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Research paper

Prediction of food effects on the absorption of celecoxib based on biorelevant dissolution testing coupled with physiologically based pharmacokinetic modeling

Yasushi Shono [a,b], Ekarat Jantratid [a], Niels Janssen [a,1], Filippos Kesisoglou [c], Yun Mao [c], Maria Vertzoni [d], Christos Reppas [d], Jennifer B. Dressman [a,*]

[a] Institute of Pharmaceutical Technology, Goethe University, Frankfurt am Main, Germany
[b] Analytical Development Laboratories, Takeda Pharmaceutical Company, Ltd., Osaka, Japan
[c] Department of Pharmaceutical Research, Merck & Co., Inc., West Point, PA, USA
[d] Laboratory of Biopharmaceutics and Pharmacokinetics, University of Athens, Greece

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ABSTRACT

Since the rate-determining step to the intestinal absorption of poorly soluble drugs is the dissolution in the gastrointestinal (GI) tract, postprandial changes in GI physiology, in addition to any specific interactions between drug and food, are expected to affect the pharmacokinetics and bioavailability of such drugs. In this study, *in vitro* dissolution testing using biorelevant media coupled with *in silico* physiologically based pharmacokinetic (PBPK) modeling was applied to the prediction of food effects on the absorption of a poorly soluble drug, celecoxib, from 200 mg capsules. A PBPK model was developed based on STELLA® software using dissolution kinetics, solubility, standard GI parameters and post-absorptive disposition parameters. Solubility, dissolution profiles and initial dissolution rate from celecoxib 200 mg capsules were measured in biorelevant and compendial media. Standard GI parameters (gastric emptying rate and fluid volume) were varied according to the dosing conditions. Disposition parameters were estimated by fitting compartmental models to the oral PK data, since intravenous data are not available for celecoxib. Predictions of food effects and average plasma profiles were evaluated using the AUC and C_{max} and the difference factor (f_1). An approximately 7-fold difference in the maximum percentage dissolved was observed in *in vitro* dissolution tests designed to represent the fed and fasted states. By contrast, the food effect estimated by simulating the plasma profiles with the PBPK model predicted only a slight delay in the peak plasma level (~1 h), and modest increases in the C_{max} and AUC of ~1.9-fold and 1.3-fold in the fed state, respectively. The PBPK approach, combining *in silico* simulation coupled with biorelevant dissolution test results, thus corresponds much better to the food effect observed for celecoxib *in vivo*. Additionally, point estimates of AUC and C_{max} as well as f_1 calculations demonstrated clear advantages of using results in biorelevant rather than compendial media in the PBPK model.

1. Introduction

The first step in the intestinal absorption process from an orally administered dosage form is drug dissolution in the gastrointestinal (GI) tract. For poorly soluble drugs, especially those with high intestinal permeability, dissolution can be considered as the rate-determining step to drug absorption.

In vivo dissolution of the drug depends not only on the physicochemical characteristics of the drug (particle size, molecular size, solubility, etc.), but also on the physiological conditions (motility, available fluid volume, fluid viscosity, food components, etc.).

One approach to improve the oral bioavailability of lipophilic drugs is to administer them with a meal [1,2]. However, the food effect on drug absorption is rather complex, as it can involve specific interactions between the drug and food as well as the physiological changes in the GI tract between the prandial states [3,4]. Various strategies for predicting food effects on intestinal absorption have been reported in the literature [5]. Using the Biopharmaceutics Classification System (BCS), about 70% of compounds could be correctly categorized into three groups according to the food effect observed (no effect, negative effect and positive effect) [6]. On the other hand, Custodio et al. suggested utilizing the Biopharmaceutical Drug Disposition Classification System (BDDCS) to predict food effects on the extent of drug availability, since these can be influenced by the inhibition of transporters and metabolism with

* Corresponding author. Institute of Pharmaceutical Technology, Goethe University, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany. Tel.: +49 69 798 29680; fax: +49 69 798 29694.
E-mail address: dressman@em.uni-frankfurt.de (J.B. Dressman).
[1] Present address: BAG Health Care GmbH, Lich, Germany.

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high-fat meals [7]. Gu et al. established a more predictive statistical model based on physicochemical parameters of the drug and dosing conditions [8]. Since all these predictions are based solely on drug substance properties, they may well be useful in the early drug screening stage, but formulation effects on dissolution kinetics and physiological factors in the GI tract which can also affect intestinal absorption of the drug are not addressed.

In vitro dissolution tests using biorelevant media has proven to be a useful tool to predict *in vivo* performance of drug products [9]. Dissolution media simulating pre- and post-prandial states in the small intestine, Fasted State Simulated Intestinal Fluid (FaSSIF) and Fed State Simulated Intestinal Fluid (FeSSIF) have been applied to establish *in vitro–in vivo* correlations (IVIVC) of oral dosage forms [10–12]. Recently, the media compositions have been updated, based on human aspirate data, to reflect the physiology in the GI tract more closely than the previous versions [13]. Combined with rational selection of apparatus, the hydrodynamics and physiologically reasonable media volumes, it should be possible to mimic the conditions in the GI tract more closely and investigate the *in vivo* performance of drug products in the fasted and fed states. Recently, successful predictions of orally administered drugs using *in silico* PBPK modeling softwares, e.g., GastroPlus®, PK-Sim®, etc., have also been reported [14–16]. These *in silico* techniques use physiological parameters based on the prandial states and available *in vitro* data of drug (solubility, dissolution, permeability, metabolism and disposition of the drug) to simulate the plasma profiles. They are becoming a powerful tool in drug research and development for the quantitative prediction of PK profiles.

In this study, the *in vitro* biorelevant dissolution tests were coupled with *in silico* PBPK modeling using the STELLA® software to simulate plasma profiles and hence predict food effects on the absorption of a lipophilic model compound in humans. Celecoxib, a cyclooxygenase-2 (COX-2)-specific inhibitor with poor aqueous solubility, was chosen as the model compound in this study. Although it is categorized as BCS Class II (low solubility–high permeability) [17], there is only a modest increase in bioavailability when the capsules are ingested in the fed state to healthy adult volunteers [18]. This behavior deviates from the positive food effect generally observed for the BCS Class II compounds and thus provided a useful case example for combining *in vitro* with *in silico* techniques.

2. Materials and methods

2.1. Chemicals and reagents

Celebrex® 200 mg capsules (Pfizer Inc., lot C080351) were purchased commercially. Celecoxib drug substance (lot 070901) was purchased from Kemprotec Ltd., Middlesbrough, UK. Long-life, heat-treated and homogenized milk (UHT milk) containing 3.5% fat (Milfina Hochwald, Kaiserslautern, Germany) was purchased commercially. Glyceryl monooleate (GMO, Rylo M19 Pharma®, 99.5% monoglyceride, lot 173403-2202/107) was kindly donated by Danisco Specialities, Brabrand, Denmark. Egg phosphatidylcholine (Lipoid E PC®, 99.1% pure, lot 108015-1/42) was kindly donated by Lipoid GmbH, Ludwigshafen, Germany. 37% hydrochloric acid (conc. HCl) and pepsin (Ph. Eur., 0.51 U/mg, lot 1241256) were obtained from Fluka Chemie AG, Buchs, Switzerland. Maleic acid (99% pure, lot 4039128) was purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Sodium oleate (82.7% pure, lot 51110) was obtained from Riedel-de Haën, Seelze, Germany. Sodium taurocholate (NaTC, 97% pure, lot 2007100274) was used as received from Prodotti Chimici Alimentari SpA, Basaluzzo, Italy. Sodium hydroxide solution (0.1 N NaOH) and hydrochloric acid solution (0.1 N HCl) were purchased from VWR International GmbH (Darmstadt, Germany). Dichloromethane, acetonitrile, glacial acetic acid, sodium acetate trihydrate, sodium chloride (NaCl), potassium dihydrogen phosphate and NaOH pellets were all of analytical grade and purchased from Merck KGaA (Darmstadt, Germany).

2.2. Media preparation

The compositions and the preparation procedures of the media used for dissolution tests and solubility have been described previously [13,19,20]. Fasted State Simulated Gastric Fluid (FaSSGF), as described by Vertzoni et al. [20], was used to represent the fasted gastric conditions. A recently developed medium, Fed State Simulated Gastric Fluid (FeSSGF), which is a mixture of buffer solution and UHT milk (50:50), was used to simulate the gastric conditions postprandially [13]. Simulated Gastric Fluid without pepsin (SGF_{sp}) (USP 31) was used as a control. For the upper small intestine, updated versions of Fasted State Simulated Intestinal Fluid (FaSSIF-V2) and Fed State Simulated Intestinal Fluid (FeSSIF-V2) in addition to their predecessors (FaSSIF and FeSSIF) were used. The compositions of these media were recently revised to be more biorelevant, based on analysis of human aspirate samples [13]. Simulated Intestinal Fluid without pancreatin (SIF_{sp}) (USP 31) was also used as a compendial control. Tables 1 and 2 summarize the compositions of the simulated gastric and intestinal media used in this study.

2.3. Analytical methods

2.3.1. The high-performance liquid chromatography (HPLC) system

The samples obtained from solubility and dissolution tests were quantitatively analyzed for celecoxib concentration using an isocratic HPLC system. The HPLC system consisted of a pump (Merck Hitachi L7100), an autosampler (Merck Hitachi L-7200) and a UV detector (Merck Hitachi L-7400). The chromatograms were evaluated with EZChrom Elite™ Version 2.8 Software (Biochrom Ltd., Cambridge, UK). Analytical column used was YMC-Pack Pro C18 100 mm × 4.6 mm I.D., S-3 μm (YMC Co., Ltd., Kyoto, Japan). The mobile phase comprised 55% acetonitrile and 45% water with the flow rate 1.0 mL/min. The injection volume was 10 μL. The detection wavelength was set at 254 nm. The analysis was performed under ambient conditions.

2.4. Solubility measurements

The shake-flask method was employed for celecoxib solubility determination in all media. Measurement was performed by adding an excess amount of the drug substance to a medium in a glass vial. The vial was incubated in a water bath at 37 °C and shaken vigorously at appropriate intervals. Samples were taken after at

Table 1
Composition of the gastric media.

	SGF_{sp}	FaSSGF	FeSSGF
Composition			
Sodium taurocholate (mM)	–	0.08	–
Lecithin (mM)	–	0.02	–
Pepsin (mg/mL)	–	0.1	–
Sodium chloride (mM)	34.2	34.2	237.0
Hydrochloric acid (mM)	71.0	25.1	–
Glacial acetic acid (mM)	–	–	17.1
Sodium acetate (mM)	–	–	29.8
Milk/buffer	–	–	1/1
Characteristic parameter			
pH	1.2	1.6	5.0
Osmolality (mOsm kg^{-1})	–	120.7 ± 10	400 ± 10
Buffer capacity (mmol L^{-1} ΔpH^{-1})	–	–	25 ± 2

Table 2
Composition of the small intestinal media.

	SIF_{sp}	FaSSIF-V2	FaSSIF	FeSSIF-V2	FeSSIF
Composition					
Sodium taurocholate (mM)	–	3	3	10	15
Lecithin (mM)	–	0.2	0.75	2	3.75
Glycerol monooleate (mM)	–	–	–	5	–
Sodium oleate (mM)	–	–	–	0.8	–
Maleic acid (mM)	–	19.12	–	55.02	–
Monobasic sodium phosphate (mM)	–	–	28.36	–	–
Monobasic potassium phosphate (mM)	49.97	–	–	–	–
Glacial acetic acid (mM)	–	–	–	–	144.05
Sodium hydroxide (mM)	45	34.80	8.7	81.65	101
Sodium chloride (mM)	–	68.62	105.85	125.5	203.18
Characteristic parameter					
pH	6.8	6.5	6.5	5.8	5.0
Osmolality (mOsm kg^{-1})	–	180 ± 10	270 ± 10	390 ± 10	670 ± 10
Buffer capacity (mmol L^{-1} ΔpH^{-1})	–	10 ± 2	10 ± 2	25 ± 2	76 ± 2

least 6 h and filtered through PVDF membrane filters having a pore size of 0.45 μm (25 mm GD/X, Whatman). The filtrate was diluted immediately with the mobile phase and then analyzed by HPLC. For FeSSGF, with milk as a major component, the use of filters with a small pore size was not possible. Glass syringe filters with a pore size of 2.7 μm (25 mm GD/X, Whatman) were used to separate non-dissolved drug from the samples. Then, 1 mL of acetonitrile was added to 0.5 mL of the filtrate, mixed well and the mixture centrifuged at 7500 rpm for 3 min. The supernatant was used for the assay.

2.5. Dissolution testing of celecoxib capsules

2.5.1. USP apparatus II (paddle assembly)

The dissolution conditions consisted of a medium volume of 500 mL per vessel with a paddle revolution speed of 50 rpm. The temperature in the vessels was 37 ± 0.5 °C throughout each dissolution run. Experiments were conducted at least in triplicate. A wire helix sinker was used to prevent the capsule from floating. Sampling was performed manually using glass syringes connected with the stainless steel sampling devices. The samples were withdrawn through cylindrical polyethylene filter sticks having a porosity of 10 μm fitted directly on the end of sampling devices. The volume withdrawn was approximately 5 mL for each sampling time point. The samples were then filtered through a 0.45 μm PVDF filter (25 mm GD/X, Whatman) into the test tubes, after discarding the first 2 mL, and the subsequent filtrate was assayed by HPLC.

Pretreatment for FeSSGF samples was performed as the same procedure as in the solubility measurements.

2.5.2. Mini-paddle assembly

To simulate the volume availability in the fasted stomach, the mini-paddle apparatus was applied for dissolution testing in FaSSGF and SGF_{sp}. The mini-paddle apparatus is based on the USP paddle setup but scaled down geometrically with respect to the dimensions [21], so that hydrodynamics remain essentially similar at a given rpm. The dissolution conditions consisted of a medium volume of 250 mL per vessel with a paddle revolution speed of 50 rpm.

2.6. Determination of initial dissolution rates

The initial dissolution rates were estimated from additional dissolution experiments with more intensive sampling using the mini-paddle assembly for FaSSGF and SGF_{sp}, and the compendial paddle assembly for the other media. The dissolution conditions were the same as mentioned above. For the experiments in FeSSGF, celecoxib granules were removed from the capsule shell and dispersed into the vessel, since milk is known to delay the disintegration time of some capsule-type drug products [22].

2.7. Available pharmacokinetic data and assessments

The plasma drug concentration–time profiles were taken from the literature [18]. In this study, celecoxib 200 mg capsules were administered to 24 healthy adult subjects under fasting conditions or immediately after ingestion of a high-fat breakfast (1000 kcal) in a single-dose, randomized crossover study. Each dose was administered with 210 mL of water. Plasma drug concentrations were estimated directly from the profile, and disposition parameters were estimated based on the oral two-compartment model (WinNonlin® Model 12) as shown in Table 3.

Pharmacokinetic analysis was performed using WinNonlin® Professional Edition 4.1 software (Pharsight Corporation, Mountain View, CA, USA). Since intravenous administration data for celecoxib are not available, post-absorptive disposition parameters needed for the simulation were estimated by fitting for *in vivo* mean plasma concentration–time curves after oral administration. Both observed and simulated plasma celecoxib concentration–time profiles were evaluated to determine the following pharmacokinetic parameters based on non-compartmental analysis: area under the plasma concentration–time curve (AUC), the peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}).

2.8. Computer simulation of plasma celecoxib profiles

The plasma celecoxib profiles were simulated using the STELLA® 9.0 software (Cognitus Ltd., North Yorkshire, UK). The model structure in this simulation was the same as that published previously [23]. Negligible absorption from the stomach, simultaneous solid and liquid emptying from the stomach, and no intestinal permeability restrictions were assumed. In this study, the initial volume of fluid in the stomach was estimated to be 210 mL, which is the volume of co-administered water in the fasted state, and 700 mL, which is approximately the volume of co-administered water plus the meal in the fed state, based on the study protocol from the literature [18]. First-order gastric emptying rate in the fasted state at 2.8 h^{-1} and zero-order gastric emptying rate in the fed state at 4 kcal/min were used in accordance with the average population values [24]. The amount of drug entering the plasma, i.e., contributions from dissolved drug emptied from the stomach and drug dissolved in the small intestine, was translated into plasma concentration using the volume of distribution (V), taking into account the bioavailability factor (F). In this study, V/F values estimated with WinNonlin® from oral administration data were used for the simulation.

Table 3
Post-absorptive disposition parameters after oral administration of celecoxib 200 mg capsules in the fasted and fed states.

	Correlation[1](observed *vs.* predicted)	Disposition parameters			
		V/F[2](mL)	k_{10} (h^{-1})	k_{12} (h^{-1})	k_{21} (h^{-1})
Fasted	0.998	118,037	0.258	0.234	0.082
Fed	0.995	91,538	0.289	0.122	0.081

[1] Correlation between *in vivo* observed data and predicted profile estimated based on oral two-compartment model (WinNonlin® Model 12).
[2] Volume of distribution (corrected for the bioavailability) obtained from PK profiles after oral administration.

To estimate *in vivo* dissolution kinetics, an *in vitro* dissolution parameter was introduced into the model by applying a modification of the Noyes–Whitney equation [9]. This model is based on the assumption of dissolution of isometric, similarly sized particles, occurring under continuously decreasing surface area conditions with the ratio D/δ, where D is the diffusion coefficient and δ is the diffusion layer thickness, which is assumed to remain constant during the dissolution process. Dissolution rate is given by the following Eq. (1):

$$\frac{dW_t}{dt} = \frac{D\Gamma N^{1/3}}{V\delta\rho^{2/3}} W^{2/3}(X_s - W_t) = zW^{2/3}(C_s - C) \quad (1)$$

where W_t is the amount dissolved at time t, W is the amount of drug remaining to be dissolved, Xs is the amount of drug which saturates the volume V of the dissolution medium, C_s is the solubility of drug, C is the concentration of the dissolved drug at time t, ρ is the particle density, Γ is the shape factor, N is the number of particles to be dissolved and z is the dissolution parameter, which is a constant equal to $D\Gamma N^{1/3}/\delta\rho^{2/3}$. Whereas sink conditions were assumed in the small intestine, dissolution in the stomach was assumed to occur until the drug concentration reached its solubility (since it is assumed that there is no absorption from the stomach).

2.9. Plasma concentration profile comparison

The difference factor (f_1), which is a model-independent approach, was applied for the comparison of the plasma concentration–time profiles of celecoxib capsules obtained in the fed and fasted states. Eq. (2) was used to calculate the f_1 value for the plasma concentration–time profiles obtained in this study as follows:

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right\} \times 100 \quad (2)$$

where n is the number of time points, R is the *in vivo* plasma drug concentration at time t, and T is the simulated plasma drug concentration at time t.

3. Results and discussion

3.1. Solubility of celecoxib in biorelevant and compendial media

Fig. 1 shows the solubility of celecoxib in biorelevant and compendial media. Celecoxib is lipophilic (log $P = 3.5$) and poorly soluble (practically insoluble in water). Although it is an acidic compound, its high p*K*a at 11.1 renders it essentially neutral under GI physiological pH range [25]. Solubility in SGF_{sp}, SIF_{sp} and even in FaSSGF, which contains small amounts of bile salt and lecithin, was poor (less than 0.003 mg/mL). Solubility in FeSSGF, which is the buffer solution containing 50% milk, is much higher than in FaSSGF. Celecoxib is likely to be incorporated into casein micelles and possibly bound to milk lipids and proteins, all of which may contribute to solubility *in vivo*. For the small intestinal media, both versions of FaSSIF and FeSSIF enhanced the drug solubility, with the main effect being the mixed-micelle levels. The solubility ratio of FeSSIF-V2/FaSSIF-V2 and FeSSIF/FaSSIF was 8.9 and 2.4, respectively. Solubility of celecoxib in FaSSIF was about 3-fold higher than in FaSSIF-V2. As the only difference between the two media is the lecithin concentration (FaSSIF-V2: 0.2 mM, FaSSIF: 0.75 mM), it appears that lecithin plays a crucial role in celecoxib solubility. On the other hand, no significant difference was observed between FeSSIF-V2 and FeSSIF. The effect of the mixed-micelles of bile salt and lecithin combined with the lipolysis products (glyceryl monooleate and sodium oleate) in FeSSIF-V2 appear to compensate for the decrease in sodium taurocholate concentration.

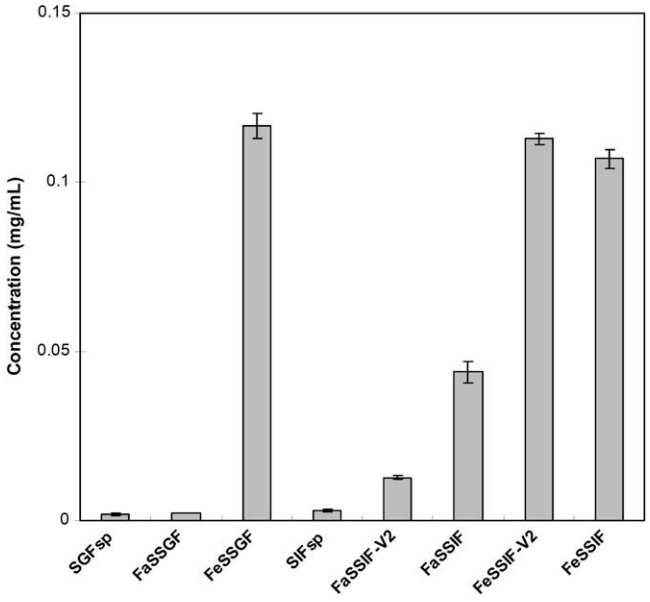

Fig. 1. Solubility of celecoxib in biorelevant and compendial media at 37 °C.

3.2. Dissolution of celecoxib in biorelevant and compendial media

Fig. 2a shows the dissolution profiles of celecoxib in the gastric media simulating the preprandial and postprandial gastric conditions in this study. Dissolution of celecoxib in FaSSGF and SGF_{sp} (control) was negligible. While the extent of dissolution in FeSSGF was much higher than FaSSGF, disintegration and dissolution rate of celecoxib capsules in FeSSGF proceeded more slowly than in other media. Milk is known to delay the disintegration time of other drug products [22,26].

Fig. 2b shows the dissolution profiles of celecoxib in SIF_{sp} (control) and both versions of FaSSIF and FeSSIF. Dissolution profiles in FaSSIF-V2 and FaSSIF were markedly different from each other. FeSSIF-V2 gave a comparable dissolution profile to FeSSIF. The plateau concentrations in both versions of FaSSIF and FeSSIF were in good agreement with the solubility data. All dissolution profiles in the small intestinal media reached the plateau phase within 30 min. The difference in the extent of dissolution between FaSSIF and FeSSIF reflects the typical pattern of poorly soluble drugs with positive food effects (e.g., danazol [10], atovaquone [10], troglitazone [27]. It is reasonable that the dissolution of hydrophobic compounds such as celecoxib (log $P = 3.5$) is dependent on the mixed-micelle levels in the medium: the maximum concentration of celecoxib in FeSSIF-V2 was about 7-fold higher than that in FaSSIF-V2. However, results of *in vivo* food effect studies in healthy volunteers are not consistent with the extent of dissolution in biorelevant media *in vitro*. When celecoxib 200 mg capsules are administrated with a high-fat breakfast, the systemic exposure of celecoxib as measured by the AUC and the C_{max} increased only slightly, i.e., to 110.7% and 125%, respectively, compared with administration in the fasted state. The extent of dissolution *in vitro* appears to have been limited by the solubility of celecoxib combined with the fixed volume of media available. *In vivo*, by contrast, it is likely that sink conditions were generated by the high permeability of celecoxib (estimated bioavailability at least 73% [28]) shifting the rate-limiting factor to absorption from the solubility of the compound to its dissolution rate. To compensate for the closed system conditions in the *in vitro* experiments, the initial dissolution rates were determined in each medium and coupled with an *in silico* model, as described in the next section.

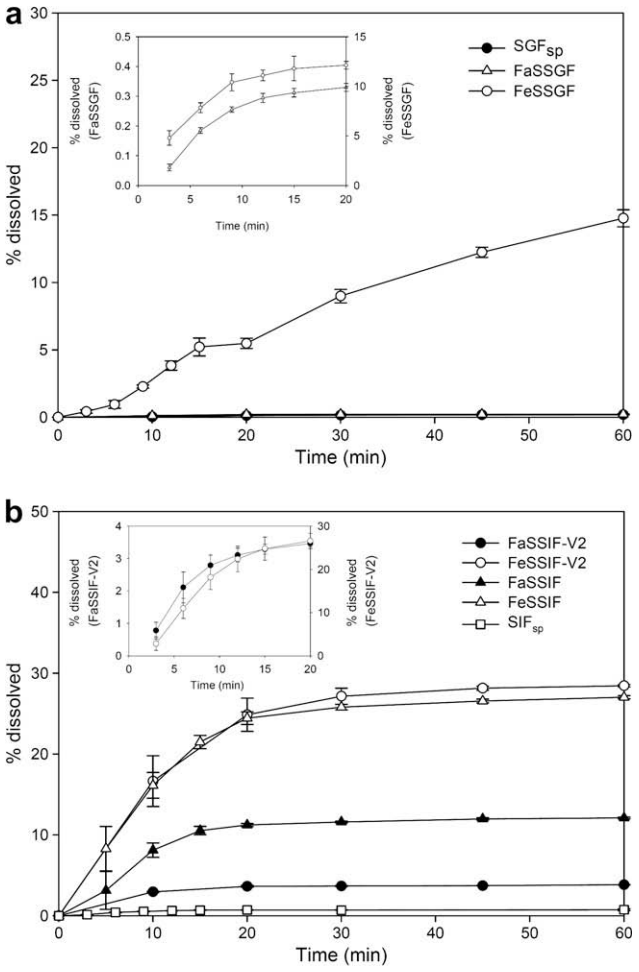

Fig. 2. Dissolution of celecoxib 200 mg capsules in (a) the gastric and (b) the small intestinal media. The inset depicts the initial dissolution profiles obtained with more intensive sampling of celecoxib 200 mg capsules (with the granules removed from the capsule shell in the case of FeSSGF), in order to estimate the *z* value.

3.3. Simulation of the plasma profiles using STELLA® software

To quantitatively predict the food effect on oral absorption of celecoxib capsules in humans, STELLA® software was coupled with the dissolution results to simulate the plasma profiles. This model requires *in vitro* dissolution parameter (*z* value), solubility, standard GI parameters in accordance with dosing conditions and post-absorptive disposition pharmacokinetic parameters. The *z* value was estimated based on an initial dissolution curve obtained with more intense sampling (see Fig. 2 inserts). Additionally, "infinity" values from dissolution studies were also determined by performing the dissolution test at 250 rpm for at least 30 min after the final time point and employed as the effective solubility of celecoxib in the formulation. The *z* values and celecoxib solubilities are given in Table 4. Standard GI parameters were estimated according to the literature [18]. As shown in Table 3, post-absorptive PK parameters had to be estimated from the observed PK profiles after oral administration using the WinNonlin® software. This model further assumes that the intestinal permeability of the compound categorized into BCS Class II is very high (for celecoxib, it is classified as 'highly permeable' based on Caco-2 permeability data [17]), and therefore poses no restriction to absorption.

Fig. 3 shows the mean observed plasma concentration data and the STELLA® simulated profiles of celecoxib 200 mg capsules in the fasted and fed states. Pharmacokinetic parameters calculated from

Table 4
Dissolution parameter (*z* value) and solubility of celecoxib in several media.

Medium	*z* value(mL/mg$^{2/3}$/h)	Solubility(mg/mL)
SGF_{sp}	0.425 ± 0.057	0.0025 ± 0.0001
FaSSGF	0.891 ± 0.171	0.0023 ± 0.0001
FeSSGF	0.096 ± 0.017	0.1015 ± 0.0002
SIF_{sp}	0.266 ± 0.053	0.0032 ± 0.0001
FaSSIF-V2	0.319 ± 0.063	0.0153 ± 0.0002
FaSSIF	0.380 ± 0.043	0.0462 ± 0.0003
FeSSIF-V2	0.300 ± 0.069	0.1131 ± 0.0011
FeSSIF	0.265 ± 0.008	0.1033 ± 0.0005

the simulated profiles generated by the STELLA® program are summarized in Table 5. It was found that the simulated profile in the fasted state fit well with the observed profile, with the AUC, C_{max} and T_{max} in good agreement with the *in vivo* data. On the other hand, although the profile in the fed state was also predicted well by the simulation, the AUC and C_{max} values calculated from the simulated profile slightly overestimated the observed values. This might be due to the 'highly permeable properties' of celecoxib [17] slightly diverging from the assumption of 'no intestinal permeability restrictions' applied for the STELLA model.

The food effect estimated by coupling the *in vitro* data with the STELLA® program suggests that peak plasma levels would be delayed for about 1 h, with an increase in the AUC and C_{max} of up to about 1.4- and 1.9-fold, respectively. The simulation approach coupled with biorelevant dissolution tests thus corresponds much better to the actual *in vivo* data.

As mentioned earlier, the underlying reason for the apparent discrepancy between predictions based solely on dissolution and the STELLA®-based predictions is that the dissolutions were performed in a closed volume setup, which limits the extent of dissolution. However, the dissolution process, which is the rate-determining step for poorly soluble–highly permeable drugs *in vivo*, likely occurs under sink conditions. The STELLA® model, which combines the dissolution rates *in vivo* with the physiological kinetics, can thus come closer to the *in vivo* processes.

3.3.1. Biorelevant or compendial media?

Fig. 4a shows a comparison of the simulated profiles of plasma celecoxib concentrations generated from different media combinations in the fasted state. The simulated profile using dissolution results in FaSSGF/FaSSIF-V2 is in good agreement with the *in vivo* profile. When SGF_{sp} instead of FaSSGF was used for the simulation

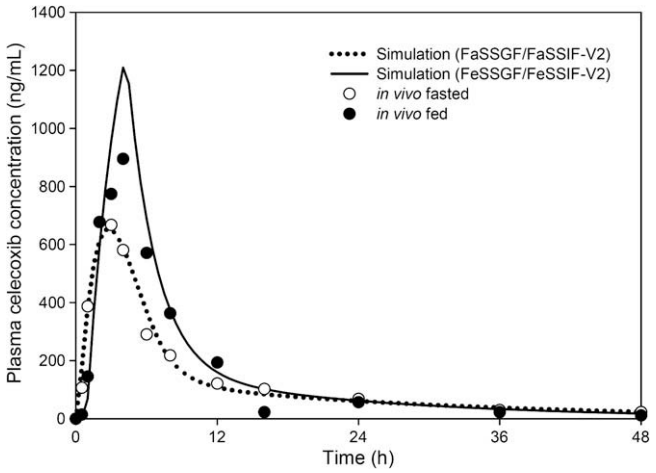

Fig. 3. Simulated plasma celecoxib concentration–time profiles in the fasted and fed states.

Table 5
Summary of pharmacokinetic parameters and food effects.

	Fasted				Fed				Ratio(fed/fasted)		
	T_{max}	C_{max}	AUC_{0-48}	AUC_{inf}	T_{max}	C_{max}	AUC_{0-48}	AUC_{inf}	C_{max}	AUC_{0-48}	AUC_{inf}
Reported[1]	2.4	806	5994	6564	3.4	1042	7141	7318	1.3	1.2	1.1
Calculated[2]	2.0	677	6064	6522	4.0	895	6961	7131	1.3	1.1	1.1
Predicted[3]	3.0	654	5958	6560	4.0	1210	8160	8506	1.9	1.4	1.3

[1] From the literature [18].
[2] Values calculated from *in vivo* data using WinNonlin® (non-compartmental analysis).
[3] Values calculated from the simulated profiles using WinNonlin® (non-compartmental analysis).

in the fasted state, essentially similar dissolution profiles in SGF_{sp} and FaSSGF led to the same simulated profiles (data not shown). On the other hand, using FaSSIF data, with 3-fold higher celecoxib solubility than FaSSIF-V2, the simulated profile shows a shorter T_{max} and an increased C_{max}. Simulated profiles generated with the combinations of FaSSGF/SIF_{sp} obviously underestimated the *in vivo* profiles due to poor solubility of celecoxib in SIF_{sp}. These results indicate that simulation of celecoxib plasma profiles in the fasted state is highly dependent upon the solubility in the medium chosen to represent the composition of fluids in the small intestine (SIF_{sp} *vs.* biorelevant media).

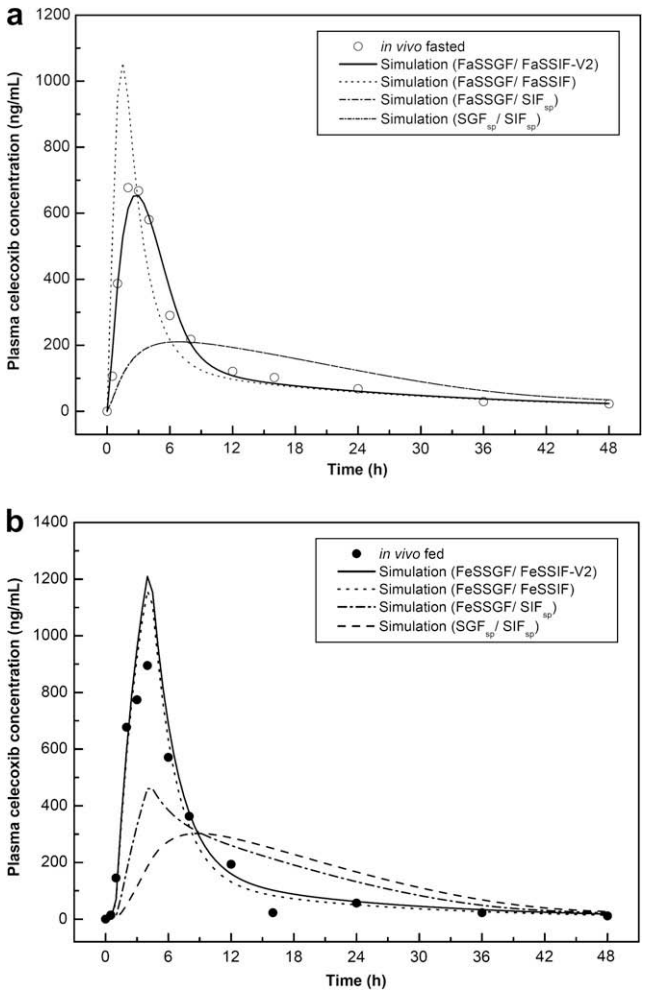

Fig. 4. Simulated plasma celecoxib concentration–time profile generated from the various combinations of dissolution media in (a) the fasted state(noting that simulated profiles generated from FaSSGF/SIF_{sp} and SGF_{sp}/SIF_{sp} are superimposable) and (b) fed state.

Fig. 4b shows the comparison of simulated and observed plasma celecoxib profiles following administration of celecoxib 200 mg capsules in the fed state. Like in the fasted state, the simulated profiles were generated from the various combinations of the media. It was found that even when the fed gastric medium, FeSSGF, was replaced by the compendial medium, SGF_{sp}, in which celecoxib exhibited extremely poor dissolution, no significant change was observed in the simulated profiles (data not shown). This result suggests that dissolution from celecoxib capsules in the stomach does not play an important role in the intestinal absorption of celecoxib. On the other hand, the profiles simulated from the combinations of FeSSGF/SIF_{sp} and SGF_{sp}/SIF_{sp} are much different from the observed data *in vivo*. Only when FeSSIF media (V2 and previous versions) were used did the results concur with the *in vivo* profiles. In summary, it is clear that the biorelevant media combinations provide strong advantages over compendial media in both the prandial states.

3.3.2. In vitro–in vivo–in silico relationships (IVIVIS-R)

To assess the simulation approach in this study further, mean observed plasma celecoxib concentration–time profiles (*in vivo vs. in silico*) were compared with simulated profiles both directly [using the difference factor (f_1)] and after estimation of major pharmacokinetic parameters (AUC and C_{max}) (Table 6).

To give a preliminary indication of bioequivalence, the ratios of AUC(test)/AUC(reference) and C_{max}(test)/C_{max}(reference) should be between 0.80 and 1.25 [29]. In the fasted state, the AUC and C_{max} ratios met the acceptance criteria (Table 6). In the fed state, the AUC and C_{max} ratios were borderline or just beyond the acceptance criteria (Table 6). Although bioequivalence criteria for f_1 have not been defined, it is worth noting that comparison of results using f_1 leads to similar conclusions if one uses the 20% difference between the two profiles as the cut-off limit (Table 6).

The *in vivo* Fa (fraction drug absorbed in the time-frame from the end of the lag time to the time at which 90% had been absorbed) and the *in silico* Fa values in the each prandial state were also correlated on a real-time basis. The regression analysis results are summarized in Table 6. The correlation between observed and simulated data was linear in each prandial state and represented a point-to-point relationship.

Table 6
Predictability of food effect based on *in silico* PBPK modeling.

	f_1[1]	Ratio		Correlation[4] (Observed Fa *vs.* simulated Fa)	
		C_{max}[2]	AUC_{0-48}[3]	Regression curve	Coefficient (R)
Fasted	9	0.96	0.98	$y = 0.98x + 0.01$	0.999
Fed	23	1.35	1.17	$y = 1.08x - 0.06$	0.997

[1] The difference factor (actual *in vivo* data *vs.* simulated data at the same time-points).
[2] C_{max} (predicted)/C_{max} (calculated).
[3] AUC_{0-48} (predicted)/AUC_{0-48} (calculated).
[4] Fraction absorbed (Fa) was compared between observed and simulated profiles in the time-frame from immediately after the lag time to Fa = 90%.

3.3.3. Sensitivity analysis using the STELLA model

During the preformulation stage of poorly soluble drugs, improvement of dissolution is one approach to overcome the rate-limiting step to oral absorption. Formulation technologies such as nanosizing, amorphous system and lipid formulation, all of which are intended to result in very fast dissolution, are well known to enhance bioavailability and minimize food effect for BCS Class II compounds. Fig. 5 shows the effect of dissolution rate of the intestinal dissolution on the simulated profiles. Simulated profiles were generated with various z values in the fasted and fed states, expressed relative to the z values used for the simulations (z value in FaSSGF/FaSSIF-V2 in the fasted state and FeSSGF/FeSSIF-V2 in the fed state, respectively). The results show that the simulated profiles in the fasted state are highly dependent upon the z value of intestinal dissolution as well as solubility in the media simulating small intestinal fluid. If the z value were smaller than the value in FaSSIF-V2, C_{max} in the fasted state would be clearly decreased and hence the food effect is likely to be more significant. On the other hand, profiles generated with different z values are almost the same in the fed state. These results indicate that dissolution rate is no longer a limiting factor to the intestinal absorption in the fed state. By contrast, when profiles in the fed state were generated with various gastric emptying rates (Fig. 6), intestinal absorption of celecoxib in the fed state was highly influenced.

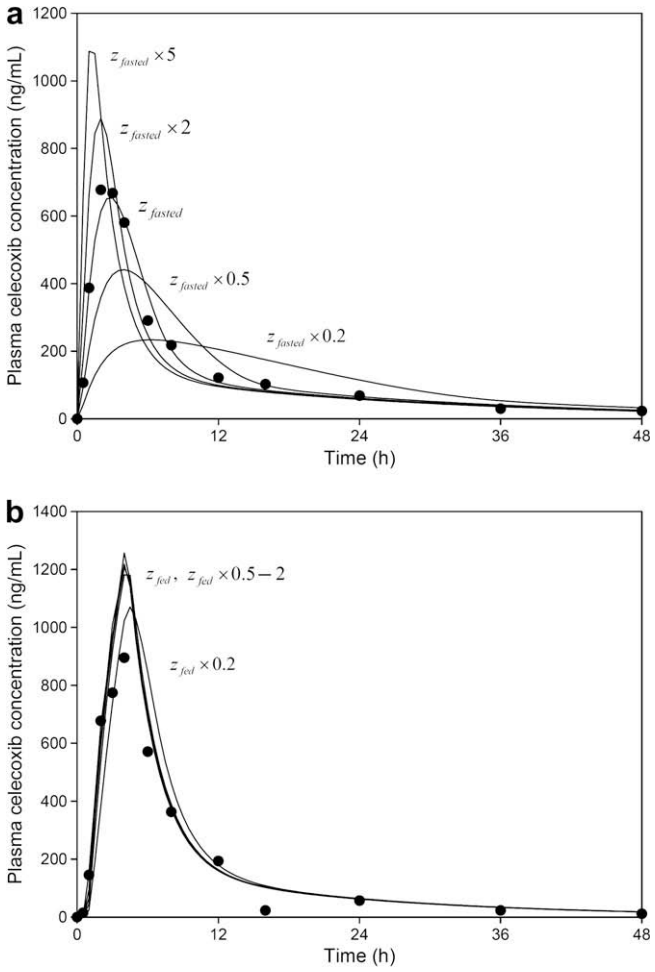

Fig. 5. Effect of dissolution rate on simulated plasma concentration–time profiles of celecoxib in (a) the fasted state and (b) fed state. z_{fasted} = ratio of the z value used in the simulation to the z value observed in the dissolution tests in FaSSIF-V2. z_{fed} = ratio of the z value used in the simulation to the z value observed in the dissolution tests in FeSSIF-V2.

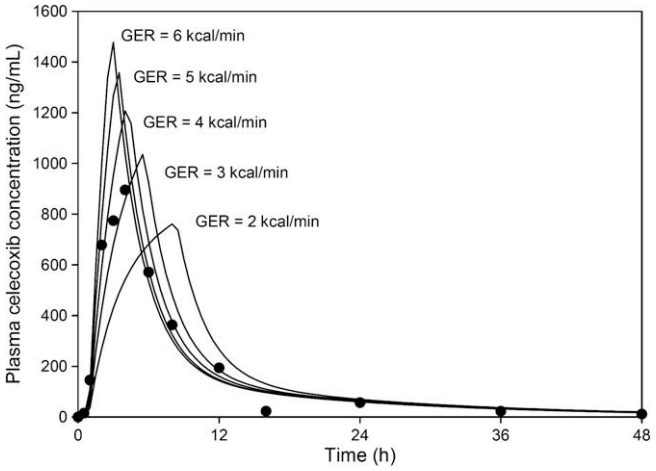

Fig. 6. Effect of the gastric emptying rate on simulated plasma concentration–time profiles of celecoxib in the fed state.

In summary, relatively fast drug release from the capsule formulation in the fasted state coupled with high absorbability throughout the GI tract appears to result in the rather moderate food effect observed for celecoxib.

4. Conclusions

In vitro biorelevant dissolution testing coupled with *in silico* simulation technology was applied successfully to predict the *in vivo* performance of celecoxib capsules in this study. *In vitro* results in biorelevant media predicted very different extents of celecoxib dissolution in the fasted and fed states, owing to different mixed-micelle levels in the media. However, biorelevant testing alone proved inappropriate to predict the *in vivo* behavior based only on the extent of dissolution in a closed setup system. By contrast, dissolution testing (combination data of the gastric and small intestinal media) coupled with the simulation software predicted the plasma profiles of celecoxib well. The results also indicate that the simulated plasma profiles generated from biorelevant media combinations show strong advantages over using the compendial media and should therefore be especially useful in course of formulation development. Simulation models additionally provide the possibility of investigating the effects of variations in formulation/GI physiology without extensive additional *in vivo* or *in vitro* testing.

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