmaceutical Preparations, 2006) and BCS class II drugs (eligible for biowaiver if the dose-to-solubility ratio at pH 6.8 is 250 mL or less and high permeability is at 85% absorbed) (WHO Expert Committee on Specifications for Pharmaceutical Preparations, 2006). Also, it was pointed out that the biowaiver concept concerning BCS II and III drugs should be further relaxed (e.g. BCS class II drugs eligible for biowaiver under the assumption that the drug dissolves completely during the GI passage (Yu et al., 2002), and BCS class III compounds eligible if rapidly dissolving (Tsdume and Amidon, 2010)).

Several examples from the literature describe how GIST can be used to identify BCS class(es) of drugs eligible for biowaiver. In the previously mentioned in vitro-in silico study of GLK IR tablets, simulation results demonstrated that differences in GLK in vitro dissolution kinetics, such as 85% drug dissolved within the 15 to 60 min time frame, are not expected to reflect on the in vivo PK profile. These results support the assumption that, in the case of BCS class II drugs, complete in vivo dissolution might occur at later time points than for highly soluble BCS class I drugs. This would allow wider biorelevant in vitro dissolution specification, than very rapid/rapid in vitro dissolution, to be set. In addition, in vitro results indicated that GLK solubility and dissolution from IR tablets are not expected to be the rate-limiting factors for GLK in vivo absorption, and since this was a highly permeable drug, there was a rationale to postulate that biowaiver extension might be applicable in the case of GLK IR tablets (Grbic et al., 2011).

Another example is the work of Okumu et al. (2009), who combined in vitro results with in silico simulations using GastroPlus™, in order to support biowaiver for IR etoricoxib solid oral dosage forms. They used in vitro measured solubility and dissolution data in different media, along with caco-2 assessed drug permeability as input functions in the program in order to predict etoricoxib absorption profile. The simulation results revealed that drug absorption after tablet administration was similar to the absorption of an oral solution, indicating fast and complete drug absorption. Furthermore, solubility results indicated etoricoxib behaves like a BCS class I drug in an acidic environment, and the dissolution transfer model confirmed that the drug stays in solution when transferred from the acidic environment of the stomach into the small intestine. Therefore, it was concluded that etoricoxib might be a suitable candidate for biowaiver.

In our CBZ study (Kovacevic et al., 2009), biowaiver justification for this BCS class II drug was elaborated upon. The GastroPlus™ generated CBZ-specific absorption model was used to predict

drug plasma concentration-time profiles based on different in vitro dissolution rates as input function. The results revealed that high dissolution rates (i.e. > 85% of drug dissolved in < 10 min) were not related to the significant increase in Cmax in comparison to the in vivo observed values, thus indicating that the predicted plasma concentration profiles were rather insensitive to the differences in drug input kinetics. Following these results, it was concluded that there is a rationale for considering CBZ biowaiver extension. However, it was stressed that, at present, other factors such as CBZ narrow therapeutic index and vital indication are the limitations for granting marketing authorization based on the in vitro data alone.

Tubic-Grozdanis et al. (2008) also demonstrated that GI simulation of oral drug absorption can aid in identification of BCS class II biowaiver candidate drugs. They used several weakly acidic (i.e. ibuprofen, ketoprofen, diclofenac, mefenamic acid, and piroxicam) and weakly basic (i.e. verapamil, miconazole, and terbinafine) BCS class II model compounds, and GIST as a tool to study how differences in dissolution rates would affect drug bioavailability and other PK properties. Theoretical dissolution profiles of two IR drug products, namely 'rapid IR' (released > 90% of the dose within 10 min) and 'slow IR' (released 80% in 45 min) were designed and used to predict plasma concentrations vs. time and absorption curves for each compound used in the simulations. Depending on the drug properties, either GastroPlus™ Single Simulation Mode or Virtual Trial (e.g. for verapamil, which is a highly variable drug) were selected for the simulations. PSA was performed in order to assess the influence of drug properties (i.e. particle size, solubility, precipitation time) on the fraction of drug absorbed. According to the obtained results, and supported by previously published biopharmaceutical data on the selected model drugs, it was deduced that ibuprofen, ketoprofen, diclofenac, piroxicam, and terbinafine could be considered as candidates for biowaiver. However, GI simulation indicated that mefenamic acid and miconazole were not eligible for granting a biowaiver. According to the predictions, mefenamic acid exhibited solubility and dissolution limited absorption in the small intestine. Moreover, this drug lacked a predictive dissolution method which would indicate its biopharmaceutical properties. In the case of miconazole, it was found that oral drug absorption was limited by dissolution rate and by the saturated solubility, indicating that a highly dosed drug would probably precipitate in the GI milieu.

Tsume et al. (2010) investigated the ability of GIST to predict oral absorption of the selected BCS class I (propranolol and metoprolol) and BCS class III drugs (cimetidine, atenolol, amoxicillin), and performed in silico BE studies to estimate the feasibility of extending biowaivers to BCS class III drugs. In addition, the significance of 'rapid dissolution' and 'very rapid dissolution' criteria for BCS class III drugs was evaluated. The GastroPlus™ Virtual Trial model was used to assess the influence of drug dissolution kinetics on oral drug absorption, Cmax, AUC, and BE. A set of virtual in vitro dissolution data (corresponding to 85% release in 15, 30, 45, 60, 90, 120, and 180 min) was used as input function in GastroPlus™ to predict the drug PK profile. For each BCS class III drug, virtual trial (500 subjects) with T85% = 15 min ('very rapid dissolution'), and virtual trials (24 subjects) at different release rates (from T85% = 30 min to T85% = 180 min) were performed as 'reference' and 'samples', respectively. The results of the predictions (mean Cmax and AUC0-inf ± SDs), with different release rates used as 'samples,' were compared with the reference results to determine BE. The results demonstrated BE up to T85% = 45 min (for amoxicillin) or T85% = 60 min (in the cases of cimetidine and atenolol) compared to the reference result of T85% = 15 min, including BE between very rapid (> 85% solubility in 15 min) and rapid dissolution (> 85% solubility in 30 min). These findings indicated that the permeability of BCS class III compounds was the rate-limiting step for oral drug absorption rather than their dissolution. Overall, the obtained results suggested that extending the biowaiver to include IR dosage forms of BCS class III drug products is feasible, and moreover,

that biowaivers for BCS class III drug products with suitably rapid dissolution would ensure 'bioperformance' of these pharmaceutical products.

Crison et al. (2012) employed in silico modeling to justify biowaiver for BCS class III drug metformin hydrochloride. GastroPlus™ modeling was performed within the range of gastric transit times expected in human subjects, to show the broad range of release rates that are expected to have no impact on AUC and Cmax, and therefore result in drug products BE. It should be noted that, although metformin exhibits nonlinear pharmacrokinetics with respect to dose, the absorption model developed in this study was based on 500 mg data, so the simulation results were limited to that dose. Two clinical studies for IR formulations were used in the model development and additional clinical studies, one for IR and one for ER formulation, were used to confirm that the model was predictive over a wide range of drug release times. Drug release profiles representing 100% of metformin released in 5 min up to 14 h were used as inputs for the model. The simulations to predict plasma concentrations of metformin corresponding to different release rates were performed as virtual trials, so that inter-subject variability could be introduced into the predictions. In order to prove model predictability, the results of virtual trial simulations (defined as 'test') were compared with the observed clinical data (defined as 'reference'). According to the simulation results, metformin release rates within 100% of drug, dissolved in 5 min up to 2 h did not have a statistically significant effect on Cmax and AUC0-t. In addition, it was shown that within this range of dissolution rates, metformin products are expected to be bioequivalent, irrespective of the results of the f2 test. In conclusion, the results illustrated that the described in vitro-in silico approach might be used to waive in vivo BE studies for metformin drug product. Furthermore, it was deduced that in silico modeling and simulation, which includes all the key parameters that fully define the absorption of BCS class III compounds (i.e. dissolution, permeability, and GI residence time), should be more mechanistically accurate and robust for BE evaluation than statistical comparison of in vitro release profiles.

6.9 Conclusions

The various examples presented demonstrate that GI modeling has become a powerful tool to study oral drug absorption and pharmacrokinetics. This method offers a distinctive opportunity to mechanistically interpret the influence of the underlying processes on the resulting PK profile. Namely, by understanding the complex interplay between drug physicochemical and PK properties, formulation factors, and human physiology characteristics, we might gain an insight into the influence of a particular factor or set of factors on drug absorption profile, and understand possible reasons for poor oral bioavailability. In this context, PSA is particularly useful, since it allows identification of critical factors affecting the rate and extent of drug absorption prior to formulation development. In addition, PSA can be used to optimize parameter values for which accurate data are not available. Other features, such as the Virtual Trials and PBPK modeling, enable even more advanced predictions of, for example, inter-individual variability or factors contributing to variability in disposition, thus further enhancing the reliability of in silico absorption modeling.

The examples also demonstrate that the in vitro-in silico approach can be successfully used to identify biorelevant dissolution specifications for the in vitro assessment of the drug product of interest, and facilitate the choice of the relevant in vitro test conditions for the prediction of the drug release process in vivo. Finding the in vitro dissolution test conditions that best predict drug in vivo performance is a substantial part of product development and quality testing strategy, thus implying that mechanistically based absorption modeling might facilitate the QbD approach in drug

development. In addition, it was illustrated that GI simulation, in conjunction with IVIVC, might contrive identification of biowaiver candidate drugs.

In view of the complexity of the described GastroPlus™ model and a number of data required for simulation, it is evident that the reliability of the modeling results is dependent on both the model and the selected data set. Therefore, the necessary input data have to be carefully selected and/or experimentally verified. However, with the right selection of input data, well-validated absorption model, and correct interpretation of modeling results, GI simulation shows great promise in assessing biorelevant features of formulated drugs.

In summary, computational absorption modeling offers an efficient and cost-effective way to assess drug bioperformance in a relatively short time frame, thus becoming an indispensable tool that facilitates formulation development process. However, certain gaps still exist, mostly concerning the lack of relevant information on drug and dosage form properties required for accurate prediction of drug PK profile. Also, lack of confidence in in silico predictions is one of the reasons why these methods have not yet been adequately exploited by the industry. With the new information regarding drug biopharmaceutical properties being collected, it is expected that GI modeling will be more often used by formulation scientists. In this context, it should be stressed that large amounts of valuable data on drug biopharmaceutical properties still lie within pharmaceutical companies and regulatory agencies, and even partial access to these data would be helpful to generate and/or validate in silico absorption models. Published examples of the successful application of in silico techniques would also assist in promoting their wider acceptance.