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RESEARCH PAPER

Pharmacokinetics of Paracetamol in Göttingen Minipigs: In Vivo Studies and Modeling to Elucidate Physiological Determinants of Absorption

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ABSTRACT

Purpose Onset and rate of gastric emptying are important determinants of drug absorption after oral dosing. Therefore, robust estimates of these parameters are needed in physiologically based absorption models to predict reliably plasma concentration time profiles. For human and some other laboratory animals, reasonable parameterization of gastric emptying has been established. However gastric emptying is less well characterized in minipigs, a large animal model rapidly gaining importance in pharmaceutical research.

Methods A pharmacokinetic crossover study using different dosage forms of paracetamol (intravenous and oral solution, capsule and tablet) was conducted in four male and four female Göttingen minipigs after an overnight fast. Deconvolution analysis was performed to determine the absorption kinetics. Estimated lag times and first order gastric emptying parameters were incorporated in a previously published PBPK model of the minipig and simulations verified. Postmortem assessments of minipig stomachs were made after different fasting protocols.

Results Fraction of dose absorbed *vs.* time profiles showed high interindividual variability, comparable to human fed state absorption. Mean gastric transit times were determined to be 0.63 h, 1.36 h, and 0.73 h for solution, capsules, and tablets, respectively. Postmortem assessment confirmed that minipig stomachs were not empty after an overnight fast.

Conclusions Gastric transit times in overnight fasted minipigs are longer than those observed in humans. This is most likely caused by delayed and incomplete food emptying and further work is needed to develop feasible and effective fasting protocols for minipigs.

KEY WORDS absorption · fasting protocol · gastric emptying · minipig · paracetamol · pharmacokinetics · physiologically based pharmacokinetic modeling · validation

INTRODUCTION

In small molecule drug development, efforts are made to enable oral administration as this is the safest, most cost-effective route and is generally associated with good patient compliance. However, due to the complex interplay of molecular and physiological factors which drive drug dissolution and permeation, oral pharmacokinetics can be difficult to predict (1).

Most drugs are poorly absorbed in the stomach but transit from stomach to small intestine, *i.e.*, gastric emptying, is a fundamental factor for predicting drug absorption. Especially for highly water soluble compounds where dissolution is not rate-limiting, gastric emptying determines the speed of reaching the small intestinal absorptive surface and thus influences the maximal plasma concentrations. Thus, gastric emptying can have direct implications for drug safety and efficacy as the therapeutic window of a compound might be missed. Alterations in stomach emptying, which can occur in pathological and physiological states have to be taken into account (2,3).

An established technique to anticipate and simulate oral pharmacokinetics in drug research and development is physiologically-based pharmacokinetic (PBPK) modeling. For a given drug, specific models are usually developed and verified in preclinical species before projection to human (4). Such predictions of absorption depend on reliable model parameterization with physiological gastrointestinal data, like

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intestinal dimensions, pH and transit times. For most commonly used laboratory animals such databases have been reasonably well established (5).

The use of the minipig as a large animal model is relatively novel in pharmaceutical research but has the potential to replace dog and monkey as it seems similar to human in many aspects of physiology, anatomy and pathophysiology (6–9). Our group has recently proposed a preliminary PBPK model of the minipig and first validations showed that the model performed encouragingly (10). However, we have also discovered some gaps in our understanding of absorption relevant parameters. The porcine stomach is monogastric as in humans, but some species-specific differences exist, such as a protrusion in the cardiac region of the stomach (*diverticulum ventriculi*) with yet unclear physiological function (11,12). Another unique feature of the pig stomach is the pylorus, which consists of a semilunar sphincter and a fibromuscular protuberance (torus pyloricus). These components are suspected to act together to regulate passage into the duodenum (13) and are unique to pig since in other laboratory species the pylorus consists of a complete circular sphincter. The functional consequences of this anatomical deviation is still debated but there is evidence that passage of large, non-dissolving particles is markedly slower than in other species (14). *In vivo* studies using telemetric devices to elucidate gastric pH and transit times, reported high inter study and inter individual variability (5,15–19) with gastric pH ranging between 1.2 and 6 (14,17) and gastric residence times extending to more than 24 h (18,19). What remains unclear is to which extent the reported results apply for dissolving drugs which might pass more easily from the stomach to the duodenum (14).

In order to improve our preliminary PBPK model of the minipig, we aimed to establish more drug-relevant estimates of gastric physiology (10). Therefore, we performed a cross-over pharmacokinetic study in Göttingen minipigs with paracetamol, a drug often used clinically to assess gastric emptying (20). At therapeutic doses, paracetamol is a highly soluble and permeable BDDCS class I drug (21). This weak acid (pKA= 9.5), is poorly absorbed in the stomach but rapidly permeates the small intestinal mucosa (22). Since the absorption phase of plasma drug concentrations correlates with gastric emptying it has been used to determine gastric lag times and empting rates (23–26). In addition we aimed to study formulation effects and therefore dosed paracetamol as solution, capsule and tablet. Lag times and gastric emptying rates were determined by numerical deconvolution of the pharmacokinetic profiles and were then used to re-parameterize the gastric emptying rate of the preliminary PBPK model (10). To verify these updates we simulated oral pharmacokinetic profiles of midazolam, caffeine, warfarin and omeprazole and compared the results to published *in vivo* data (27).

MATERIAL AND METHODS

In Vivo Studies

Minipigs were obtained from Ellegaard A/S (DK) and were allowed to acclimatize for several weeks before any experimental procedure was performed. All animals used in this study were fed 120 g of a standard minipig pellet diet twice daily and had *ad libitum* access to tap water. Under maintenance conditions, minipigs had access to straw as bedding and environmental enrichment. For the PK assessment, eight minipigs between 5 and 6 months of age were fitted with jugular vein catheters. Animals were allowed to recover from surgery for 24 h before entering the study. Animals showed a mean weight of 8.2 kg +/− 1.4 kg SD at the first dose and were growing during the 4 weeks of study. Therefore, animals were weighed prior to each dosing and the dose adjusted. Animals gained on average 0.7 kg (+/− 0.1 kg SD) between the intravenous bolus and oral solution administration (1 week of washout), 0.9 kg (+/− 0.2 kg SD) between solution and capsule (2 weeks of washout) and 0.2 kg (+/− 0.2 kg SD) between capsule and tablet administration (1 week of washout). Minipigs were fasted overnight (12 h) before drug administration. Pellet meal (120 g) and bedding (straw) were offered 4 h after dosing and the next meal was offered again 7 h post-dose. For intravenous administration and oral solution Perfalgan 500 mg infusion solution for children was used, at a concentration of 10 mg/mL. Capsules were filled in house with micronized API. For tablets, we used commercially available 325 mg tablets of Tylenol (oval shape).

Animals were put in sex-matched groups of 4 and received 10 mg/kg paracetamol as an intravenous bolus and as oral administrations of 30 mg/kg. The oral solution was administered by gavage and to assist correct dosing and minimize stress for the minipigs, solid dosage forms were hidden in a treat (a spoon of pudding). Venous blood (1 ml) was withdrawn before dosing and at 0.0833, 0.25, 0.5, 1, 2, 4, 7 h post dosing. A washout period of at least 7 days was allowed between different administrations.

For the post mortem assessment, we used animals which had to be sacrificed for other reasons than the present study. To assess efficiency of fasting protocols we designed a set of pilot studies (Control, Pilot 1 to 3, see Table I). Male minipigs of 4.5–5 months of age were pair housed in groups of 4, according to their corresponding treatment group. All animals were humanely sacrificed using a mixture of zoletil and pentobarbitone after an overnight fast. Stomachs were clamped on cardia and pylorus, removed from the body and weighed. Thereafter stomachs were opened, washed and reweighed. Empty stomach weight as well as the weight of gastric content (assessed as the difference from full to empty stomach) of treatment groups were compared by one way ANOVA.

Table I Summary of Fasting Protocols Applied to Minipigs

Study	Enrichment	Food (day before necropsy)	Metoclopramid i.m.
Control	Straw	Pellet diet	–
Pilot 1	Cotton towels	Pellet diet	–
Pilot 2	Cotton towels	Fluid diet	–
Pilot 3	Cotton towels	Fluid diet	0.2 mg/kg, 2 h prior to sacrifice

The control group had free access to straw throughout the study. In all other study arms, straw was replaced by alternative enrichment (cotton towels). In pilot study 1, animals received twice daily a commercially available minipig feed. In pilot 2 and 3 the pellets were replaced by a fluid diet (milk powder in water) the day before necropsy. In pilot study 3, animals received an intra muscular injection of the prokinetic drug metoclopramide, 2 h prior to necropsy

The following treatments were applied: The control group had free access to autoclaved straw as enrichment and were fed a commercially available minipig pellet diet (SDS, Essex, UK) twice daily. In pilot study 1, cotton towels were used to enrich pens as an alternative for straw 4 days prior to necropsy. Pellet diet was given as in the control group. In pilot study 2, animals had alternative enrichment for 4 days (cotton towels, no straw) and pellet diet was replaced by two fluid meals the day before necropsy. The fluid diet consisted of 150 g standard milk powder (27 g protein, 41 g carbohydrate, 26 g fat per 100 g) dissolved in 0.75 L of tap water. The same fluid diet was offered in pilot 3. In addition, 2 h before necropsy, animals received 0.2 mg/kg of the prokinetic drug metoclopramide intra muscularly. All procedures where approved by the Swiss or Danish Animal Experimental inspectorate.

Bioanalytics

Quantification of paracetamol levels in plasma was accomplished by LC-MS/MS. Plasma was precipitated in three volumes of methanol containing [(2H4)-Acetaminophen (250 $ng{\cdot}mL^{-1}$)] as internal standard, mixed and centrifuged (10 min, 3°C, 5889*g*). Thereafter, 1uL of the supernatant diluted 50:50 with water was injected onto a YMC – UltraHT Pro C18 50×2 mm column operating with mobile phases A (H2O pH5.5 ammonium acetate + acetic acid solution) and B (Methanol) at 500 $\mu L{\cdot}min^{-1}$ flow. The outlet of the column was coupled to AB Sciex Qtrap 5500 mass spectrometer with TurboIonSpray source. Detection was carried out using multiple reaction monitoring mode with positive ion detection focusing on the transitions 152.0/109.9 for Acetaminophen and 157.0/115.0 for deuterated acetaminophen. Calibration curves were established by weighted ($1/\times^2$) linear regression from peak area ratios (peak area of analyte/peak area of internal standard) *versus* nominal concentration. The calibration curve consisted of at least 6 valid levels and the back calculated concentration did not differ from the nominal concentration of the calibration standards by more than 15%, except at the lower limit of quantification level, where a difference of up to 20% was tolerated. The lower limit of quantification was 10 ng/ml.

Deconvolution

Pharmacokinetic profiles following intravenous administration of paracetamol were described individually by compartmental modeling to serve as a basis for the deconvolution analysis of the oral profiles. Cumulative percentage of dose absorbed *versus* time profiles were derived and were characterized in terms of time to onset of absorption and time to half maximal absorption. Lag times were estimated as the time of the first non-zero input rate from the deconvolved individual plots of input rate *vs.* time.

Non Compartmental Analysis

The maximum concentration (Cmax) and time to reach maximum concentration (Tmax) were obtained directly from the plasma concentration time data. Area under the plasma concentration time curve (AUC), elimination half-life (t1/2), volume of distribution (Vss) and total body clearance (CL) were calculated non-compartmentally. AUC was calculated by the trapezoidal method with extrapolation from the last time point measured to infinity. Bioavailability was determined as the ratio of the dose corrected AUC0-∞ values for each individual oral application and the intravenous route.

PBPK Model of the Minipig

A full description of our preliminary minipig PBPK model was previously published in (10). The minipig absorption model was implemented according to the ACAT model framework (28) and a detailed description is given in supplementary material. The structure and parameterization was not changed, except for the gastric emptying times.

Paracetamol pharmacokinetics were simulated based on compound specific inputs, summarized in Table II (10). Due to the scarcity of available information on intestinal dimensions, the small intestine model was implemented as compartments of equal length and uniform diameter with equal transit

Table II Overview of Input Parameters Used for PBPK Modeling of Paracetamol, Caffeine, Omeprazole, and Warfarine

Compound	Paracetamol	Caffeine	Omeprazole	Midazolam	Warfarine
Molecular weight (g/mol)	151	194	345	326	308
Dose (mg/kg)	i.v. : 10 p.o. : 30	p.o.: 1[24]	p.o.: 1[24]	p.o.: 1[24]	p.o.: 1[24]
LogD (at pH 7.4)	0.47 measured at pH 7.5[a]	0.02 [29]	2.23[c]	3.10[a]	2.15[a]
pKa (A = Acidic, B=Basic)	A:9.5[a][19]	0.92[30]	A:9.29; B:4.77; B:1.57[c]	B: 6.57[c]	B:-6.60; A: 6.33[c]
Solubility (mg/ml)	16.0 at pH 6.5[a]	18.70 at pH 4[b]	0.25 at pH 7[c]	0.03[31] at pH 7.4	5.0 at pH 7[c]
B/P ratio	1[28]	–	–	–	–
Fup (%)	75–80%[28]	–	–	–	–
Intestinal permeability (cm/s *10E-4)	0.81 [26] (scaled from CACO-2)	4.0[32] (scaled from CACO-2)	Caco-2: 3.92[33] (scaled from CACO-2)	1.72[a] (scaled from LLCPK)	2.8 [34] (scaled from CACO-2)
Mean clearance (l/h*kg)	0.408	0.047	0.649	1.020	0.009

[a] In house measurements

[b] Information according to manufacturers data sheet

[c] Information retrieved from Drugbank

times. Stomach dimensions were not available from the public domain, so post mortem stomach length and radius were measured in two in-house minipigs. We used the mean reported values for stomach pH of 3.6. As no reliable data for porcine intestinal permeability was available, we used permeability scaled from CACO-2 experiments using an in-house correlation to human jejunal permeability (29) and assumed that porcine and human values would be the same. Regional scaling of effective permeability was performed using the GastroPlus Opt logD SA/V6.1 model (SimulationsPlus, GastroPlusTM manual, 2010b). Emptying from the stomach compartment in the default ACAT model was treated as a first order process without lag time and is implemented as a mean gastric transit time (MGTT). Thus the time to reach half maximal gastric emptying, t_fabs50, is given by:

$$MGTT = \frac{t\_fabs50}{\ln(2)}$$

To account for drug partitioning into different tissues in the distribution model, we used an adaptation of the approach proposed by Rodgers to calculate partition coefficients (30). Clearance in the model was added as hepatic clearance based on the mean observed total systemic clearance determined by non-compartmental analysis.

The absorption model was updated with the newly determined MGTT values and validated on a set of published minipig crossover PK studies for caffeine, omeprazole, midazolam, and warfarin (27). Although the data source used a different minipig strain, the experimental conditions were comparable to those used in our study. To avoid confounding the performance of the absorption model with the PBPK disposition model, we described disposition by a compartmental model fit to the plasma concentration profiles after an i.v. dose. Model performance was estimated by visual inspection of observed *vs.* predicted plots. A deviation within two-fold was deemed acceptable (4). Input parameters are summarized in Table II.

Software

Deconvolution, PK model fitting and non-compartmental analysis was performed in Phoenix WinNonlin (Version 1.2, Pharsight Corporation, Mountain View, CA, USA). The PBPK model for minipig was implemented in Gastroplus (Version 8, Simulation plus, Lancaster, CA, USA). Basic statistics for mean weights of full and empty stomachs in minipigs and their variation were compared by single factor ANOVA using R (version 2.15.0 [2012-03-30]). I.v. and p.o. pharmacokinetic profiles for caffeine, omeprazole, midazolam and warfarin were digitized from publications using in house software (27).

RESULTS

Paracetamol was well tolerated by all animals at the administered doses. Mean concentration time profiles after intravenous and oral dosing are shown in Fig. 1.

Non-compartmental Analysis

Means of individual pharmacokinetic parameters calculated by non-compartmental analysis are given for male and female animals in Tables III and IV. The change in body weight was in line with the expected growth of the minipigs (31).

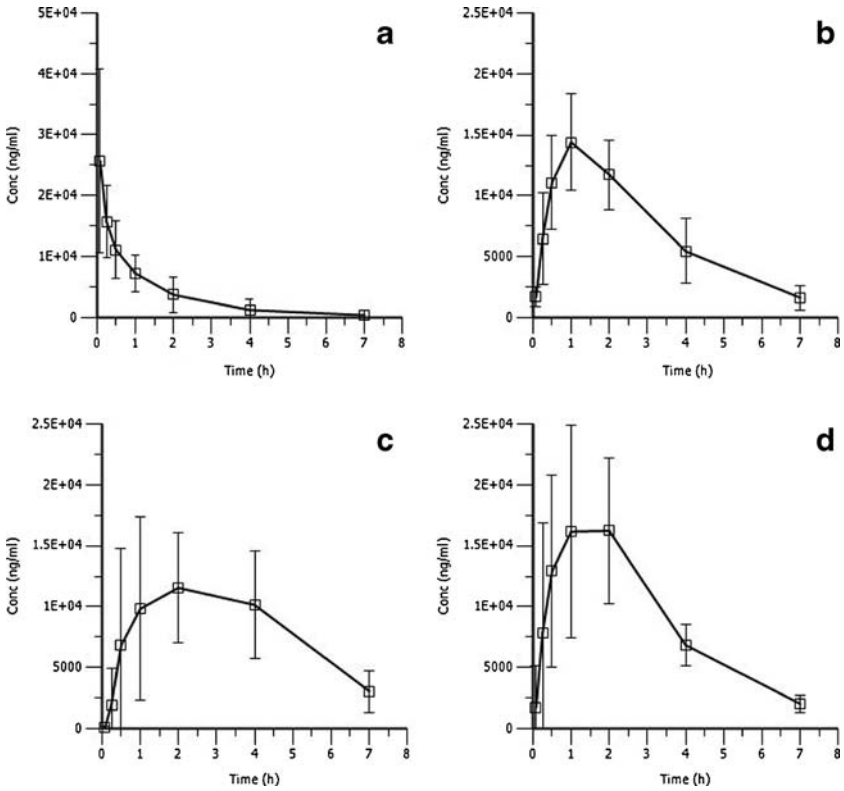

Fig. 1 Mean +/− standard deviations of paracetamol plasma concentrations in 4 male and 4 female Göttingen minipigs. (**a**) Intravenous administration as solution at 10 mg/kg. (**b**) Oral administration as solution at 30 mg/kg. (**c**) Oral administration as capsule at 30 mg/kg. (**d**) Oral administration as tablet at 30 mg/kg.

Deconvolution of Pharmacokinetic Profiles

Profiles describing the change in absorption rate over time derived by numerical deconvolution in individual minipigs showed high inter-individual variability, especially for the solid dosage forms (Fig. 2, upper panel). Mean +/− standard deviation for cumulative fraction of dose absorbed are shown in the lower panel of Fig. 2. To minimize variability due to different bioavailabilities the fractions are expressed relative to the total absorbed dose in each individual minipig.

Mean time to reach half maximal fraction of dose absorbed (t_fabs50) and lag times are summarized in Table V and Fig. 3. By dividing t_fabs50 by ln(2) we derived mean gastric emptying times (MGTT) which were used to parameterize the PBPK model (Table V). Of all oral administrations, capsules showed the longest lag times and highest variability.

Assessment of Stomach Content

Results of minipig stomach post mortem assessment is summarized in Table VI and visualized in Fig. 4. All animals in the control group and pilot study 1 exhibited a significant amount of semi-solid stomach content despite the overnight fasting period. When a liquid diet was applied the day before

Table III Results of Non-compartmental Analysis of Pharmacokinetic Profiles as Well as Administered Doses and Mean Body Weight for Male Minipigs ($N = 4$) are Summarized

Male minipigs	1	2	3	4
Route of administration	I.v.	p.o. (solution)	p.o.(capsule)	p.o.(tablet)
Nominal Dose (mg/kg)	10	30	30	30
Mean weight (kg)+/−SD	8.2+/−1.7	8.8 +/−1.7	9.6+/− 1.8	9.8+/−1.9
Cmax (ng/ml)	21700 ± 2110	14800 ± 2800	15800 ± 2550	15500 ± 5770
Tmax (h)	–	1.13	3.50	1.38
Half-life (h)	2.71 ± 0.55	3.63 ± 0.20	3.57 ± 0.43	3.84 ± 0.26
AUC(0-inf)	32300 ± 6130	76300 ± 23900	103000 ± 8930	80400 ± 27600
Volume of distribution (L/kg)	0.74 +/− 0.10	–	–	–
Clearance (ml/[min*kg])	5.31 ± 1.07			
Bioavailability (%)	–	78.6 ± 24.6	106 ± 9.1	82.9 ± 28.5

Bioavailability was calculated by dividing intravenous and oral area under the concentration time curve (AUC)

Table IV Results of Non-compartmental Analysis of Pharmacokinetic Profiles as Well as Administered Doses and Mean Body Weight for Female Minipigs ($N = 4$) are Given

Female minipigs	1	2	3	4
Route of administration	I.v.	p.o. (solution)	p.o.(capsule)	p.o.(tablet)
Nominal Dose (mg/kg)	10 mg/kg	30 mg/kg	30 mg/kg	30 mg/kg
Mean weight (kg)+/−SD	8.15+/−1.5	8.8+/−1.3	9.8+/−1.3	9.9+/−1.3
Cmax (ng/ml)	30100 ± 4450	14700 ± 4650	16200 ± 5620	24400 ± 4340
Tmax (h)	–	1.13	1.88	1.50
Half-life (h)	5.14 ± 3.04	4.25 ± 0.39	4.79 ± 1.39	4.21 ± 0.51
AUC(0-inf)	27100 ± 4700	54700 ± 12800	69200 ± 4480	84100 ± 10500
Volume of distribution (L/kg)	0.68 +/−0.10	–	–	–
Clearance (ml/[min*kg])	6.27 ± 0.95			
Bioavailability (%)	–	67.3 ± 15.8	85.2 ± 5.5	104 ± 12.9

Bioavailability was calculated by dividing intravenous and oral area under the concentration time curve (AUC)

necropsy (Pilot 2), stomachs tended to be macroscopically empty. After i.m. application of 0.2 mg/kg metoclopramid 2 h before necropsy (Pilot 3), all animals in the treatment group exhibited empty stomachs. However, no significant difference was determined between treatment groups as the study was not sufficiently powered to reveal a significant effect, considering the high variability in remaining food amount.

Comparison of PBPK Model Simulations to Observed Data

We used input parameters summarized in Table II and simulated the disposition pharmacokinetics of paracetamol using our minipig PBPK model. Simulations were in good agreement with observed intravenous plasma concentration time profiles (Fig. 5)

For modeling absorption of paracetamol we re-parameterized the ACAT model using the newly determined mean gastric transit time (MGTT). Paracetamol is categorized as a BDDCS class 1 compound with high permeability (21). Under this assumption, it is valid to relate observed concentration time profiles to gastric emptying. Scaling from CACO-2 experiments resulted in a moderate permeability of 0.8 cm/s*10^-4. However, we knew from simulating human paracetamol absorption that permeability of 10 cm/s*10^-4 is needed to fit observed plasma concentrations (data not shown). Further support for high intestinal permeability of the drug came

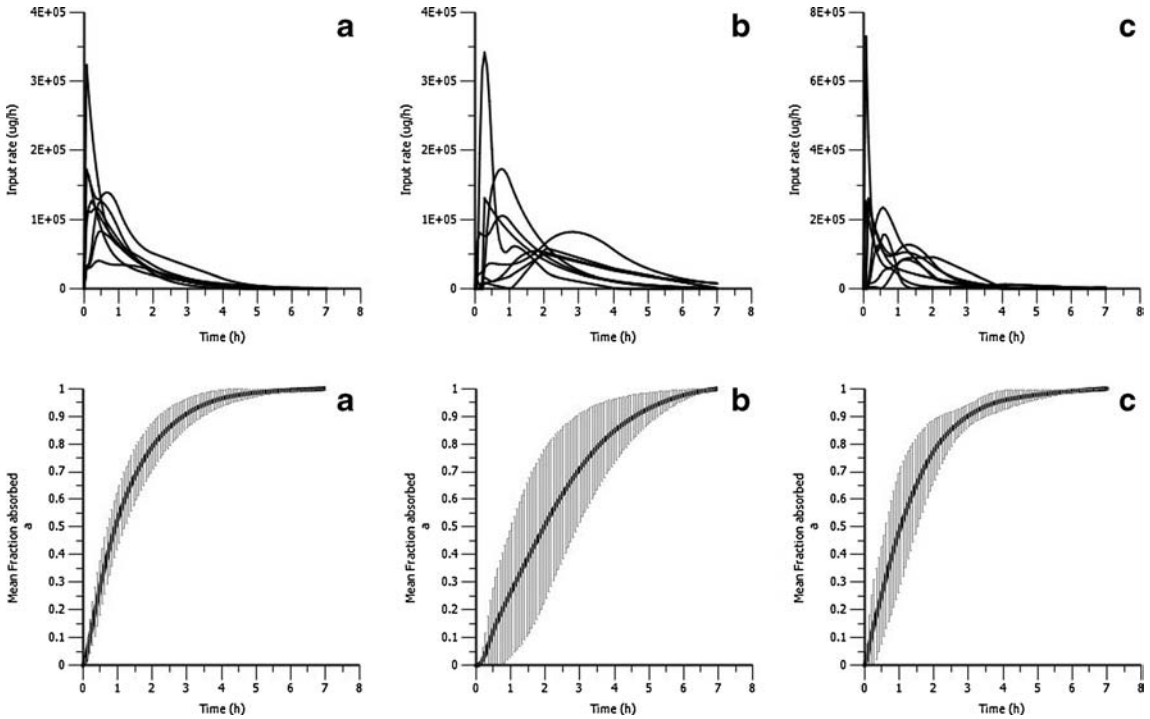

Fig. 2 *Upper panel* shows paracetamol input rates as a function of time of 8 Göttingen minipigs. *Lower panel* shows mean cumulative fraction of dose absorbed. *Grey bars* indicate standard deviation. (**a**) Solution (**b**) Capsule (**c**) Tablets.

Table V Mean Times and Standard Deviations to Reach 50% of Maximal Fraction Absorbed (t_fabs50), Mean Gastric Transit Times (MGTT) and Lag Times (t_lag) of Paracetamol Absorption in Solution, Capsule and Tablets are Listed

	Solution		Capsule		Tablet	
	Mean	SD	Mean	SD	Mean	SD
t_fabs50 (h)	0.91	0.1	1.96	0.28	1.05	0.21
MGTT (h)	0.63		1.36		0.73	
t_lag (h)	0.02	0.02	0.1	0.1	0.05	0.03

Table VI Minipigs Underwent Different Starving Protocols and Empty Stomach Weight and Content (g) were Assessed After Overnight Starving Period

Study protocol	Control	Pilot 1	Pilot 2	Pilot 3
Empty stomach weight (g) (+/− SD)	128 +/− 15	116 +/− 16	112 +/−15	150 +/−6
Stomach content (g) (+/− SD)	159 +/− 190	76 +/− 37	25 +/− 18	25 +/−5

Mean weights (g) and standard deviations are summarized

from independent work of Levitt (32) and from Ussing chamber experiments using porcine jejunum (33). As we were lacking scaling factors for permeability measured in Ussing chambers, we assumed the same permeability in pigs as in humans and used a value to 10 cm/s * 10^-4. Using this value in our simulations we achieved good agreement with observed data for all administration forms of paracetamol. Results are summarized in Fig. 6.

Validation

We validated the re-parameterized PBPK model using published minipig pharmacokinetic studies of caffeine, omeprazole, midazolam, and warfarin (27). As the focus was on the absorption model performance we used compartmental model fits to intravenous data to account for disposition pharmacokinetics and applied the ACAT model with the newly determined mean gastric emptying times (Table V). Input values used for the validation are summarized in Table II. Overlays of observed and simulated profiles of caffeine, omeprazole, warfarin and midazolam are given in Fig. 7.

DISCUSSION

Reported gastric pH and gastric emptying show high inter-study and inter-individual variability in minipigs (5,10,15–19). Some of the variation in reported pH might be explained by error associated with experimental measurement since the gastrointestinal tract is susceptible to fast post mortem changes and rapid bacterial degradation of mucosa (34). Furthermore, *in vivo* experiments using solid non-dissolving devices, such as capsule transponders might disturb the physiological gut function. Such measurements might be less useful to predict *in vivo* behavior of dissolving drugs. To get more relevant estimates of gastric emptying for drug development, we performed a cross over pharmacokinetic study with paracetamol in 8 Göttingen minipigs. The animals received doses in the usual therapeutic range (*i.e.*,10–30 mg/kg for pigs) to avoid side effects or significant involvement of active processes in absorption (22,35,36). Generally, paracetamol distributes rapidly throughout most body tissues and fluids. The apparent volume of distribution in our minipigs (0.7 L/kg) was close to the volume observed in humans (0.9 L/kg) and slightly lower than the value reported in landrace pigs (1.5 L/kg) (36). Non-compartmental analysis revealed mean paracetamol systemic clearance of 5.31 ± 1.07 ml/(min*kg) in males and 6.27 ± 0.95 ml/(min*kg) in females, while in landrace pigs a higher clearance of 14.6 ml/(min*kg) was reported (36). Differences in metabolic turnover between conventional farm pigs and the laboratory purpose bred minipigs have been reported by others (37). The bioavailability for different formulations in minipig was high, which is in line with observations in human

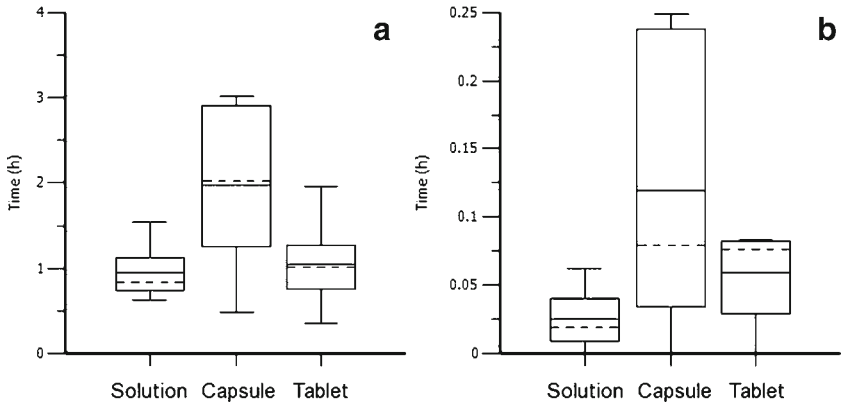

Fig. 3 Box plots of mean (*solid line*, +/− standard deviations) and median (*broken line*) time to reach half maximal fraction absorbed (t_fabs50) (**a**) and corresponding lag times (**b**) for the three different oral administrations of paracetamol.

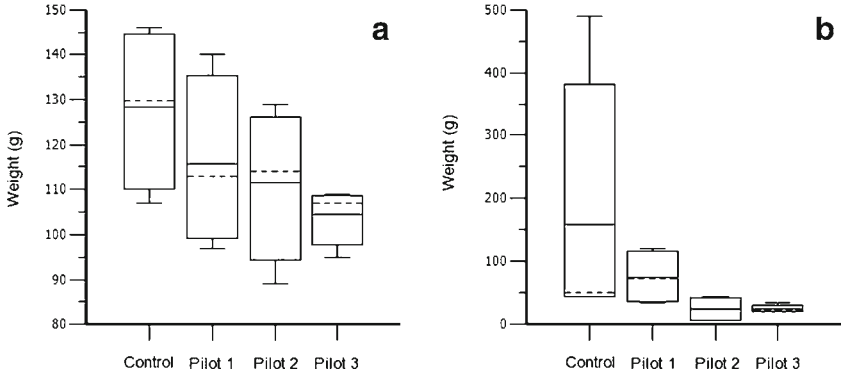

Fig. 4 Results of minipig stomach post mortem assessments. Control animals (N=4) had free access to straw throughout the study and were fed a standard pellet diet. In pilot study 1 (N=4) straw was withdrawn for 4 days, but minipigs received the same solid food as the control group. In pilot study 2 (*N* = 4) animals had no access to straw and were fed a liquid diet. In pilot 3 (N=4) the same conditions as in pilot study 2 were applied, in addition, the animals received 0.2 mg/kg metoclopramide i.m., 2 h prior to necropsy. Plots show mean (*solid line*) and median (*broken line*) weight (g) of empty stomachs (**a**) and residual stomach content after overnight fasting (**b**) in the four different study settings. *Error bars* indicate standard deviations.

(70% to 90%) (22). Incomplete and variable bioavailability in humans is believed to be largely due to variable first pass metabolism (38–40).

A tendency to higher paracetamol clearance was seen in female minipigs compared to males although more animals would have to be tested for a statistically significant conclusion. However, others have reported that Göttingen minipigs can exhibit sex-related differences in metabolism (37). In humans, the oxidative pathway of paracetamol is mediated by CYP2E1 and there are reports of significantly higher turnover of the two typical CYP2E1 substrates chloroxazone and p-nitrophenol, in incubations with female minipig microsomes than in those of male animals (41). The high sequence identity of porcine and human CYP2E enzyme would support that paracetamol is also a CYP2E substrate in pigs (41). In addition, paracetamol undergoes extensive phase II metabolism (42). *In vitro* experiments with pooled liver microsomes of different species revealed that at therapeutic concentrations landrace pig ranged between monkey and humans in terms of glutathione metabolite production (43). Interestingly, at toxic concentrations, pig microsomes showed the highest turnover of all species, which is in line with reports that pig is a readily conjugating species (44). To date, active transporters, such as P-glycoprotein are very poorly described in the minipig. However, we believe that the influence of active transport on paracetamol absorption in minipigs is minor.

The input rate *vs.* time profiles obtained by numerical deconvolution showed high variability. A trend towards a lag time followed by an early, fast component, superimposed with a prolonged slow absorption could be observed. Interestingly, such gastric emptying patterns were also observed in paracetamol studies performed in humans. Emptying patterns showed a very fast initial absorption phase, followed by a slower first order phase or two consecutive first order absorption phases with quiescent interval (25). This seemed to be mainly the case for smaller fluid volumes while large volumes emptied by first order kinetics (45).

The advanced CAT model implements stomach emptying as a simple first order process without lag time (28). Mean gastric transit times (MGTT) derived from the time to reach half maximal fraction absorbed (t_fabs50) were used to parameterize the stomach compartment. The assumption that gastric emptying determines paracetamol absorption rate is valid when drug permeability is high and not limiting. However, scaling from CACO-2 experiments resulted in only moderate permeability values (0.8 cm/s*10^-4). An explanation for the discrepancy may be that paracetamol is a rather hydrophilic (LogD 0.47 at pH 7.5), low molecular weight

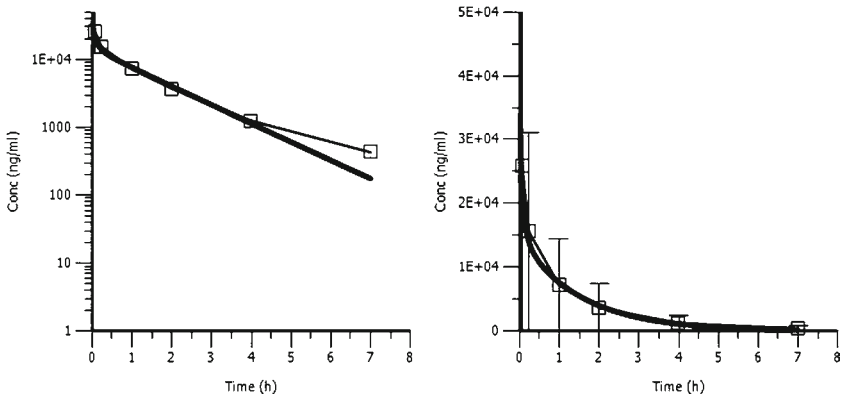

Fig. 5 Linear and logarithmic representations of observed (*squares*, +/− SD) and simulated (*bold line*) plasma concentration time profiles after intravenous bolus administration of 10 mg/kg paracetamol.

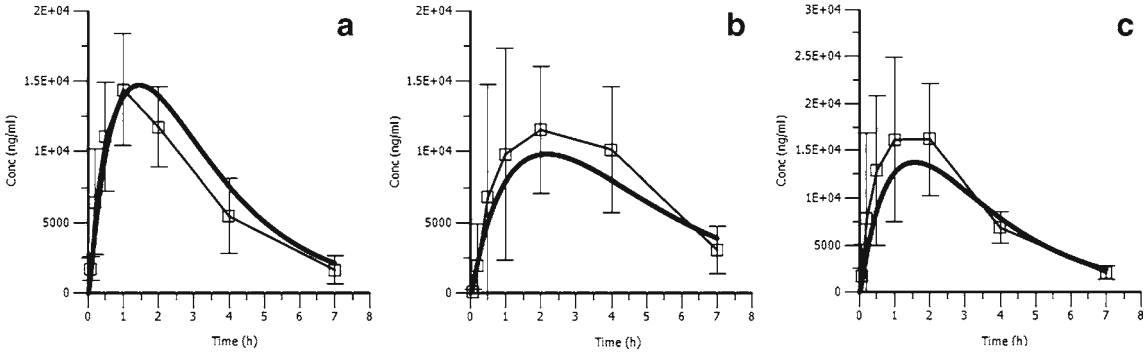

Fig. 6 Overlays of observed (*squares*, +/− SD) and simulated (*bold line*) plasma concentration time profiles after oral administration of 30 mg/kg paracetamol. (**a**) Solution. (**b**) Capsule. (**c**) Tablet.

compound and might permeate significantly *via* the paracellular route which is often underestimated by the CACO-2 data (46). As the drug exhibits a high bioavailability, is classified as a BDDCS class I compound and is commonly used as a marker for gastric emptying (21,32,35), we decided that the value scaled from CACO-2 experiments was not a realistic estimate of *in vivo* permeability. A recent interspecies comparison study with Ussing chamber experiments also supported a high intestinal permeability for paracetamol in pigs (33). However, since we lacked scaling factors to relate these Ussing chamber experiments to *in vivo* permeation we decided to use a value obtained from modeling human paracetamol absorption. Using this value, the simulations for minipig agreed well with the observed data (Fig. 6). We further validated the re-parameterized model on an external data set for caffeine, omeprazole, midazolam and warfarin administered as oral solutions. Although the data source did not use Göttingen minipigs, the study and animal housing conditions employed were comparable to the present study design (27).

Simulations for caffeine, using the scaled permeability values from CACO-2 experiments, fitted the *in vivo* data reasonably (Fig. 7a) (47). In the case of omeprazole, we included a first pass loss in the range of that observed in other preclinical species (90%) to fit the *in vivo* data (Fig. 7b). Omeprazole shows very low bioavailability in rats (6.4, 9.6, and 12.6% at the doses of 10, 20, and 40 mg/kg (48)) and dogs (15% (49)) in contrast to humans, where bioavailability is intermediate (40–50%) (50). The model also gave reasonable simulations for midazolam when we accounted for a high first pass loss, in line with other preclinical species (Fig. 7d). Fraction absorbed times fraction escaping intestinal metabolism (Fa × Fg) is only 0.02–0.09 in cynomolgus monkey (51) and

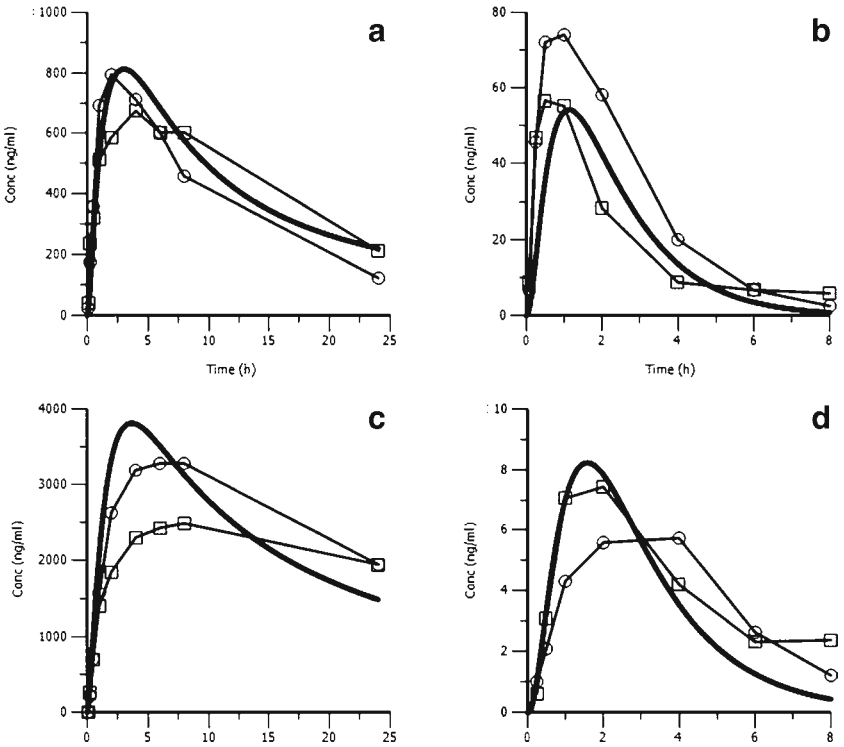

Fig. 7 Overlay plots of simulations and observed data of our external validation set. Simulations of re-parameterized minipig PBPK model are given as bold lines. Observed data are mean profiles of male (*squares*, $N = 4$) and female (*circles*, $N = 4$) micro-minipigs. Panel (**a**), (**b**), (**c**) and (**d**) show results of caffeine, omeprazole, warfarin, and midazolam.

0.03 in rat (52) while moderate first pass is seen in human (Fa × Fg of 0.46–0.57) (53). Active efflux of omeprazole and midazolam might partly account for the low bioavailability observed in preclinical species but to date, drug transporters are very poorly described in minipigs (54,55). In simulations of warfarine pharmacokinetics, bioavailability was reasonably matched but Tmax was under predicted (Fig. 7c). Overall, the validation showed that the estimated mean gastric transit times reflect a realistic parameterization for the minipig PBPK model.

In our *in vivo* paracetamol study, minipigs showed a lag time between oral administration of paracetamol and onset of absorption. This is generally interpreted as reflecting gastric food milling (56). Observed lag times were relatively short (0.03–0.12 h) compared to values measured in humans for different test meals (0.6–1.4 h) (57). This could be an indication that food milling and hence the motoric stomach function, is less pronounced in pigs than in humans. This hypothesis is supported by the observation that in the porcine stomach, food is deposited in layers depending on the size of food particles (58). Further, we found prolonged food retention in our post mortem assessments of minipig stomachs. Interestingly, overnight pellet food withdrawal was not sufficient to obtain a true fasted state and all control animals had remaining semisolid food (mean 159 g) in their stomachs. Although this amount could be reduced to a mean of 76 g by straw depletion from the pens, the consistency of stomach content did not differ from the control group. By switching to a liquid diet, offered the day before necropsy, we found two out of four animals having macroscopically empty stomachs. However, complete food depletion was only achieved when we applied a prokinetic drug shortly before sacrificing the animals. Although our results suggest that fasted state can be achieved, we feel that the applied procedure will be of limited use in a drug research and development setting. Usually co-administration or pretreatment with drugs such as metoclopramid is avoided to not confound study readout by potential drug-drug interactions. Moreover, keeping minipigs for long times in fasted state might make them more prone to stomach ulcerations. In fact, we observed signs of gastric mucosa erosion in one animal of pilot study 3. More animals would have to be tested to clarify whether this was a sporadic observation.

In line with the observation that minipigs were not in fasted state during our paracetamol study, drug emptying seemed to agree with human data on gastric emptying of liquid (1.3 h (+/− 0.3 SD)) and solid (2.4 h (+/− 0.9 SD)) test meals (57,59). Gastric drug emptying in humans was reported to be much shorter (0.15 to 0.6 h) and clinical fasted state paracetamol studies revealed emptying half-times of 0.35 h (25). Human fed state pharmacokinetic studies exhibit increased variability compared to fasted state studies (60) which would be in line with our observations in minipig, where we found high variability in absorption rate and speed. Whether the hydrophilic drug paracetamol binds to remaining food and hence empties more erratically from the stomach, is unclear. *In vivo* studies in other species indicate that the compound would bind to the stomach content (61,62) while *in vitro* studies in standardized test meals could not confirm this observation (63). It can be assumed that the impact of food binding would be stronger in more lipophilic compounds. Considering the drug space which is currently explored in pharmaceutical industry, heading clearly towards highly lipophilic and poorly soluble compounds, food effects and hence variability might be even more pronounced in pharmacokinetic studies.

CONCLUSIONS

This work aimed to determine gastric emptying times in minipigs under standard housing conditions by means of paracetamol pharmacokinetics to update a recently proposed PBPK model. Overall, our results indicate that the minipig has a weaker stomach milling function (*i.e.* gastric motility) than human, which is reflected in prolonged food retention and shortened lag times. Simulations for several drugs using the updated model confirmed the prolonged gastric emptying. However, further work is needed to better characterize the effect of feeding on gastric emptying in minipigs and to define appropriate fasting protocols.

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H. Lorentsen is employed by the minipig provider Ellegaard Göttingen Minipigs A/S.

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