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FULL PAPER

Pharmacology

Effect of the liquid form of traditional Chinese medicine, Hozen-S, on gastric motility in dogs

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ABSTRACT. Juzen-taiho-to, a traditional Chinese herbal medicine, is used for patients with anorexia and fatigue in human medicine. In our previous study, granulated Juzen-taiho-to improved vincristine-induced gastrointestinal adverse effects through increasing gastric motility in dogs. As the effect of Hozen-S, the sweet liquid form of Juzen-taiho-to, on dog gastric motility has not been investigated, we examined the effect of administration of Hozen-S on gastric motility. Furthermore, we assessed dog plasma ghrelin level to further elucidate the mechanism of the effect of Hozen-S on gastric contraction. Finally, we assessed the palatability of Hozen-S compared to granulated Juzen-taiho-to and its effect on body weight in dogs. Administration of Hozen-S significantly increased gastric motility, plasma ghrelin concentration, and body weight. A palatability evaluation revealed that the dogs preferred Hozen-S to granulated Juzen-taiho-to. In conclusion, Hozen-S administration to dogs promoted gastric motility by raising plasma ghrelin levels. Considering these functional and palatability data, Hozen-S may replace granulated type Juzen-taiho-to and become a prominent traditional Chinese veterinary medicament.

KEYWORDS: dog, gastric motility, ghrelin, Hosen-S, traditional Chinese medicine

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Traditional Chinese medicines (kampo) are widely used in Japanese medicine and recently in veterinary practice. Some owners of cancer-affected dogs prefer treatment using these medicines [10]. Juzen-taiho-to, a traditional Chinese medicine, consists of 10 crude drugs such as Atractylodes lancea rhizome (So-jutsu), cinnamon, and Panax ginseng. It is used for patients with anorexia, fatigue, and other conditions in human medicine [3]. Juzen-taiho-to was reported to reduce the hematological side effects of anti-cancer drugs in mice [16]. In our previous study, Juzen-taiho-to stimulated gastric contraction and had protective effects against vincristine-induced gastrointestinal toxicity in dogs when administered at 450 mg/kg/day [14]. Considering these reports, Juzen-taiho-to may be useful in veterinary medicine to reduce the side effects of anti-cancer drugs. However, the mechanism of the protective effects of Juzen-taiho-to against vincristine-induced gastrointestinal toxicity in dogs is still unclear. Since there have been very few studies on traditional Chinese medicines, some veterinarians are hesitant to prescribe these medicines to animals.

Although gastrointestinal contraction is regulated by several factors, ghrelin is reported to be one of the factors that play important roles in the gastrointestinal contraction. Ghrelin is a 28-amino acid peptide predominantly produced by endocrine cells in the oxyntic mucosa of the stomach. Ghrelin is also reported to increase gastrointestinal motility in humans, rats, and so on [17]. Recent studies showed that intravenous injection of ghrelin promoted appetite and gastric motility in dogs [22, 23]. However, few studies have been conducted to assess the relationship between Juzen-taiho-to and ghrelin in dogs.

Using traditional Chinese medicine in veterinary medicine involves changing the administration method for dogs. Commonly used traditional Chinese medicines are granulated and have a unique smell and taste; thus, many dogs and cats will not take these medicines willingly, which makes it difficult for veterinarians and owners to administer a sufficient dose.

Therefore, we focused on Hozen-S, which is sold as a sweet liquid form of Juzen-taiho-to and used to improve anorexia

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and fatigue in humans. Although the basic ingredients of Hozen-S and Juzen-taiho-to are same, they are different not only in properties and taste but also in ratio and volume of crude drugs. Since Hozen-S is sweet and liquid form of Juzen-taiho-to, it may be administered to dogs more easily than generally used granulated Juzen-taiho-to. However, no studies have demonstrated the efficacy and palatability of Hozen-S in dogs.

In the present study, we examined the effects and possible mechanisms of Hozen-S on gastric motility in the dogs. We also examined whether the dogs preferred the palatability of Hozen-S to granulated Juzen-taiho-to.

MATERIALS AND METHODS

Animals

Five clinically healthy female beagle dogs (6 years of age and weighing 9.0–10.0 kg) were purchased from the Research Institute for Animal Science in Biochemistry and Toxicology (Tokyo, Japan). They were acclimatized for one month before the experiments. The dogs were housed separately in stainless steel cages and in a controlled standard environment. They were fed on a commercial pelleted diet and canned dog's food at a fixed time daily. Water was provided *ad libitum*. Animals were handled following the recommendations of the Guide for the Care and Use of Laboratory Animals and approved by the Faculty of Agriculture, Tokyo University of Agriculture and Technology (approval no. R03-118).

Chemicals and reagent

Hozen-S was obtained from Meiji Yakuhin Co., Ltd. (Tokyo, Japan). Granulated Juzen-taiho-to was obtained from TSUMURA (Tokyo, Japan). Active Ghrelin ELISA Kit was obtained from DENIS PHARMA (Tokyo, Japan).

Experimental design

Three experiments were performed in this study. In the first experiment, five dogs were orally administered Hozen-S at 0.8 ml/kg daily with food for 42 days. The dose was calculated by referring to the dosage of Juzen-taiho-to in human medicine [5]. Although Hozen-S at 0.8 ml/kg is less than granulated Juzen-taiho-to at 450 mg/kg/day, which we used in the previous study, Juzen-taiho-to at 450 mg/kg/day was a relatively high quantity referring to the dosage in human medicine. We therefore set the dosage of Hozen-S closer to the dosage in human medicine in this study [5]. Ultrasonographic assessment of gastric motility in dogs was performed at day 0, 7, 14, 21, 28, 35, and 42 after administration. A 56-day washout period was carried out between the first and second experiments. In the second experiment, five dogs were orally administered Hozen-S at 0.8 ml/kg or 4.0 ml/kg daily with food for 42 days. Blood samples were taken from the cephalic vein of the five dogs at day 0, 14, 28, and 42 to measure the plasma ghrelin level. Before performing these experiments, we gave the dogs the same amount of food without Hozen-S for 42 days and confirmed no change in their motility index, ghrelin and body weight. General status (vigorousness, appetite, vomiting, and diarrhea) was observed each day during these experiments. We set a 56-day interval between the second and third experiments. After that, we performed a third experiment to assess the palatability of Hozen-S compared with granulated Juzen-taiho-to in the same five dogs.

Ultrasonographic assessment

To assess gastric motility in dogs, we used a previously described technique with some modifications [20]. Motility was assessed using ultrasonography (EUB-7500; Hitachi Medical Corp., Chiba, Japan) with a 6.5-MHz phased array convex transducer. Ultrasonography was carried out by a single operator. Dogs were restrained in the standing position. The probe position was adjusted to obtain maximum visualization of the transverse image of the gastric antrum close to the left lobe of the liver. A cross-section of the antral area was measured by tracing the serosal side of the antrum with the built-in caliper. In both the contracted and relaxed phases, the antral region was evaluated three times. The number of contractions was counted in a 3-min period. The percentage of the amplitude was calculated using the following formula: (mean area relaxed−mean area contracted)/mean area relaxed. The number of antral contractions in 3 min was regarded as the frequency. The motility index, an indicator of gastric motility, was expressed as amplitude multiplied by frequency. Dog gastric motility can be evaluated in a short time non-invasively by assessing the motility index. When the motility index increases, gastric motility is considered to be promoted [19]. Commercially canned food (10 g/kg) and Hozen-S were provided 30 min before ultrasonographic evaluation. After the evaluation, the dogs were provided with an additional commercial dried pellet diet.

Measurement of the plasma ghrelin level

Blood samples were taken from the cephalic vein of the five dogs about 3 hr after feeding. The blood samples were transferred into chilled tubes containing ethylenediaminetetraacetic acid-2Na and aprotinin and promptly centrifuged at 4°C and 1,500 × *g* for 15 min. The supernatants were then acidified with 1 mol/l HCl (1/10 volume). All plasma samples were stored at −80°C until hormonal analyses were performed. Plasma ghrelin concentrations were determined using Active Ghrelin ELISA Kit (DENIS PHARMA), following instructions provided by the manufacturer. Data of absorbance were measured at a wavelength of 450 nm using a pectrophotometer (Bio-Rad iMark Microplate Readers, Bio-Rad, Hercules, CA, USA). Ghrelin concentration in the sample was calculated based on the calibration curve created from the absorbance of the standard product.

Palatability assessment of Hozen-S in dogs

To evaluate the palatability of Hozen-S in dogs, we first evaluated whether Hozen-S changed the daily feed intake because dogs sometimes refuse to eat their feed when medicines alter their taste or smell. We recorded the daily intake of feed mixed with Hozen-S (0.8 ml/kg) for 42 days. We also evaluated their body weight (BW) every week.

Next, we performed the two-bowl test for five dogs with some modifications. This test is the most common method for dog feed palatability assessment [1, 18]. Briefly, two bowls were prepared, and the same amount of feed was mixed with Hozen-S (0.8 ml/kg) or granulated Juzen-taiho-to (450 mg/kg) in respective bowls. The two bowls were presented 1 meter apart simultaneously to the dogs. The bowls were left with the dogs until one of the bowls had been completely consumed. After that, the consumed food amounts of the two respective bowls were quantified and the intake ratio (IR) of each bowl was calculated using the following equation (1) to determine whether one bowl or the other one was consumed in a higher proportion. The two-bowl test was conducted for two days with the bowls switched between days to override a side-bias (left or right bowl preference).

Intake ratio=Consumed amount of A/(Consumed amount of A + Consumed amount of B) (1)

Statistical analysis

The results are expressed as mean ± standard deviation (S.D). Paired *t*-test was adopted to analyze motility index, plasma ghrelin level and body weight. Welch's *t*-test was adopted to analyze Intake Ratio. Values of $P<0.05$ were considered significant.

RESULTS

Effects of Hozen-S on gastric motility in dogs

After Hozen-S at 0.8 ml/kg was administered to five dogs for 42 days, the motility index was assessed. The motility index gradually increased, and at day 35, it was significantly higher than at day 0 (Fig. 1) without affecting their general status. These results suggest that the administration of Hozen-S promoted gastric motility.

Effects of Hozen-S on the plasma ghrelin level in dogs

To elucidate the mechanism of the effect of Hozen-S on gastric contraction in dogs, we assessed the effect of Hozen-S on plasma ghrelin level. When Hozen-S was administered to dogs at 0.8 ml/kg, there were no changes in their plasma ghrelin level (data not shown). When Hozen-S was administered to dogs at 4.0 ml/kg for 42 days, the plasma ghrelin level gradually increased and at day 42 was significantly higher compared to day 0 (Fig. 2). These results suggest that the administration of Hozen-S promoted gastric motility by stimulating the secretion of ghrelin in dogs.

Palatability assessment of Hozen-S in dogs

Although traditional Chinese medicine has the possibility of being useful in veterinary medicine [14, 15], it is sometimes difficult to administer to animals because of its unique smell and taste. Thus, we investigated Hozen-S palatability in the dogs. First, we investigated whether Hozen-S (0.8 ml/kg) affected daily food intake and BW of dogs when administered with food for 42 days. Since the dogs were fed a specified amount of feed and ate up everything with or without Hozen-S, the daily intake of food remained unchanged. However, the BW of dogs after day 7 was significantly heavier than day 0 (Fig. 3).

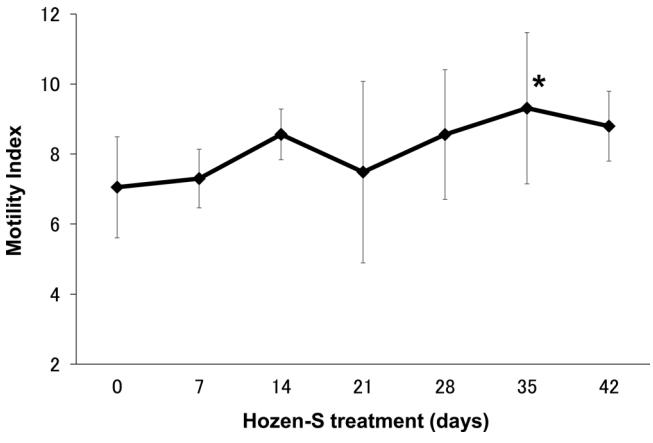

Fig. 1. Effect of Hozen-S on the gastric motility index in dogs. Hozen-S (0.8 ml/kg/day, Per os (PO)) was administered with food to five dogs for 42 days. Ultrasonography for gastric function was performed at day 0, 7, 21, 28, 35 and 42 after the administration of Hozen-S. The motility index for each dog was analyzed. The results are expressed as the mean ± standard deviation. *Significant difference ($P<0.05$) from baseline (day 0).

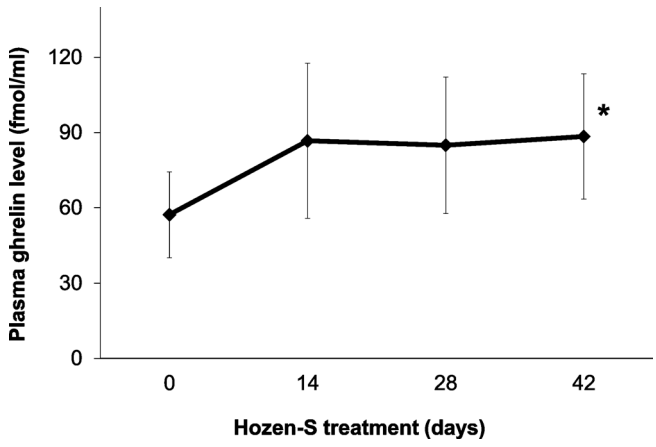

Fig. 2. Effect of Hozen-S on plasma ghrelin level in dogs. Hozen-S (4.0 ml/kg/day, PO) was administered with food to five dogs for 42 days. Blood was taken from the cephalic vein of each dog at day 0, 14, 28, and 42 after the administration of Hozen-S and the plasma ghrelin level was measured. The results are expressed as the mean ± standard deviation. *Significant difference ($P<0.05$) from baseline (day 0).

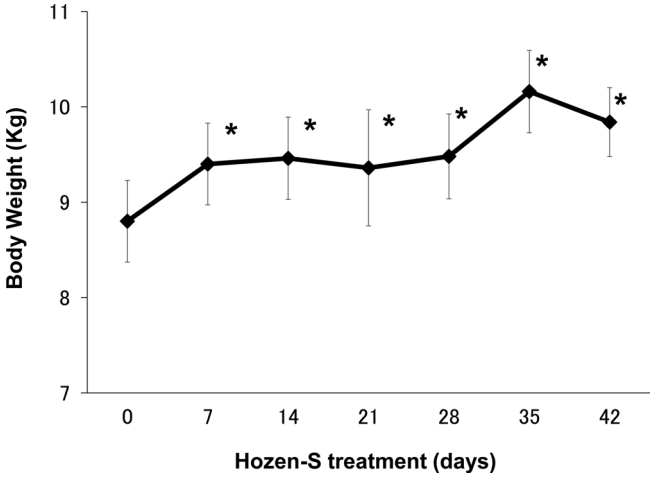

Fig. 3. Effect of Hozen-S on body weight in dogs. Hozen-S (0.8 ml/kg/day, PO) was administered with food to five dogs for 42 days. The body weight (BW) of dogs was examined at day 0, 7, 14, 21, 28, 35, and 42 after administration. The results are expressed as the mean ± standard deviation. *Significant difference (P<0.05) from baseline (day 0).

Table 1. Intake Ratio of the food mixed with Hozen-S or granulated Juzen-taiho-to in five dogs

Parameter	Hozen-S	Juzen-taiho-to
Intake Ratio [a]	0.74 ± 0.17 *	0.26 ± 0.17

[a] Amount consumed of A/(amount consumed of A + amount consumed of B). *P<0.05.

We next performed the two-bowl test to compare the palatability of Hozen-S and granulated Juzen-taiho-to. We assessed the intake of the feed mixed with Hozen-S or granulated Juzen-taiho-to and then calculated the IR of the feed. The IR of the feed with Hozen-S was significantly higher than that of granulated Juzen-taiho-to (Table 1), suggesting that Hozen-S was more willingly consumed by the dogs.

DISCUSSION

Juzen-taiho-to has been traditionally prescribed in Japanese medicine to patients with anorexia and fatigue. It is often administered to improve the general systemic condition of cancer patients by reducing the adverse effects of chemotherapy [13]. An experimental study reported that Juzen-taiho-to ameliorated the carboplatin-induced decrease in food intake and BW in mice [16]. In our previous study, the administration of granulated Juzen-taiho-to to dogs promoted gastric contraction and reduced vincristine-induced gastrointestinal adverse reactions [14].

First, we investigated whether the administration of Hozen-S promoted gastric motility in dogs by calculating the motility index. As shown in Fig. 1, the administration of Hozen-S increased the motility index in dogs. This suggests that Hozen-S promoted gastric motility in dogs and may be protective against anti-cancer drugs-impaired gastrointestinal motility and other similar conditions.

Traditional Chinese medicines may have beneficial effects in veterinary medicine; however, many things are still unclear. There are few studies about the mechanism of the effects of these medicines, which sometimes makes veterinarians hesitant to prescribe these medicines. Therefore, we examined the mechanism of the promotion of gastric contraction by Hozen-S in dogs. Although gastric contraction is regulated by several factors, we focused on ghrelin, a peptide discovered in endocrine cells of the stomach, which is reported to significantly increase food intake and gastric motility in dogs [22, 23]. As shown in Fig. 2, we demonstrated that administration of 4.0 ml/kg Hozen-S significantly raised the plasma ghrelin level in dogs. Hozen-S contains 10 crude drugs including Panax ginseng and cinnamon. Ginsenoside, the major pharmacologically active ingredient of Panax ginseng was reported to increase ghrelin secretion in human cells [21]. Cinnamon extracts were also demonstrated to upregulate ghrelin gene expression in human cells [7]. Based on these reports, Panax ginseng and cinnamon may also increase ghrelin secretion in the cells of dogs and raise the plasma ghrelin level. Further investigations are needed on the effects of the respective crude drugs included in Hozen-S on dogs. However, we assessed the effects of two doses of Hozen-S on plasma ghrelin level in this study and only a higher dose of it increased plasma ghrelin level in dogs. According to the prior research, although administration of lower, middle or higher doses of Rikkunshito, a traditional Chinese herbal medicine, to dogs promoted their gastrointestinal contractions, the plasma ghrelin level in dogs was increased only by the administration of a higher dose [22]. The results in our present study did not contradict the result of the prior research. A certain amount of traditional Chinese medicines might be needed to observe the changes in the plasma ghrelin level.

Since ghrelin is considered to increase food intake and gastric motility in dogs [17, 22] and intravenous injection of ghrelin is reported to promote gastric contraction in dogs [23], Hozen-S might promote gastric motility in dogs by raising the plasma ghrelin level. On the other hand, it was reported that ghrelin had no effects on gastric emptying [12] and gastrointestinal contraction [11]. In the present study, gastric motility and the serum ghrelin level tended to increase, while gastric motility significantly increased

at day 35. However, gastric motility did not change significantly at day 42. Collectively, it is necessary to clarify the detailed relationship between ghrelin and gastric motility in dogs by increasing the number of dogs or injecting ghrelin to the dogs in the future study.

To apply traditional Chinese medicines to veterinary medicine, it is necessary to consider taste and the ease of swallowing medicines. Although the prescription of traditional Chinese medicines is common in Japanese medicine, many patients reported difficulty taking these medicines due to reasons such as bitterness and harshness [4]. Moreover, the granular form, which is the most popular form for dosage of traditional Chinese medicines in Japan, may choke patients, which prevents them from taking it [4]. Dogs were also reported to dislike bitterness [2]. Moreover, granular or powdered form medicines sometimes make it difficult for owners to administer these medicines to dogs. Although they were sometimes mixed with feed as a method to administer them to dogs, there was a risk that dogs may not want to eat the feed if these medicines altered its taste. In human medicine, the bitterness and difficulty of swallowing traditional Chinese medicines was reported to be improved when mixed with sweet foods such as ice cream [4]. Thus, we focused on Hozen-S, which is a sweet liquid form of Juzen-taiho-to, and examined the ease of administration of Hozen-S to dogs compared with the commonly used granulated Juzen-taiho-to. Hozen-S did not change the daily intake of the feed when mixed with it. Moreover, as shown in Table 1, the intake ratio of Hozen-S mixed with feed was significantly higher than that of granulated Juzen-taiho-to. These results suggest that the dogs preferred Hozen-S to granulated Juzen-taiho-to. Thus, Hozen-S may be administered more easily than granulated Juzen-taiho-to.

As shown in Fig. 3, the BW of dogs significantly increased without change of daily food intake. As most sweet liquid-type traditional Chinese medicines contain white sugar, the calorific content of Hozen-S, which was not published, may affect the weight gain of dogs. However, because Hozen-S was administered at 0.8 ml/kg in this experiment, its calories may not be so high. Therefore, other factors may be responsible. For example, Hozen-S may improve digestion and absorption; thus, as digestion and absorption are closely related to BW [9], the recorded increase in dog's BW may be attributed to the fact that Hozen-S promoted gastric motility and increased ghrelin level. Considering that Hozen-S is often administered with anti-cancer drugs, the weight-increasing effect of Hozen-S may be helpful for cancer-bearing dogs that lose weight and have decreased survival time due to cancer-related cachexia syndrome [6, 8].

In conclusion, we demonstrated that Hozen-S promoted gastric contraction and raised the plasma ghrelin level in dogs. Hozen-S might promote gastric contraction through increasing the ghrelin level. Therefore, it was implied that Hozen-S has a protective effect against anti-cancer drug-induced gastrointestinal adverse effects, including weight loss. As the administration of Hozen-S was easier than granulated Juzen-taiho-to, Hozen-S may replace the granulated form and become a more prominent traditional Chinese veterinary medicine.

CONFLICTS OF INTEREST. The authors have nothing to disclose.

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