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Research paper

Characterization of the GI transit conditions in Beagle dogs with a telemetric motility capsule

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ABSTRACT

In preclinical research, Beagle dogs are an important model for formulation development and for evaluation of food effects on drug absorption. In this study, the gastrointestinal transit conditions in Beagle dogs were studied with a telemetric motility capsule at different intake conditions. In a cross-over study design, the SmartPill® was given to six Beagle dogs to measure transit times, pH values, pressures and temperatures in the different parts of the canine GI tract. Moreover, the effects of commonly applied pre-treatments as with pentagastrin and famotidine on GI transit conditions were investigated. The gastric transit time in fasted state was short (0.57 ± 0.37 h) and only slightly affected by the pre-treatments. In fed state, gastric transit was clearly prolonged (2.94 ± 0.91 h). The mean intestinal transit time was in the range of 1–2 h and not affected by the intake conditions. The gastric pH values in fasted and fed Beagle dogs were highly variable, but pre-treatment with pentagastrin and famotidine clearly decreased variability. Pre-treatment with pentagastrin resulted in minimum pH values around 0.5 pH units lower than without pre-treatment. Oral administration of famotidine led to constantly elevated pH values of pH 7–8. The maximum pressures in the canine GI tract did not vary significantly between the study arms and typically, maximum pressures of up to 800 mbar were observed in the stomach. The comparison of the data from this study with recent SmartPill® data from humans revealed that major differences could be observed with respect to gastric transit times in fed state, small intestinal transit times as well as maximum pressures arising during GI transit. These differences should be kept in mind if the dog model is used to assess the *in vivo* performance of solid oral dosage forms intended for use in humans.

1. Introduction

In pharmaceutical research and development, dogs are commonly used to study the performance of oral dosage forms since human and canine physiology are regarded as comparable in many aspects. Therefore, the dog model represents an important model for the development and optimization of oral formulations. The possibility to administer even larger oral dosage forms (e.g. extended release tablets) is a great advantage over other commonly used laboratory animals such as mice, rats or rabbits [1]. Among the different breeds of dogs, the Beagle dog is the most commonly used dog for preclinical studies due to the ease handling of this breed [2].

Owing to its great importance in preclinical research, the gastrointestinal conditions in Beagle dogs have been subject of various studies, which revealed that its GI physiology shows various similarities with human GI physiology [3–7]. However, due to differences in absorption and subsequent metabolism between species none of the laboratory animal models is directly predictive for the oral bioavailability in humans [8]. In addition, some differences between canine and human GI physiology have to be considered to prevent from wrong conclusions from studies with Beagle dogs [6,9–11]. For instance, in case of drugs with pH-dependent solubility or pH-sensitive dosage forms, the gastric pH is a key parameter for the resulting pharmacokinetic (PK) profile. Owing to differences in gastric acid secretion, gastric pH is generally expected to be higher in dogs compared to humans [4,12]. In order to minimize this effect, pre-treatment with pentagastrin is commonly performed to simulate the low human gastric pH levels and its effect on drug release and absorption [13]. The pentapeptide pentagastrin is a synthetic analogue of gastrin, which is typically administered intramuscularly and which causes similar effects as

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endogenous gastrin (i.e. stimulation of gastric acid secretion) [14]. Another modification of gastric pH in the Beagle dog commonly applied in preclinical research is based on the administration of pH-elevating drugs [13,15–17]. Pre-treatment with antacids such as the H_2-receptor antagonist famotidine shall elevate the gastric pH consistently. This pre-treatment allows to study the effect of an elevated gastric pH on the oral absorption of weakly basic drugs. The elevation of gastric pH can also be used to investigate oral drug delivery under conditions simulating hypo- and achlorhydric subjects or subjects on medication with proton pump inhibitors and H_2-receptor antagonists or taking antacids.

Furthermore, the dog model is used to study the effect of concomitant food intake on oral drug absorption. For instance, Wu and colleagues have used the dog model to demonstrate the improved bioavailability and diminished food effect of a nanoparticulate formulation of aprepitant [18]. Also Lentz and colleagues have proposed a dog model, which shall allow to predict the food effect on oral drug bioavailability in humans [19]. In order to validate this model, Lentz et al. investigated in dogs various different drugs with known food effects in humans and compared the results with human data.

It should be kept in mind that a thorough understanding of GI physiology is a prerequisite for the interpretation of results from in vivo studies in both, animals and humans. The GI physiology of animals and humans as well as the behavior of dosage forms in the gut can be studied with a range of different techniques, which include the sampling of aspirates, telemetric capsules such as the Heidelberg pH capsule or the Bravo pH system as well as imaging techniques such as scintigraphy [20–22]. Within the last years, several interesting techniques became commercially available that can be used to study GI transit conditions *in vivo* in a non-invasive way. One of these techniques is the SmartPill®, a telemetric motility capsule (TMC). This ingestible and freely moveable system has the size of a large capsule (26 × 13 mm) and allows the quantification of pH, temperature and pressure with high temporal resolution. In recent studies, our group has used this system to explore GI transit conditions in healthy human volunteers in fasted and fed state in order to describe the physiological GI conditions occurring in clinical trials as these form the basis of the majority of the published PK data [23,24]. Therefore, these investigations followed a study protocol that resembled the protocol of bioavailability and bioequivalence studies according to FDA and EMA guidelines [25–27]. With respect to dogs, the SmartPill® has already been used in privately owned veterinary patients [28], but to the best of our knowledge not in Beagle dogs and not under conditions simulating preclinical dog studies.

The primary aim of this study was to study the GI transit times and conditions in fasted and fed Beagle dogs with the aid of a telemetric motility capsule (SmartPill®) under conditions of preclinical dog studies. These data were compared with human data gathered recently with the same system. The second aim of this work was to study the effects of the pre-treatments with pentagastrin and famotidine on GI transit and luminal pH values. The protocol used in this study was based on protocols published in the years 2005 and 2011 by Zhou, Fancher and their co-workers [13,17].

2. Materials and methods

This animal study described in this work was approved by the Regierungspräsidium Tübingen (17–002-A) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

2.1. Materials

Pentagastrin was purchased from Sigma-Aldrich (Steinheim, Germany). Pentagastrin was lyophilized and stored at 2–6 °C until application. Prior to use it was reconstituted with isotonic 0.9% NaCl solution to the concentration of 0.2 mg/ml with a resulting pH value of pH 6–7. The aseptically manufactured lyophilisate consisted of 0.936% (w/w) Pentagastrin, 35.101% (w/w) sodium hydroxide 0.1 M, 17.161% (w/w) hydrochloric acid 0.1 M, and 46.802% (w/w) mannitol. Famotidine (FAMOTIDIN 1A 40 mg tablets) was from 1A-Pharma (Oberhaching, Germany). The ingredients for the test meal (eggs, bacon, butter, toast and milk) used in this study were purchased at a local supermarket.

2.2. Telemetric motility capsule (TMC) system

The SmartPill® GI Monitoring system (Given Imaging Ltd., Yoqneam, Israel) was used to measure gastrointestinal pH (measuring frequency 0.2 Hz), temperature (measuring frequency 0.05 Hz) and pressure (measuring frequency 2.0 Hz) [29,30]. A data receiver was fixed outside the cage to record the data packages sent by the capsule.

2.2.1. Calibration

Before ingestion, the proper functioning of each capsule was checked in terms of pH, pressure and temperature. For this purpose, a one-point calibration was performed for pH (at pH 6.0) and temperature (at room temperature). Additionally, the integrity of the pressure sensor was tested over a range of 0–400 mbar with the aid of a manometer. Due to the known drift of the pH sensors, an extended post-calibration procedure was conducted after capsule excretion in order to account for the drift. For this purpose, the pH recordings of the SmartPill® were compared at five different pH values ranging from pH 1.0 to pH 10.0 with recordings from a calibrated pH electrode (model MP230, METTLER TOLEDO, Germany). Pressure (0–500 mbar) and temperature sensors (one-point calibration at room temperature) were also checked again after excretion.

2.2.2. Data analysis

Temperature compensation for pH value and pressure was performed automatically by the corresponding MotiliGI® software. Also the baseline of pressure was automatically corrected by the software. However, we abstained from using these baseline corrected pressure data as these were only relative data, which did not represent the real values measured *in vivo*. Thus, only temperature compensated pressure and pH data as well as the original temperature data were used for data analysis. All datasets were analyzed with the aid of Origin 8.5.1G (OriginLab Corp., Northampton, USA). As illustrated in Fig. 1, gastric emptying time (GET), small intestinal transit time (SITT) and colonic arrival time (CAT) were determined by considering significant pH changes. Gastric emptying was identified by a significant and permanent pH change to values of pH 5 or higher. In case of famotidine, due to elevated gastric pH values, the increase was less clear and therefore,

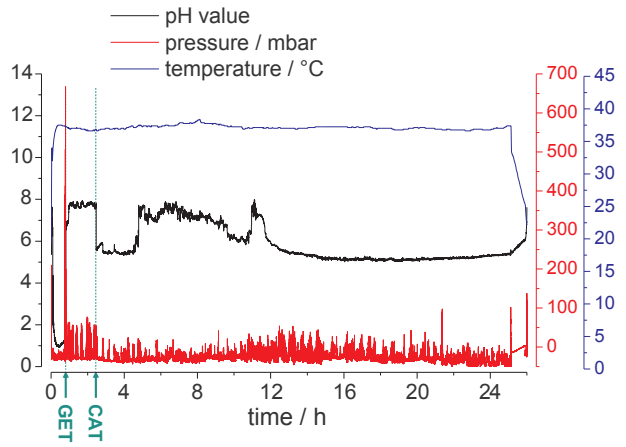

Fig. 1. Exemplary pH, temperature and pressure profile obtained after TMC administration in a fasted beagle dog. GET – gastric emptying time, CAT – colon arrival time.

we also considered the occurrence of maximum pressure events above 300 mbar as these are typically limited to the stomach. Colonic entry was defined by a sharp pH decrease of at least 0.5 pH units occurring at least 30 min after gastric emptying. Capsule excretion was identified by a drop in temperature.

As a consequence of the observed pH drift of the TMC sensors, the pH values were corrected based on the results of the post-calibration. The following equation was used:

$$pH_{corr} = pH_m - \left(\frac{\Delta\ pH}{WGTT} \cdot t\right) \quad (1)$$

where pH_m is the pH value measured by the TMC, ΔpH is the mean pH drift calculated from the values determined during calibration, which was performed before ingestion and after excretion, WGTT is the whole gut transit time in hours and t is the time in hours after the TMC intake.

The small intestinal transit time ($SITT_{norm}$) was normalized by the following Eq. (2) to enable the comparison of individual pH profiles with differences in their transit times:

$$SITT_{norm} = \frac{t - GET}{CAT - GET} \quad (2)$$

where $SITT_{norm}$ is the normalized small intestinal transit time in hours, t is the time in hours after TMC intake, *GET* is the gastric emptying time in hours and *CAT* is the colon arrival time in hours. The normalized SITT was used to compare the values in proximal and distal parts. Proximal parts were defined by a $SITT_{norm}$ of 0–0.1 h and distal parts were defined by $SITT_{norm}$ of 0.9–1 h. The same procedure was already applied in a recent publication [31].

The data were characterized by minimum, maximum, interquartile range, median and arithmetic mean including the standard deviation where appropriate.

2.3. Animals

Six Beagle dogs (3 male/3 female) aged 2–4 years and weighing 13–18 kg were included in this study. During the different treatments, the dogs were located in metabolic cages. All dogs had free access to water throughout the study. Animal identification: The first digit of the animal number indicates the study phase and the following digits sex (0 = m/5 = f) and individual (xx1–xx3).

2.4. Study design

The cross-over study comprised of four different arms, in which the TMC was administered after an overnight fast together with 50 mL of water at different conditions (Table 1). Before each study arm, the dogs were fasted for at least 18 h. After ingestion of the telemetric motility capsule with 50 mL water per animal, no food was allowed for further 4 h. After this time, dry food (Trockenfutter Kliba 3363, Provimi Kliba AG, Kaiseraugust Switzerland) was presented to the dogs as well as canned food (Ideal Balance Canine Adult with Chicken and Vegetables, Hill's Pet Nutrition GmbH Hamburg, Germany) adjusted to their individual body weight.

Table 1
Description of the study arms.

Arm	Study phase	Description
A	1	Fasted, without pre-treatment
B	2	Fasted, pretreatment with pentagastrin (6 µg/kg, i.m.) 30 min prior to TMC administration
C	3	Fasted, pretreatment with famotidine (40 mg, oral) 60 min prior to TMC administration
D	4	Fed (aliquot of 9 mL/kg of a modified FDA standard meal given 5 min prior to TMC administration, approx. 11 kcal/kg body weight kcal)

For study arm B, a pentagastrin dose of 6 µg/kg body weight was injected intramuscularly (i.m.) 30 min prior to the TMC administration. For study arm C, one tablet containing 40 mg of famotidine was given orally 60 min prior to the TMC administration. In study arm D, each dog received a homogenized and mushy aliquot of 9 mL/kg body weight (~11.3 kcal/kg body weight) of a test meal, which was administered orally by gavage 5 min prior to the TMC administration. This test meal represented a modified FDA standard meal (i.e. FDA standard meal without hash brown potatoes) and was prepared in form of a mastermix that was used for all dogs. This mastermix consisted of 144.6 g eggs, 39 mL butter, 53.24 g bacon, 211.7 g toast, 307 mL of milk and 436 mL of water. The meal was cooked one day prior to administration, homogenized in a house hold blender (Gastroback®) and kept cool at 4–8 °C in a fridge. On day of the experiment the slurry was slowly heated up in a water bath to reach body temperature and was subsequently administered by oral gavage. Based on the product specifications, the total caloric values of this meal per dog were between ~150 and 200 kcal depending on body weight. Based on the composition and the product specifications, around 50% of the calories arose from fat.

3. Results

No significant differences were observed between male and female animals. Therefore, therefore the results are presented as the mean of all animals. However due to the low animal numbers no final conclusions about sex differences can be drawn.

3.1. Transit times

The transit times for the different study arms are summarized in Table 2. It can be seen that gastric emptying was clearly prolonged in fed dogs, whereas the small intestinal transit time as well as the colon transit time were not relevantly affected. In comparison to fasted state conditions without any pre-treatment, the administration of pentagastrin led to a slight delay of gastric emptying. On the other hand, famotidine slightly decreased the GET.

3.2. pH values

3.2.1. Gastric pH values

The profiles in Fig. 2 demonstrate that the pH values in fasted and fed Beagle dogs were highly variable, whereas pre-treatment with pentagastrin or famotidine clearly decreased variability in gastric pH. The minimum pH values after pre-treatment with pentagastrin were around 0.5–1 pH units lower than without pre-treatment. It can be seen from Fig. 2 that even after pre-treatment with pentagastrin, the dogs 01 and 03 experienced higher gastric pH values prior to gastric emptying. This increase in gastric pH was probably due to the retropulsion of neutral contents from the small intestine into the stomach as was already shown in other studies [32,33]. We excluded the possibility of localization within the small intestine, because high pressures of up to 650 mbar as well as shorter phases of low pH could be observed and were indicative of localization within the stomach.

After administration of the TMC together with water, a reduced gastric pH could be observed as well, but the levels were more variable compared to the gastric pH after a pentagastrin pre-treatment. After the famotidine pre-treatment, the pH values were almost constantly elevated to levels of pH 7–8.

3.2.2. Small intestinal pH values

In Fig. 3, the pH values in proximal and distal parts of the small intestine are depicted. The pH value ranges from pH 6.5–7 in proximal parts, whereas pH 7.5–8 was measured in the distal parts. The pre-treatment with pentagastrin as well as the administration of the TMC together with food caused a slight acidification of the proximal small intestine in two cases. This effect was probably due to the emptying of

Table 2
Transit times of the TMC in six beagle dogs at different intake conditions (means ± SD). GET – gastric emptying time, SITT – Small intestinal transit time, CTT – Colon transit time, WGTT – Whole gut transit time.

	GET/h	SITT/h	CTT/h	WGTT/h
Fasted (A)	0.57 ± 0.37	1.37 ± 0.59	25.4 ± 3.3	27.3 ± 3.3
Fasted + pentagastrin (B)	0.92 ± 0.06	1.67 ± 0.81	22.4 ± 5.5	25.0 ± 5.9
Fasted + famotidine (C)	0.39 ± 0.18	1.91 ± 0.86	21.7 ± 5.3	24.0 ± 4.8
Fed (D)	2.94 ± 0.91	1.94 ± 0.27	28.2 ± 4.7	33.0 ± 4.1

highly acidic gastric contents into the small intestine.

3.2.3. Colonic pH values

As can be seen in Fig. 4, the different study treatments had no effect on colonic pH values. The colonic pH values showed large variations with values from pH 5 to pH 8.

3.3. Pressures

The maximum pressures measured in stomach and small intestine in different study arms are shown in Fig. 5. The maximum pressures obtained in this study did not vary in a relevant manner between the different study arms. Typically, the maximum pressures were observed in the stomach and amounted to values of up to 800 mbar.

4. Discussion

4.1. Transit times

The fasted state gastric transit times 0.57 ± 0.37 h measured in this study with the large, indigestible capsule in Beagle dogs were comparable to recent data obtained with the Bravo pH system by Mahar and colleagues [34]. In contrast, in studies published by Lui et al. and Sagawa et al., in which other telemetric capsules were used in Beagle dogs, mean gastric transit times of longer than 1 h were reported for fasted state intake [35,36]. However, in both studies some dogs showed significantly delayed gastric emptying times (> 5 h) that certainly have contributed to the higher mean GET compared to our study. In the present study, there was only one dog that showed a delayed GET in fasted state (1.01 h). With respect to gastric transit times in humans, the GET obtained in this study corresponds well with transit times obtained

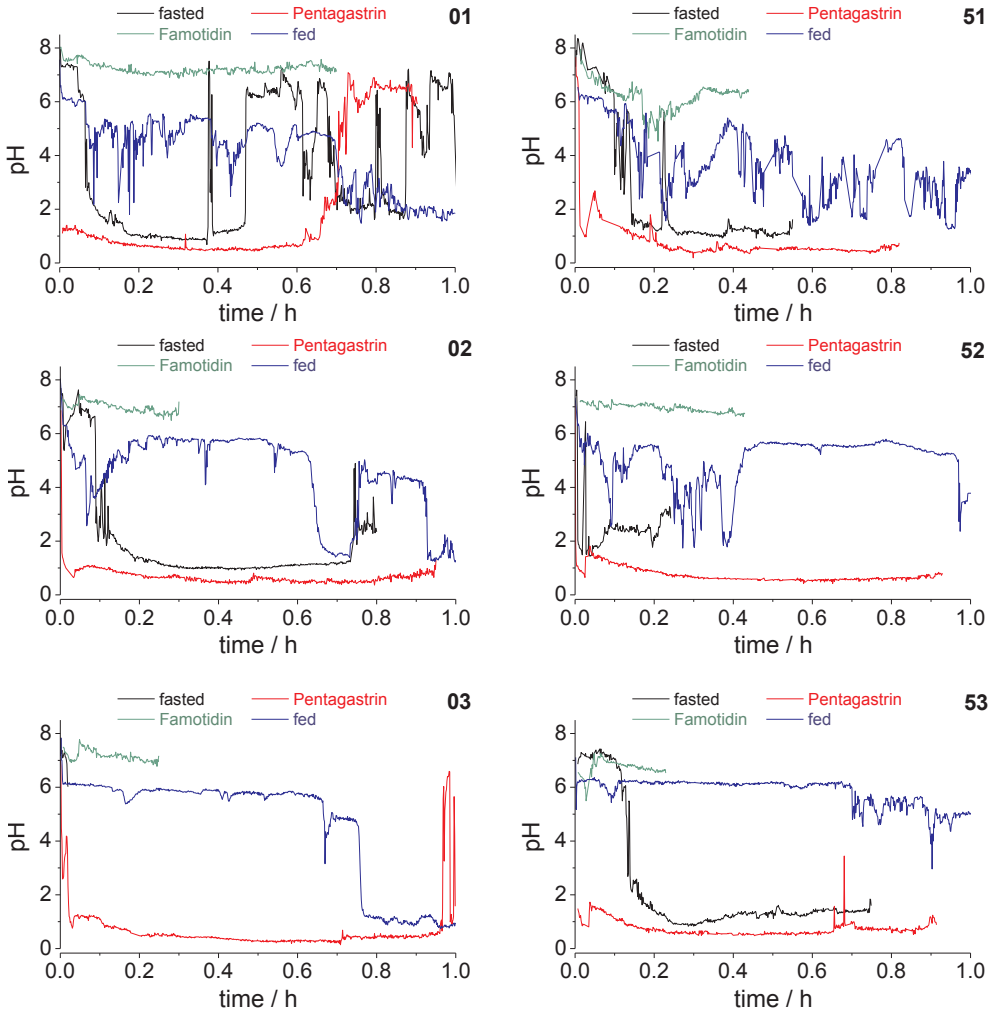

Fig. 2. Individual gastric pH values over the first hour after TMC intake or until gastric emptying of the TMC in six beagle dogs (left: male dogs, right: female dogs) at different intake conditions.

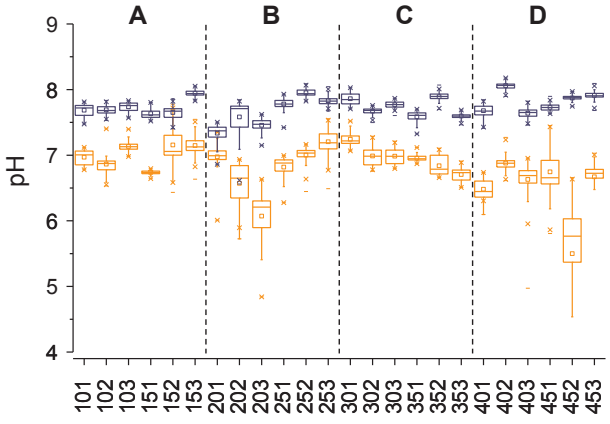

Fig. 3. Comparison of pH values in proximal (orange) and distal parts (blue) of the small intestine measured in six beagle dogs at different intake conditions (A–D). The differentiation into proximal and distal was based on the normalization of the small intestinal transit time. For pH values in proximal parts and distal parts, only pH values were considered that were measured during the normalized transit time of 0–0.1 and 0.9–1, respectively. Box: 50%, whisker: 5–95%, square: mean, asterisks max/min. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

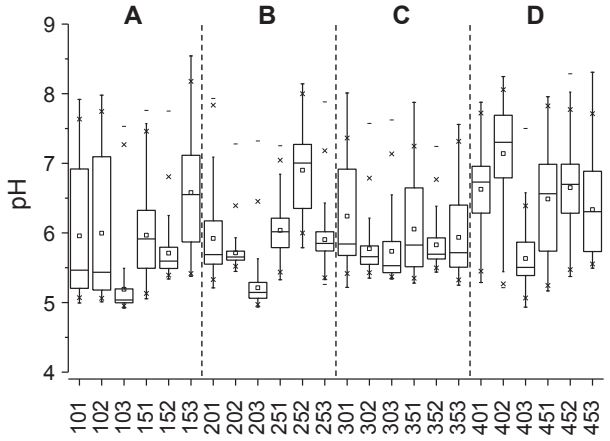

Fig. 4. Comparison of colonic pH values measured in six beagle dogs at different intake conditions (A–D). Box: 50%, whisker: 5–95%, square: mean, asterisks max/min.

in fasted healthy humans [24,31,37].

The pre-treatments with pentagastrin and famotidine seemed to have slight effects on the GET in the fasted state. This effect was probably caused by the changed gastric pH value. It was shown in different studies that the instillation of the stomach with acidic contents as well as the administration of antacids can delay gastric emptying [38,39]. In addition, Cooke and colleagues have also shown that pentagastrin decreases gastric emptying of a liquid test meal in dogs and increases the volume of luminal contents [40].

With respect to the effect of food intake on gastric emptying, it can be stated that food significantly delayed the GET of the indigestible TMC in this study and others [34,36]. Comparable to the situation in humans, large, non-digestible objects can only be emptied by the intense peristalsis of the migrating motor complex (MMC) - the dominant motility pattern in the fasted upper GI tract [35,37]. Due to the interruption of the MMC by food intake, the TMC could only be emptied after recurrence of the MMC [3]. The GET obtained in the present study was somewhat in contrast to data from other groups. For instance, by using the Bravo pH system Sagawa and colleagues demonstrated in male Beagle dogs that increasing the amount of co-administered food (dry dog food) from 10 g to 200 g leads to an increase of gastric emptying from 9.4 h to more than 20 h [36]. Both values are clearly above the GET determined in the present study, although the administered 10 g portion of dog food contained only around 40 kcal, which was clearly lower than the caloric value (150–200 kcal) of our test meal. One explanation for this difference might be the different consistency of the meals. Whereas the human food used in our study was cooked and homogenized, Sagawa and colleagues used dry dog food or wetted dry dog food for their studies. It is known that the consistency of ingested food affects the process of digestion and also the gastric transit time. Liquid and semi-solid meals are generally emptied faster than isocaloric solid food [3]. In another study with dogs, Ehrlein and Pröve have shown that the gastric emptying of a highly viscous meal is significantly slower compared to meals with lower viscosity [41]. Nonetheless, the different consistencies of the food used in the two studies may only partially explain the observed difference in GET. It can be further hypothesized that a higher caloric density as well as deviations in the study protocols may also have contributed to these differences as the dogs that received 10 g of dry food were fed again 6 h after capsule administration. This may certainly have caused a further delay of gastric emptying of the capsule due to interruption of the MMC. In line with this, the authors stated that in 12 out of 16 dogs the capsule was emptied after 4–5 h, whereas the GET in the other four dogs was longer than 23 h. In addition, it is also known that increased levels of stress of the dogs can cause a delayed return of the MMC [7]. This example nicely illustrates that the study protocol as well as the external conditions can have relevant consequences for the GI transit conditions and

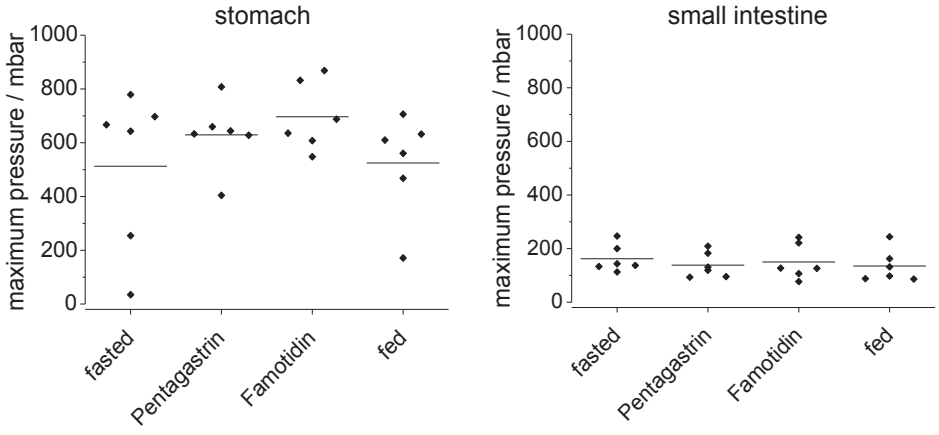

Fig. 5. Maximum pressures in stomach (left) and small intestine (right) measured at different intake conditions.

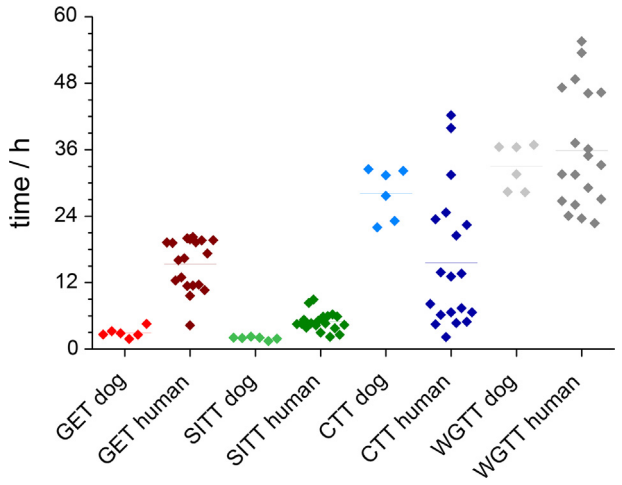

Fig. 6. Comparison of different GI transit times in beagle dogs (n = 6) and humans (n = 19) after fed state administration of the TMC. Human data were published recently by Koziolek and co-workers [23]. GET – gastric emptying time, SITT – Small intestinal transit time, CTT – Colon transit time, WGTT – Whole gut transit time.

also for the outcome of preclinical studies. Therefore, we highly recommend to aim for the highest degree of standardization and suggest proper training sessions for the animals to reduce experimental stress (for all formulation selection studies) in order to generate data sets that are comparable to each other and in which the variability is minimized.

By comparing the present data set from fed Beagle dogs with results from a recent study (Fig. 6) with fed healthy human volunteers [23], it can be seen that the TMC was emptied from the fed stomach much earlier in the dog study compared to the study in humans although the caloric value of the meal administered to the dogs (~150–200 kcal, approximately 11.3 kcal/kg BW) was comparable to the caloric value of the meal consumed by the healthy subjects (965 kcal, 13.8 kcal/kg BW). Hence, the canine stomach likely returned to MMC much faster, which allowed an earlier gastric emptying of the TMC and the meal administered was homogenized to a slurry. It was already mentioned that liquid food is emptied faster from the stomach than equicaloric solid food [3]. In the comparative study, the human volunteers were allowed to eat freely after 4.5 h, which caused a further extension of the fed state motility and delayed the recurrence of the MMC. In contrast, the dogs were not allowed to eat again after administration of the homogenized food, suggesting that the MMC returned faster in the dogs, which resulted in a shorter GET.

The small intestinal transit time was shorter in dogs than in humans. It can be seen in Table 2 and Fig. 6 that the SITT was around 1–2 h in dogs and in the range of 3–5 h in humans. Values of around 2 h were also reported in other studies performed with telemetric capsules in dogs [42]. This observation is most likely due to the different dimensions of the small intestine between both species. The length of the small intestines in dogs is approximately 225–290 cm compared to approximately 625 cm in man [4]. A shorter SITT has the consequence that less time is available for drug dissolution and absorption in the upper GI tract, which may affect the predictive power of dog studies with respect to drug absorption in humans as was discussed by Paulson and colleagues for celecoxib [43]. In order to increase the transit times of solid oral dosage forms in the canine GI tract, Yamada et al. pretreated dogs with loperamide. By doing so, the absorption of a sustained release formulation of paracetamol was increased in the dog [44]. However, this procedure will certainly cause changes of other GI functions and thus, should only be used if these changes are well characterized and do not affect drug release.

Dogs are often used to study drug absorption from immediate release (IR) dosage forms, but due to the comparable CTT to humans the dog might also be an appropriate model for extended release (ER) formulations. Colonic absorption rates of different drugs in Beagle dogs were investigated by colonoscopic perfusion in a recent study [45]. If colon absorption is almost complete, the colon transit time will have a major impact on the PK profile. It can be seen from Fig. 6, that CTT in dogs was well within the range found in human volunteers, although the CTT seems to be more variable in humans than in dogs. This might be due to the different lengths of the human (150 cm) and canine colon (60 cm) [4].

4.2. pH values

We have demonstrated in this study that mainly the gastric pH profiles were affected by the different study treatments, whereas small intestinal and colonic pH profiles were widely unaffected. The data from the SmartPill® are not in line with aspiration data from different dog studies, in which significantly higher pH values in the fasted stomach were measured [12,46]. The reason for this discrepancy might be that neither a TMC nor water was administered before the samples of gastric fluid were taken. Considering other studies with telemetric capsules, which came to results that were similar to our data, it seems likely that any additional manipulation (e.g. administration of a TMC and water for swallowing) already influences the naive gastric pH levels in dogs [47]. McArthur and colleagues have demonstrated in humans that the administration of distilled water stimulates the secretion of gastric acid to a slight extent [48]. In a study with Beagle dogs published by Akimoto et al., such an effect was not observed after administration of 10 mL of water. However, in the same study the administration of a placebo gelatin capsule filled with 500 mg lactose caused a clear drop in gastric pH [12]. Despite these effects, the administration of a telemetric capsule is the best procedure to obtain realistic pH data as it closely simulates the administration of an oral dosage form in preclinical studies.

In fasted state, the pH value quickly dropped to acidic pH values of pH 1–2 due to the mixing of the co-administered water with residual gastric contents. Again, these data correspond well with existing literature on the application of telemetric capsules in dogs [34,49]. Such a rapid pH decrease was also observed in humans in recent studies that were performed with telemetric capsules [31]. The pre-treatment with pentagastrin and famotidine caused relatively stable pH conditions with less individual fluctuations. In case of intra-muscular pentagastrin pretreatment, the gastric pH reached almost immediately the range of pH 1. The pH value constantly remained around pH 7 after oral famotidine pretreatment However, considering recent *in vivo* data in humans [24,31], it should be kept in mind that the pentagastrin pre-treatment results in a slightly lower gastric pH compared to fasted healthy volunteers [4,24,31]. Moreover, it also seems to prolong the gastric transit time of oral dosage forms (see Table 2) and it delays the gastric emptying of luminal contents, which results in higher volumes of luminal fluids in the stomach [40]. These effects may have implications especially for weakly basic compounds. Lentz and colleagues have demonstrated that the oral bioavailability of atazanavir in humans significantly increases almost fivefold after pre-treatment with pentagastrin [19]. We suggest that it was probably the combination of a favorable gastric pH along with increased luminal fluid volumes and prolonged gastric transit time after pre-treatment with pentagastrin that has caused this effect. As the drug is exposed for longer times to a higher volume of an acidic medium, more drug may dissolve [15,16]. In addition, due to highly acidic gastric contents entering the duodenum, the pH in the proximal small intestine was also slightly lowered after administration of pentagastrin as compared to the other study treatments, which may cause higher solubility of weakly basic drugs in intestinal fluids. On the other hand, for acidic compounds this lower pH value within the proximal intestine can be disadvantageous, as described recently for alendronate [50].

The pretreatment with famotidine did constantly elevate the gastric

pH to values of around pH 7. This was in line with aspiration data from Fancher and co-workers [13]. The pre-treatment with famotidine is thus suitable to simulate achlorhydric subjects or subjects medicated with proton pump inhibitors (PPI), H_2-receptor antagonists and antacids. The effect of high gastric pH on drug release from poorly soluble, weakly basic drugs has recently gained a lot of attention for oral anticancer drugs [51–53] by simulating worst-case conditions for dissolution and hence bioavailability. Therefore, dogs pre-treated with famotidine can be a valuable tool [15,16,54] to predict the impact of a broad gastric human pH range on bioavailability. However, it must be kept in mind that the pre-treatment with famotidine might lead to an overestimation of the effect of proton pump inhibitors (e.g. omeprazole, pantoprazole) in certain cases since the gastric pH in humans treated with PPI will be slightly lower than in dogs pre-treated with famotidine [55–57]. This will especially apply to weakly basic drugs with a pK_a value in the range of 2–6 as these are very sensitive to luminal pH changes in the stomach. For the simulation of patients suffering from hypochlorhydria or achlorhydria, the use of famotidine may also be valuable as the mean gastric pH value in patients suffering from true hypochlorhydria is reported to be 7.44 ± 0.11 in men and 7.65 ± 0.33 in women [58].

With respect to the fed conditions, the canine gastric pH profile largely reflected the range of pH values observed in the fed human stomach. It can be seen in Fig. 7 (left) that in the initial time after food and TMC intake, the pH values in the fed Beagle dog were clearly higher compared to corresponding pH values in humans. This difference is likely to be caused by a lower basal acid secretion in dogs compared to humans [3]. This observation should be considered especially in studies that aim for the investigation of food effects on oral PK after administration of IR dosage forms containing drugs with pH-dependent solubility or enteric coated dosage forms. However, in clinical studies, in which bioavailability or bioequivalence is investigated in humans, the drug is typically administered 30 min after begin of the meal intake. In our dog study, the TMC was administered 5 min after food intake. For future dog studies, it may be better to consider a delay of 30–45 min between the start of food administration and drug administration. It can be seen from Fig. 7 (right) that the canine gastric pH profile 30 min after food administration is closer to the corresponding human pH profile. However, a later administration may also lead to a decreased GET. A closer look to the further course of the gastric pH profiles presented in Fig. 7 reveals that the gastric pH value in dogs returned to baseline pH values much faster than for human subjects. This observation is probably a result of the faster gastric emptying of the meal in combination with higher peak acid secretion rates in Beagle dogs [4]. In contrast, in healthy human volunteers it takes around 4 h until baseline pH values are reached [23].

The comparison of small intestinal and colonic pH values in dogs and humans revealed that the differences are mainly small. The intestinal pH value typically increased from pH values of pH 6.5–7 in proximal parts to values of up to pH 8 in distal parts. Similar observations were also made for healthy humans, although the intestinal

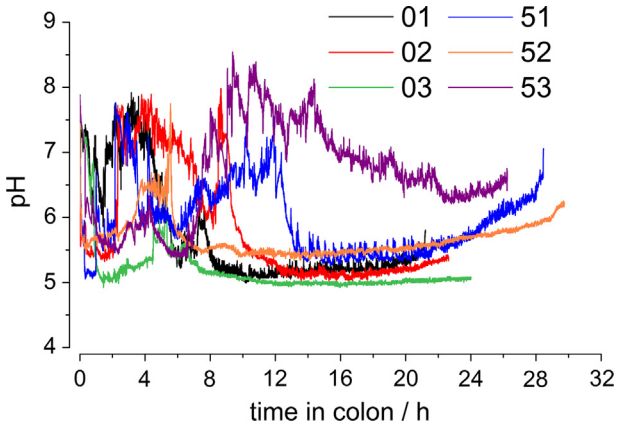

Fig. 8. Individual colonic pH profiles in beagle dogs exemplarily shown for fasted state administration (A) of the TMC.

pH values in proximal parts of the human small intestine are around one pH unit lower, which may also be related to the higher basal acid secretion in humans [4,23,31]. Although this difference is small, it may have implications on drug absorption, especially for drugs with limited, but pH-dependent solubility as well as for enteric coated formulations [35]. Unfortunately, in in vivo studies it is hard to separate this effect from the shorter SITT seen in dogs as less time is available for drug dissolution and subsequent absorption. The colonic pH values fluctuated over a range of pH 5 to pH 8. Similar pH values were measured in the human colon. The variable pH values in the colon can be explained by the regionally changing colonization with different microbiota as well as by the limited volume of free fluid. Therefore, we assume that changing pH values indicate transit through the colon, whereas constant pH values are a sign of TMC localization at a certain region within fecal contents. The relatively long periods of constant pH as shown in Fig. 8 were not found in such a distinctive manner in humans. Therefore, we think that the TMC resided in the rectum for relatively long time until excretion. Thus, the transit through areas of the colon, which provide optimal conditions for drug absorption (i.e. ascending colon) are probably shorter in dogs. This would be in line with the shorter length of the canine colon that was already mentioned earlier.

Based on the dynamic data presented in this study, the simulation of canine gastric and intestinal conditions by media with constant pH values does not describe the physiological situation and may lead to the overestimation of certain effects [59]. As for the simulation of human GI conditions, the *in vitro* simulation should either consider dynamic changes of the medium or focus on simulating worst-case scenarios. The use of mean pH values is not deemed justified in light of the TMC data. To give an example, it was shown in a recent study by Bhattachar and colleagues that the variation in gastric pH contributes to the variability in the PK profiles of a weakly dibasic drug in male Beagle dogs, which was due to the pH-dependent solubility of the drug (galunisertib) [60].

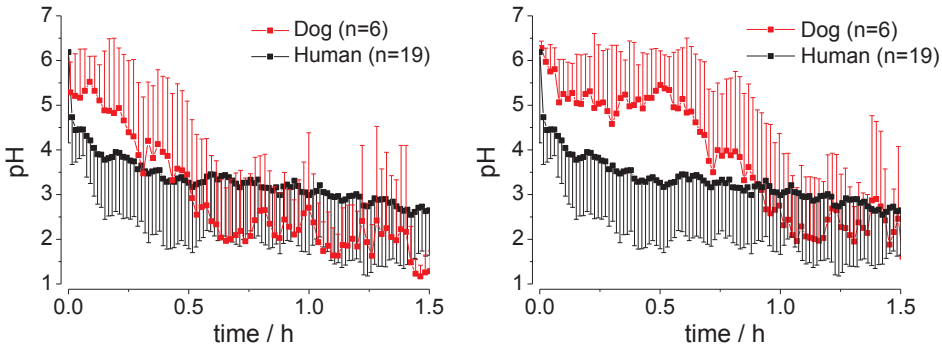

Fig. 7. Comparison of the mean gastric pH values under fed conditions in beagle dogs and humans (means ± SD). *left:* The starting point 0 h is defined in both species as the intake of the TMC (dogs: 5 min after beginning of meal intake, humans: 30 min after beginning of meal intake). *right:* The starting point 0 h was scaled in both data sets to the start of food administration.

4.3. Pressures

At all intake conditions, the maximum pressures revealed in the dog were significantly higher compared to those found in humans. In humans, the maximum pressures only amounted to values of up to 500 mbar [23,24], whereas in dogs, maximum pressures of more than 800 mbar were observed. This is in line with data reported by Kamba and colleagues, who used pressure-sensitive tablets to study mechanical forces in the human and the canine stomach under fasted and fed conditions [61,62]. The evaluation of extended release dosage forms in dogs can result in unwanted effects caused by the higher pressure, such as dose dumping [63]. However, dosage forms that show dose dumping in the dog might not necessarily experience dose dumping in the human as well. This aspect was nicely demonstrated in a recent work of McInnes and colleagues. In this work, two matrix tablet formulations were investigated in fasted and fed dogs by using scintigraphy in combination with blood sampling [64]. It could be revealed that one of the two formulations disintegrated quickly after fasted administration resulting in dose dumping. Thus, this formulation was shown to be highly sensitive towards mechanical stress. The same formulation also exhibited dose dumping in fed dogs and fed humans, but not in fasted human subjects. Hence, this is a nice example, in which the gastric environment in fasted dogs was too harsh compared to humans. The second formulation investigated in this study did not show dose dumping in the dog model and thus, was regarded as robust even under worst-case conditions. Although the canine model does not nicely reflect the mechanical stresses occurring in humans, it may represent a worst-case scenario and can therefore be used, to assess the risk of dose dumping of ER dosage forms.

4.4. Application of the dog model in formulation development

Various publications have demonstrated that animal and human oral bioavailability typically do not correlate very well to each other and that the absolute (overall) bioavailability can neither be directly compared nor can be clearly predicted for humans based solely on animal data [2,6]. This lack of correlation is often associated with interspecies differences in terms of intestinal and hepatic first-pass effects. However, with respect to the fraction absorbed good correlations were observed for various animals such as rats or cynomolgus monkeys. Although for dogs the correlation is weaker [2,65], the dog model has been successfully used in the past to assess the performance of clinical formulations and to predict the direction and extent of food effects. Therefore, pilot experiments performed with dogs are an important part of preclinical research [18]. In particular, for the evaluation of the *in vivo* behavior of innovative oral dosage forms, such as immediate release dosage forms that are based on different enabling technologies, the dog model has been proven very useful. Additionally, the dog model has also been applied to investigate the pH-dependent absorption of drugs and to evaluate formulation principles to mitigate this effect [16,66,67]. It is thus a common strategy to rank-order different oral dosage forms within the same species to select the most promising oral dosage form for phase I or phase II.

However, this and other studies have shown that some anatomical and physiological differences exist between dogs and humans that need to be considered. These include higher luminal forces acting on oral dosage forms, lower basal and higher peak acid output in the stomach as well as shorter intestinal transit times in comparison to humans. These physiological differences are likely to have an implication on drug absorption (e.g. over prediction of absorption in dogs) and should be kept in mind for data interpretation [9]. The different shortcomings described in this work raise the question of how the data of the dog model should be used and implemented in the selection of oral formulations for phase I and II trials. From our point of view, all available data (i.e. *in vitro, in silico* and *in vivo* data) should be considered at early stages of development and animal models are certainly an important piece of the puzzle. They can be useful in the selection of the most appropriate formulation by ranking different formulation prototypes with respect to differences in absolute or relative bioavailability as well as to individual variability of the different formulations within a single species. However, the final decision on the formulation should never be based solely on one specific study, but on a set of different data (i.e. *in vitro* dissolution data, supporting *in silico* investigations and *in vivo* studies comparing different prototypes). Moreover, additional aspects such as the level of development complexity of the formulation, the stability of the formulation and the cost of goods need also be considered for the final decision. If no clear decision can be made after consideration of all the factors listed above, a formulation selection study should to be performed in the target species – the human.

5. Conclusion

The dog model is commonly used in formulation development to rank-order different formulation prototypes based on their *in vivo* performance in animals. However, these dosage forms are designed for administration in humans and therefore, the evaluation and interpretation of the results from such studies should consider the physiological differences between dogs and humans that were revealed in the present work. By comparing the data from this SmartPill® study in dogs with recent data from healthy human subjects, we could show that important differences exist with respect to transit times in stomach and small intestine, luminal stomach pH values as well as pressures arising during the GI transit of dosage forms. All these factors can affect oral drug absorption and therefore, their individual contribution should be carefully considered during the decision making process towards the human formulation, supported by *in vitro* experiments and *in silico* simulations.

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