Original Research Article

The Japanese Extracted Herbal Medicine Daikenchuto Increases the Contractile Activity of the Internal Anal Sphincter Muscle in Conscious Dogs

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Abstract

Objectives: This study aimed to elucidate the effect of the extract of daikenchuto (DKT), a Japanese Kampo medicine, on the contractile activity of the internal anal sphincter in conscious dogs.

Methods: Force transducers were attached to the serosal surface of the rectum and the internal anal sphincter of male beagle dogs. In addition, the contractile activity of the rectum and the internal anal sphincter was continuously measured until 6 h after DKT administration *via* telemetry in the conscious state. The DKT dose was 1.5 g/body, and the administration route was intrarectal in the expectation of a direct effect on the rectoanal region. DKT was re-administered to the same animal after drug withdrawal, and the plasma concentrations of hydroxy-α-sanshool (HAS) and hydroxy-β-sanshool (HBS) before and after administration were measured.

Results: After DKT administration, the contractile activity of the internal anal sphincter immediately increased, peaked at 10 min, continued for ≥1 h, and had almost disappeared after 4 h. Rectal contraction differed from that of the internal anal sphincter, with no significant contraction observed. HAS and HBS were found in the plasma of animals administered with DKT and persisted up to 2 h after the administration.

Conclusions: This is the first report on *in vivo* telemetry demonstrating that DKT exhibited contractile effects on the dog's internal anal sphincter. The increased anal pressure and improvement of fecal incontinence symptoms observed in previous clinical studies may have been based on this sphincter contraction.

Keywords
daikenchuto, internal anal sphincter, fecal incontinence, telemetry system

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Introduction

Fecal incontinence (FI) is a disease defined in Rome IV as "recurrent uncontrolled passage of fecal material in an individual with a developmental age of at least 4 years"[1]. This is a frequent pathology. Although no consensus has been established, the prevalence of FI is generally considered to be >2% in adults, accounting for approximately 50% of nursing home residents[2]. One characteristic of FI is se-

vere damage to daily life, even in patients with less severe diseases, with reduced quality of life, including depression or social stigma[3].

The etiology varies with factors, including aging, delivery history, anal sphincter damage, diarrhea or constipation, and, in particular, anal sphincter dysfunction which appear critical for FI. Damage to the sphincter during vaginal delivery or sphincterectomy during rectal cancer surgery is known to be associated with severe FI[4,5]. The internal anal sphincter

(IAS) on the luminal side of the anal canal is an involuntary smooth muscle. It plays a role in maintaining constant anal tone by continuous contraction, so that defecation is normally controlled.

As of this time, no drugs have been approved for the treatment of FI, and conservative therapeutic methods, such as diet, biofeedback, antidiarrheal agents such as loperamide, or fecal bulking agents such as Psyllium, have been employed in the treatment of FI. Loperamide is a particularly effective drug[6]. However, it is not an easy-to-use drug as it requires fine dose adjustment due to the risks of constipation as an adverse effect[7]. In addition, it has been reported that topical administration of phenylephrine ointment, an α1 agonist, increases anal pressure and improves FI symptoms[8]. However, that effect is absolutely temporary and does not lead to general therapy. Furthermore, although sacral nerve stimulation has recently been used as a surgical therapy and has shown to be effective[9], the standard methods of care for FI, including medications, are still in the process of being established.

Daikenchuto (DKT) is a Japanese herbal medicine made from the botanical raw materials (BRMs) of Japanese pepper, processed ginger, and ginseng radix. It has been approved as a prescribed medicine by the Japanese National Health Insurance plan for the treatment of various abdominal symptoms, such as abdominal cold and pain with abdominal bloating[10]. Basic studies have revealed that DKT exhibits a contractile effect on the isolated intestinal tract of guinea pigs[11] and improves intestinal transit in various models of postoperative ileus (POI)[12,13]. A clinical pharmacology study has also revealed that DKT enhances small intestinal motility in healthy volunteers[14]. In addition, placebo-controlled DB-RCT for POI has also been shown to improve POI symptoms in patients with abdominal surgery[15,16]. In the United States, DKT has also been approved as an investigational drug by the Food and Drug Administration and is primarily used for the treatment of POI[15-17].

Conversely, DKT has been suggested to exert increasing effects on the resting anal pressure in patients with chronic constipation[18] and may thus enhance the contractility of the anal sphincter. In other words, DKT has been suggested as a potential pharmacotherapy for FI for the enhancement of anal sphincter contractility.

However, direct evidence of DKT being effective for anal sphincter contraction is lacking. This study aimed to elucidate the effect of DKT extract on the IAS in conscious dogs.

Methods

Materials

For this study, the extract powder of DKT (Tsumura & Co., Tokyo, Japan) was used. DKT is a mixture of three BRMs produced *via* the following process: Japanese pepper, processed ginger, and ginseng radix are mixed in a ratio of 2:5:3, extracted with hot water, then dried in a spray drying system.

Experimental animals

About 10- to 11-month-old male TOYO beagle dogs (9.5-11.0 kg) were purchased from Kitayama Labes Co., Ltd. (Iwakuni, Japan). The animals were allowed *ad libitum* access to water and standard laboratory food (DS-A; Oriental Yeast, Tokyo, Japan) and housed in an animal room kept at a temperature of 21.2°C-23.2°C, relative humidity of 44.9%-90.1%, and controlled lighting, with lights on from 07:00 to 19:00 and off from 19:00 to 07:00 the next day. All experimental procedures were conducted at the Kumamoto laboratory of LSI Medience Corporation in accordance with the "Guidelines for the Care and Use of Laboratory Animals" approved by the Laboratory Animal Committee of LSI Medience Corporation (Approval No. 2019-0393).

Preparation of animals

Dogs were treated with ketamine (10 mg/kg i.m.) and xylazine (2 mg/kg i.m.) as induction anesthesia and isoflurane and nitrous oxide inhalation as general anesthesia.

Strain gauge-force transducers (F-12IS; Star Medical, Tokyo, Japan) were implanted in the rectum and the IAS to measure the contractile activity of the rectal smooth muscle and IAS. First, the animals were laparotomized, and the transducer was secured with sutures on the serosal surface of the rectum. The perianal skin was then incised to identify the IAS from the subcutaneous muscularis based on the region and aspect. Moreover, the IAS was partially divided from the external anal sphincter, and the transducer was secured with sutures to the IAS side of the divisional area. Lead wires from the transducers were tunneled subcutaneously from the buttock to the dorsal region and exteriorized through a stab wound. The exteriorized lead wires were connected to the transmitter for telemetry and covered with a canvas jacket to protect against self-inflicted trauma. After surgery, all the dogs wore an Elizabeth collar until the surgical site was cured, so that the dog would not interfere around the anus. They were also allowed to recover for over 14 days.

The contractile activity of the rectum and IAS was continuously recorded from the transducer to an amplifier and then analyzed using computer software (Analyze II; Star Medical).
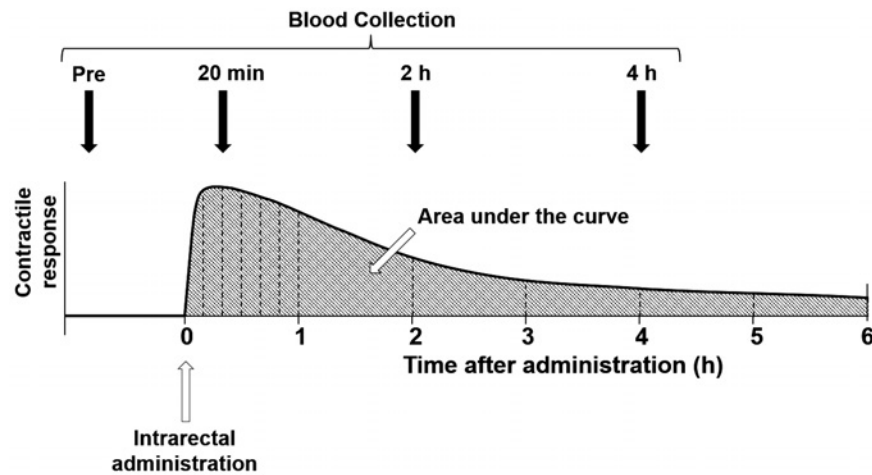
Figure 1. Schematic diagram of the study design.

Contractile activity of the rectum and anus

Animals were fasted for ≥18 h prior to administration. At more than 1 h prior to administration, the intestine was cleaned with glycerin enema, and the measurement was started by connecting a transmitter and force transducer.

After placing the animal on a hammock and confirming that it had become calm and the IAS activity had stabilized, the administration catheter was inserted into the anus for about 3-5 cm, and 5.0 mL of the dosing solution was injected into the rectum for about 1 min.

At first, 0.5 g/5.0 mL of phenylephrine hydrochloride solution was administered as a positive control, and animals with the contractile response of the IAS were selected and used in this study.

To evaluate the efficacy of DKT, 5 mL of saline containing 1.5 g of DKT was administered as an infusion for approximately 1 min (n = 3). In control animals, saline was administered in the same manner (n = 3). Approximately 1 h after administration, the animal was removed from the hammock and was allowed to move freely. In addition, the contractile response was continuously recorded until 6 h after administration. The typical experimental scheme is presented in Figure 1.

Measurement of plasma concentrations of DKT ingredients

After a washout period of ≥20 days after the administration of DKT, the same animals were administered DKT in the same manner. Blood samples (2 mL) were drawn from the cephalic vein using a heparin-treated syringe before and 20 min, 2 h, and 4 h after administration. Next, >0.5 mL of plasma was separated *via* centrifugation and preserved at −80°C. The plasma concentrations of hydroxy-α-sanshool (HAS) and hydroxy-β-sanshool (HBS), which are major components of Japanese pepper, were measured using the LC-MS/MS method (HPLC: Nexera X2 system [Shimadzu Corporation, Kyoto, Japan], MS/MS: QTRAP 5500 [SCIEX, Framingham, MA, USA]). The analytical method was properly validated, and the limit of detection for both compounds was 1.0 ng/mL.

Data analysis

Contraction of both the rectum and IAS was continuously recorded by telemetry. In addition, the efficacy of the drug was evaluated using the area under the curve (AUC) of the contractile wave.

All data are expressed as mean ± standard error of the mean of three dogs in each group. For the statistical analysis, paired Student's t test was used, with values of $p < 0.05$ regarded as significant.

Results

Effects on the contractile response of the rectum and IAS

The contractile response curves of the IAS and rectum in DKT- or saline-administered animals are presented in Figure 2 (IAS), 3 (rectum). Immediately after DKT administration, the contractile response of the IAS increased (Figure 2A), and in the saline-administered animal, only a slight spontaneous response was shown, unlike DKT. Noticeable contraction of the rectum had not been exhibited by either DKT- or saline-administered animals, with no difference between the groups (Figure 3).

The results of the AUC evaluation up to 60 min after administration in both the IAS and rectum are presented in Figure 4, and the results up to 6 h are presented in Figure 5. Figure 4a shows the AUCs every 10 min in the IAS, whereas Figure 4b shows the AUCs every 10 min in the rectum. The AUC immediately increased after DKT administration, and the maximal response was observed within 10 min ($p < 0.05$) and then continued for 1 h (Figure 4a). Similar to
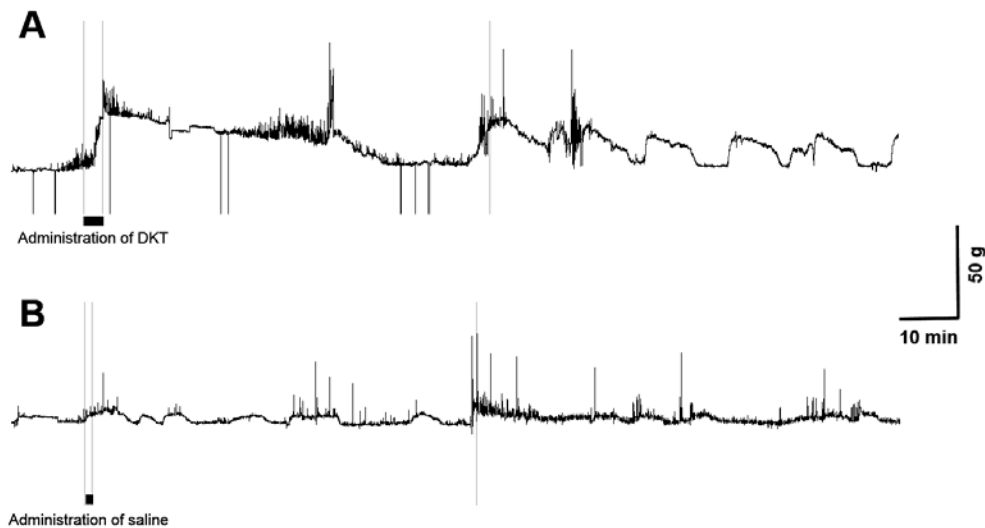
Figure 2. Trace of the contractile response of the internal anal sphincter in both treatment groups.
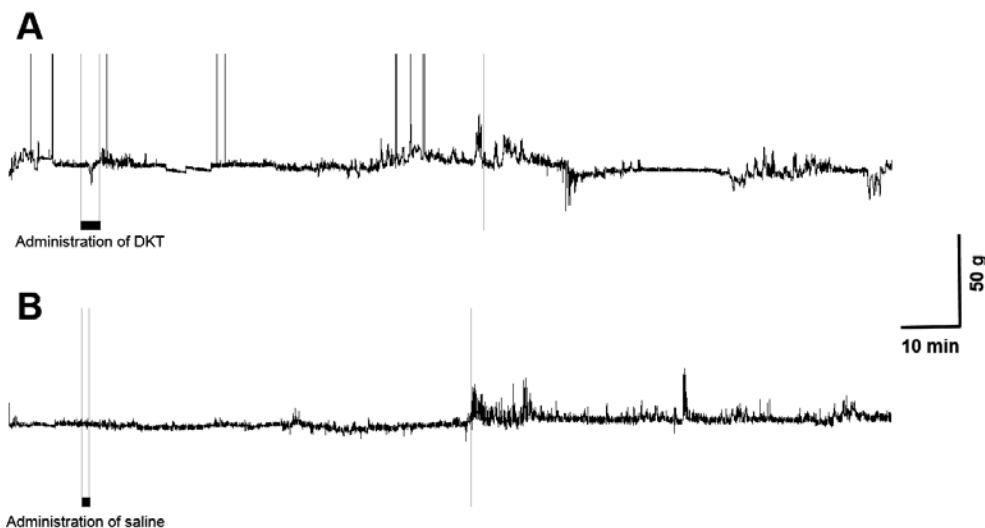A: DKT at 1.5 g/5.0 mL/dog
B: Saline at 5.0 mL/dog

Figure 3. Trace of the contractile response of the rectum in both treatment groups.
A: DKT at 1.5 g/5.0 mL/dog
B: Saline at 5.0 mL/dog

Figure 4, the AUC evaluation every 1 h up to 6 h after administration in both regions is presented in Figure 5. The Peak AUC after administration was observed within 1 h, thereafter approaching values in the control group. In addition, the effects had almost disappeared after 4 h (Figure 5 a).

For both groups, the AUC was smaller in the rectum than in the IAS (Figure 4b, 5b).

Plasma concentration of DKT ingredients

The plasma concentrations of HAS and HBS before and after DKT administration are presented in Figure 6.

The plasma concentrations of both compounds were not detected before the administration, but were detected at high levels from 20 min after administration; it persisted up to 2 h after administration (HAS, 29.3 ng/mL; HBS, 11.1 ng/mL). After 4 h, one of three animals exhibited plasma concentrations of 2.64 ng/mL in HAS and 1.49 ng/mL in HBS, respectively, whereas the remaining two were below the limit of detection. In all three animals, the maximum concentrations of HAS were higher than those of HBS.
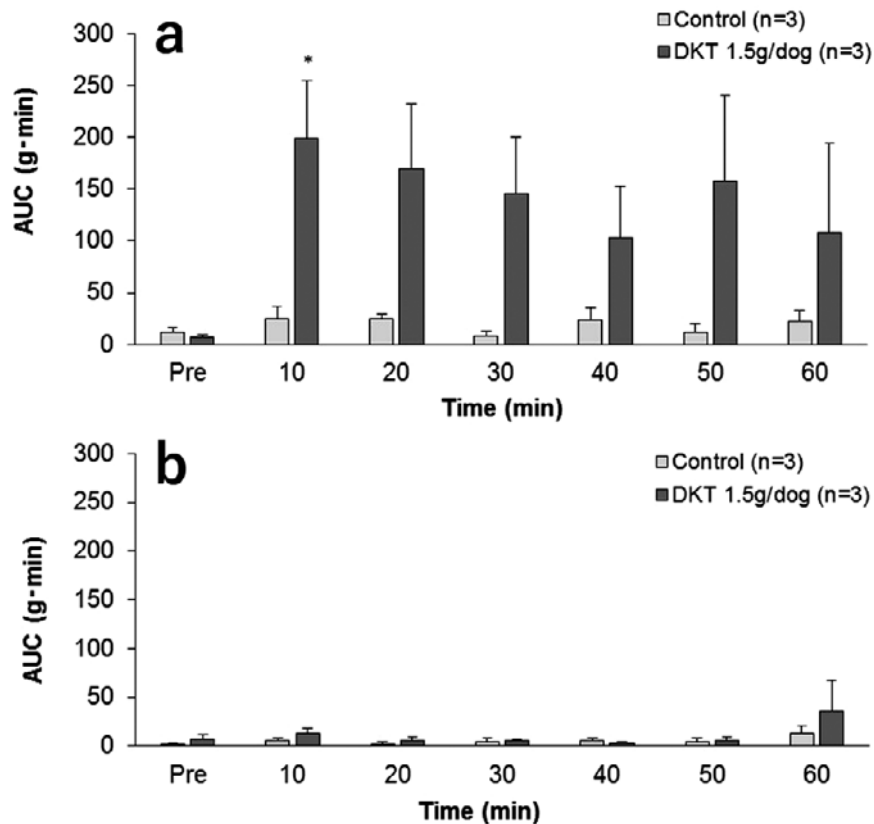
Figure 4. The area under the curve of the contractile response wave of the internal anal sphincter and rectum up to 60 min after administration.

a: internal anal sphincter; b: rectum

Data are expressed as mean ± standard error of the mean

* $p < 0.05$: Significantly different from the vehicle group (Student's t-test)

Discussion

This is the first report to examine the effects of DKT on the contractile activity of the IAS in conscious dogs by attaching a force transducer to the surface of the IAS and by measuring the contractile activity by telemetry. The contractile activity of the IAS is deeply affected by anesthesia; thus, the drug effect was determined using conscious animals to elucidate the functions *in vivo*.

The structure of the anal canal in dogs resembles that in humans, as the smooth muscle continuous with the circular muscles of the rectum thicken in the anal canal and form the IAS. The external anal sphincter, which is a striated muscle, surrounds the outside of the IAS in a ring shape with interposed rectal longitudinal muscles. Here, we identified the IAS from region and aspects and secured the transducer to the IAS.

The clinical dose of DKT in Japan is 15 g/day orally, which is equivalent to 1.25 g/day of extract used in this study. However, to clearly confirm the direct effect of DKT on the anorectal region, the drug was administered intrarectally, which is different from the clinical practice, and the dose was set at 1.5 g/5.0 mL/body, the maximum dose which is possible to prepare and administer.

The contractile activity of the IAS immediately increased after the intrarectal administration of DKT, with the maximal response observed within 10 min and continuing >1 h. Thereafter, the enhanced activity had almost disappeared by about 4 h. The plasma concentrations of HAS and HBS, as DKT ingredients, were detected at high levels from 20 min after administration and persisted up to 2 h.

Past clinical studies have reported increases in the anal resting pressure and squeeze pressure compared with placebo groups after oral administration of DKT for 4 weeks in patients with chronic constipation[18]. An observational study revealed that oral administration of DKT for 1 month to patients with FI increased the anal resting pressure[19], which is consistent with the results of Iturrino et al.[18]. Meanwhile, a pharmacokinetic study of a single oral administration of DKT in healthy Japanese volunteers and American volunteers revealed that the plasma concentrations of DKT ingredients, especially HAS and HBS, were increased[20]. Although whether the mechanisms underlying the increased anal pressure in humans as previously reported
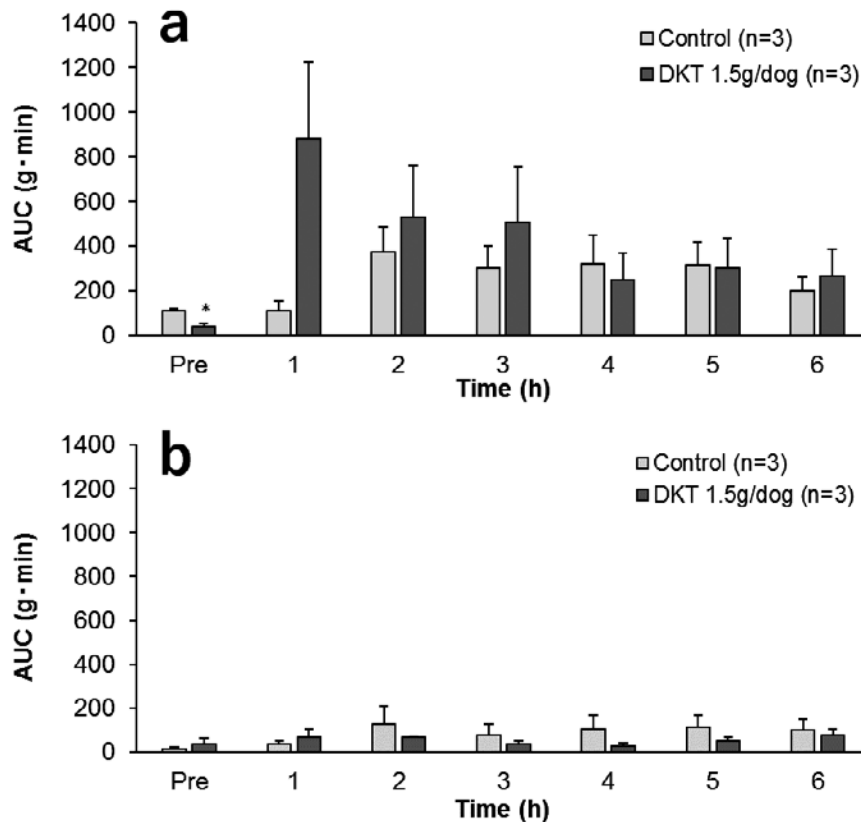
Figure 5. The area under the curve of the contractile response wave of the internal anal sphincter and the rectum up to 6 h after administration.

a: internal anal sphincter; b: rectum

Data are expressed mean ± standard error of the mean

* $p < 0.05$: Significantly different from the vehicle group (Student's t-test)

and in dogs observed in the present study are the same remains unclear, the ingredients (i.e., HAS and HBS) are likely to affect the contractility of the IAS.

Both HAS and HBS derived from Japanese pepper as one of the BRMs, and DKT caused the contraction of the isolated intestines of guinea pigs and rats[11,21], thus exerting direct effects on intestinal smooth muscle. The IAS is a smooth muscle, as well as the intestine, and a similar contractile response may thus be observed. Therefore, this study evaluated the more direct effects of DKT on the IAS *in vivo*. To confirm the above, the administration route was set as intrarectal. As a result, the contractile response was clearly increased immediately after administration and continued for over 1 h. In the case of oral administration of DKT to humans, HAS and HBS were immediately absorbed and reached maximum plasma concentrations about 15 min after administration[20]. In the results from the intrarectal administration to dogs, both HAS and HBS were detected even 20 min after the administration. This indicates that both ingredients were absorbed from the rectum immediately after administration. However, whether the immediate contractile response was caused by the absorbed ingredients

remains unclear. Conversely, in the background of contractions continuing >1 h, the plasma concentrations of each ingredient persisted up to 2 h. This suggests that the absorbed ingredients may exert effects from the blood vessel side.

The anal tone generated by IAS contraction at rest is mainly controlled by sympathetic nerves[22]. In addition, the clinical studies on therapeutic agents for FI using α agonists have been conducted, but the effects of DKT on sympathetic nerves have not been reported. In the future, further detailed studies are expected to elucidate the mechanisms of DKT and the relationships between the effects of DKT and ingredients other than HAS and HBS.

Interestingly, in the present study, a clear rectal contraction has not been observed in animals treated with DKT. Rectal innervation is reported to be dominated by enteric motor neurons, unlike the sympathetic dominant IAS[22] and thus may represent an important key in elucidating the mechanisms of action of DKT. Moreover, clinical results have reported that DKT reduces the sensory threshold of the rectum[18,23], which may support the hypothesis that the action on the rectum is primarily sensory sensitization rather than contraction.
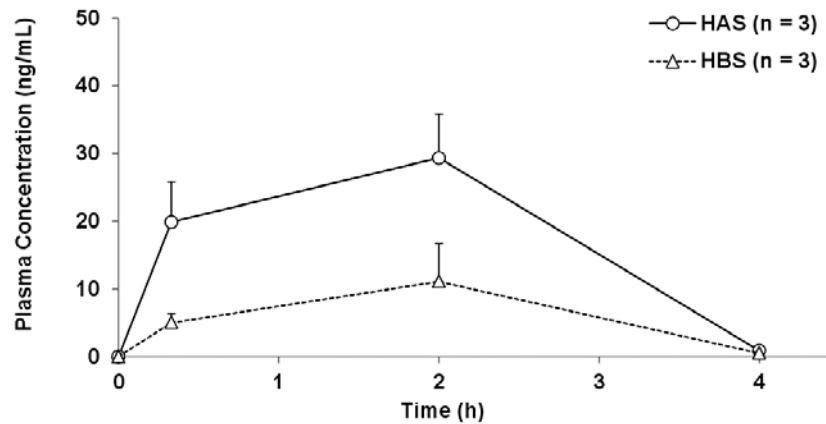
Figure 6. Mean plasma concentrations of HAS and HBS after intrarectal administration of DKT.

HAS, hydroxy-α-sanshool; HBS, hydroxy-β-sanshool.

Data are expressed as mean ± standard error of the mean

This study had several limitations. First, the intra anal pressure of conscious animals was not directly measured; therefore, anal manometry should be employed to elucidate whether DKT increases the anal pressure in further research. Second, the administration route for this study differs from the application of DKT in daily clinical practice. Therefore, it was impossible to determine in this study whether oral administration of DKT causes IAS contraction. Finally, the mechanism of action has not been elucidated, and further studies are expected.

In conclusion, *in vivo* telemetry revealed that DKT exhibited contractile effects on the IAS of dogs. The increased anal pressure and improvement of FI symptoms observed in clinical studies may be based on this sphincter contraction. In the future, we hope to provide conclusive clinical evidence that DKT administration improves FI symptoms in FI patients *via* the increase in IAS contractility.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Kotaro Maeda, Hidetoshi Katsuno, and Toru Kono. The first draft of the manuscript was written by Kotaro Maeda, and all authors commented on the previous versions of the manuscript. All authors read and approved the final manuscript.

Approval by Institutional Review Board (IRB)

All experimental procedures were performed at the Kumamoto laboratory of LSI Medience Corporation in accordance with the "Guidelines for the Care and Use of Laboratory Animals" approved by the Laboratory Animal Committee of LSI Medience Corporation (Approval No. 2019-0393).

Disclaimer

Hidetoshi Katsuno is one of the Associate Editors of Journal of the Anus, Rectum and Colon and on the journal's Editorial Board. He was not involved in the editorial evaluation or decision to accept this article for publication at all.

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