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Research paper

Gastric pH profiles of beagle dogs and their use as an alternative to human testing

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Abstract

Gastric pH levels were measured in samples of gastric aspirates from eight fasted beagle dogs. The gastric pH in fasting dogs fluctuated from 2.7 to 8.3, with a mean of 6.8 ± 0.2 (SE). Each dog received the following four treatments in randomly-assigned order: (A) distilled water; (B) a placebo capsule; (C) pentagastrin, and (D) ranitidine. The gastric pH remained relatively constant after distilled water administration. In contrast, the treatments with pentagastrin and placebo capsule each lowered gastric pH. Pretreatment with pentagastrin was more successful in lowering gastric pH than that with placebo capsule. On the other hand, the pH rose above 7.0 in all dogs by the first hour after treatment with ranitidine. This animal model may be helpful in evaluating the biopharmaceutics of drugs exhibiting pH-dependent dissolution or decomposition.

Keywords: Gastric pH; Placebo capsule; Pentagastrin; Ranitidine; Animal model

1. Introduction

Although there are many physiological differences between animals and humans, new products and dosage forms are frequently evaluated in animals to determine oral bioavailability prior to the introduction of products into humans [1,2]. Beagle dogs are commonly used as a convenient animal species for testing oral dosage forms because of their ability to ingest human-scale dosage forms and their ease of handling. Although the dog is a very useful model, many instances exist for which there is a large discrepancy between the oral bioavailability observed in dogs and that observed in humans [3]. Consideration of differences in gross physiology, e.g. motility, pH, and surface area, between the gastro-intestinal (GI) tracts of dogs and humans may provide an explanation for some of the differences observed in drug absorption [4,5]. It is not surprising that the species-dependent gastric emptying and intestinal transit times can result in differences in the rate and/or extent of absorption. For example, the reduction of the bioavailability of acetaminophen from sustained-release granules in dogs would result from the shorter transit time in the GI tract compared with humans [6]. In addition, species differences in gastric and intestinal pH may also contribute to the differences in drug absorption because the rate of GI secretion is known to be widely different among species [4,5]. For example, humans are good acid secretors, whereas dogs are poor secretors [4]. When the solubility of a drug is pH-dependent, species differences in absorption of the drug are expected.

Gastric pH- controlled dogs are considered to be useful as an animal model to predict the bioavailability of drugs and formulations with pH-dependent dissolution profiles at various acidity levels, because of the difficulty of identifying and recruiting normal and achlorhydric subjects [7]. Some studies have tried to evaluate the effect of administering pentagastrin [8], H_2-receptor antagonists [9] which control gastric pH and which may alter drug absorption. However, to our knowledge, few detailed studies have been available on the gastric pH in dogs under various conditions. In addition, little is known about the effect of mechanical stimulations, e.g. water and dosage forms, on the pH.

The primary aim of this preliminary study was to further clarify the gastric pH in dogs pretreated with distilled water, a gelatin capsule (placebo), ranitidine and pentagastrin. The study was also designed to evaluate the usefulness of dog models which have modified gastric pH as an alternate to human testing.

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2. Materials and methods

2.1. Chemicals

Ranitidine hydrochloride (Zantac injection, 50 mg/ml) was obtained from Glaxo (UK). Pentagastrin (lot 123H5831) was purchased from Sigma (St. Louis, MO). Lactose mono-hydrate, gelatin capsules and distilled water were obtained from Wako Pure Chemical Industry (Osaka), Kasho Co. Ltd. (Tokyo), and Kanto Chemical Co. Inc. (Tokyo), respectively. All other chemicals were standard commercial products of analytical grade.

2.2. Animals

Eight healthy 2-year-old male beagle dogs weighing 9–11 kg were used. The dogs were maintained on a standard solid meal of commercial diet (Pulina Co. Ltd., Japan). During the experimental period, all dogs were allowed free access to water, but no food was given.

2.3. Treatment

The dogs were randomized to receive four treatment sequences in a four-way crossover design, with a minimum recovery period of 1 week separating each treatment. Treatment A, which served as the control, consisted of 10 ml of distilled water administered by intubation. In treatment B, dogs were orally administered a placebo gelatin capsule containing 500 mg lactose with 10 ml of distilled water. In treatment C, pentagastrin was injected intramuscularly at a dose of 6 μg/kg. Treatment D consisted of injecting 50 mg/dog of ranitidine intravenously. Prior to each treatment, the dogs underwent an overnight fast of at least 18 h, which was continued for an extra 4 h after treatments.

2.4. Measurement of the gastric pH

The pH-time profiles were analyzed for the gastric fluids. A tube was inserted into the stomach through a catheter, and a small volume of gastric fluid was aspirated through the tubing. The pH of the gastric fluid was determined using a pH meter (ACT pH meter D-13, Horiba, Japan). Measurements were carried out at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, and 4 h after the treatment.

2.5. Evaluation

Areas under the pH level vs. time curve were calculated by the trapezoidal rule from the start of the treatment up to 4 h after the treatment. The maximum or minimum gastric pH and the time to reach the pH were recorded according to observations. Treatment differences were analyzed by multiple comparison (Dunnett's multiple range test) based on untransformed data, and on the ordinary linear model (SAS, GLM). The value of significance was set at a P value of less than 0.05.

3. Results and discussion

Eight individual dog profiles are shown in Fig. 1 to illustrate the range of behavior seen in the gastric pH profiles. The pH of the gastric fluids in fasting dogs fluctuated, with a range of 2.7–8.3, but the majority of animals had a basal pH of around 7. The pHs were always steady around 7.0 in three dogs when the gastric fluids were sampled over 5 times in eight dogs. Gastric pHs were less than 5.0 in only six of the total 40 samples studied. The overall mean gastric pH was 6.8 ± 0.2 (mean $\pm$ SE), and was similar to the results reported in the literature [10,11]. On the other hand, a discrepancy was observed between the results obtained using an intubation method in this study and those obtained using a radio-telemetric device (Heidelberg capsule) [12]. The reason for this discrepancy is unclear. However, possible explanation of it is that aspirates may have been obtained from the anterior region in the stomach. The anterior portion has a higher pH than the posterior portion of the stomach since the parietal cells tend to be localized in the lower part of the stomach [5]. One of the limitation of this study was that the location of the tip of the intubation tube in the stomach was not checked.

Our results also indicated that the average gastric pH in the dogs was markedly higher than the published data in humans [13]. Dogs are known to have a very low basal gastric acid secretion, which is ~0.1 μmol/min per kg [4]. Therefore, the gastric pH of the dog can be as high as its duodenal contents in the unstimulated state [5]. The differences of gastric pH profiles between dogs and humans are consistent with reported results for the lower rate of basal acid secretion in the dog [4,5].

Fig. 2 illustrates the gastric pH-time profiles with four treatments, and Table 1 summarizes GI pH parameters.

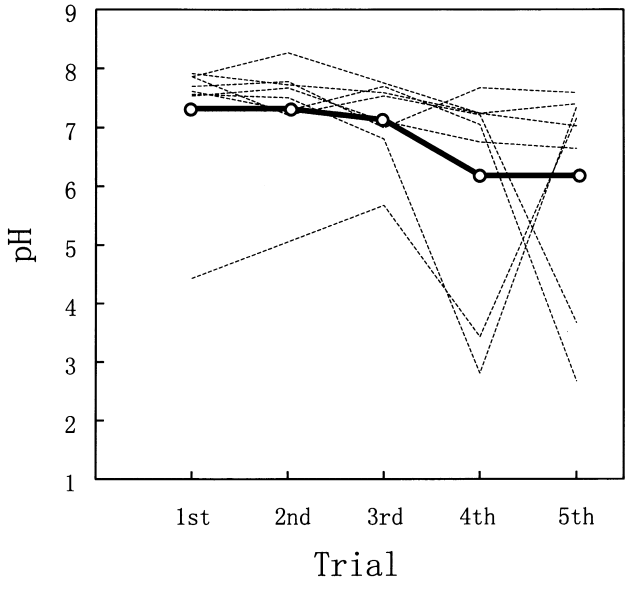

Fig. 1. Fluctuation of gastric pH in fasting beagle dogs ($n = 8$). - - - - -, individual; -o-, mean.

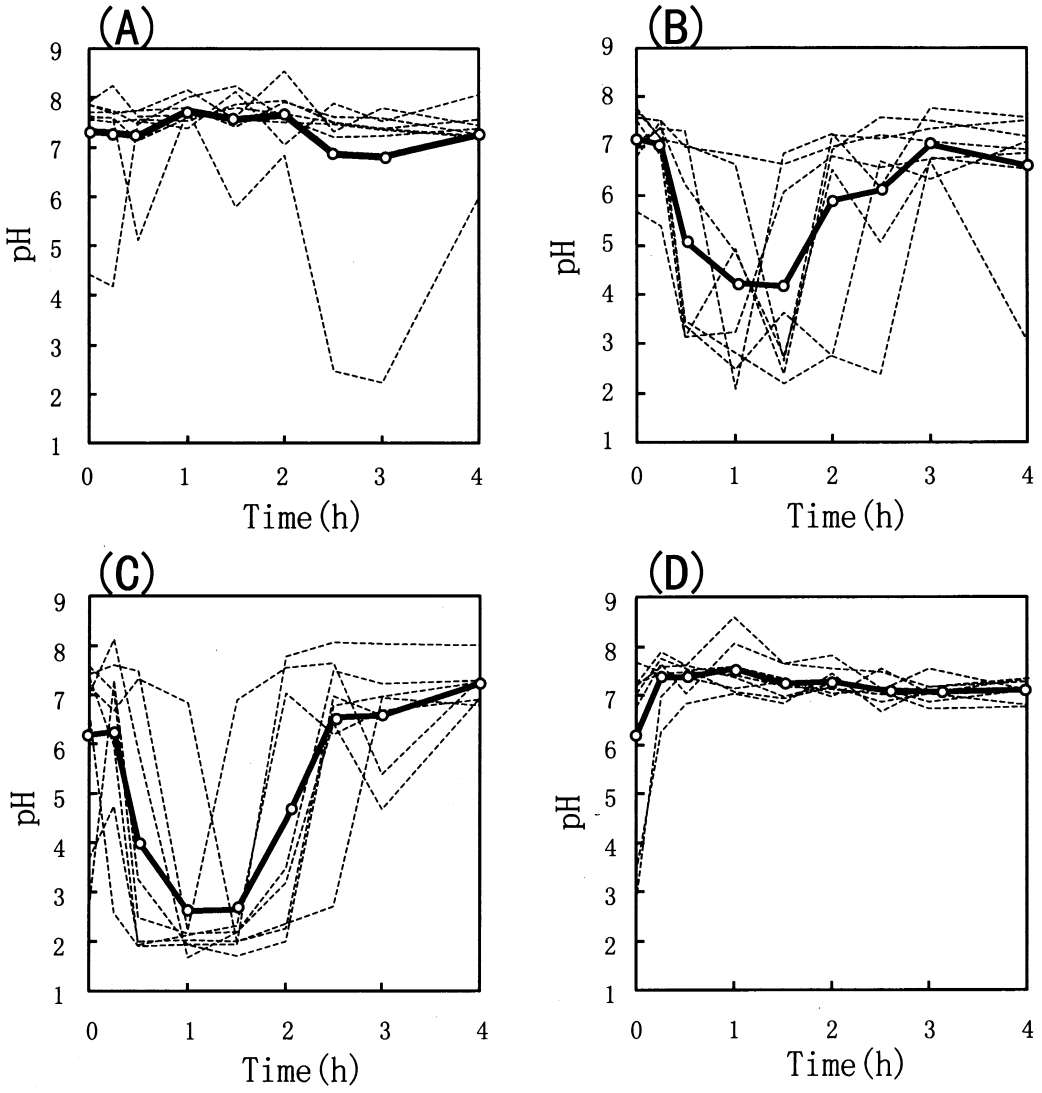

Fig. 2. Gastric pH-time profiles in fasting beagle dogs following treatment with distilled water (A), placebo capsule (B), pentagastrin (C) and ranitidine (D). - - - - -, individual; ⊸, mean.

The gastric pH in dogs remained relatively constant after receiving distilled water. Feldman and Grossman reported that the introduction of isotonic saline caused only a slight increase in gastric acid secretion in dogs [14]. Thus, it is expected that the gastric pH in dogs will be unchanged after receiving an aqueous formulation. However, the average gastric pH in dogs was decreased markedly after the administration of a gelatin capsule which contained 500 mg lactose. Within about 1 h after oral administration of this capsule to dogs, the average trough pH level exhibited 3.0.

Table 1
Gastric pH parameters in eight dogs with various pretreatment

Pretreatment	Gastric pH		Time to reach minimum or maximum pH (h)		AUC of pH-time curve (pH × h)
	pH_{max}	pH_{min}	T_{max}	T_{min}	
Distilled water (p.o.)	8.07 ± 0.09	6.24 ± 0.69	1.34 ± 0.21	2.47 ± 0.50	29.09 ± 1.19
Gelatine capsule (p.o.)	–	3.02 ± 0.53	–	1.44 ± 0.20	23.57 ± 1.55[a]
Pentagastrin (i.m.)	–	1.95 ± 0.07	–	1.06 ± 0.15	20.51 ± 0.88[b]
Ranitidine (i.v.)	7.77 ± 0.14	–	0.78 ± 0.22	–	28.81 ± 0.29

[a] $P < 0.05$ compared to distilled water.
[b] $P < 0.01$ compared to distilled water.

The AUC for the pH vs. time curve was significantly lower in the treatment with the capsule than in the treatment with the distilled water ($P < 0.05$). However, the gastric pH returned to a high level 1.5 h after the administration, and the pH reading ranged from 2.2 to 6.6. Gastric acid secretion rates in dogs following food ingestion exceed those in humans [5]. This decrement in gastric pH can be explained by assuming that the secretion was stimulated mechanically in response to the capsule administration even though the stimulation was not so potent.

Beagle dogs pretreated with pentagastrin, an analogue of gastrin that reproducibly stimulates gastric acid secretion, have been used to screen different drugs and formulations. After intramuscular administration of pentagastrin, a more profound reduction in the gastric pH was observed compared to that in the capsule group. In eight dogs, which initially had gastric pH values ranging from 3.7 to 7.6, pH values within 1.7–2.2 were attained in 0.5–1.5 h, but this effect disappeared up to about 2.5 h after dosing. The time to return to the original values for all pH values examined was slower in the dogs pretreated with pentagastrin compared to that with the placebo capsule. The comparison of the area of the pH-time curves confirmed the significant effect of the pentagastrin ($P < 0.01$). In addition, pentagastrin might be expected to exert a prolonged duration of action during a repeated administration [15]. The pretreatment with pentagastrin was successful in lowering gastric pH to the desired value similar to that of humans having a normal gastric condition.

Large populations of humans, e.g. those treated with antacid, H_2-blockers or proton pump-inhibitors, patients with achlorhydria, many elderly patients, patients after gastrectomy, and those receiving other medical treatments affecting acid secretion in the stomach, have an increased gastric pH. Beagle dogs whose gastric acidity is controlled with ranitidine appear to be useful animal models to evaluate the biological absorption behavior of drugs at low acidity levels. Ranitidine have been chosen over other H_2-receptor antagonists, because it has been less likely to cause potential drug-drug interaction via the inhibitory effect on the cytochrome P450 systems [16]. Before the ranitidine treatment, the pH ranged from 2.8 to 7.7, with all but six readings being 7.0 or above. A wide variation in the gastric pH was not found, and the mean gastric pH induced by ranitidine 1 h after the intravenous administration was 7.5, with a range of 7.0–8.6. In addition, this level of pH was maintained consistently for 4 h. The results of this study demonstrated that the pretreatment with ranitidine might be very useful in developing an achlorhydric dog model as an alternative to human testing.

In conclusion, the gastric pH in dogs appeared to be dissimilar to that in humans and the fluctuation could not be ignored in the fasted state. The higher gastric pH observed in dogs might partly explain the difference of absorption characteristics between the species and would be of concern for those drugs with a half-maximal absorption pH in the pH 5–7 range and for the evaluation of enteric-coated and sustained-release products with a dissolution pH in this range. However, the dog model might prove to be useful as a means to provide for controlled gastric pH, and as an experimental system to screen the effect of gastric pH on the absorption of drugs from dosage forms exhibiting pH-dependent dissolution.

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