Effects of the neurokinin-1 antagonist maropitant on canine gastric emptying assessed by radioscintigraphy and breath test

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Keywords
Stomach, motility, dog, prokinetic, cisapride

Summary

Objective: Delayed gastric emptying is a well-recognised phenomenon in a number of canine disease conditions. Only a limited number of drugs have been reported to have some gastrokinetic effect in the dog. The aim of this study was to investigate prokinetic effects of maropitant. **Material and methods:** In a cross-over study 24 healthy adult Beagle dogs were randomised to receive either maropitant (2 mg/kg q24 h PO), cisapride (1 mg/kg q12 h PO) or placebo (vitamin-$B_{12,}$ 10 µg/dog q24 h PO) for 7 days with a 7-day washout period between treatments. Gastric emptying was measured simultaneously via ^{99m}Technetium radioscintigraphy and ^{13}C-sodium acetate breath testing for 6 hours post-feeding. The decrease in radioactive counts in the stomach and the increase in $^{13}CO_2$ concentration in exhaled breath (measured via gas chromatography) were plotted against time. The area under the curve was determined for each test and the time to 25%, 50% and 75% gastric emptying was calculated for each method. Friedman test was used to compare gastric emptying times. **Results:** With both methods, no difference for gastric emptying times was observed for any treatment. **Conclusion and clinical relevance:** Neither maropitant nor cisapride were shown to have an effect on gastric emptying in healthy beagles using radioscintigraphy or breath test when compared to placebo. Consequently, neither drug can be recommended as a gastric prokinetic in dogs.

Schlüsselwörter
Gastrisch, Motilität, kanin, prokinetisch, Cisaprid

Zusammenfassung

Gegenstand und Ziel: Eine verzögerte Magenentleerung ist bei verschiedenen Erkrankungen des Hundes bekannt. Nur eine begrenzte Anzahl von Medikamenten wurden hinsichtlich ihres prokinetischen Effekts beim Hund untersucht. Das Ziel dieser Studie war, einen möglichen gastroprokinetischen Effekt von Maropitant zu untersuchen. **Material und Methoden:** Im Rahmen einer Cross-over Studie erhielten 24 gesunde Beagle randomisiert jeweils für 7 Tage Maropitant (2 mg/kg p. o. einmal täglich), Cisaprid (1 mg/kg p. o. zweimal täglich) und ein Plazebo (Vitamin-$B_{12,}$ 10 µg/Hund p. o. einmal). Zwischen den Behandlungen lag jeweils eine Ruhephase von 7 Tagen. Am Ende jeder Medikamentengabe wurde die Magenentleerung simultan mittels ^{99m}Technetium-Radioszintigraphie und ^{13}C-Natriumazetat-Atemtest über 6 Stunden nach der Fütterung gemessen. Die „Area-under-the-curve" der radioaktiven Counts bzw. der Konzentration von $^{13}CO_2$ in der Ausatemluft (bestimmt mittels Gaschromatographie) wurde gegen die Zeit aufgetragen und anhand dieser Kurve die 25-, 50- und 75%ige Magenentleerungszeit berechnet. Zum Vergleich der Magenentleerungszeiten diente der Friedman-Test. **Ergebnisse:** Mit beiden Methoden ließ sich zwischen den Magenentleerungszeiten für die eingesetzten Medikamente im Vergleich zum Plazebo kein signifikanter Unterschied feststellen. **Schlussfolgerung und klinische Relevanz:** Weder Maropitant noch Cisaprid zeigten einen prokinetischen Effekt auf den Magen. Der Einsatz dieser Substanzen zur Förderung der Magenentleerung beim Hund ist daher nicht empfehlenswert.

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Introduction

Gastric emptying is a complex part of the physiology of digestion. Reflex mechanisms ensure that the rate of gastric emptying is adapted to the amount of nutrients delivered to the duodenum; and gastric contractions, pyloric relaxation and duodenal motility are strictly coordinated (1). Delayed gastric emptying and disturbed antropyloroduodenal coordination have been described in the dog with mechanical (gastric neoplasia, foreign body, polyp) or functional obstruction (inflammation, ulceration, infection) (2) or

secondary to pancreatitis, electrolyte disturbances, metabolic diseases, septicaemia, drug administration (especially anticholinergics), and other conditions (2–6). Diagnosing and treating gastric emptying disorders is important, as gastric retention and excessive gastric distension can lead to vomiting/regurgitation and potentially aspiration pneumonia, which carries a guarded prognosis. In the authors' experience, especially in dogs with significant systemic diseases (septicaemia, peritonitis, severe metabolic derangements like diabetic ketoacidosis), prokinetics decrease the risk of aspiration pneumonia and hence morbidity and mortality. In addition, two recent studies documented delayed gastric emptying in dogs undergoing abdominal surgery (7) and the effect of anaesthesia on gastrointestinal transit times (8).

Investigations into the effect of a variety of drugs on canine gastric emptying have given equivocal results (2). To date, no drug with sufficient prokinetic effect on the canine stomach to be of clinical benefit is available or tested sufficiently. Several classes of drugs have been investigated so far: There is only weak evidence that dopaminergic D_2 antagonists (metoclopramide, domperidone) accelerate gastric emptying in dogs. Metoclopramide reversed gastric relaxation by dopamine infusion in dogs in an experimental setting (9) and accelerated emptying of liquids measured via gastric cannulation at high oral doses (10). Metoclopramide had no effect on gastric emptying of solids when measured by gastric cannulation or strain-gauge technique (10, 11), however, it improved antropyloroduodenal coordination in one study (11). Domperidone increased the pyloric diameter (assessed via ultrasound) in healthy dogs and humans, which was interpreted as improving antropyloroduodenal coordination (12).

Serotonergic 5-hydroxytryptamine 4 (5-HT_4) receptor agonists have potent prokinetic effects (13): Cisapride has been studied most extensively in dogs. It stimulates pyloric and duodenal motor activity, enhances antropyloroduodenal coordination and increases duodenal contractions (13). Unfortunately, since cisapride has been withdrawn from the European market due to cardiac side effects in humans and dogs, there is a lack of an effective prokinetic drug alternative for dogs. Data on the prokinetic effect of novel 5-HT_4 receptor agonists (i. e., velusetrag [14], mosapride [15, 16], prucalopride [17]) are limited. Motilin agonists (erythromycin and its derivatives) accelerate gastric emptying in the dog (18) by inducing phase III interdigestive contractions, which might not be ideal in a clinical setting and lead to "dumping" of poorly pre-digested gastric contents into the duodenum; whereas histamine H_2-antagonists (ranitidine, nizatidine), which stimulate intestinal motility by inhibiting acetylcholinesterase activity (19) only show a mild prokinetic effect on the canine stomach.

There has been speculation that the neurokinin (NK)-1 receptor antagonist maropitant might have prokinetic effects in dogs (20, 21). It is licensed as an anti-emetic for dogs and cats, and its effectiveness in treating and preventing vomiting due to a variety of causes has been shown in multiple studies (22–24). Both NK-1 receptors (25) and their natural ligand, the tachykinin substance P (SP) (26) have been identified in the canine stomach. There have been conflicting reports about the effects of tachykinins and their antagonists on gastric emptying in rodent models, with increased (27), decreased (28) and unchanged (29) gastric emptying rates documented. However, these studies are difficult to compare with each other, as SP was administered by different routes (aortic versus intra-peritoneal injection), at different dosages and different time-points in relation to feeding. To the authors' knowledge, no study has assessed the effect of maropitant on postprandial patterns of canine gastric emptying.

Thus, the aim of this current study was to investigate the effect of maropitant on gastric emptying times in healthy dogs in an open label positive (cisapride) and placebo-controlled, randomised, cross-over design using the gold-standard of radioscintigraphy and the ^{13}C-sodium acetate breath test, an indirect method that has been shown to correlate well with scintigraphic values (30).

Materials and methods

Study dogs

The study population consisted of 24 healthy Beagle dogs; eight female spayed and 16 male castrated. A power calculation had determined that 20 dogs would be sufficient to detect a change of 25% in gastric emptying times. The median age of the dogs was 4.5 years (range 1.4–6.6 years), mean weight was 12.8 kg (SD ± 3.6 kg) and body condition score of all dogs was in the range 3–4/5. Three dogs were privately owned and 21 dogs belonged to the research colony of the Small Animal Hospital in Giessen, Germany. Written informed owner consent was obtained for the three privately owned dogs before the start of the study. Ethical approval of the study protocol was obtained from the regional council board (approval number V54–19c20–15(1)GI 18/17).

Prior to the start of the study, dogs were deemed healthy based on physical examination and full haematology and biochemistry panels including normal results of a bile-acid stimulation test. In addition, all dogs were treated with fenbendazole at a dose of 50 mg/kg daily for 3 days within the 2 weeks before the start of the study. Daily clinical examinations were performed on each dog during the study period. Exclusion criteria were the presence of any clinical signs attributed to gastrointestinal disease (vomiting, diarrhoea, weight loss, and inappetence) in the 6 months before the study, a history of abdominal surgery or the administration of any drugs (except antiparasitics) within the last 14 days before the start of the study.

Study design

Two weeks before the study, a commercially available canned food[1] was gradually introduced into the dogs' diet. This was fed

[1] Intestinal canned diet, Royal Canin

exclusively from week –1 until the end of the study. Each dog was randomly assigned to receive one of six possible predefined sequence of treatments (first day of treatment defined as day 0, see ▶ Table 1). The dosages used were: Maropitant[2] 2 mg/kg q24 h PO, cisapride[3] 1 mg/kg q12 h PO or a commercially available vitamin B_{12} preparation[4] 10 µg/dog q24 h PO. Each drug was given for 7 days with a washout period of 7 days in between each treatment. On the last day of each treatment period, a test meal (see below) was fed 1 hour (± 10 minutes) after drug dosing, and gastric emptying was determined simultaneously by scintigraphy and ^{13}C-sodium acetate breath test (see below).

Test meal. Half of the daily caloric requirements were calculated individually for each dog, based on the following formula: 70 x kg bodyweight^{0,75} x 1.6 = kcal/day. Two-hundred MBq ^{99m}Technetium bound to albumin[5] (to avoid absorption) as well as 100 mg unbound ^{13}C-sodium acetate[6] were added to each test meal, which was then mixed thoroughly using a household blender. A scintigraphic image of the food was visually inspected to confirm homogenous spread of the radioactive tracer within the test meal before feeding (data not shown). At least 75% (by weight) of the food had to be consumed within 15 minutes of it being offered to each dog, otherwise no measurement of gastric emptying was performed and that leg of the study for that dog was repeated at a later time (out of the assigned sequence). Drinking water was available ad libitum at all times, except when scintigraphic imaging and breath testing were being performed.

^{99m}Technetium radioscintigraphy. As previously described (30), dogs were kept in sternal position above the gamma camera with minimal restraint. Image acquisition time was 60 seconds. Ventral images were taken immediately after intake of the test meal, every 15 minutes for the first 2 hours, every 30 minutes for the following 2 hours, and finally every 60 minutes for another 2 hours. These time points have been shown to be sufficient for optimal calculation of gastric emptying in an earlier study (31). The area of the stomach (region of interest, ROI) was defined manually based on the morphological features of the stomach visible, and all pixels within this region were counted automatically. Subsequently, decay-corrected radioactive counts were plotted against time. Total area-under-the-curve (AUC) was calculated and, using the percentiles of the AUC, gastric emptying times at different stages (25%, 50% and 75% of emptying) were determined as described previously (30).

^{13}C-sodium acetate breath test. A baseline sample was obtained from each dog immediately before each test meal was ingested. Subsequent breath samples were taken at the same time points as used for the scintigraphic measurements. These time points have been validated in earlier studies (7, 30, 32). Samples were collected via a commercially available anaesthetic face mask[7] for small animals that fitted tightly around the dogs' muzzles. The mask was connected to a three-way valve (allowing the dogs to breathe normally throughout the process) and attached to a commercially available glass vial[8]. Filling of each vial was ascertained by the build-up of condensation; they were then sealed immediately, labelled and analysed within 14 days. A pilot study conducted before the start of sampling had shown that breath samples were stable at room temperature for up to 3 weeks after collection (data not shown). The $^{13}CO_2$ content of breath samples from each time point was assessed in duplicates using gas chromatography with Isotope Ratio Mass Spectrometry. Ion currents consistent with the mass of ^{12}C [44] and ^{13}C [45] were measured against a certified International Atomic Energy Agency (IAEA) reference material. To allow Craig correction for ^{17}O, ^{18}O (mass 46) was also determined (33). The $^{12}CO_2/^{13}CO_2$ ratio (Δ^{13}C) was calculated using the integrated software[9] and Excel 2007-SP1[10]. Delta ^{13}C was expressed in permille, where Δ^{13}C is the ^{12}C/^{13}C isotope ratio in relation to a primary reference isotope ratio (PDB standard) (33). The AUC and thus quartiles of gastric emptying (25%, 50% and 75%) were determined similarly to the scintigraphic measurements.

Statistical analysis

Statistical analysis was performed using SPSS statistical software[11]. Data were assessed for normal distribution by inspection of histograms and found to be normally distributed. Median values of 25%, 50% and 75% gastric emptying times between the three

2 Cerenia®, Zoetis Germany, Berlin, Germany
3 PropulsidTM, Pharmacia & Upjohn Co., a division of Zoetis (Pfizer), New York, USA
4 Vitamin B12-ratiopharm, Ratiopharm GmbH, Ulm, Germany
5 Solco-Nanocoll, Sorin-Biomedica, Munich, Germany
6 1,13C-sodium acetate, cat. # 298042, Sigma Aldrich, Munich, Germany
7 Anaesthetic mask for small animals, Heiland Vet (today: Henry Schein Vet), Hamburg; Germany
8 Non-evacuated Exetainer, Labco, Wycombe, United Kingdom
9 IonVantage 1.1., GV Instruments, Wytenshawe, United Kingdom
10 Excel 2007-SP1, Microsoft, Unterschleissheim, Germany
11 IMB SPSS Statistics 19.0, IBM Corp., Armonk, New York, USA

Table 1 Treatment regimen in this study. Each treatment was given for 7 days with a 7-day washout period. Each group (A–F) contained four dogs.
Tab. 1 Verwendetes Behandlungsregime. Jedes Medikament wurde für 7 Tage verabreicht mit einer 7-tägigen Ruhephase dazwischen. Jede Gruppe (A–F) umfasste vier Hunde.

Group of dogs	Treatment 1	Treatment 2	Treatment 3
A	Maropitant	Cisapride	Placebo
B	Maropitant	Placebo	Cisapride
C	Cisapride	Placebo	Maropitant
D	Cisapride	Maropitant	Placebo
E	Placebo	Maropitant	Cisapride
F	Placebo	Cisapride	Maropitant

Table 2 Median (range) of gastric emptying times (minutes) at different stages (25%, 50%, 75%) in 24 dogs with different treatments, measured via radioscintigraphy (sc) and ^{13}C-sodium acetate breath test (b).

Tab. 2 Effekt unterschiedlicher Medikamente auf die Magenentleerungszeit (Minuten; Medianwert und Steuung) zu unterschiedlichen Stadien (25%, 50%, 75%) ermittelt anhand von Szintigraphie (sc) und ^{13}C-Natriumazetat-Atemtest (b) bei 24 Hunden

Method	Gastric emptying	Gastric emptying time (minutes)		
		Placebo	Maropitant	Cisapride
Radioscintigraphy	25%	37.4 (26.6–45.7)	36.3 (28.1–49.2)	35.99 (27.8–45.6)
	50%	80.2 (56.9–98.8)	78.5 (58.7–105.8)	76.2 (62.3–97.9)
	75%	140.6 (97.4–173.8)	137.6 (99.3–182.5)	132.6 (107.4–169.8)
^{13}C-sodium acetate breath test	25%	88.7 (79.1–117.0)	91.6 (66.8–115.9)	92.6 (72.3–119.0)
	50%	155.7 (125.2–191.9)	156.3 (110.1–190.6)	157.1 (117.7–180.8)
	75%	231.7 (179.9–265.4)	231.1 (164.2–268.8)	222.5 (171.1–257.0)

treatments were compared using Friedman test (as data were not normally distributed). In addition, the effect of any treatment throughout the open label placebo-controlled cross-over study was investigated separately for each stage of gastric emptying (25%, 50% and 75%) using a linear mixed effect model. To accomplish this, three dummy variables were created for each treatment sequence (compare to ▶Table 1) in each individual dog (0 = no treatment, 1 = treatment). This was done for each drug (maropitant, cisapride and vitamin B_{12}) separately. Setting the dog ID as random effect, these "treatment" dummy variables were – together with the current treatment effect – included into the model as fixed main effects. Significance was set at $p < 0.05$.

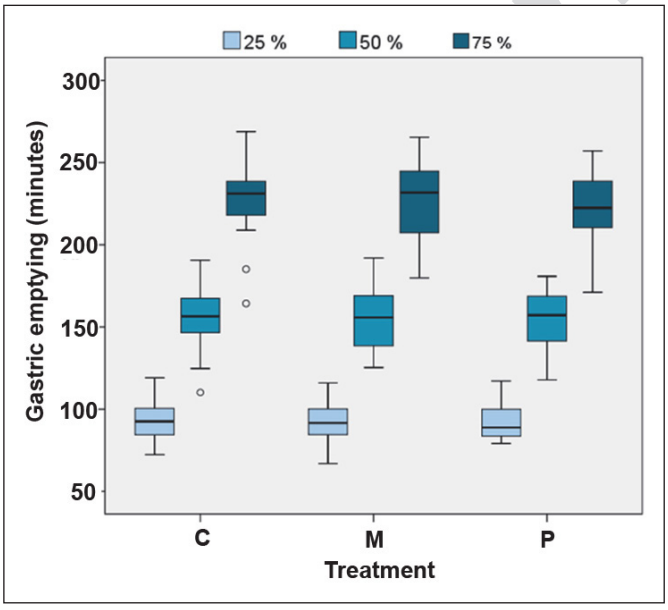

Fig. 1 Gastric emptying times for different treatments assessed via radioscintigraphy in 24 dogs. C = cisapride, M = maropitant, P = placebo.

Abb. 1 Szintigraphisch ermittelter Effekt verschiedener Medikamente auf die Magenentleerungszeit bei 24 Hunden. C = Cisaprid, M = Maropitant, P = Plazebo.

Results

Study dogs and test meal. All dogs accepted the test meals voluntarily at the first time of offering. One dog vomited approximately 1 hour after feeding. A subsequent clinical examination of the dog did not reveal any possible cause nor any sign of ill health. The test was aborted for this animal and this leg of the analysis repeated 2 weeks later. This dog exhibited no further vomiting during the second test.

^{99m}Technetium radioscintigraphy. Gastric emptying showed a sigmoidal pattern in all dogs. Lag phases varied in duration and were absent in six dogs. Gastric emptying times are summarized in ▶Table 2. No significant difference could be detected between treatments at any stage: $p = 0.607$ at 25%, $p = 0.214$ at 50% and $p = 0.167$ at 75% gastric emptying (▶Fig. 1). Treatment had no significant effect across all groups for all time points (▶Table 3).

Table 3 Effect of pre-treatment with different drugs (maropitant, cisapride or placebo) on gastric emptying times (minutes) at different emptying stages (25%, 50%, 75%) measured via radioscintigraphy (sc) and ^{13}C-sodium acetate breath test (b) in a mixed linear model.

Tab. 3 Effekt verschiedener Vorbehandlungen (Maropitant, Cisaprid, Plazebo) auf die Magenentleerungszeit (Minuten) in verschiedenen Stadien (25%, 50%, 75%) ermittelt anhand von Szintigraphie (sc) und ^{13}C-Natriumazetat Atemtest (b) mittels gemischter linearer Statistik.

Method	Gastric emptying	Effect of pre-treatment		
		Maropitant	Cisapride	Placebo
Radio-scintigraphy	25%	0.067	0.416	0.170
	50%	0.057	0.484	0.195
	75%	0.060	0.646	0.226
^{13}C-sodium acetate breath test	25%	0.508	0.767	0.174
	50%	0.290	0.242	0.380
	75%	0.199	0.370	0.237

^{13}C-sodium acetate breath test. The measured rate of exhaled $^{13}CO_2$ showed a typical pattern with increase, peak and exponential decline. Gastric emptying times are summarized in ▶Table 2. No significant difference could be detected between treatments at any stage: p = 0.959 at 25%, p = 0.847 at 50% and p = 0.093 at 75% gastric emptying (▶Fig. 2). Previous treatment (changeover from one drug to another) had no significant effect across all groups for all time points, indicating that the washout period was sufficient (▶Table 3).

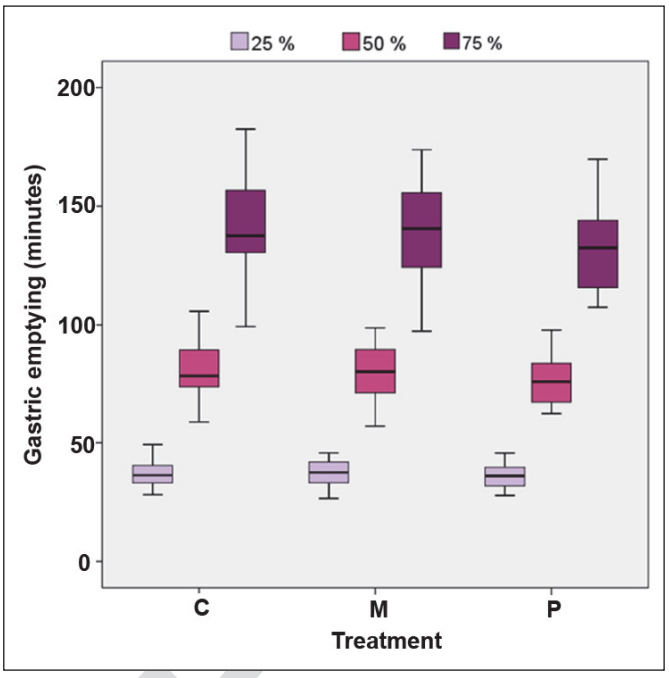

Fig. 2 Gastric emptying times for different treatments assessed via ^{13}C-sodium acetate breath test. C = cisapride, M = maropitant, P = placebo.

Abb. 2 Mittels ^{13}C-Natriumazetat-Atemtest ermittelter Effekt verschiedener Medikamente auf die Magenentleerungszeit bei 24 Hunden. C = Cisaprid, M = Maropitant, P = Plazebo.

Discussion

In the present study, both a direct (radioscintigraphy) and an indirect method (breath testing) were used to evaluate the solid phase gastric prokinetic effects of orally administered maropitant and cisapride compared to placebo in healthy Beagle dogs. Neither method was able to detect a significant difference in gastric emptying times between the three treatments. These findings are similar to two preliminary studies investigating the effect of maropitant on gastric motility by wireless motility capsule (20) or ultrasound (21). In both of these studies, maropitant and other prokinetic agents did not accelerate gastric emptying time when compared to placebo (20) and only metoclopramide (and not maropitant) had an effect on antral motility (21).

The fact that maropitant has no effect on gastric emptying in the dog is interesting since tachykinins in general, and SP in particular, have been reported to both stimulate and inhibit motility of the stomach and the pyloric sphincter via NK receptors in different studies (34–36). For example, although SP was shown to cause a dose-dependent contraction of the canine stomach in vivo, this response declined with subsequent SP injections and could be fully blocked by atropine (36). In other studies, SP was found to stimulate only circular contractions of the antrum and fundus, with no effect on the gastric corpus or pylorus when measured using strain-gauge techniques (34), and thus possibly no effect on overall gastric emptying either. Another study described a potential dual role for SP in the canine stomach, where it was found to increase basal tone of the gastric smooth muscles in vivo, but to inhibit peristaltic contractions (mainly spike potentials) (35). In this study it was concluded that SP was a modulator of gastric motility rather than a main driver of contractions (35). Thus antagonising SP with an NK-1 receptor antagonist could in theory have the opposite effect; decreasing basal tone and promoting contractions.

The overall effect of NK-1 receptor inhibition on gastric contractions may be too small to measure in an in vivo situation compared to the effect exhibited by other naturally occurring neurotransmitters (especially acetylcholine) or intestinal peptides (for example pentagastrin or gastrin-releasing peptide) (37, 38), which might "overrule" modulation by SP or other tachykinins. Other reasons why we were not able to demonstrate an effect of maropitant on gastric emptying may lie with the complicated mechanism of NK receptor regulation and signalling: There is some evidence that NK-1 receptor signalling is differentially regulated by binding of its ligands – SP-binding and binding of other tachykinins can cause different effects (39). The presence of different NK-1 receptor subtypes with different affinities to antagonists has also been proposed (39). Alternatively, as neurokinin receptors are located on smooth muscle cells, neurons and epithelial cells, their regulation might elicit different responses depending on the site of activation or blockage. It is also possible that the main tachykinin receptor in the intestine of the dog is not NK1, but NK2, as there is some evidence for this in other species (40), but this has not been investigated in the dog to date. A recent study documented a 12% variation coefficient of gastric emptying times measured using scintigraphy (41). Hence, even though an effort was made to ensure adequate power of the present study to detect a difference (estimate of gastric emptying variation was based on the available literature at the time and the experience of the authors), it cannot be ruled out that the number of dogs was too small, especially in the light of moderate individual variations.

In the current study, it was interesting that cisapride, which was chosen as the "positive control", did not have a prokinetic effect when compared to placebo. This might appear puzzling, as 5-HT_4 agonists are in general considered strong prokinetic drugs in the entire gastrointestinal tract (9). Similarly, cisapride and other 5-HT_4 agonists have been shown to consistently accelerate gastric emptying in rodents and humans (42, 43). This might be due to the dosing regimen used here or the formulation of the drug used.

There are only few studies documenting the effects of cisapride on gastric emptying times using non-invasive techniques. It was shown to accelerate total gastroduodenal emptying and improve antropyloroduodenal coordination in one study (18). However, no effect of cisapride on gastric motility was observed via tracking of orally ingested barium sulphate spheroids (6) or scintigraphy (44) in healthy dogs. In addition, some recent studies assessing antral contraction amplitude, frequency and motility index showed that the effect of cisapride on these parameters was inferior to that of metoclopramide (21, 45). Some synthetic 5-HT_4 agonists have been shown to be more effective in promoting both liquid and solid phase gastric emptying in the dog (43). It might be concluded then that cisapride is not as effective as other 5-HT_4 agonists in stimulating canine gastric emptying in healthy dogs, thus it may not be ideal as a positive control in experimental studies or even in a clinical setting. However, at the time this study was conducted, no other 5-HT_4 agonist was commercially available, and cisapride was considered the best option for a positive control.

We consider it unlikely that the failure to detect any prokinetic effects of maropitant or cisapride in the present study was due to the techniques used to measure gastric emptying. In general, non-invasive direct techniques are considered superior to invasive or indirect methods, as gastric emptying can be observed in real time, the test meal resembles "normal" food, there is no need for general anaesthesia/permanent restraint and preferential monitoring of either liquid or solid phase gastric emptying can be observed; which is why radioscintigraphy is considered the gold-standard against which other methods should be compared (31). Invasive methods such as those using strain-gauges (intragastric balloons and fixed intragastric volumes of saline) are for obvious reasons not a feasible option in a clinical setting. Indirect methods (plasma tracers, breath test) assume that the rate-limiting step to detection of the tracer substance is gastric emptying (i. e. normal absorption and metabolism of the substrate is assumed), if this requirement is fulfilled, these techniques are usually much easier to perform (with a test meal resembling normal food) and do not require specialist equipment or handling of radioactive material (31). Here we used the ^{13}C-sodium acetate breath test as an indirect alternative to radioscintigraphy, as this method has been described as useful in measuring gastric emptying in the dog (32), and it correlates well with measurements obtained by scintigraphy (30). Both techniques give different average gastric emptying times, as one (scintigraphy) is a direct method and another a direct one (breath test) relying on metabolism of a marker substance before it can be detected non-invasively. Hence, reference values for normal versus abnormal gastric emptying times always also depend on the method used. In the present study, the absence of significant differences in gastric emptying times between treatments demonstrated with either technique, strengthen the validity of these observations. In addition, there was a good correlation between both methods in the present study (data not shown).

Overall, it seems necessary to draw a careful distinction between drug effects in an experimental setting (in vivo or in vitro), where effects on single muscle layers or gastric contraction patterns may be observed, especially when other mediators of gastrointestinal motility are artificially blocked, and clinical settings. In addition, it is likely that the induction of muscle contractions in the stomach alone is not enough to significantly increase total gastric emptying, but that sequential contractions and antropyloroduodenal coordination are more important (46). Even though some improvement in this coordination has been observed in the dog with 5-HT_4 agonists (18), it is possible that it is mediated by other mediators entirely. In rats, this coordination has been found to be mediated via ghrelin (46). This gastrointestinal peptide has also been used successfully to accelerate gastrointestinal motility in dogs with post-operative gastric ileus (47). While the goal of the present study was not to elucidate if and how maropitant or cisapride influence antropyloroduodenal coordination, this may be an interesting topic for further research.

In conclusion, neither maropitant nor cisapride demonstrated clinically relevant effects on different stages of gastric emptying in healthy dogs in this study. As this study did not include dogs with gastric dysmotility, recommendations regarding their use in naturally occurring gastric emptying disorders is difficult. It is worth noting, however, that the lack of a prokinetic effect of maropitant may be beneficial in individual cases where the cause of vomiting is unclear and gastric obstruction cannot be entirely ruled out. Studies assessing the effect of maropitant in dogs with abnormal gastric motility are necessary to further elucidate its effects in these situations.

Conclusion for practice

This study could not show a consistent prokinetic effect of the neurokinin-1 receptor antagonist maropitant on canine gastric emptying. This does however, not exclude that this drug can potentially improve gastropyloroduodenal coordination or has prokinetic properties in other parts of the gastrointestinal tract. It is noteworthy that the positive control drug chosen for this study – the 5-HT4 receptor antagonist cisapride – also had no prokinetic effect (similar to placebo). Hence, the usefulness of both drugs to improve gastric emptying times in the dog have to be assessed critically.

Conflict of interest

The authors declare not to have any conflict of interest. All consumables used in this study were sponsored and supplied by Zoetis (Paris, France).

References

1. Ehrlein H, Kaske M. Motorik des Magen-Darm-Kanals. In: Physiologie der Haustiere. Engelhardt W, Breves G, Hrsg. Stuttgart: Enke 2000.
2. Washabau RJ. Gastrointestinal motility disorders and gastrointestinal prokinetic therapy. Vet Clin North Am Small Anim Pract. 2003; 33 (5): 1007–1228, vi.

3. Cullen JJ, Titler S, Ephgrave KS, Hinkhouse MM. Gastric emptying of liquids and postprandial pancreatobiliary secretion are temporarily impaired during endotoxemia. Dig Dis Sci 1999; 44 (11): 2172–2177.
4. Fioramonti J, Bueno L. Gastrointestinal myoelectric activity disturbances in gastric ulcer disease in rats and dogs. Dig Dis Sci 1980; 25 (8): 575–580.
5. Johnson CS, Fales-Williams AJ, Reimer SB, Lotsikas PJ, Haynes JS. Fibrosing gastrointestinal leiomyositis as a cause of chronic intestinal pseudoobstruction in an 8-month-old dog. Vet Pathol 2007; 44 (1): 106–109.
6. Takeda M, Mizutani Y, Yamano M, Tsukamoto K, Suzuki T. Gastric emptying in diabetic gastroparetic dogs: Effects of SK-951, a novel prokinetic agent. Pharmacology 2001; 62 (1): 23–28.
7. Schmitz S, Jansen N, Failing K, Neiger R. 13C-sodium acetate breath test for evaluation of gastric emptying times in dogs with gastric dilatation-volvulus. Tierärztl Prax 2013; 41 (K):87–92.
8. Boscan P, Cochran S, Monnet E, Webb C, Twedt D. Effect of prolonged general anesthesia with sevoflurane and laparoscopic surgery on gastric and small bowel propulsive motility and pH in dogs. Vet Anaesth Analg 2014; 41 (1): 73–81.
9. Hall J, Washabau R. Gastrointestinal prokinetic therapy: Dopaminergic antagonist drugs. Compend Contin Educ Pract Vet 1997; 19 (2): 214.
10. Gué M, Fioramonti J, Bueno L. A simple double radiolabeled technique to evaluate gastric emptying of canned food meal in dogs. Application to pharmacological tests. Gastroentérologie Clin Biol 1988; 12 (5): 425–430.
11. Hall JA, Solie TN, Seim HB, Twedt DC. Effect of metoclopramide on fed-state gastric myoelectric and motor activity in dogs. Am J Vet Res 1996; 57 (11): 1616–1622.
12. Platteborse R, Hermans C, Loots W, Van Loon J, Van Gorp A, Smet F, et al. The effect of domperidone on pyloric activity in dog and in man. Postgrad Med J 1979; 55 Suppl 1: 15–18.
13. Edelbroek M, Schuurkes J, De Ridder W, Horowitz M, Dent J, Akkermans L. Effect of cisapride on myoelectrical and motor responses of antropyloroduodenal region during intraduodenal lipid and antral tachygastria in conscious dog. Dig Dis Sci 1995; 40 (4): 901–911.
14. Manini ML, Camilleri M, Goldberg M, Sweetser S, McKinzie S, Burton D, et al. Effects of Velusetrag (TD-5108) on gastrointestinal transit and bowel function in health and pharmacokinetics in health and constipation. Neurogastroenterol Motil 2010; 22 (1): 42–49, e7–8.
15. Seto Y, Yoshida N, Kaneko H. Effects of mosapride citrate, a 5-HT4-receptor agonist, on gastric distension-induced visceromotor response in conscious rats. J Pharmacol Sci 2011; 116 (1): 47–53.
16. Tsukamoto A, Ohno K, Maeda S, Nakashima K, Fukushima K, Fujino Y, et al. Effect of mosapride on prednisolone-induced gastric mucosal injury and gastric-emptying disorder in dog. J Vet Med Sci 2012; 74 (9): 1103–1108.
17. Briejer MR, Prins NH, Schuurkes JAJ. Effects of the enterokinetic prucalopride (R093877) on colonic motility in fasted dogs. Neurogastroenterol Motil 2001; 13 (5): 465–472.
18. Tanaka T, Mizumoto A, Mochiki E, Suzuki H, Itoh Z, Omura S. Effects of EM574 and cisapride on gastric contractile and emptying activity in normal and drug-induced gastroparesis in dogs. J Pharmacol Exp Ther 1998; 287 (2): 712–719.
19. Bertaccini G, Coruzzi G, Poli E. Histamine H2 receptor antagonists may modify dog intestinal motility independently of their primary action on the H2 receptors. Pharmacol Res Commun 1985; 17 (3): 241–254.
20. McCord K, Boscan P, Dowers K, Bradley A, Cosgrove S, Dame K, et al. Comparison of gastrointestinal motility in dogs treated with metoclopramide, cisapride, erythromycin or maropitant using the SMART-Pill™. J Vet Int Med ACVIm Forum Proceedings 2009; 735–736.
21. Bogard K, Gaschen F, Olsen E, Waddle M, Stout R, Gaschen L. Double-blind, placebo-controlled ultrasonographic evaluation of the effects of antiemetic drugs on antral motility and gastric emptying in healthy dogs. J Vet Int Med ACVIM Forum Proceedings 2015; 1184.
22. Conder GA, Sedlacek HS, Boucher JF, Clemence RG. Efficacy and safety of maropitant, a selective neurokinin 1 receptor antagonist, in two randomized clinical trials for prevention of vomiting due to motion sickness in dogs. J Vet Pharmacol Ther 2008; 31 (6): 528–532.
23. Gardner CJ, Twissell DJ, Dale TJ, Gale JD, Jordan CC, Kilpatrick GJ, et al. The broad-spectrum anti-emetic activity of the novel non-peptide tachykinin NK1 receptor antagonist GR203040. Br J Pharmacol 1995; 116 (8): 3158–3163.
24. Ramsey DS, Kincaid K, Watkins JA, Boucher JF, Conder GA, Eagleson JS, et al. Safety and efficacy of injectable and oral maropitant, a selective neurokinin 1 receptor antagonist, in a randomized clinical trial for treatment of vomiting in dogs. J Vet Pharmacol Ther 2008; 31 (6): 538–543.
25. Mantyh PW, Mantyh CR, Gates T, Vigna SR, Maggio JE. Receptor binding sites for substance P and substance K in the canine gastrointestinal tract and their possible role in inflammatory bowel disease. Neuroscience 1988; 25 (3): 817–837.
26. Pearse AG, Polak JM. Immunocytochemical localization of substance P in mammalian intestine. Histochemistry 1975; 41 (4): 373–375.
27. Mangel AW, Koegel A. Effects of peptides on gastric emptying. Am J Physiol 1984; 246 (4 Pt 1): G342–345.
28. Holzer P. Stimulation and inhibition of gastrointestinal propulsion induced by substance P and substance K in the rat. Br J Pharmacol 1985; 86 (1): 305–312.
29. Valdovinos MA, Thomforde GM, Camilleri M. Effect of putative carcinoid mediators on gastric and small bowel transit in rats and the role of 5-HT receptors. Aliment Pharmacol Ther 1993; 7 (1): 61–66.
30. Schmitz S, Failing K, Neiger R. Solid phase gastric emptying times in the dog measured by 13C-sodium-acetate breath test and 99mTechnetium radioscintigraphy. Tierärztl Prax 2010; 38 (K): 211–216.
31. Wyse CA, McLellan J, Dickie AM, Sutton DGM, Preston T, Yam PS. A review of methods for assessment of the rate of gastric emptying in the dog and cat: 1898–2002. J Vet Intern Med 2003; 17 (5): 609–621.
32. Wyse CA, Preston T, Love S, Morrison DJ, Cooper JM, Yam PS. Use of the 13C-octanoic acid breath test for assessment of solid-phase gastric emptying in dogs. Am J Vet Res 2001; 62 (12): 1939–1944.
33. Craig H. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim Cosmochim Acta 1957; 12 (1–2): 133–149.
34. Allescher HD, Kostolanska F, Tougas G, Fox JE, Regoli D, Drapeau G, et al. The actions of neurokinins and substance P in canine pylorus, antrum and duodenum. Peptides; 10 (3): 671–679.
35. Milenov K, Oehme P, Bienert M, Bergmann J. Effect of substance P on mechanical and myoelectrical activities of stomach and small intestines in conscious dog. Arch Int Pharmacodyn Ther 1978; 233 (2): 251–260.
36. Nakazato Y, Sekine H, Isogaya M, Ito S, Ohga A. Atropine- and hexamethonium-resistant motor response to greater splanchnic nerve stimulation in the dog stomach. J Auton Nerv Syst 1987; 20 (1): 35–42.
37. Fox JE, McDonald TJ. Motor effects of gastrin releasing peptide (GRP) and bombesin in the canine stomach and small intestine. Life Sci 1984; 35 (16): 1667–1673.
38. Itoh Z, Takeuchi S, Aizawa I, Couch EF. Inhibitory effect of pentagastrin and feeding natural and motilin-induced interdigestive contractions in the stomach of conscious dogs. Gastroenterol Jpn 1977; 12 (4): 284–288.
39. Maggi CA, Schwartz TW. The dual nature of the tachykinin NK1 receptor. Trends Pharmacol Sci 1997; 18 (10): 351–355.
40. Holzer P, Holzer-Petsche U. Tachykinin receptors in the gut: physiological and pathological implications. Curr Opin Pharmacol 2001; 1 (6): 583–590.
41. Boillat CS, Gaschen FP, Gaschen L, Stout RW, Hosgood GL. Variability associated with repeated measurements of gastrointestinal tract motility in dogs obtained by use of a wireless motility capsule system and scintigraphy. Am J Vet Res 2010; 71 (8): 903–908.
42. Richards RD, Valenzuela GA, Davenport KG, Fisher KL, McCallum RW. Objective and subjective results of a randomized, double-blind, placebo-controlled trial using cisapride to treat gastroparesis. Dig Dis Sci 1993; 38 (5): 811–816.
43. Takeda M, Tsukamoto K, Yamano M, Uesaka H. Effects of SK-951, a benzofuran derivative, as a prokinetic agent in rats and dogs. Jpn J Pharmacol 1999; 81 (3): 292–297.

44. Iwanaga Y, Wen J, Thollander MS, Kost LJ, Thomforde GM, Allen RG, et al. Scintigraphic measurement of regional gastrointestinal transit in the dog. Am J Physiol 1998; 275 (5 Pt 1): G904–910.
45. Peterson L, Gaschen L, Otamendi A, Stout R, Gaschen F. Ultrasound evaluation of the effects of various prokinetics administered intravenously on gastric and duodenal motility in healthy dogs. Journal of Veterinary Internal Medicine ACVIM Forum Proceedings 2013; 709.
46. Ariga H, Nakade Y, Tsukamoto K, Imai K, Chen C, Mantyh C, et al. Ghrelin accelerates gastric emptying via early manifestation of antro-pyloric coordination in conscious rats. Regul Pept 2008; 146 (1–3): 112–116.
47. Trudel L, Bouin M, Tomasetto C, Eberling P, St-Pierre S, Bannon P, et al. Two new peptides to improve post-operative gastric ileus in dog. Peptides 2003; 24 (4): 531–534.