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Utility of PBPK Absorption Modeling to Guide Modified Release Formulation Development of Gaboxadol, a Highly Soluble Compound With Region-Dependent Absorption

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ABSTRACT

Given the complexity of controlled release (CR) formulations, selecting the right preclinical tools is important to enable decision making on the *in vivo* performance of these formulations during development. In recent years, with the advancements of absorption/physiologically based pharmacokinetic (PBPK) modeling, such computational approaches play an increasing role in guiding formulation development. Development of PBPK models for CR formulations requires additional information compared with immediate release (IR) products. Perhaps the most important aspect is the need to simulate absorption in the lower intestine. Relatively few publications have investigated the use of PBPK models for compounds with region-dependent absorption. In this manuscript, we use gaboxadol as a model compound with region-dependent absorption. We first explored gaboxadol regional absorption in dogs to develop a PBPK model for absorption in the large intestine. Two matrix-based CR formulations were subsequently developed and tested in minipigs and demonstrated distinctly different pharmacokinetic profiles from the IR formulation. A minipig absorption PBPK model successfully predicted the observed plasma concentration data, with the predictions based on the *in vitro* dissolution being within the observed experimental variability. Finally, we demonstrate the development of an *in vitro–in vivo* correlation for the preclinical data using the same PBPK model.

Introduction

Preclinical evaluation of modified release (MR) formulations historically has been composed of a combination of *in vitro* dissolution testing and preclinical animal models, most commonly studies in dogs. The primary goals of such preclinical studies are to allow for early development of an *in vitro–in vivo* relationship (IVIVR) or an *in vitro–in vivo* correlation (IVIVC) for the dissolution method, investigation of mechanism of release, for example, via imaging, and selection of prototype formulations for further clinical testing.[1] In recent years with the advancements of absorption/physiologically based pharmacokinetic (PBPK) modeling, such computational approaches play an increasing role into guiding formulation development and such models have been successfully demonstrated also for MR dosage forms.[2-4]

Given the complexity of MR formulations, the right selection of preclinical tools is important to enable correct decision making on the *in vivo* performance of these formulations during development. Animal models selected need to accommodate the prolonged release from the formulations. For example, dogs, the most common preclinical species for screening of immediate release (IR) formulations, are known to exhibit significantly shorter small intestinal transit time compared with humans as well as the length of the colon is relatively small.[5] This has led to consideration of minipigs, which have longer intestinal transit times, as an alternative animal model for formulation screening. However, only a few studies have been reported in pigs with MR formulations.[6-9] Similarly, development of PBPK models requires additional information compared with standard PBPK models for IR compounds. The most important aspect is perhaps simulation of absorption in the lower intestine that needs to be taken into account; thus understanding of any region-dependent absorption is critical to the successful development of such models. Again limited reports are available with using PBPK models that account for region-dependent absorption.[3,4]

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Gaboxadol is a $GABA_A$ receptor agonist that was previously in clinical development for treatment of chronic pain and insomnia.[10] It is a zwitterion with pK_a values of 4.3 (acidic) and 8.3 (basic) and log P of −0.61. It is dosed as the hydrochloride (HCl) salt. The compound solubility is more than 30 mg/mL in the physiological pH range. Although gaboxadol exhibits moderate permeability in Caco-2 cells (P_{app} ~ 6–8 × 10^{-6} cm/s),[10] it exhibits high fraction absorbed (84%–93%) in both preclinical species and in humans,[11] thus can be categorized as a Biopharmaceutics Classification System (BCS) Class I compound. Absorption of gaboxadol is rapid with a short T_{max} of approximately 0.5 h and with a half-life of 1.5–2.0 h.[12] Recently, it has been demonstrated that intestinal absorption of gaboxadol is likely mediated by the human proton-dependent amino acid transporter 1 (hPAT1).[10,13] This transporter-mediated uptake can result in region-dependent absorption. Broberg et al.[13] showed that in rats, absorption of gaboxadol from the colon is only 4.2% relative to almost complete absorption (81.3%–91.3%) after administration in the stomach, duodenum, or jejunum. Thus, although the BCS Class I classification would normally be considered positive for the development of a controlled release (CR) formulation, this regional-dependent absorption poses a challenge in optimizing the release profile of a CR formulation.

Given the regional absorption of gaboxadol, it represents an interesting model compound to assess the utility of absorption PBPK modeling in informing possibility of success for CR formulation in a preclinical setting. In this manuscript, we present the development of an absorption model based on preclinical information to project behavior of two matrix-based CR formulations of gaboxadol and comparison of the projections with the outcome of minipig studies evaluating these two formulations with different release rates. Finally, we demonstrate the development of an IVIVC for the preclinical data using the PBPK model.

Methods

Formulations

The formulation used for the dog regional absorption studies was a simple aqueous solution of 0.2 mg/mL gaboxadol. For the minipig studies, CR formulations tested were 15 mg potency hydroxypropyl methylcellulose (HPMC) K100M-based matrix tablets. One of the formulations (fast CR, 15.5% HPMC) was designed with a complete release in approximately 6 h to maximize small intestinal absorption but still allow for reduction of C_{max}. The second formulation (slow CR, 58.5% HPMC) was designed with a prolonged release (complete release post 12 h) to be tested for further model verification. Dissolution for each formulation was measured in 900 mL deaerated water using a United States Pharmacopeia (USP) II apparatus at 50 rpm (Fig. 1). IR formulation was a simple dry filled capsule (DFC). Dissolution profiles in other media (e.g., 0.1 N HCl) resulted in similar differences between tested formulations; given the simplicity of the deaerated water as media, it was selected for the final formulation testing for this study.

Dog Regional Absorption Pharmacokinetic Study

Fasted male Beagle dogs (Marshall Farms, North Rose, NY), weighing approximately 10 kg, with ileal and colonic ports were used of these studies. All animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility in accordance with United States Department of Agriculture guidelines. The Guide and Animal Welfare regulations were followed in the conduct of the animal studies. Veterinary care was given to any animals requiring medical attention. Formulation dosing studies were conducted under a protocol approved by the Merck Institutional Animal Care and Use Committee (IACUC).

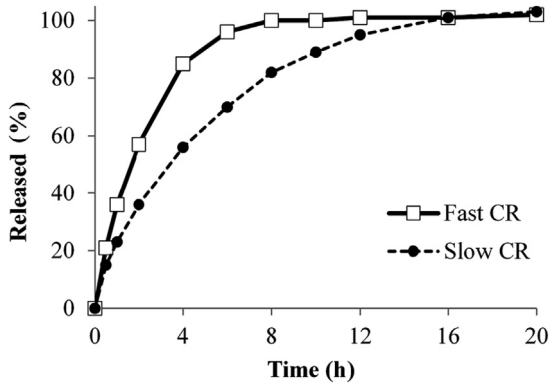

Figure 1. Dissolution of gaboxadol matrix CR formulations.

In the morning of the study, six animals were dosed with 0.2 mg/kg of gaboxadol (1 mL/kg) either orally or via the ileal or colonic port followed by 5 mL rinse with sterile water. Study was a crossover study (i.e., each animal was dosed via all three administration routes) and was completed over a period of 3 weeks (animals were dosed every week). Animals had been fasted overnight prior to dosing and food was returned at 4 h after dosing. Blood was drawn from a 21 g catheter placed into the cephalic vein at pre-dose and 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h after dosing. The plasma was separated by centrifugation (15 min at 2500g) and kept frozen at −70°C until analysis by LC–MS/MS. The analytical method for gaboxadol has been published before.[14] The assay used had a lower limit of quantitation (LOQ) of 0.1 ng/mL for gaboxadol based on 150 μL aliquots of plasma. The calibration curve dynamic range was 0.1 to 1000 ng/mL.

Minipig Formulation Comparison Pharmacokinetic Study

Castrated male, vascular access port implanted Yucatan minipigs (Sinclair Research Center Inc., Auxvasse, MO) weighing approximately 50 kg were used for the study. The Guide and Animal Welfare regulations were followed in the conduct of the animal studies. Veterinary care was given to any animals requiring medical attention. All formulation dosing studies were conducted under a protocol approved by the Merck IACUC. The study design was a full crossover with three periods utilizing eight animals dosed every week. Animals were fasted overnight prior to the day of the study. On the day of the study, animals were dosed orally with 15 mg potency formulations followed by a 3.5 mL/kg water rinse. Water was withheld for 1 h prior to dose to 1 h post-dosing. Food was returned 4 h post-dosing. Blood was collected at pre-dose and 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, and 24 h post dosing. The plasma was separated by centrifugation (15 min at 2500g) and kept frozen (−70°C) until analysis by LC–MS/MS.

Pharmacokinetic Analysis

Area under the curve (AUC_{last}), observed maximum plasma concentration (C_{max}), and time of C_{max} (T_{max}) were calculated using a linear trapezoidal, non-compartmental model in Phoenix WinNonlin (Certara USA, St. Louis, MO). As plasma concentrations at 24 h were below LOQ, only data up to 12 h data are used for calculations in this manuscript.

Absorption PBPK Modeling/IVIVC

All simulations were conducted in GastroPlus™ software (v8.6; Simulations Plus, Lancaster, CA). The corresponding species default Opt-logD v6.1 model was used initially for either dog, human, or minipig simulations. Pharmacokinetic parameters for systemic disposition were estimated either in Phoenix WinNonlin or with the PK-Plus module in GastroPlus™. The following physicochemical properties were used for all simulations: molecular weight 140.14, pK_a values of 4.3 (acidic) and 8.3 (basic) and log *P* of −0.61. Solubility was set at 30 mg/mL.

Modeling of Dog Pharmacokinetic Data

The regional absorption study data were utilized to develop a regional absorption model in GastroPlus™. First the oral pharmacokinetic dog, data were fit to a compartmental model that was assumed to represent maximal bioavailability after oral dosing. The following PK parameters were used: CL/F = 0.85 L/(h kg), Vc/F = 0.69 L/kg, K12 = 0.21 1/h, K21 = 0.52 1/h. The upper intestinal effective permeability (P_{eff}) was estimated also from the dosing of the oral solution and the fitted plasma–concentration time profile. To simulate intra-colonic dosing, a PO simulation was run but transit times of the Advanced Compartmental Absorption and Transit (ACAT) model prior to caecum were set at 0.0001 h. This setting allows for essentially "instantaneous" arrival of the dose in the caecum to simulate intra-colonic administration at time zero and was only used for the intra-colonic dosing simulation. Based on the observed plasma-concentration profiles after oral and colonic dosing in dogs, the caecum/colon permeability/transit times in the software were optimized to capture the observed very quick and minimal absorption of the compound following intra-colonic administration. It should be noted that in the context of this simulation caecum/colon compartments do not need to translate directly to the corresponding anatomical regions but are used to simulated lower gut absorption. This adjustment is analogous to adjustment of absorption scale factor (ASF) values in previous publications.[3]

Simulation of Human Pharmacokinetic Data

The following PK parameters were used for human simulations[12]: CL = 0.43 L/(h kg), Vc = 0.22 L/kg, K12 = 3.00 1/h, K21 = 1.65 1/h, FPE = 8% (based on reported 92% bioavailability), fu = 98%. P_{eff} was set at 5×10^{-4} cm/s (same setting as dogs) given reported oral T_{max} of 0.5 h. For IR, a solution was simulated. The permeability/caecum transit time settings derived from the dog simulations were used for the human simulations based on the assumption that regional absorption in dogs is representative of humans (i.e., caecal transit time of 0.2 h was used while total colonic transit time was modified accordingly to maintain the default total colonic transit time). The dog model is generally considered as a suitable surrogate for colonic absorption estimation.[15] Subsequently projections for the two hydrophilic matrix formulations were carried out. Formulations were simulated as CR: integral tablets with input of the entire dissolution profile as seen in Figure 1.

Modeling of Minipig Pharmacokinetic Data

The default minipig ACAT model was used with modification of the permeability/transit time values in the same manner as discussed for the dog and clinical simulations again under the assumption that regional absorption differences were similar between species. Pharmacokinetic parameters were obtained from the oral DFC arm under the assumption it represents maximal oral absorption; the human PK parameters were used as a starting point and optimized to fit the observed data. Final PK parameters used were: CL/F = 0.47 L/(h kg), Vc/F = 0.29 L/kg, K12 = 3.00 1/h, and K21 = 1.65 1/h. Projections for the two hydrophilic matrix formulations were carried out with input of the entire dissolution profile (CR: integral tablets).

Table 1
Summary of Mean (±SD, $n = 6$) Pharmacokinetic Parameters for Gaboxadol in Male Bi-Port Beagle Dogs Following Administration of 0.2 mg/kg of Gaboxadol (1 mL/kg Water Solution) via Oral Dosing; Ileal Port and Colonic Port

Dosing Route	AUC_{last} (ng h/mL)	C_{max} (ng/mL)	T_{max} (h)	Relative Bioavailability
Oral	223 ± 52.4	174 ± 61.1	0.5 (0.25–0.5)	–
Ileal port	233 ± 70.7	187 ± 15.9	0.25 (0.25–0.5)	104%
Colonic port	13.4 ± 6.54	11.7 ± 3.87	0.25 (–)	6.00%

For T_{max}, median and range, if applicable, are reported.

PBPK-Based IVIVR/IVIVC

To enable the development of an IVIVC, the *in vivo* dissolution/release profile (i.e., the release vs. time profile used for the simulation) was fit to the observed plasma pharmacokinetic data within the GastroPlus™ software using the Optimization Module. The following optimization settings were used: parameters selected were the time scale and shape of the Weibull function, objective function weight was set as unity and the concentration–time profile was used as the observations weight. These settings resulted in best fit of the entire plasma concentration profiles with r^2 values of 0.98 and 0.99 for the two formulations. The resulting *in vivo* dissolution profile was plotted against the *in vitro* dissolution data in Microsoft Excel (2013) to assess if an IVIVR/IVIVC was present.

Results

Dog Regional Absorption Pharmacokinetic Data

Mean dog pharmacokinetic parameters for gaboxadol following oral, intra-ileal, or intra-colonic dosing are summarized in Table 1. The oral and intra-colonic administration plasma concentration time profiles are shown in Figure 2. Exposures following intra-colonic dosing were approximately 6% of that of oral and intra-ileal administration ($p < 0.05$, paired t-test). Intra-ileal dosing resulted in similar mean AUC compared with oral dosing. Similar

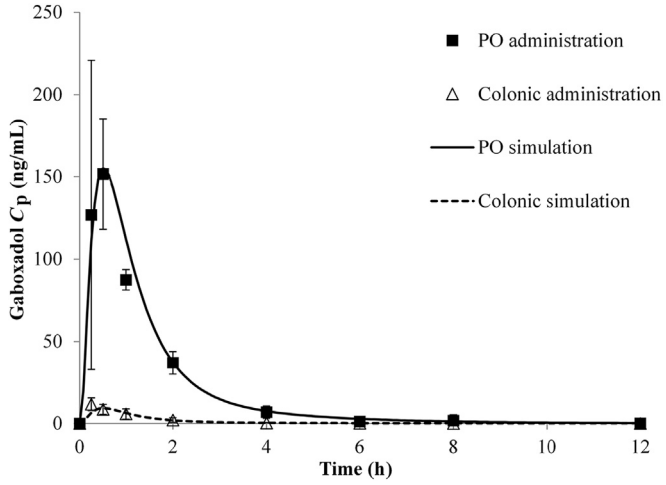

Figure 2. Observed (mean ± SD, $n = 6$) and simulated gaboxadol plasma–concentration profiles from regional absorption study (only PO and colonic administration shown) after dosing of 0.2 mg/kg gaboxadol solution to dogs.

Table 2
Mean (±SD, $n = 8$) Pharmacokinetic Parameters for Gaboxadol in Minipigs Following Oral Administration of a Gaboxadol IR Dry-Filled Capsule and Two Controlled Release Formulations (Fast CR and Slow CR, Respectively) at a Dose of 15 mg

Formulation	AUC_{last} (ng h/mL)	C_{max} (ng/mL)	$C_{8\ h}$ (ng/mL)	$C_{12\ h}$ (ng/mL)	T_{max} (h)	Relative Bioavailability
IR DFC	635 ± 113	247 ± 69.0	7.41 ± 3.05	1.64 ± 0.80	1.0 (0.5-2.0)	–
Fast CR	479 ± 180	103 ± 41.4	19.2 ± 10.8	3.94 ± 2.85	3.0 (0.5-4.0)	75.4%
Slow CR	377 ± 91.5	88.4 ± 26.8	17 ± 11.8	4.84 ± 6.14	2.0 (1.0-4.0)	59.4%

For T_{max}, median and range are reported.

trends were observed for mean C_{max} estimates. Mean C_{max} following oral and intra-ileal dosing were similar; mean C_{max} following colonic dosing was significantly lower ($p < 0.05$, paired t-test). The lower colonic absorption was consistent across all animals tested.

Minipig Formulation Comparison Pharmacokinetic Data

Both CR formulations (fast CR and slow CR) were able to blunt the mean C_{max} and exhibited delayed T_{max} compared with the IR formulation. Mean C_{max} was reduced by ~58% and 64% in case of the fast release and slow release CR formulations respectively ($p < 0.05$ for both comparisons, paired t-test). At the same time, the mean plasma concentrations at 8 and 12 h post-dosing were about 2.3–3.0-fold higher for both the CR formulations compared with the IR DFC although variability was high. Overall exposures for the CR formulations trended lower than the IR DFC ($p < 0.05$ for slow CR vs. IR, whereas the fast CR vs. IR difference did not reach statistical significance). It is worth noting that in the fast CR formulation arm, one single animal showed significantly lower exposures than the rest (57.3 ng h/mL vs. average of 539 ng h/mL for the remaining 7 animals; C_{max} difference was also ~fourfold). There was no identifiable cause for the low exposure to exclude the animal from the analysis but it is possible that because of that animal, the exposures of the fast CR formulation are somewhat under-predicted. However, the overall conclusions from the study are not meaningfully altered even when data are reanalyzed only with seven animals. Summary pharmacokinetic parameters are shown in Table 2.

Absorption Modeling

Modeling of Dog Pharmacokinetic Data

The estimated intestinal P_{eff} was 5×10^{-4} cm/s. The caecum and colon P_{eff}/transit time values were subsequently optimized to capture data of the intra-colonic administration as described in *Methods* sections. The final model that resulted in reasonable description of the observed Fa used a transit time of 0.2 h in caecum and a permeability of 0.05×10^{-4} cm/s in colon (colon transit time was increased to account for unchanged total caecal + colonic transit time; the rest of the ASF settings were the default). The model fits are shown in Figure 2. Intra-ileal administration data are not shown in Figure 2 as a modification of the ACAT model was not implemented given the similar exposure between ileal and oral administration.

Table 3
Projections of Clinical Pharmacokinetics of 15 mg Test Gaboxadol CR Formulations and Comparison of Relative Exposure to IR to Data From the Minipig Study

Variable	Simulated Human AUC (ng h/mL)	Simulated Human C_{max} (ng/mL)	Simulated CR/IR Ratio (AUC, C_{max})	Observed Pig CR/IR ratio (AUC, C_{max})
IR	455	240	–	–
Fast CR	336	80.6	0.74, 0.34	0.75, 0.42
Slow CR	223	50.2	0.49, 0.21	0.59, 0.36

Simulation of Human Pharmacokinetic Data

The permeability/caecum transit time settings derived from the dog simulations were used for the human simulations. Based on these settings, projection of human colonic Fa is 3.9%, slightly lower than the value in dogs because of lower ASFs in humans versus dogs in the ACAT model. Based on the refined lower GI absorption model, the model projections of bioavailability for 15 mg dose (same dose as manufactured for minipig studies) are shown in Table 3 and Figure 3. The inset in Figure 3 shows also the comparison of the IR model against observed clinical IR data (10 mg),[12] verifying adequate performance of the upper small intestinal absorption model for the purpose of exploratory simulations for CR development. The simulations project a loss of 20% and 46% bioavailability for the fast and slow CR formulations relative to IR (solution) administration. The results are qualitatively similar to what was observed in minipigs where a 25% and 41% loss of bioavailability was observed for the two formulations respectively.

Modeling of Minipig Pharmacokinetic Data

Minipig simulations (15 mg) based on the *in vitro* dissolution data are shown in Figure 4. Based on the dissolution data, the simulations projected somewhat larger difference between the two MR formulations, especially on C_{max}, compared with what was observed. However, the projections can be considered generally aligned with the observations, projecting the plasma concentration profiles within the observed variability of the individual time points of the experimental data (error bars included in Fig. 4).

PBPK-Based IVIVR/IVIVC

The relationship between the dissolution data and the estimated *in vivo* release from the PBPK model is shown in Figure 5. Overall there was a good agreement between the *in vitro* data and the observed *in vivo* release. Especially for the slow CR formulation,

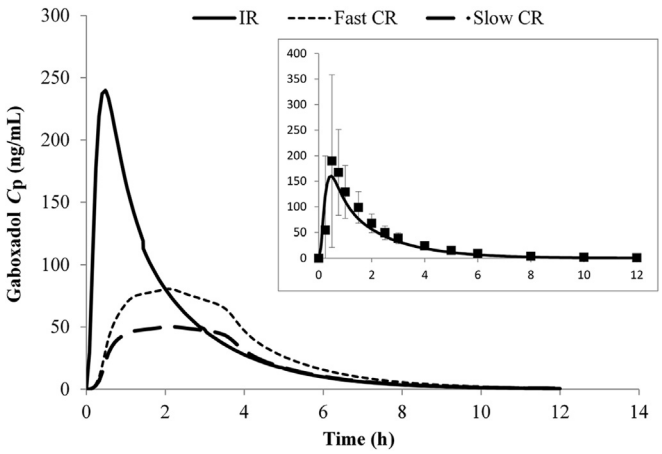

Figure 3. Projected gaboxadol clinical plasma concentration versus time profiles for 15 mg IR and CR formulations. Inset: comparison of the IR model against observed clinical data (10 mg).

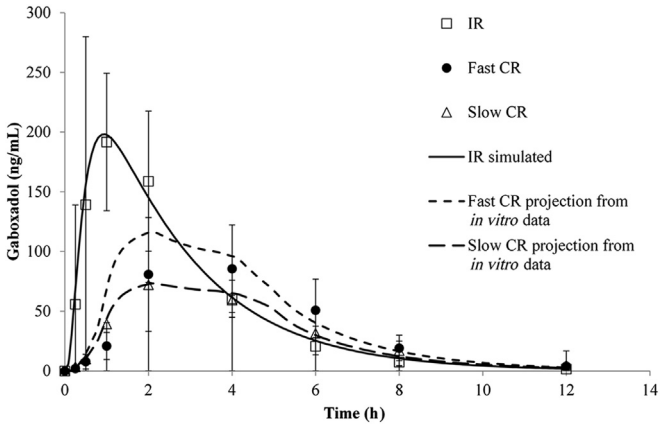

Figure 4. Observed (mean ± SD, $n = 8$) versus simulated minipig data for 15 mg IR and CR gaboxadol formulations.

the relationship appears very close to 1:1, whereas the fast CR formulation was somewhat slower *in vivo* compared with *in vitro*. The overall (combined formulations) R^2 value for the regression was 0.96.

Discussion

Development of MR formulations represents a challenge for formulators and biopharmaceutics scientists who need to balance the compound intrinsic physicochemical properties and absorption characteristics, the ability of the selected formulation technology to provide the selected release rate and the inherent physiological variability in transit times and regional differences in absorption to achieve the desired pharmacokinetic profile. Historically, the biopharmaceutics strategy for development of MR formulations has focused on dissolution studies in combinations with preclinical and mostly clinical studies.[1] In the most recent years, absorption/PBPK modeling is increasingly being used to guide formulation development. However there are relatively few reports specifically discussing the application of such models to MR products. Gaboxadol, a BCS I compound with region-dependent absorption, which has been previously demonstrated, at least in rats, to be due to transporter-mediated uptake, represented an interesting model compound to test the ability of PBPK models in combination with preclinical data to inform *in vivo* behavior of MR formulations. Because of the high solubility, it can be formulated in matrix tablets

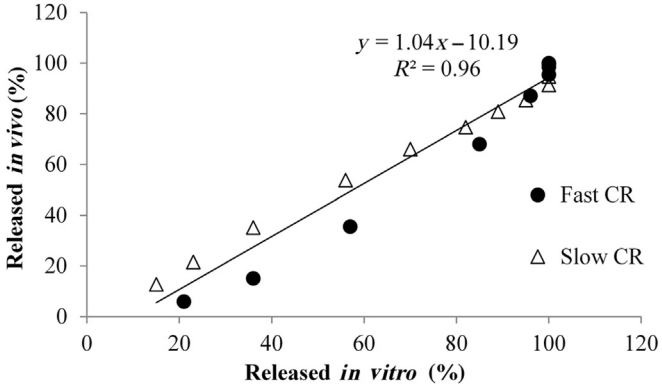

Figure 5. IVIVR/IVIVC curve for gaboxadol CR formulations tested in minipigs.

that can provide well-defined release rates for *in vivo* testing. The high solubility also ensures that the *in vivo* absorption is controlled only by the combination of release of the drug from the formulation and the regional permeability without concerns for solubilization/precipitation.

The absorption/PBPK model for MR formulations needs to take into account absorption in the large intestine. For the development of the region-dependent absorption model for gaboxadol, a regional absorption study in dogs was conducted. In dogs, gaboxadol was well absorbed after oral or intra-ileal administration, indicating good absorption across the small intestine. However, absorption in colon was very low (Table 1 and Fig. 2). The results are very similar to what Broberg et al.[13] reported in rats where absorption of gaboxadol from the colon was only 4.2% relative to almost complete absorption (81.3%-91.3%) after administration in the stomach, duodenum, or jejunum. Although our study does not verify the role of PAT1 in regional absorption of gaboxadol in dogs, it raises the possibility of similar behavior in the colonic absorption of the compound between rats and dogs. The possibility that the low colonic absorption in the dog study was because of the expected low colonic water volumes appears quite low. Given the solubility of gaboxadol and the extended colonic transit time, nearly complete absorption would be expected if permeability was high (confirmed by simulations using default permeability settings—data not shown). Although we cannot exclude any instability of gaboxadol in the dog colonic environment, we are not aware of any literature suggesting this possibility.

The region absorption data were subsequently implemented in the PBPK model. The dog model is generally considered as a suitable surrogate for human colonic absorption estimation, although that is most typically applied for passively absorbed compound.[15] It is interesting that in the case of gaboxadol simulated in this paper, the same colonic absorption models appear to work well for the minipig simulations, suggesting quite good translatability of the region-dependent absorption between species (at least rats, dogs, and minipigs). Perhaps this is not surprising given the transporter-mediated uptake of gaboxadol. If a different mechanism is responsible for region-dependent absorption, such as P-gp efflux or paracellular permeability changes, larger differences may be observable between species.

Subsequently to the development of the regional absorption model, we simulated clinical exposures of two matrix tablets designed to provide sufficiently different *in vivo* dissolution time course to interrogate the model behavior. This step would represent a typical step during CR formulation development in an industrial setting. The simulations were compared to the results of the minipig study conducted with these two CR formulations. As shown in Figure 3 and Table 3 simulations of clinical exposures where in qualitative agreement with the preclinical data. However, as clinical data were not available to confirm the model predictions, to better understand the suitability of PBPK modeling to guide formulation development, we subsequent attempted the simulation of the minipig data. Recently, a PBPK model for minipigs was reported.[16] In this manuscript, we were able to use the PBPK model in the GastroPlus™ software to accurately simulate the behavior of two CR formulations. To our knowledge, this is the first reported application of the model for CR formulations in minipigs and for a compound with region-dependent absorption. As shown in Figure 4, predictions with the *in vitro* profile as input resulted in adequate prediction of the observed plasma concentration profiles, with predictions being within the observed variability of the individual time points of the experimental data (error bars included in Fig. 4), supporting the utility of PBPK modeling.

IVIVCs are often sought at early stages of CR formulation development to further guide dissolution method refinement and

formulation development. However, establishment of IVIVCs for MR formulations with region-dependent absorption, using the traditional deconvolution/convolution approach may be challenging as the resulting input function from deconvolution is reflective of both the *in vivo* dissolution time course and the permeability limitations. PBPK modeling may represent an alternative approach in these cases as it allows to model the two processes separately. Indeed for gaboxadol, we developed an IVIVR/IVIVC model (we use the term IVIVR/IVIVC instead of just IVIVC, as this was not intended for regulatory application) using the developed absorption/PBPK model. The relationship between *in vitro* and *in vivo* release rate appeared close to linear (Fig. 5).

The primary intent of the simulations conducted in this manuscript was to assess the predictability of the PBPK model against the observed minipig data and demonstrate the utility of such a PBPK approach. However, we have included simulations of clinical data that would be typically conducted at the early stages of formulation development. As indicated earlier in the discussion, our study did not investigate the functional involvement of PAT1 in the colonic absorption of gaboxadol in dogs and pigs. The observations from the studies presented in this manuscript, namely the very low relative colonic bioavailability in the dog study and the reasonable agreement of the PBPK model projections in pigs against experimental data that showed loss of bioavailability for CR formulations (Fig. 4), and the previously reported data in rats,[13] raise the possibility that the region-dependent absorption mechanism is conserved between the three species. Simulations in humans were conducted under the assumption of a similar behavior. PAT1 mRNA expression in humans has been reported throughout the gastrointestinal tract with higher levels in the small intestine.[17] PAT1 in small intestinal enterocytes is localized at the brush border.[17] However no studies have specifically looked at the functional role or the localization of the transporter in colonocytes and no regional absorption studies, or studies with MR formulations, have been reported to verify the level of absorption of gaboxadol in human colon. Thus, a final verification of the human PBPK model would require similar data as those generated in minipigs and used for the verification of the minipig PBPK model. Although the preclinical data presented demonstrate the potential application of PBPK models in early CR formulation development before any clinical data are available, it is important to keep in mind that further model verification with clinical data would be important to allow for use of the PBPK model for further clinical formulation development, as species differences in transporter expression may need to be accounted for in the models.

The data from the minipig study on each own are interesting in understanding more about the applicability of this model for formulation development. Although in the recent years, minipigs have attracted attention as an alternative model for the preclinical screening of formulations, there are relatively limited published reports on evaluation of CR dosage forms in minipigs. To our knowledge, this is the first report studying a compound with such pronounced region-dependent permeability in the model. In a previous paper, we had studied a BCS III compound with ~30% relative colonic bioavailability.[9] To date, there has been no reported detailed study of region-dependent absorption in minipigs. Although additional studies are needed for more compounds, based on the indirect assessment of colonic absorption from this and our previous study, it would appear that minipig may be suitable model for compounds with region-dependent absorption. In the past, variability in pig gastric emptying has been highlighted as a potential limitation for the use of this model for formulation screening. In this study, the variability seen was generally low-moderated for all formulations (Table 2), with the exception of a single animal tested with the fast CR tablets. The variability for the IR DFC was quite low (<20% CV for AUC), thought this may not be too surprising given the very high solubility of gaboxadol that may allow for quick gastric emptying with the dosed water volume. However variability in this study was moderate also for both CR formulations. This is in line with our previous study with another BCS III compound where we also did not observe any unusual variability or significant outliers across six different CR tablet formulations. Thus, as we suggested before, the concern around prolonged gastric emptying may need to be looked at on a case-by-case basis with both formulation and compound properties considered.

Conclusions

Application of PBPK modeling to guide formulation development has attracted significant attention in recent years. Significant efforts both in academia and industry are being undertaken to refine existing models, such as the efforts from the Oral Biopharmaceutics Tools (OrBiTo) IMI project.[18] For gaboxadol, a BCS Class I compound with region-dependent absorption, we report in this manuscript how the combination of PBPK models with *in vitro* dissolution and preclinical data can be used to guide formulation development. We further demonstrate the utility of a recently described minipig PBPK model to project formulation performance and develop an IVIVC that can be useful to guide subsequent steps in development. This case study supports the notion that combination of the preclinical data and *in silico* tools appears a valid approach to informing biopharmaceutics aspects of CR formulations.

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