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Pharmacology

Efficacy of Juzen-taiho-to against vincristine-induced toxicity in dogs

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ABSTRACT. Vincristine, one of the anti-cancer drugs used in veterinary practice, has adverse hematological and gastrointestinal effects in dogs. Juzen-taiho-to is a traditional Chinese medicine used for patients with anorexia in human medicine. However, the protective effects of Juzen-taiho-to against anti-cancer drug-induced toxicity in dogs have not been investigated. We therefore examined whether the administration of Juzen-taiho-to to dogs affects gastric motility, and vincristine-induced gastrointestinal and hematological toxicity. The study was composed of three trials. In the first trial, Juzen-taiho-to (450 mg/kg/day) was orally administered to five dogs. In the second and third trials, vincristine (0.75 mg/m^2) was intravenously administered to each dog in the absence or presence of Juzen-taiho-to (450 mg/kg/day). During these trials, gastric motility and blood parameters were assessed. Juzen-taiho-to increased gastric motility and improved vincristine-induced gastrointestinal, but not hematological, adverse effects in dogs. This study suggested that Juzen-taiho-to may be applicable for gastrointestinal care in dogs receiving chemotherapy.

KEY WORDS: dog, gastric motility, Juzen-taiho-to, vincristine

To treat cancer, surgical therapy, radiotherapy and chemotherapy are mainly selected in veterinary medicine. Although several anti-cancer drugs are used in chemotherapy, they are known to have adverse effects in dogs [12]. Vincristine is an anti-cancer drug used as chemotherapy for lymphoma and hematopoietic tumors in dogs [7, 14]. The administration of vincristine to dogs is associated with adverse gastrointestinal, hematological and neurologic effects [7]. Among them, the gastrointestinal events are common [12]. Marked gastrointestinal toxicity may lead to the weakening of cancer-bearing dogs and poor chemotherapy outcomes.

Traditional Chinese medicines are widely used in human and veterinary medicine in Japan. Juzen-taiho-to, a traditional Chinese medicine, is composed of ten kinds of crude drugs, such as Atractylodes lancea rhizome (So-jutsu), cinnamon and Panax ginseng, and is used for patients with anorexia, etc., in human medicine. Juzen-taiho-to was reported to reduce the hematological side effects of anti-cancer drugs in mice [10]. However, there are no reports demonstrating that Juzen-taiho-to reduces anti-cancer drug-induced adverse gastrointestinal and hematological effects in dogs.

We therefore examined whether the administration of Juzen-taiho-to to dogs affects gastric motility and blood parameters. In addition, the protective effects of Juzen-taiho-to against vincristine-induced gastrointestinal and hematological toxicity in dogs were investigated.

MATERIALS AND METHODS

Animals

Five healthy female beagle dogs (45 months of age and weighing 8.0–9.0 kg) were purchased from the Research Institute for Animal Science in Biochemistry and Toxicology (Tokyo, Japan). A one-month acclimatization period was allowed before the experiments. The dogs were housed individually in stainless steel cages in a controlled environment. Commercial dried pellet diet and canned dog food were given at a fixed time each day. Water was given ad libitum.

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Chemicals and reagents

Juzen-taiho-to was obtained from TSUMURA (Tokyo, Japan). Vincristine was obtained from Nippon Kayaku Co., Ltd. (Tokyo, Japan). Atropine was obtained from FUSO Pharmaceutical Industries, Ltd. (Osaka, Japan).

Ultrasonographic assessment

To assess the gastric motility in dogs, we used a previously described technique with some modifications [14]. Briefly, gastric antral 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, and 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. The cross-section of the antral area was measured by tracing the serosal side of the antrum with the built-in caliper (Fig. 2A). In both the contracted and relaxed phases, the antral region was evaluated 3 times. The number of contractions was counted in a 3-min period. The percentage of the amplitude was calculated by 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. Commercial canned food (10 g/kg) was provided 30 min before the ultrasonographic evaluation. After the evaluation, the dogs were provided additional commercial dried pellet diet.

Experimental design

This study was composed of three trials (Fig. 1). First, Juzen-taiho-to (450 mg/kg, PO) alone was administered with food to the dogs for 14 days. This dose was calculated by referring to the usage of Juzen-taiho-to in human medicine [3]. In the previous study, we reported that administration of Juzen-taiho-to (450 mg/kg, PO) showed the anti-oxidative effects without affecting the general and hematological statuses in dogs [8]. The ultrasonographic assessment was performed on days 0, 7 and 14. Blood samples were taken from the cephalic vein of five dogs on days 0, 7 and 14 to analyze blood parameters. In the second trial, vincristine (0.75 mg/m^2, IV) alone was administered to five dogs 30 min before the ultrasonographic assessment on day 1. The dose of vincristine is clinically used in veterinary medicine [2]. Ultrasonographic assessment was performed on days 0–6. Blood parameters were assessed on days 0, 2, 3, 4 and 6. A 28-day washout period was carried out between the first and second trials. In the third trial, vincristine (0.75 mg/m^2, IV) was administered to five dogs 30 min before the ultrasonographic assessment on day 1 and Juzen-taiho-to (450 mg/kg, PO) was administered with food to the same dogs from 7 days before the administration of vincristine for 13 days. Ultrasonographic assessment was performed for 7 days, and the blood parameters were assessed on days 0, 2, 3, 4 and 6. A 42-day washout period was carried out between the second and third trials. Before the second and third trials, we confirmed that the five dogs had normal clinical signs. Blood parameters (red blood cell (RBC), hematocrit (HCT), hemoglobin (Hgb), platelet (Plat), white blood cell (WBC), lymphocyte (Lym), neutrophil (Neu) and eosinophil (Eos) values) in the three trials were measured by IDEXX Laboratories (Tokyo, Japan). The general status (vomiting, diarrhea and appetite) was also observed each day during the trials. Experiments and animal care were performed in accordance with the recommendations in the 'Guide for the Care and Use of Laboratory Animals' approved by the Faculty of Agriculture, Tokyo University of Agriculture and Technology.

Statistical analysis

The results are expressed as the mean ± S.D. Statistical evaluations were performed using a paired *t*-test or Welch's *t*-test. Values of $P<0.05$ or $P<0.01$ were considered significant.

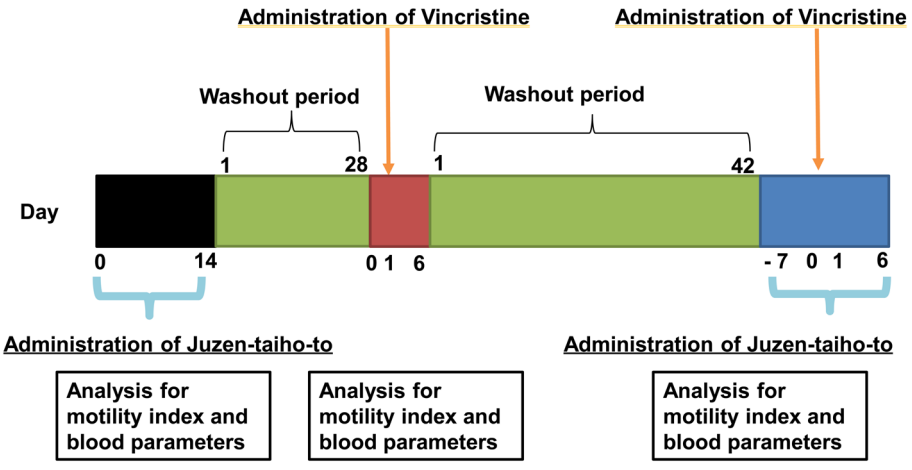

Fig. 1. Schematic experimental design of the time course of Juzen-taiho-to administration to dogs and ultrasonographic assessment and the blood collection. Firstly, Juzen-taiho-to (450 mg/kg, PO) alone was administered to five dogs for 14 days. After the washout period, vincristine (0.75 mg/m^2, IV) alone was administered to five dogs at Day 1. After the washout period again, Juzen-taiho-to (450 mg/kg, PO) and vincristine (0.75 mg/m^2, IV) were administered to five dogs. Juzen-taiho-to was administered from Day −7 to Day 6 and vincristine was administered at Day 1. In each trial, the motility index and the blood parameters were measured regularly.

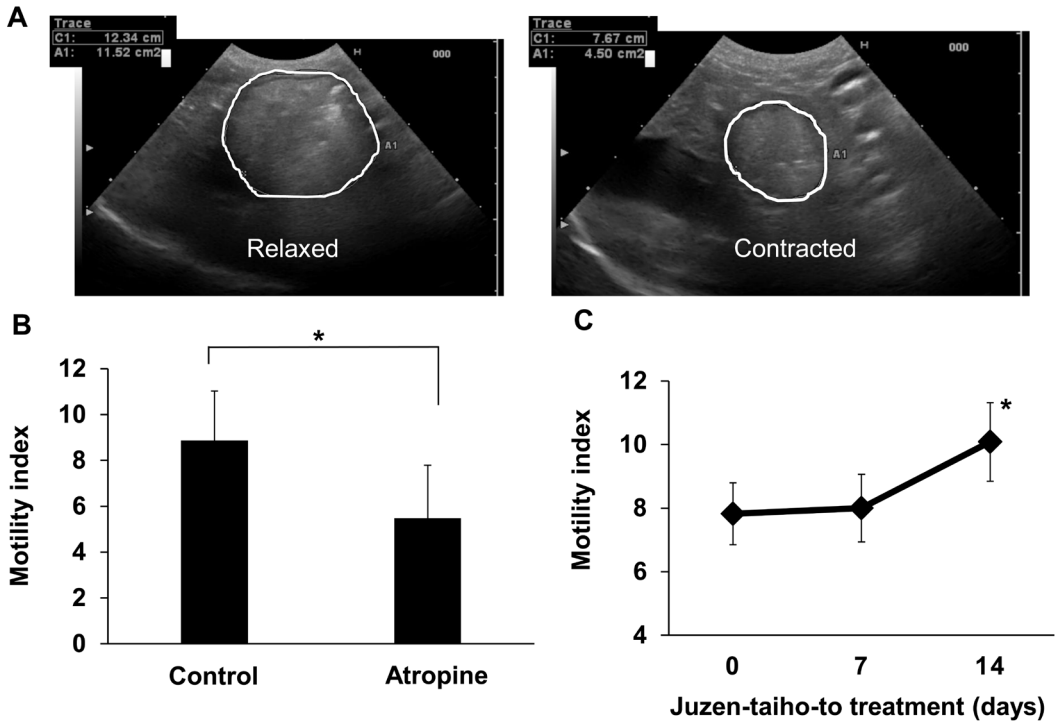

Fig. 2. Ultrasonographic assessment of the cross-section of the gastric antrum. Representative images of contracted (right) and relaxed (left) conditions are shown (A). Effects of Atropine on the gastric motility index in dogs (B). Atropine (0.04 mg/kg, IM) was administered with food to five dogs. Ultrasonography for the gastric function was performed and the motility index for each dog was analyzed. The results are expressed as the mean ± S.D. *Significant difference (P<0.05) compared with Control. Effects of Juzen-taiho-to on the gastric motility index in dogs (C). Juzen-taiho-to (450 mg/kg/day, PO) was administered with food to five dogs for 14 days. Ultrasonography for the gastric function was performed on days 0, 7 and 14 after the administration of Juzen-taiho-to. The motility index for each dog was analyzed. The results are expressed as the mean ± S.D. *Significant difference (P<0.05) from baseline (day 0).

RESULTS

Effects of Juzen-taiho-to on gastric contraction in dogs

Juzen-taiho-to has been used to improve anorexia in humans. However, it remains unclear whether Juzen-taiho-to affects gastrointestinal events in dogs. Therefore, we first investigated the effects of Juzen-taiho-to on gastric contraction in dogs as an indicator of their gastrointestinal events. We measured gastric motility by ultrasonography (Fig. 2A). To quantify gastric motility, we calculated the motility index based on the diameter of the gastric antrum using a previously described formula [14]. We confirmed that administration of atropine (0.04 mg/kg, IM) significantly decreased the motility index in dogs (Fig. 2B). After Juzen-taiho-to was administered to five dogs at 450 mg/kg each day for 14 days, the motility index was assessed on days 0, 7 and 14. The motility index on day 14 was significantly higher than that on day 0 (Fig. 2C), suggesting that the administration of Juzen-taiho-to promoted gastric contraction in dogs.

Effects of Juzen-taiho-to on blood parameters in dogs

We next assessed the effects of Juzen-taiho-to on changes in blood parameters as an indicator of blood toxicity. We collected blood from each dog on days 0, 7 and 14 after the administration of Juzen-taiho-to. The RBC, HCT, Hgb and Plat values were not affected by Juzen-taiho-to (Fig. 3A–D). Furthermore, the WBC, Lym, Neu and Eos values were unchanged (Fig. 4A–D).

Effects of Juzen-taiho-to on vincristine-induced attenuation of gastric contraction and blood toxicity in dogs

In veterinary practice, vincristine is widely used to treat dogs with several cancers, and it is known to have several adverse effects, such as gastrointestinal and hematological adverse reactions, in dogs [7]. To clarify the effects of Juzen-taiho-to on the vincristine-induced gastrointestinal and hematological adverse effects in dogs, vincristine (0.75 mg/m^2, IV) was administered to five dogs in the presence or absence of Juzen-taiho-to, and the motility index and blood parameters were assessed. We first confirmed that vincristine treatment significantly reduced the motility index (Fig. 5A), and the RBC (Fig. 5B), HCT (Fig. 5C), Hgb (Fig. 5D), Plat (Fig. 5E), WBC (Fig. 6A), Lym (Fig. 6B), Neu (Fig. 6C) and Eos (Fig. 6D) values compared with before treatment. Of the five vincristine-treated dogs, vomiting was observed in one on day 6, and diarrhea was observed in one on days 2 and 3.

Juzen-taiho-to treatment significantly improved the vincristine-induced decrease in the motility index (Fig. 5A), but it had little

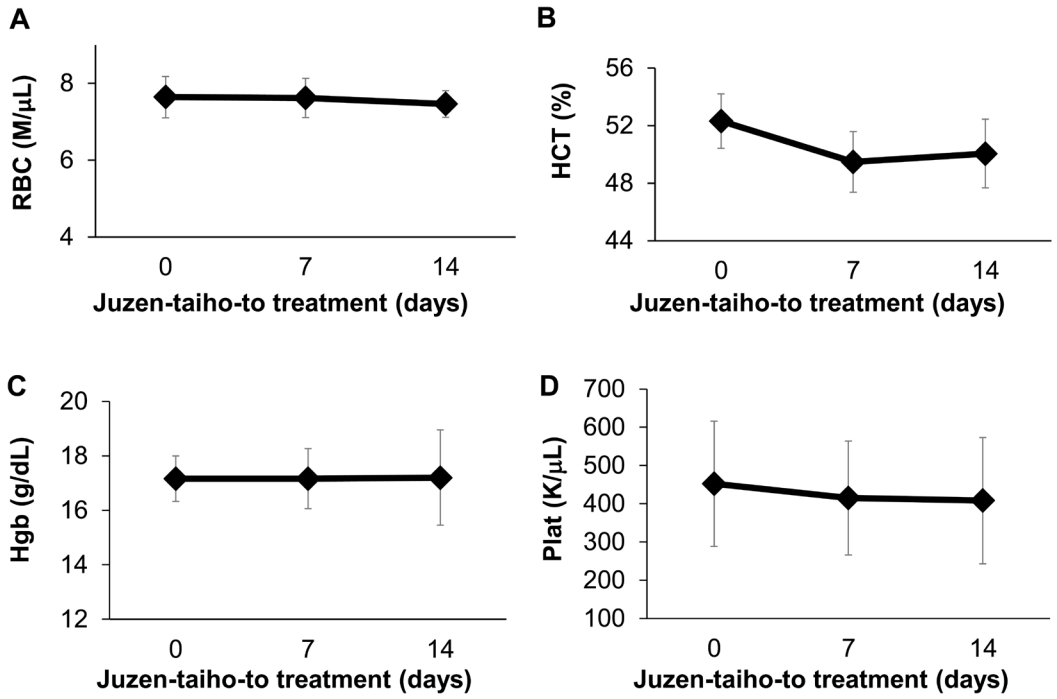

Fig. 3. Effects of Juzen-taiho-to on the red blood cell (RBC), hematocrit (HCT), hemoglobin (Hgb), and platelet (Plat) values in dogs. Juzen-taiho-to (450 mg/kg, PO) was administered with food to five dogs for 14 days. Blood samples were taken from the cephalic vein of each dog on days 0, 7 and 14. RBC (A), HCT (B), Hgb (C), and Plat (D) values on days 0, 7 and 14 were analyzed. The results are expressed as the mean ± S.D.

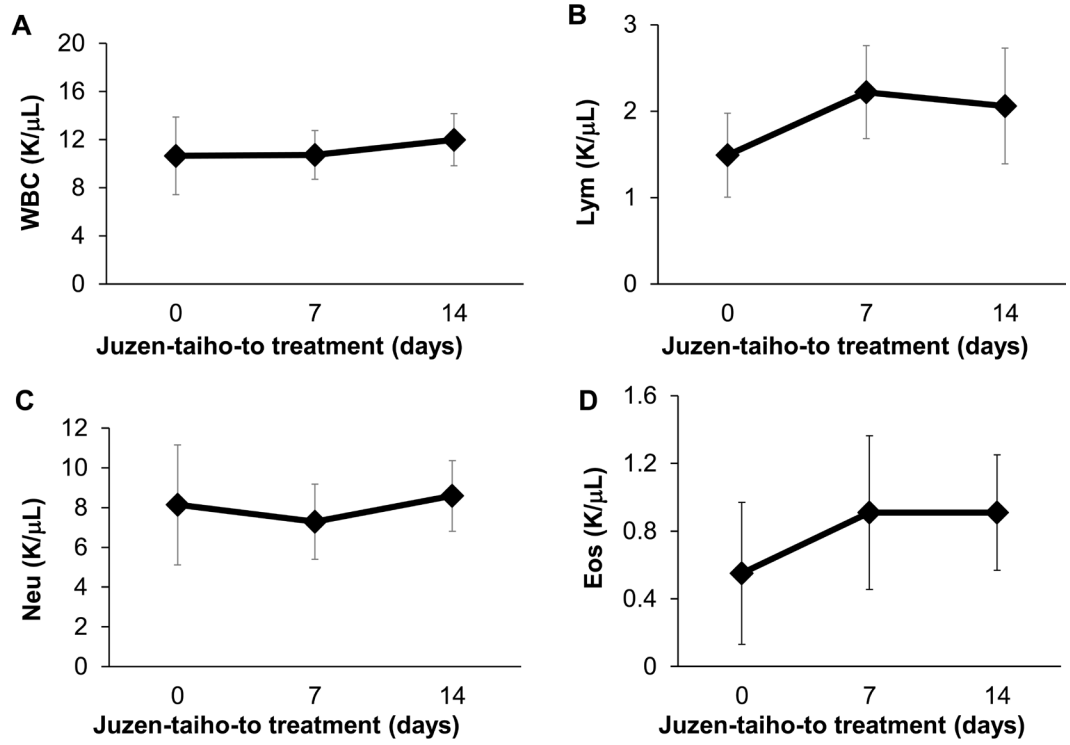

Fig. 4. Effects of Juzen-taiho-to on the white blood cell (WBC), lymphocyte (Lym), neutrophil (Neu), and eosinophil (Eos) values in dogs. Juzen-taiho-to (450 mg/kg/day, PO) was administered with food to five dogs for 14 days. Blood samples were taken from the cephalic vein of each dog on days 0, 7 and 14. WBC (A), Lym (C), Neu (B), and Eos (D) values on days 0, 7 and 14 were analyzed. The results are expressed as the mean ± S.D.

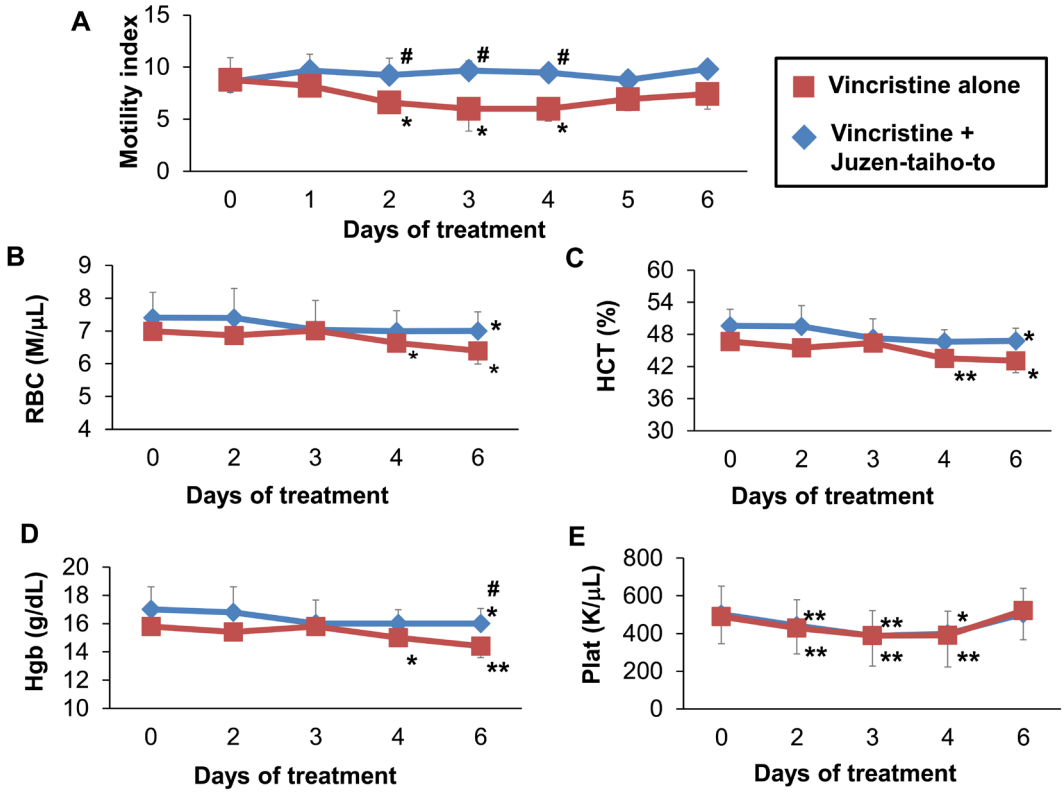

Fig. 5. Effects of Juzen-taiho-to on the vincristine-induced attenuation of gastric contraction and blood toxicity in dogs. Vincristine (0.75 mg/m^2, IV) was administered to five dogs in the presence or absence of Juzen-taiho-to (450 mg/kg/day, PO). Ultrasonography for gastric function was performed each day (on days 0–6) after the administration of vincristine. The motility index for each dog was analyzed (A). Blood samples were taken from the cephalic vein of each dog on days 0, 2, 3, 4 and 6. The red blood cell (RBC) (B), hematocrit (HCT) (C), hemoglobin (Hgb) (D), and platelet (Plat) (E) values were analyzed. Red indicates the administration of vincristine alone. Blue indicates vincristine + Juzen-taiho-to. The results are expressed as the mean ± S.D. *Significant difference (P<0.05) from baseline (day 0). **Significant difference (P<0.01) from baseline (day 0). $^{\#}$Significant difference (P<0.05) between the two trials on the same day.

effect on the decrease in the RBC (Fig. 5B), HCT (Fig. 5C), Hgb (Fig. 5D), Plat (Fig. 5E), WBC (Fig. 6A), Lym (Fig. 6B), Neu (Fig. 6C) and Eos (Fig. 6D) values. In the co-treated five dogs, vomiting was observed in one on day 3 and diarrhea was observed in one on day 4. This suggests that co-treatment of vincristine and Juzen-taiho-to reduces vincristine-induced gastrointestinal, but not hematological, adverse effects in dogs.

DISCUSSION

In the current study, we demonstrated that the administration of Juzen-taiho-to to dogs promoted gastric contraction and reduced vincristine-induced gastrointestinal adverse reactions.

Juzen-taiho-to has been used to improve anorexia in humans. It was also reported to improve the cisplatin-induced decrease in food intake and body weight in mice [10]. In our previous study, we found that the administration of Juzen-taiho-to to dogs reduced oxidative stress [8]. However, there were no reports demonstrating the protective effects of Juzen-taiho-to on anti-cancer drug-induced gastrointestinal toxicity in dogs. We therefore investigated whether the administration of Juzen-taiho-to improves the vincristine-induced decrease in gastric motility in dogs. To examine the effects of Juzen-taiho-to on gastric motility, we used ultrasonography to assess gastric motility in dogs non-invasively as following the previous study [13] (Fig. 2A) and calculated the motility index. As shown in Fig. 2C, administration of Juzen-taiho-to increased the motility index in dogs. Moreover, it prevented the vincristine-induced decrease in the motility index (Fig. 5A). This suggests that Juzen-taiho-to has protective effects against gastrointestinal toxicity induced by anti-cancer drugs in dogs and may be useful as supplemental medicine in cancer chemotherapy. Although it was shown in the vincristine treated dogs that the decrease in the motility index was related to the expression of gastrointestinal clinical signs such as vomiting and diarrhea [14], we hardly observed vincristine-induced vomiting and diarrhea in our dogs and could not check whether the administration of Juzen-taiho-to to dogs affects the gastrointestinal toxicity.

Mechanistically, gastric contraction is regulated by several factors. Among them, the dopamine D2 receptor and ghrelin are known to regulate gastric motility in humans and dogs [1, 14, 16]. Juzen-taiho-to contains ten kinds of crude drugs, such as Atractylodes lancea rhizome (So-jutsu), cinnamon and Panax ginseng, which are related to the dopamine D2 receptor and ghrelin. For example,

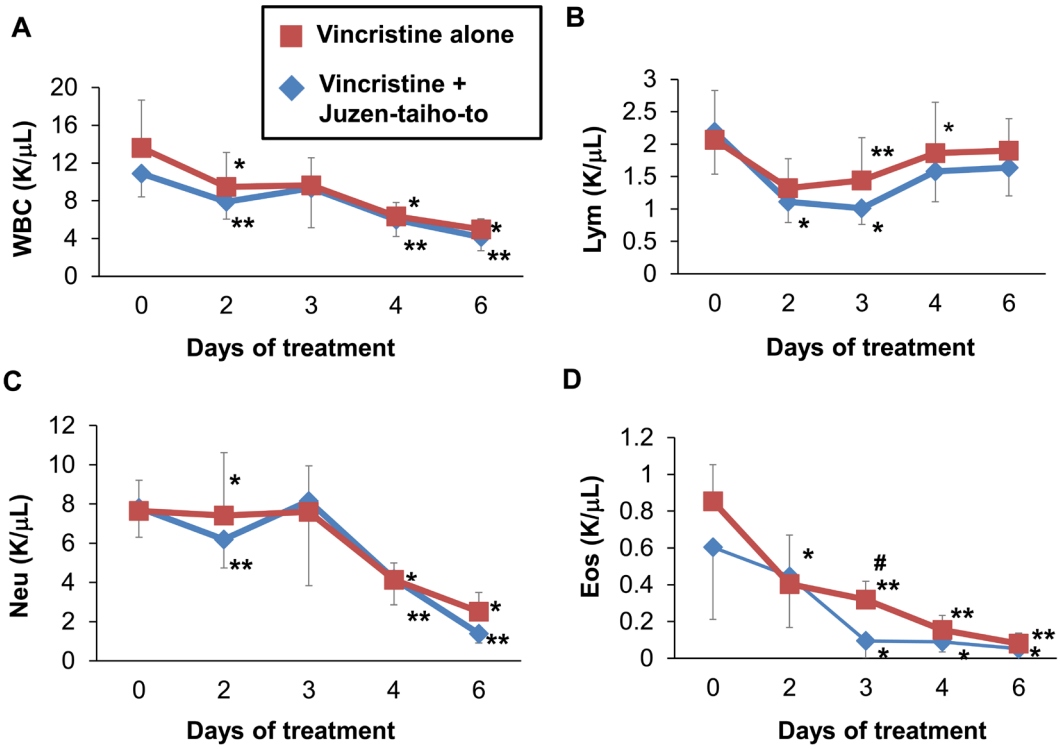

Fig. 6. Effects of Juzen-taiho-to on the vincristine-induced decrease in the WBC, Lym, Neu and Eos values in dogs. Vincristine (0.75 mg/m^2, IV) was administered to five dogs in the presence or absence of Juzen-taiho-to (450 mg/kg/day, PO). Blood samples were taken from the cephalic vein of each dog on days 0, 2, 3, 4 and 6. The white blood cell (WBC) (A), lymphocyte (Lym) (B), neutrophil (Neu) (C), and eosinophil (Eos) (D) values were analyzed. Red indicates the administration of vincristine alone. Blue indicates vincristine + Juzen-taiho-to. The results are expressed as the mean ± S.D. *Significant difference (P<0.05) from baseline (day 0). **Significant difference (P<0.01) from baseline (day 0). $^{\#}$Significant difference (P<0.05) between the two trials on the same day.

Atractylodes lancea rhizome stimulates gastric emptying by inhibiting the dopamine D2 receptor [4]. Ghrelin, a peptide hormone produced in the stomach, is known to function in the regulation of gastric contraction in humans and rodents [11]. Cinnamon extracts induced ghrelin gene expression in human cells [5]. Ginsenoside, the major pharmacologically active ingredient of ginseng, increased ghrelin secretion in human cells [15]. Based on these reports, the administration of Juzen-taiho-to to dogs may promote gastric motility by inhibiting the dopamine D (2) receptor and increasing ghrelin secretion. To clarify this, further investigations are needed. Furthermore, there are other methods such as the rate of acetaminophen method and ^{13}C-breath test to assess gastric motility [9, 13]. Although we assessed gastric motility only using ultrasonography because of its non-invasiveness, facility limitations, and technical issues in this study, multiple analyses are required for a better understanding of gastric motility in dogs.

Although vincristine is used in chemotherapy for dog lymphoma and hematopoietic tumors [7], it sometimes causes blood/bone marrow adverse events in dogs, which restrict its clinical application [12]. Juzen-taiho-to is known to exhibit protective effects against anti-cancer drug-induced hematological adverse effects. For example, Juzen-taiho-to had protective effects against carboplatin-induced hematological adverse effects by attenuating the decrease in white blood, platelet and bone marrow cell counts in mice [10]. It was also reported to have protective effects against myelosuppression induced by the anti-cancer drug TS-1 in mice [6]. We therefore investigated whether the administration of Juzen-taiho-to improves vincristine-induced blood toxicity in dogs. However, Juzen-taiho-to had little effect on the vincristine-induced decrease in the RBC (Fig. 5B), HCT (Fig. 5C), Hgb (Fig. 5D), Plat (Fig. 5E), WBC (Fig. 6A), Lym (Fig. 6B), Neu (Fig. 6C) and Eos (Fig. 6D) values. In mouse experiments, Juzen-taiho-to exhibited protective effects against carboplatin-induced blood toxicity at 1.7 g/kg/day [10]. On the other hand, 450 mg/kg of Juzen-taiho-to was orally administered to dogs in the present study. The difference in concentration may have attenuated its effects on vincristine-induced hematological adverse effects in dogs.

In conclusion, we demonstrated that the administration of Juzen-taiho-to to dogs stimulates gastric contraction and has protective effects against gastrointestinal toxicity. Further studies on the effects of Juzen-taiho-to may improve gastrointestinal care for dogs receiving chemotherapy.

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