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Research Article

Theme: Advancements in Dissolution Testing of Oral and Non-Oral Formulations
Guest Editor: Sandra Klein

Physiologically Based Absorption Modeling of Salts of Weak Bases Based on Data in Hypochlorhydric and Achlorhydric Biorelevant Media

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Abstract. Physiologically based absorption modeling has been attracting increased attention to study the interactions of weakly basic drug compounds with acid-reducing agents like proton-pump inhibitors and H2 blockers. Recently, standardized gastric and intestinal biorelevant media to simulate the achlorhydric and hypochlorhydric stomach were proposed and solubility and dissolution data for two model compounds were generated. In the current manuscript, for the first time, we report the utility of these recently proposed biorelevant media as input into physiologically based absorption modeling. Where needed, data collected with the biorelevant gastrointestinal transfer (BioGIT) system were used for informing the simulations in regard to the precipitation kinetics. Using two model compounds, a HCl salt and a semi-fumarate co-crystal which as expected dissolve to a greater extent in these media (and in gastric and intestinal human aspirates) compared to what the pH–solubility profile of the free form would suggest, we demonstrate successful description of the plasma concentration profiles and correctly predicted the lack of significant interaction after administration with pantoprazole or famotidine, respectively. Thus, the data reported in this manuscript represent an initial step towards defining biorelevant input for such simulations on interactions with acid-reducing agents.

KEY WORDS: physiologically based absorption modeling; hypochlorhydria; achlorhydria; biorelevant media; GastroPlus.

INTRODUCTION

Physiologically based absorption modeling is being increasingly utilized as an integral part of the biopharmaceutics toolkit to facilitate drug product development, along with dissolution, preclinical, and clinical studies. Looking at published literature, the most common application of the models is in the formulation development field and in the prediction of food effects. However, there is increased interest to expand use of these models in other clinical-related questions that are dictated by physicochemical properties of the compound/formulation; as such interactions with acid-reducing agents have been attracting increased attention.

In the last few years, there has been a number of physiologically based pharmacokinetics (PBPK) publications specifically looking at the impact of gastric pH on absorption. Mitra et al. reported the use of physiologically based absorption modeling, along with preclinical studies, to investigate the impact of gastric pH on absorption of a proprietary BCS II weak base and to facilitate development of an acid-resistant formulation (1). The free base pH–solubility profile and dissolution data in dilute HCl-based media (pH 3.0 HCl/NaCl) were used as input. Bhattachar et al. utilized similar models, using the pH–solubility profile as input, to understand impact of gastric pH on absorption for a weak base, LY2157299 (2). Similar approaches were undertaken by Chung et al. for ARRY-403 and Parrot et al., for alectinib, again relying on the pH–solubility profile as the primary input (3,4). A solubility-based simulation was also used for FARYDAK® (panobinostat), in the first example for which such a simulation was used to inform drug labeling (5). Cristofoletti et al. reported PBPK models for ketoconazole and posaconazole incorporating the knowledge of the measured stomach pH in the clinical trial in their assessment (6). More recently, Lu et al. described a combination of PBPK and population PK (popPK) approaches to build

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models for the impact of gastric pH on pharmacokinetics of pictilisib; adjustment of the gastric pH and the pH–solubility profile were the main inputs in the models (7).

These publications indicate that simulating the impact of gastric pH on absorption, based on the pH–solubility profile (coupled with the API particle size), is a viable option for free base compounds formulated as fast-dissolving IR products. However, one would anticipate that incorporation of more detailed dissolution data would be critical for such simulations when dissolution kinetics of the formulation include aspects of supersaturation in the gastric fluids. This may be applicable to formulations that incorporate acidifying agents such as the one reported by Mitra et al. (1) or salts that transiently supersaturate. This transient supersaturation may result in differential absorption under the achlorhydric or hypochlorhydric conditions, as demonstrated for example for prasugrel by Seiler et al. (8). Dissolution data would also be critical for simulation of modified release systems with pH-dependent release characteristics which may be conveyed by the excipients and not just the API (9). However, there are limited reports available on such models and the few available utilize different media for the simulation of the hypochlorhydric or achlorhydric stomach. For example, while Mitra et al. (1) utilized a pH 3.0 HCl/NaCl medium, Doki et al. recently utilized data generated in buffer systems at pH 5.0, 6.0, and 7.0 for levothyroxine IR and pH 4.5 with 1% SDS and pH 6.8 for nifedipine CR tablets as input for their simulations (9).

In a recent publication, we proposed new "standardized" biorelevant media to simulate the hypochlorhydric and achlorhydric stomach, based on luminal data in humans collected after administration of pantoprazole or famotidine in a protocol simulating typical drug–drug interaction studies (10). We further compared equilibrium solubility and apparent solubility (*i.e.*, observed supersaturated concentrations during dissolution experiments) in these media and aspirates, for two salts of lipophilic, highly permeable weak bases, pioglitazone hydrochloride and semi-fumarate co-crystal of Compound B (a proprietary compound). Human gastric fluid (HGF) and human intestinal fluid (HIF) were from samples collected for the characterization of the human gastric fluids from healthy adults in the fasted state without any pretreatment (control phase), after partial inhibition of gastric acid secretion using pantoprazole (hypochlorhydric conditions, pantoprazole phase) and after complete inhibition of gastric acid secretion using famotidine (achlorhydric conditions, famotidine phase) (10).

In the current manuscript, we investigate the utility of the apparent solubility data generated in the newly proposed media (Table I) (10) to serve as input to physiologically based absorption models and assess the ability to retrospectively predict the lack of a significant interaction of salts of pioglitazone and Compound B with pantoprazole or famotidine despite the steep pH-dependent solubility of the corresponding free base.

MATERIALS AND METHODS

Materials

Compound B (semi-fumarate co-crystal) was from Merck & Co., Inc., Kenilworth, NJ, USA. Acetonitrile and water of HPLC-grade were from Sigma-Aldrich® (Chemie GmbH, Steinheim, Germany). Sodium dihydrogen phosphate dihydrate, sodium chloride, and sodium hydroxide were of analytical grade and purchased from Sigma-Aldrich® (Chemie GmbH, Steinheim, Germany). Pepsin from porcine gastric mucosa (15.8% protein) was purchased from Sigma-Aldrich (Saint Louis, USA). FaSSIF/FeSSIF/FaSSGF powder was kindly donated by biorelevant.com.

BioGIT Experiments

Biorelevant gastrointestinal transfer (BioGIT) system is an *in vitro* setup that allows the estimation of upper small intestinal concentrations of high-permeability APIs after administration in the fasted state (11). For the experiments presented, the previously described configuration was used. Specifically, the initial volume of the gastric compartment was 250 mL [a mini vessel with 500-mL capacity from Erweka (Heusenstamm, Germany) was used] and the duodenal volume was maintained at 40 mL during the entire experiment [a mini vessel with 100-mL capacity from Distek (New Brunswick, NJ, USA) was used].

One capsule containing 100 mg free base equivalent of Compound B semi-fumarate co-crystal (Merck & Co., Inc., Kenilworth, NJ, USA) was added to the gastric compartment containing 250 mL of Level III fasted state simulating gastric fluid (FaSSGF) (12). Level II fasted state simulating intestinal fluid (FaSSIF) (12) was employed in the duodenal compartment. Level III FaSSGF simulates the pH, buffer capacity, total sodium taurocholate concentration, total (lyso)phosphatidylcholine concentration, and pepsin concentration in the bulk gastric contents after administration of a glass of water in the fasted state (12). Level II FaSSIF simulates similar characteristics in the fasted upper small intestine without taking into account the presence of proteins or enzymes (12). A series of phosphate buffer solutions containing sodium chloride, sodium taurocholate, and phosphatidylcholine were employed in the reservoir compartment so that the composition of contents in the duodenal compartment (pH, buffer capacity, osmolality, sodium taurocholate concentration, and phosphatidylcholine concentration) remained unaltered during an experiment (11). The mini-paddles in gastric and duodenal compartments rotated at 75 rpm. The emptying of contents of the gastric compartment (on a volume basis) followed first-order kinetics with a half-emptying time of 15 min as previously described (11). Experiments were performed at 37°C for 45 min using a three-channel peristaltic pump (Reglo ICC pump, part ISM 4308, Ismatec®). Incoming flow rates (from the gastric and the reservoir compartment to the duodenal compartment) were changed every 10 min and sampling was performed at midpoint (11). Upon collection, each sample from the duodenal compartment was immediately filtered through regenerated cellulose filters (Titan 3, 17 mm, 0.45 μm, SUN SRi, Rockwood, USA). Adsorption on to these filters has been found to be negligible (10). Compound B was assayed using HPLC-UV method using a reversed-phase XTerra RP-C_{18} column (50 mm × 4.6 mm, 3.5-μm particle size). The mobile phase used was phosphate buffer (NaH_2PO_4, 10 mM, pH 2.5) and acetonitrile (70:30 *v*/*v*) at a flow rate of 1 mL/min. Absorption was measured at 319 nm and the limit of quantification was 0.08 μg/mL (10).

Table I. Composition of Level III Biorelevant Media for Simulating Hypochlorhydric and Achlorhydric Stomach Conditions (10)

	Hypochlorhydric conditions		Achlorhydric conditions	
	$FaSSGF_{hypoc\text{-}maleates}$	$FaSSGF_{hypoc\text{-}phosphates}$	$FaSSGF_{achlo\text{-}maleates}$	$FaSSGF_{achlo\text{-}phosphates}$
Pepsin (mg/mL)	0.1	0.1	0.1	0.1
Sodium taurocholate[1] (μM)	80	80	80	80
Phosphatidylcholine[1] (μM)	20	20	20	20
Sodium chloride (mM)	15.7	9.7	7.0	15.7
Maleic acid[2] (mM)	1.0	-	3.0	-
Sodium dihydrogen phosphate[2] (mM)	-	6.0	-	0.25
Sodium hydroxide	q.s pH 5.0	q.s pH 5.0	q.s pH 7.0	q.s pH 7.0
pH	5.0	5.0	7.0	7.0

[1] Added by using FaSSIF/FeSSIF/FaSSGF powder
[2] Hyphen means not applicable

Simulations Software

All simulations were conducted in GastroPlus™ software (v9.5, Simulations Plus, Lancaster, CA). The default Opt-logD v6.1 model was used. Key physicochemical and biopharmaceutics properties used as input for the two compounds are summarized in Table II.

Modeling of Pioglitazone Data Under Achlorhydric Conditions

A GastroPlus™ model to describe the pharmacokinetics of pioglitazone, after oral administration of pioglitazone hydrochloride in the fasted state, has been previously described. The model used as input the pH-dependent equilibrium solubility and particle size (10 μm) of the API, and assumed no precipitation (13). The intestinal supersaturation assumed in the model is in line with what has been observed in *in vitro* experiments using the gastrointestinal simulator (GIS) (14) and the BioGIT system (data not shown). The same baseline model was employed in this manuscript with the focus on the prediction of the achlorhydric and hypochlorhydric conditions relative to the control phase (*i.e.*, standard fasted administration). The apparent solubility input for these simulations was obtained directly from the dissolution data in the newly proposed media (Table II). The average of the dissolved concentration for the first 20 min of the experiment was used as the estimate of the apparent solubility. Given the gradual decline of the dissolved concentration, we considered that timepoints past 20 min may be reflective of some precipitation (10). Given that in the dissolution the first timepoint represented plateau of dissolution, particle size used in the simulations was the same as the previously published model (13).

Modeling of Compound B BioGIT Data to Estimate Precipitation Time

Unlike pioglitazone, a physiologically based absorption model for Compound B has not been described before. Therefore, model development included the prediction in the standard fasted state (default fasted state in the software). Data to estimate mean precipitation time for the normal gastric conditions were generated in the BioGIT transfer

Table II. Physicochemical and Biopharmaceutics Parameters Used as Input for GastroPlus™ Simulations

Input parameter*		Pioglitazone hydrochloride	Compound B semi-fumarate co-crystal
pKa (basic)		5.6 (13)	4.4 (MSD data)
logP		2.94 (13)	2.9 (MSD data)
Human Intestinal Permeability		4×10^{-4} cm/s (13)	3.5×10^{-4} cm/s (MSD data)
Solubility data			
Gastric	Level III FaSSGF	0.38 mg/mL (equilibrium solubility) (13)	2.5 mg/mL (equilibrium solubility (MSD data)
	Level III $FaSSGF_{hypoc\text{-}phosphates}$**	Not used	240 μg/mL
	Level III $FaSSGF_{hypoc\text{-}maleates}$**	Not used	305 μg/mL
	Level III $FaSSGF_{achlo\text{-}phosphates}$**	55 μg/mL	Not used
	Level III $FaSSGF_{achlo\text{-}maleates}$**	8.7 μg/mL	Not used
Intestinal	Level II FaSSIF	16 μg/mL** 0.5 μg/mL (free base equilibrium solubility in FaSSIF) (13)	27 μg/mL**

*In all cases, data are presented in pioglitazone free base and Compound B free base equivalent concentrations; pKa and LogP values refer to the free base
**Refers to apparent solubility, estimated from dissolution data in reference (10)

system as described earlier in the "BioGIT Experiments" section. To model the BioGIT data in GastroPlus™, an ACAT file was generated to simulate the conditions of the BioGIT experiment. For the gastric compartment, the volume was set to 250 mL and the duodenal compartment at 40 mL based on the experimental setup used. Mean transfer transit times were 0.36 h out of the gastric compartment and 0.06 h out of the duodenal compartment. Since first-order processes are assumed in the software, mean times are equivalent to reciprocal of the corresponding rate constants. Since in BioGIT the transfer out of the duodenal compartment represents both intestinal transit and absorption of a highly permeable compound (11), permeability values in GastroPlus™ in both compartments were set at zero (*i.e.*, no additional removal rate from these two compartments). Ding et al. took a similar approach previously to compare GastroPlus™ simulations to artificial stomach-duodenum (ASD) model data for galunisertib although in their paper a detailed modeling of the *in vitro* compartments was not fully presented (15). A 5-min delay in capsule shell rapture was incorporated based on the observed dissolution lag time. To simulate the BioGIT data in the duodenal compartment, an effective mean particle size was first fit to dissolution data generated in Level III FaSSGF to estimate the input dissolution kinetics for the system (data not shown). The resulting particle size estimate was 100 μm. A range of precipitation times (100 to 10,000 s) were simulated to represent fast, slow, or practically no precipitation, and the simulation results were compared to the observed concentrations in the duodenal compartment. The range of precipitation times used is in line with precipitation times used in other publications.

Modeling of Compound B Pharmacokinetic Data

Data used for the simulations were obtained from a clinical study comparing the exposure of semi-fumarate co-crystal of Compound B (100 mg free base equivalent dose) in fasted healthy volunteers or after pantoprazole treatment (40 mg pantoprazole dosed for 5 days in the morning; in the fifth day, the pantoprazole dose was administered 2 h prior to semi-fumarate co-crystal of Compound B). The study was conducted as part of the clinical development of Compound B following necessary institutional review board and regulatory authority approvals.

The control arm of the study was used to generate pharmacokinetic parameters for the model as follows: CL/F = 0.164 L/h/kg, Vc/F = 0.535 L/kg, K_{12} 0.111 1/h, and K_{21} 0.137 1/h using the PKPlus module. These PK parameters were considered to represent maximum oral bioavailability; the assumption was supported by earlier data in the single rising dose study where linear pharmacokinetics was observed at 100 mg relative to the 5-mg starting dose. PK parameters estimated from the single rising dose study are similar to the ones estimated from the control arm of this drug–drug interaction study (data not shown); for better accuracy, taking into account the potential between study variability, parameters from the current study are used. In addition, BioGIT data conducted for Compound B also suggested low propensity for precipitation as discussed in the "RESULTS" section. For both the control and the pantoprazole arm simulations, API particle size of 30 μm (used for the capsule batch tested in the clinical study) and colon solubility 11 μg/mL (colon solubility has little impact in the simulation since absorption is essentially complete in the small intestine) were used for the simulations. Blood to plasma ratio = 1 and plasma protein fu = 4% were used, while subject body weight was assumed to be 70 kg.

For the pantoprazole co-administration arm, simulations were set based on the observed dissolution data from our previous manuscript (10). As shown in Table II, apparent gastric solubility was set at 305 or 240 μg/mL (average dissolved concentration across the plateau of the dissolution curve; typically that would be the 10–50 min timepoints) for the hypochlorhydria maleate based buffer (Level III $FaSSGF_{hypoc\text{-}maleates}$) and the phosphate based buffer (Level III $FaSSGF_{hypoc\text{-}phosphates}$), respectively. Intestinal solubility was set at 27 μg/mL based on the previously reported measurements in Level II FaSSIF (average dissolved across the experiment).

RESULTS

Simulation of Pharmacokinetic Data After Pioglitazone Hydrochloride Administration

Pioglitazone hydrochloride has been reported not to exhibit sensitivity to a change in gastric pH despite the pronounced pH dependency of the equilibrium solubility of the free base. Ranitidine (a H2 blocker) co-administration only results in 13% reduction of AUC and 16% reduction of C_{max} on average (ACTOS prescribing information, http://general.takedapharm.com/content/file.aspx?filetypecode=actospi, accessed Dec. 13, 2017). Figure 1 compares the predictions of plasma concentrations based on the apparent solubility obtained in media simulating the achlorhydric conditions relative to the predictions based on apparent solubility in media simulating the conditions in the control fasted state. Using either the Level III $FaSSGF_{achlo\text{-}phosphates}$ or Level III $FaSSGF_{achlo\text{-}maleates}$, apparent solubility values in the initial simulation resulted in projection of total exposure and C_{max} similar to the control fasted state, although the Level III $FaSSGF_{achlo\text{-}maleates}$ predicted a slower absorption. However, a closer inspection of the simulation indicated that given the relatively low dose of pioglitazone (30 mg), a good portion of the predicted absorption is coming from intestinal solubilization driven by the reasonably high apparent solubility measured during the dissolution experiments in Level II FaSSIF relative to the very low previously reported equilibrium solubility of the free base. As an additional check of the biorelevant media, two additional simulations were conducted where the intestinal solubility was set to the equilibrium solubility of the free base (0.5 μg/mL). Under this scenario which would represent complete neutralization of the salt *in vivo* upon dissolution in the gastric environment, only the Level III $FaSSGF_{achlo\text{-}phosphates}$ apparent solubility (which as shown in Table II was much higher than the apparent solubility observed in $FaSSGF_{achlo\text{-}maleates}$, *i.e.*, 55 *vs.* 8.7 μg/mL, respectively) resulted in predictions consistent with the small effect of acid-reducing agents reported before.

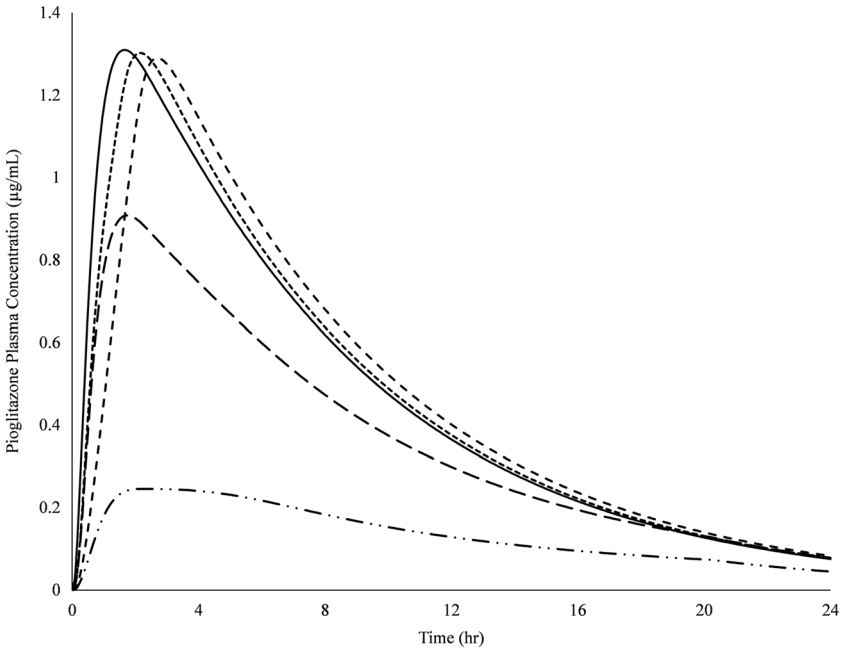

Fig. 1. Simulation of pioglitazone pharmacokinetic data under achlorhydric conditions using different biorelevant solubility data as input. The control simulation previously reported in (11) is shown as a reference. Control simulation (———), apparent solubility based on dissolution data in Level III $FaSSGF_{achlo\text{-}phosphates}$ and Level II FaSSIF (— — — —), apparent solubility based on dissolution data in Level III $FaSSGF_{achlo\text{-}maleates}$ and Level II FaSSIF (- - - -), apparent solubility based on dissolution data in Level III $FaSSGF_{achlo\text{-}phosphates}$ and solubility of free base in FaSSIF (–– ––), and apparent solubility based on dissolution data in Level III $FaSSGF_{achlo\text{-}maleates}$ and solubility of free base in FaSSIF (– ·· –)

Estimation of Precipitation Time of Compound B Using BioGIT Data

Figure 2 shows the observed data from the BioGIT experiment (mean ± SD, $n = 3$) along with model-predicted concentrations in the "duodenal" compartment of the BioGIT as a function of precipitation time. A very fast precipitation time (100 s) clearly underpredicts the observed concentration data. The default precipitation time (900 s) appears to reasonably capture the overall concentration curve although it somewhat underpredicts the first measured point and starts to trend lower at the 45-min timepoint. Longer precipitation times (3000 and 10,000 s) accurately predict the 15- and 45-min timepoints but somewhat overpredict the two intermediate timepoints.

Simulation of Pharmacokinetic Data After Administration of Semi-fumarate Co-crystal of Compound B

Figure 3 shows the simulation (against observed data) for the control subjects (normal gastric pH) as a function of the precipitation time, using the same precipitation times used to simulate the BioGIT data. It is evident that the longer the precipitation time, the better the prediction of the C_{max}. The long precipitation times (3000 and 10,000 s) allow for a C_{max} prediction within the variability of the clinical data.

In the clinical drug–drug interaction study, co-administration of Compound B with pantoprazole had a minimal, non-clinically significant impact on Compound B exposures. More specifically, AUC and C_{max} values were reduced by 10 and 19% on average. The plasma concentration profile after co-administration with pantoprazole is shown in Fig. 4, along with simulations based on two different solubility inputs: (a) Level III $FaSSGF_{hypoc\text{-}maleate}$ and (b) Level III $FaSSGF_{hypoc\text{-}phosphate}$.

Simulations with either of the newly proposed hypochlorhydric biorelevant media in general do a decent job in predicting the observed plasma concentration profile. The predicted plasma concentration curve is within the variability of the observed clinical data; the total AUC is generally well predicted although the C_{max} is somewhat under-predicted.

DISCUSSION

Predicting the impact of acid-reducing agents on the bioavailability of weak bases appears to be gaining increased attention given the number of recent publications (1–7,9). This drug–drug interaction is often limited to the impact of the changing gastric pH, which is easily incorporated in available commercial software, and the absorption impact is directly related to the differential solubility. Thus, it is not surprising that there has been reasonably good success in predicting the effect (or lack thereof) when the free base is used in the drug product. However, for drug products where formulations are employed to allow for dissolution/solubilization of the API to an extent greater than what is suggested by the pH–solubility profile, it would seem that additional dissolution data would be needed to inform such simulations.

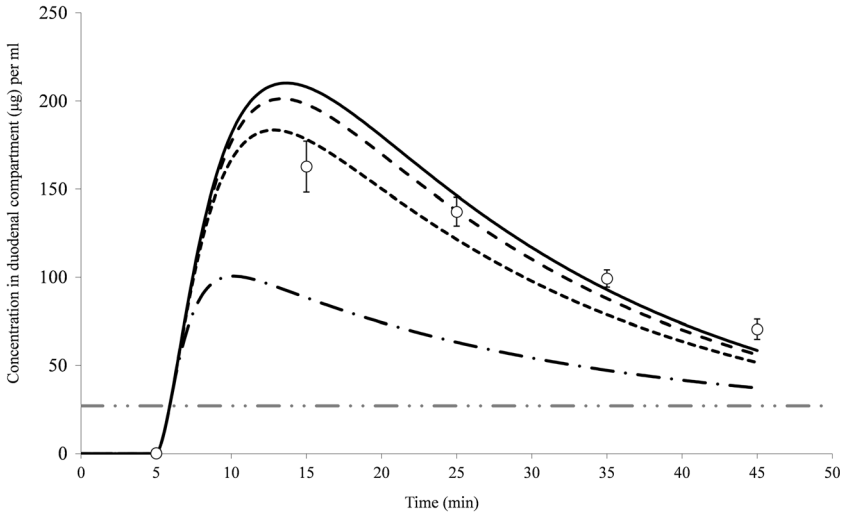

Fig. 2. Compound B BioGIT data under conditions simulating the normal gastric pH conditions in the fasted state. Simulations as a function of precipitation time: Tprep = 100 s (– · –), Tprep = 900 s (- - - -), Tprep = 3000 s (–– ––), Tprep = 10,000 s (–––––); actual (mean ± SD) data (•); apparent solubility obtained by direct dissolution of Compound B in Level II FaSSIF (– ·· –)

In this manuscript, we report for the first time simulations of two formulations using a salt (pioglitazone hydrochloride) and a co-crystal with a counter ion (Compound B semi-fumarate co-crystal) in achlorhydric or hypochlorhydric conditions by using as input apparent solubility measurements in previously proposed "standardized" achlorhydric or hypochlorhydric biorelevant media. The media were recently proposed based on the characterization of intestinal aspirates from a clinical study where a proton-pump inhibitor and a H2 blocker were used (16). While in our

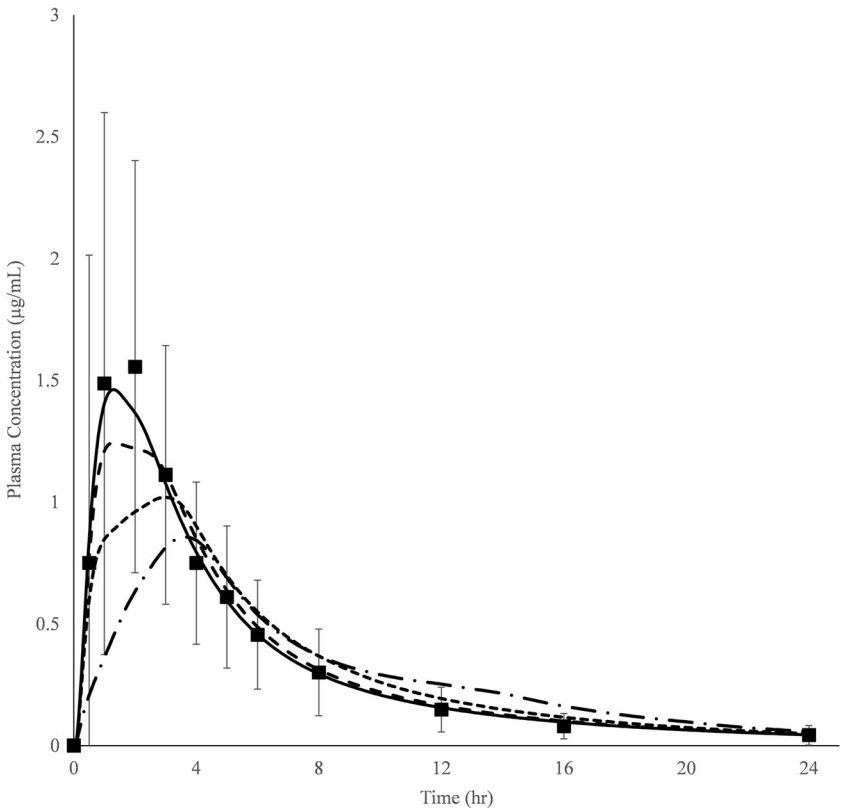

Fig. 3. Simulation of Compound B pharmacokinetic data under standard fasting conditions. Lines represent simulations as a function of precipitation time and squares are observed data (mean ± SD). Tprep = 10,000 s (————), Tprep = 3000 s (–– ––), Tprep = 900 s (- - - -), and Tprep = 100 s (– · –)

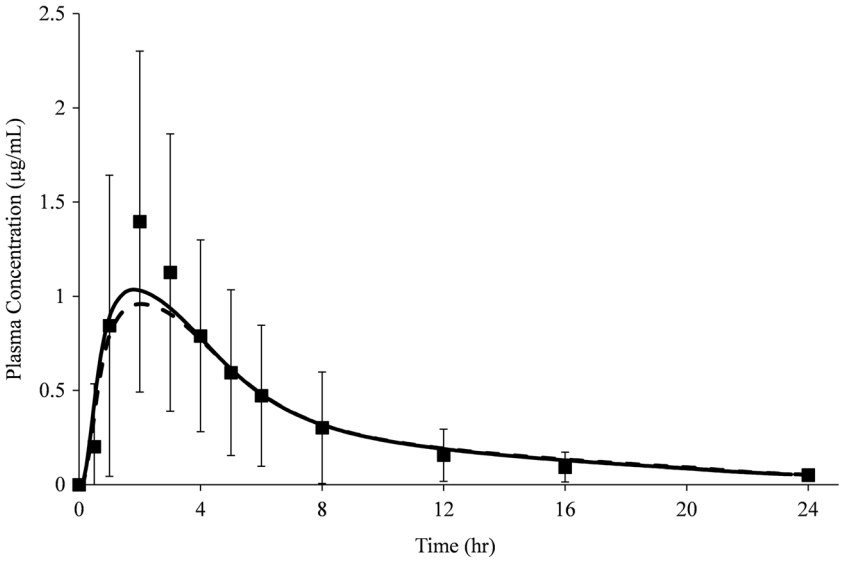

Fig. 4. Simulation of Compound B pharmacokinetic data after co-administration with pantoprazole using different biorelevant solubility data as input. Squares represent observed data (mean ± SD). Apparent solubility based on dissolution data in Level III $FaSSGF_{hypoc\text{-}phosphates}$ (———) and apparent solubility based on dissolution data in Level III $FaSSGF_{hypoc\text{-}maleates}$ (– – – – –)

previous manuscript we compared the dissolution of the same two APIs in these newly proposed media and in human aspirates (10), a question remained on whether the measurements could be further utilized as input to predictive oral absorption *in silico* models. As shown in this manuscript for both pioglitazone (Fig. 1) and Compound B (Fig. 4), incorporating data from the *in vitro* measurements in the simulations resulted in generally successful predictions of the lack of significant drug–drug interaction. For Compound B for which detailed plasma concentration data were also available under both administration conditions, the projected average plasma concentrations were within the variability of the clinical data. Although for the examples shown here the analysis was retrospective (*i.e.*, after the clinical study was conducted), such projections could be conducted ahead of clinical studies to better understand the risk of such interactions and inform design of the studies (e.g., selection of the acid-reducing agent).

Although the simulations for the prediction of the pH effect could be considered "bottom-up" as the solubility input is not based on clinical data, it should be acknowledged that the entire simulation still represents a middle-out approach which is commonly employed during pharmaceutical development. Specifically, precipitation settings for the simulations in the two examples provided were at least partially informed by the clinical data (although an initial estimate was observed from the dissolution data), and both compounds tested did not appear to suffer from significant precipitation. It is possible that simulations would be more difficult for compounds where significant precipitation is observed. For precipitating compounds, this may also require estimation of different precipitation time settings under different dosing conditions as the degree of supersaturation obtained may alter the precipitation kinetics. Since for these two compounds concentrations during dissolution experiments were relatively stable for up to 45 min under most conditions tested (10), and given that even in the control fasted arm (highest degree of supersaturation) BioGIT data did not indicate pronounced precipitation, this did not represent an issue.

While not the primary focus of this manuscript, we also report the first example of detailed incorporation of data from the BioGIT system into a GastroPlus™ simulation which was accomplished by creating a new model (ACAT) file. In general, estimation of precipitation settings prior to clinical data has been highlighted as an area of uncertainty for oral absorption physiologically based models and an opportunity for adoption of new *in vitro* tools (17). The BioGIT system represents one of the potential transfer models that have shown promise in the literature to study precipitation of weak bases. Based on the data generated for Compound B which has low but still appreciable solubility in Level II FaSSIF, we conclude that the *in vitro* data are supportive that precipitation is not pronounced. Thus, the BioGIT data are in agreement with the clinical data. However, it is also evident from Fig. 3 that multiple precipitation rates can still reasonably explain the data. It would appear that the residence time in the "duodenal" compartment of BioGIT is relatively short to allow for a further distinction between longer precipitation rates. With the availability of the *in vivo* data, the precipitation time can be fitted for further simulations as shown in Fig. 3, which is in our experience often the practice within the pharmaceutical industry.

CONCLUSIONS

In this manuscript, for the first time, we report the utility of recently proposed biorelevant media mimicking stomach and intestinal conditions after administration of proton-pump inhibitors or H2 blockers, as input into physiologically based absorption modeling. Using two model compounds, a HCl salt and a semi-fumarate co-crystal which as expected dissolve to a greater extent in these media (and in aspirates)

compared to what the pH–solubility profile of the free form would suggest, we demonstrate reasonably successful predictions of the plasma concentration profiles. While data from more compounds would be needed to understand the applicability of this approach on compounds with differing characteristics such as fast-precipitating compounds, the data reported in this manuscript represent an initial step towards defining biorelevant input for such simulations on interactions with acid-reducing agents.

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