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ORIGINAL ARTICLE

Depolarizing Effects of Daikenchuto on Interstitial Cells of Cajal from Mouse Small Intestine

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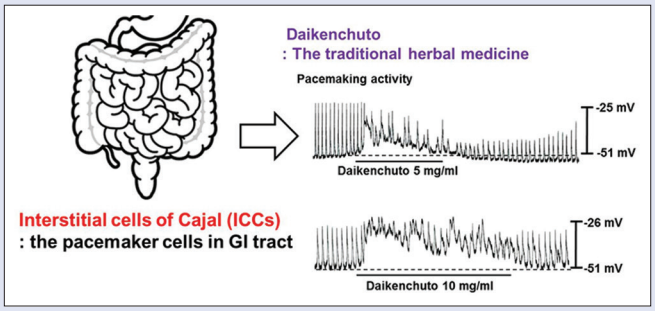
ABSTRACT

Background: Daikenchuto (DKT; TJ-100, TU-100), a traditional herbal medicine, is used in modern medicine to treat gastrointestinal (GI) functional disorders. Interstitial cells of Cajal (ICCs) are the pacemaker cells of the GI tract and play important roles in the regulation of GI motility. **Objective:** The objective of this study was to investigate the effects of DKT on the pacemaker potentials (PPs) of cultured ICCs from murine small intestine. **Materials and Methods:** Enzymatic digestions were used to dissociate ICCs from mouse small intestine tissues. All experiments on ICCs were performed after 12 h of culture. The whole-cell patch-clamp configuration was used to record ICC PPs (current clamp mode). All experiments were performed at 30–32°C. **Results:** In current-clamp mode, DKT depolarized and concentration-dependently decreased the amplitudes of PPs. Y25130 (a 5-HT_3 receptor antagonist) or SB269970 (a 5-HT_7 receptor antagonist) did not block DKT-induced PP depolarization, but RS39604 (a 5-HT_4 receptor antagonist) did. Methoctramine (a muscarinic M_2 receptor antagonist) failed to block DKT-induced PP depolarization, but pretreating 4-diphenylacetoxy-N-methylpiperidine methiodide (a muscarinic M_3 receptor antagonist) facilitated blockade of DKT-induced PP depolarization. Pretreatment with an external Ca^{2+}-free solution or thapsigargin abolished PPs, and under these conditions, DKT did not induce PP depolarization. Furthermore, *Ginseng* radix and *Zingiberis* rhizomes depolarized PPs, whereas Zanthoxyli fructus fruit (the third component of DKT) hyperpolarized PPs. **Conclusion:** These results suggest that DKT depolarizes ICC PPs in an internal or external Ca^{2+}-dependent manner by stimulating 5-HT_4 and M_3 receptors. Furthermore, the authors suspect that the component in DKT largely responsible for depolarization is probably also a component of *Ginseng* radix and *Zingiberis* rhizomes.

Key words: Daikenchuto, gastrointestinal tract, interstitial cells of Cajal, pacemaker potentials

SUMMARY

- Daikenchuto (DKT) depolarized and concentration-dependently decreased the amplitudes of pacemaker potentials (PPs)
- Y25130 (a 5-HT_3 receptor antagonist) or SB269970 (a 5-HT_7 receptor antagonist) did not block DKT-induced PP depolarization, but RS39604 (a 5-HT_4 receptor antagonist) did
- Methoctramine (a muscarinic M_2 receptor antagonist) failed to block DKT-induced PP depolarization, but pretreating 4-DAMP (a muscarinic M_3 receptor antagonist) facilitated blockade of DKT-induced PP depolarization
- Ginseng radix and Zingiberis rhizomes depolarized PPs, whereas Zanthoxyli fructus fruit (the third component of DKT) hyperpolarized PPs.



Abbreviations used: DKT: Daikenchuto, GI: Gastrointestinal, ICCs: Interstitial cells of Cajal, PPs: Pacemaker Potentials.

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INTRODUCTION

Chinese (or Japanese) traditional herbal medicines are prescribed for the treatment of a wide array of diseases and conditions, including gastrointestinal (GI) disorders.[1] Daikenchuto (DKT; TJ-100, TU-100) is the most widely prescribed traditional Chinese herbal medicine, and it is called Kampo in Japan. DKT is a mixture of *Ginseng* radix, *Zingiberis* siccatum rhizomes, and *Zanthoxyli* fructus,[2] is used traditionally to treat abdominal bloating and a cold sensation in the abdomen,[3-5] and prescribed by physicians for the treatment of chronic constipation.[6]

Interstitial cells of Cajal (ICCs) are the pacemaker cells of the GI tract and generate rhythmic oscillations in membrane potentials known as slow waves,[7,8] and thus, ICCs play important roles in the regulation of GI motility.[9] Endogenous agents, such as neurotransmitters, hormones, and paracrine substances, modulate GI tract motility by influencing ICCs,[10-12] and it appears that some traditional herbal medicines could regulate the pacemaker potentials (PPs) of ICCs.[13-15] It has been reported that DKT accelerates small intestinal movement by directly inhibiting smooth muscle and partially by inhibiting neural activities,[16] and that it induces phasic contractions in the duodenum and proximal jejunum via cholinergic receptors.[17] These findings provide a scientific basis suggesting the therapeutic use of DKT for the treatment of GI disorders.

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However, relatively little is known about the effects of DKT on the PPs of ICCs in the GI tract. Therefore, in the present study, we investigated the effects of DKT on the PPs of cultured ICCs obtained from mouse small intestine.

MATERIALS AND METHODS

Preparation of daikenchuto and its constituents

DKT extract powder was manufactured by Tsumura and Co., Tokyo. To prepare DKT constituents, the powders obtained from the ethanol or water extracts of *Ginseng* radix (Catalog number: CA03-041), *Zingiberis* rhizome (Catalog number: CA04-001), and *Zanthoxyli* fructus (Catalog number: 029-037) were obtained from the plant extract bank at the Korean Research Institute of Bioscience and Biotechnology (Daejeon, Korea). DKT and its constituents were authenticated by Hyungwoo Kim (Division of Pharmacology, Pusan National University, School of Korean Medicine, Yangsan, Korea). The powder was then immersed in ethanol or water, sonicated for 15 min, and extracted for 72 h. The extract so obtained was filtered through nonfluorescent cotton and evaporated under reduced pressure using a rotary evaporator (N-1000 SWD, Eyela, Japan) in 45°C. The condensed extract was then lyophilized using a Modul Spin 40 dryer (Biotron Corporation, Calgary, Canada) for 24 h. The yield of lyophilized powder obtained was 12.3%. The DKT was dissolved in distilled water at a concentration of 0.5 g (crude drug)/ml and stored in a refrigerator. The extracts of *Ginseng* radix, *Zingiberis* rhizome, and *Zanthoxyli* fructus were dissolved in dimethyl sulfoxide as a stock solution at 100 mg/mL and stored at 4°C. The stock solution was diluted with medium to the desired concentration prior to use.

Preparation of cells and cell cultures

Animal care and the study protocol were in accordance with the guidelines issued by the Ethics Committee of Pusan National University (Yangsan, Republic of Korea). BALB/c mice (3–5 day-old) were used throughout the study. Small intestines were excised (from 1 cm below the pyloric ring to the cecum) and opened along the mesenteric border. Luminal contents were removed using Krebs-Ringer bicarbonate solution, and the tissues were pinned to the bases of Sylgard dishes. Mucosae were removed by sharp dissection. Small tissue strips of intestine muscle (consisting of circular and longitudinal muscles) were equilibrated for 30 min in Ca^{2+}-free Hank's solution, which contained the following; potassium chloride (KCl), 5.36 mM; sodium chloride (NaCl), 125 mM; sodium hydroxide (NaOH), 0.34 mM; sodium bicarbonate, 0.44 mM; glucose, 10 mM; sucrose, 2.9 mM; and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 11 mM (final pH of 7.4). Cells were then dispersed in an enzyme solution containing collagenase (Worthington Biochemical, Lakewood, NJ, USA; 1.3 mg/mL), bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO, USA; 2 mg/mL), trypsin inhibitor (Sigma-Aldrich; 2 mg/mL), and ATP (0.27 mg/mL), and plated onto sterile glass coverslips coated with murine collagen (2.5 mg/mL; Falcon/BD, Franklin Lakes, NJ, USA) in 35 mm culture dishes. Cells were cultured at 37°C in a 95% oxygen/5% carbon dioxide incubator in smooth muscle growth medium (Clonetics, San Diego, CA, USA) supplemented with 2% antibiotics/antimycotics (Gibco, Grand Island, NY, USA) and murine stem cell factor (5 ng/mL; Sigma-Aldrich). All experiments on ICC clusters were performed after they had been cultured for 12 h. ICCs were identified immunologically using an anti-c-Kit antibody, that is, phycoerythrin-conjugated rat anti-mouse c-Kit monoclonal antibody (eBioscience, San Diego, CA, USA), at a dilution of 1:50 for 20 min. Because the morphology of ICCs differed from other cell types in culture, it could be identified under a phase-contrast microscope after incubation with anti-c-Kit antibody.

Patch-clamp experiments

Physiological salt solution was used to bathe cultured ICC clusters (Na^+-Tyrode) and contained the following: KCl, 5 mM; NaCl, 135 mM; calcium chloride ($CaCl_2$), 2 mM; glucose, 10 mM; magnesium chloride ($MgCl_2$), 1.2 mM; and HEPES, 10 mM (adjusted to pH 7.4 with NaOH). The pipette solution used to examine pacemaker activity contained the following: KCl, 140 mM; $MgCl_2$, 5 mM; dipotassium ATP (K_2ATP), 2.7 mM; sodium GTP (NaGTP), 0.1 mM; creatine phosphate disodium, 2.5 mM; HEPES, 5 mM; and ethylene glycol tetra-acetic acid, 0.1 mM (adjusted to pH 7.2 with potassium hydroxide). Patch-clamp techniques were conducted in whole-cell configuration to record potentials (i.e., current clamp mode) from cultured ICCs using Axopatch I-D and Axopatch 200B amplifiers (Axon Instruments, Foster, CA, USA). Command pulses were applied using an IBM-compatible personal computer (Compaq; Houston, TX, USA) and pClamp software (versions 6.1 and 10.0; Axon Instruments, Foster City, CA, USA). Data were filtered at 5 kHz and displayed on an oscilloscope, a computer monitor, and/or a pen recorder (Gould 2200; Gould, Valley View, OH, USA). Results were analyzed using pClamp and Origin software (version 6.0, Microcal, Northampton, MA, USA). All experiments were performed at 30–33°C.

Drugs

The drugs used in the experiments were Y25130, RS39604, SB269970, methoctramine, 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP), and thapsigargin, and all were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions were prepared and stored according to the manufacturer's instructions. Chemicals were dissolved in physiological salt solution to their final concentrations immediately before use.

Statistical analysis

Results are expressed as mean ± standard errors. The Student's *t*-test was used to determine the significances of differences. A statistically significant difference was seen at $P < 0.05$. "*n*" values reported in the text refer to the number of cells used in patch-clamp experiments.

RESULTS

Effects of daikenchuto on the pacemaker potentials of cultured interstitial cells of Cajal from murine small intestine

The patch-clamp technique was applied to ICCs that formed network-like structures after culture for 12 h. Under current clamp mode ($I = 0$), ICCs generated PPs [Figure 1a] with a mean resting membrane potential of −51.3 ± 2.3 mV and a mean amplitude of 25.4 ± 1.5 mV. DKT (1–10 mg/ml) depolarized PPs and decreased the PP amplitudes in a concentration-dependent manner [Figure 1b-d]. In the presence of DKT, mean degrees of depolarization were 2.6 ± 0.6 mV at 1 mg/ml, 16.2 ± 0.7 mV at 5 mg/ml, and 26.1 ± 1.5 mV at 10 mg/ml [Figure 1e, $n = 24$] and mean amplitudes were 24.3 ± 1.3 mV at 1 mg/ml, 11.4 ± 1.4 mV at 5 mg/ml, and 7.2 ± 0.8 mV at 10 mg/ml [Figure 1f, $n = 24$]. Summarized values and a bar graph showing the effects of DKT on PPs are provided in Figure 1e and f. These results suggest that DKT dose dependently depolarizes ICC PPs.

Identification of daikenchuto receptor subtypes in cultured interstitial cells of Cajal from murine small intestine

To investigate the relationship between DKT and its receptors, we studied 5-HT and muscarinic receptors because they are known to

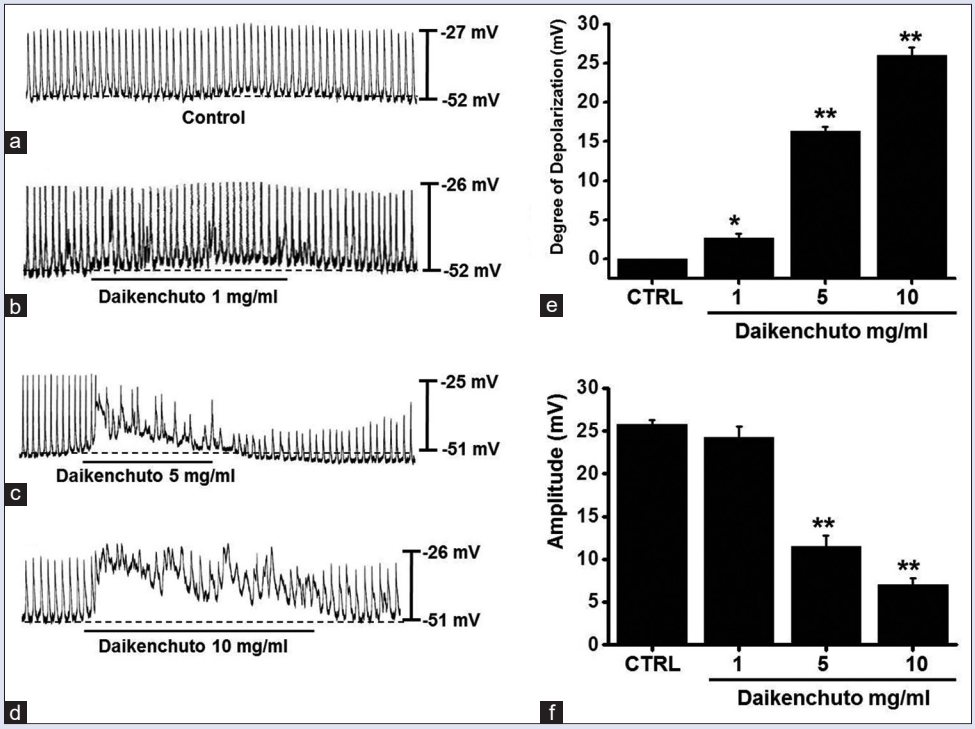

Figure 1: Effects of daikenchuto on pacemaker potentials in cultured interstitial cells of Cajal from mouse small intestine. (a-d) The pacemaker potentials of interstitial cells of Cajal exposed to daikenchuto (1–10 mg/ml) in current clamp mode (i = 0). Responses to daikenchuto (e and f). Bars represent mean ± standard error of the means. *P < 0.05, **P < 0.01. Significantly different from nontreated controls. CTRL: Control

mediate GI tract motility and to be strongly associated with prokinetic activity.[13,18,19] The stimulation of 5-HT_4 receptor (5-HT_4R) in the enteric nervous system results in the release of acetylcholine in the GI tract, which leads to the excitation of smooth muscles in myenteric plexus, and thus, 5-HT_4R is regarded a prokinetic.[18] Therefore, we investigated whether the prokinetic action of DKT involves 5-HT receptors. In ICCs, only three receptors (5-HT_3R, 5-HT_4R, and 5-HT_7R) are present.[13,20,21] To identify the 5-HT receptor subtypes involved in the effects of DKT, ICCs were pretreated with various 5-HT receptor antagonists and then treated with DKT. Y25130 (a 5-HT_3 receptor antagonist), RS39604 (a 5-HT_4R antagonist), and SB269970 (a 5-HT7 receptor antagonist) were all pretreated at 10 μM for 5 min, and then DKT was added. After pretreating Y25130 or SB269970, DKT depolarized membranes [Figure 2a and d]; membrane depolarization produced in the presence of Y25130 or SB269970 by DKT (5 mg/ml) was 15.8 ± 1.0 mV and 16.5 ± 0.6 mV, respectively [n = 4; Figure 2d]. However, pretreatment with RS39604 blocked the effect of DKT [n = 4; Figure 2b and d]. In addition, ICCs isolated from the GI tract express M_2 and M_3 subtypes of muscarinic receptors.[22] To identify the muscarinic receptor subtypes involved, ICCs were pretreated with muscarinic receptor antagonists and then treated with DKT. Methoctramine (a muscarinic M_2 receptor antagonist) or 4-DAMP (a muscarinic M_3 receptor antagonist) were pretreated at 10 μM for 5 min and then DKT (10 mg/ml) was added. Treatment with methoctramine or 4-DAMP had no effect on PPs, and pretreatment with methoctramine did not block the DKT-induced PP depolarization [Figure 3a]. In the presence of methoctramine, mean DKT-induced PP depolarization was 26.3 ± 0.5 mV [n = 4; Figure 3c], but after pretreating 4-DAMP, DKT-induced PP depolarization was blocked [Figure 3b]. After 4-DAMP pretreatment, DKT-induced PPs depolarization was 0.3 ± 0.4 mV [n = 4; Figure 3c]. These results show that DKT affects ICCs through 5-HT_4 and M_3 receptors.

Effects of external Ca^{2+}-free solution and of Ca^{2+}-ATPase inhibitor on daikenchuto-induced pacemaker potential depolarization in cultured interstitial cells of Cajal from murine small intestine

External and internal Ca^{2+} regulation plays important roles in smooth muscle contraction and in the pacemaker activities of ICCs in the GI tract.[23] To investigate the roles of external or internal Ca^{2+}, DKT was applied under external Ca^{2+}-free conditions and in the presence of thapsigargin (an inhibitor of Ca^{2+}-ATPase in endoplasmic reticulum).[24,25] Pretreatment with external Ca^{2+}-free solution abolished PPs, and under this condition, DKT did not induce PP depolarization [n = 5; Figure 4a]. In addition, pretreatment with thapsigargin abolished PPs, and similarly under this condition, DKT did not induce PP depolarization [n = 5; Figure 4b]. Summarized values and a bar graph showing the effects of DKT using an external Ca^{2+}-free solution or in the presence of a Ca^{2+}-ATPase inhibitor are shown in Figure 4c. These results show that DKT-induced PP depolarization is dependent on internal and external Ca^{2+} regulation.

Effects of daikenchuto constituents on the pacemaker potentials of cultured interstitial cells of Cajal from murine small intestine

We examined the effects of the constituents of DKT, that is, *Ginseng* radix, *Zingiberis* (ginger) rhizomes, and *Zanthoxyli* fructus[26] on the PPs of ICCs. Both *Ginseng* and dried ginger depolarized and decreased the amplitudes of PPs [Figure 5a and b]. However, *Zanthoxylum* fruit inhibited, hyperpolarized, and decreased the amplitudes of PPs [Figure 5c]. These results suggest that the main depolarizing component in DKT is probably also an ingredient of *Ginseng* radix and *Zingiberis* rhizomes.

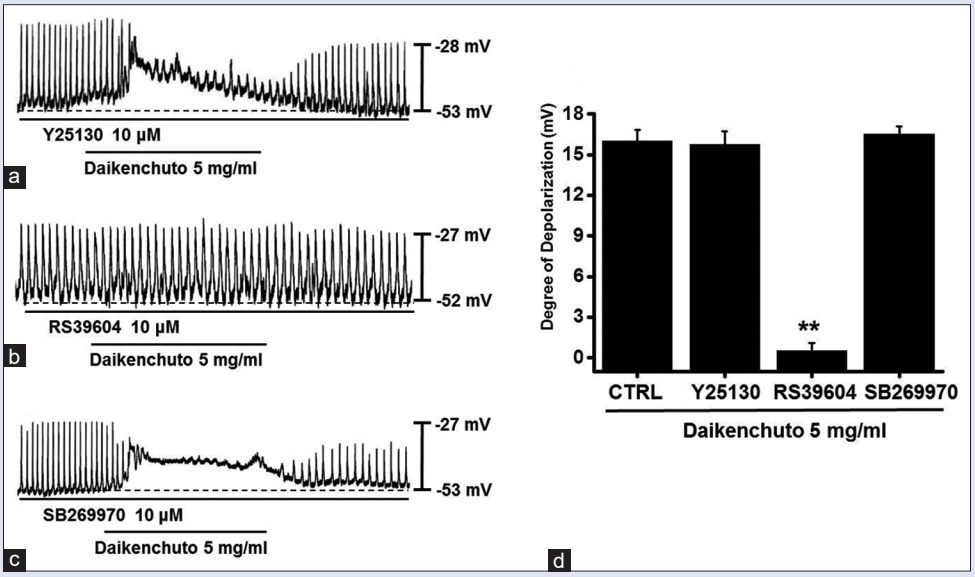

Figure 2: Effects of 5-HT receptor subtype antagonists on daikenchuto-induced pacemaker potential depolarization in cultured interstitial cells of Cajal from mouse small intestine. Pacemaker potentials of interstitial cells of Cajal exposed to daikenchuto (5 mg/ml) in the presence of 5-HT3 receptor antagonist (Y25130; 10 μM) (a), in the presence of 5-HT4 receptor antagonist (RS39604; 10 μM) (b), and in the presence of 5-HT7 receptor antagonist SB269970 (10 μM) (c). Responses to daikenchuto in the presence of different receptor antagonists (d). Bars represent mean ± standard error of the means. $^{**}P < 0.01$. Significantly different from non-treated controls. CTRL: Control

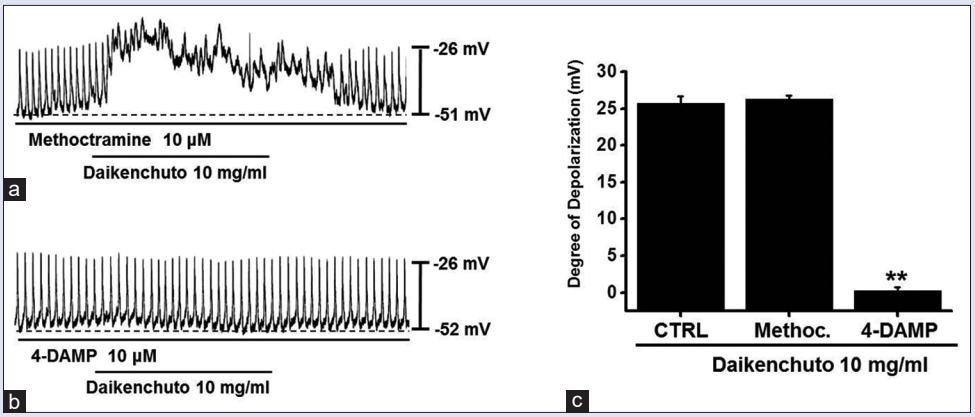

Figure 3: Effects of muscarinic receptor subtype antagonists on daikenchuto-induced pacemaker potential depolarization in cultured interstitial cells of Cajal from mouse small intestine. Pacemaker potentials were depolarized when interstitial cells of Cajal were exposed to daikenchuto (10 mg/ml) in the presence of methoctramine (a muscarinic M2 receptor antagonist; 10 μM) (a), in the presence of 4-DAMP (a muscarinic M3 receptor antagonist; 10 μM) (b). Responses to daikenchuto in the presence of different receptor antagonists (c). Bars represent mean ± standard error of the means. $^{**}P < 0.01$. Significantly different from non-treated controls. CTRL: Control, Methoct: Methoctramine

DISCUSSION

In the present study, we investigated the effect of DKT on GI motility by examining the PPs of ICCs from murine small intestine. In these cells, DKT depolarized PPs in an internal and an external Ca^{2+}-dependent manner by stimulating 5-HT_4 and M_3 receptors, which suggests that DKT offers a basis for developing novel prokinetic agents that prevent or alleviate GI motility dysfunctions. Furthermore, the study also shows that the primary depolarizing component in DKT is probably also present in *Ginseng* radix and in *Zingiberis* rhizomes.

Herbal therapy has been used in Asia for thousands of years. DKT is composed of *Ginseng* radix, *Zingiberis* siccatum rhizome, and *Zanthoxyli* fructus,[2] and it is often used to treat GI hypomotility, such as, ileus, following abdominal surgery.[27] It has been shown that DKT induces contractions in the antrum, duodenum, and jejunum by acting through cholinergic and 5-HT_3 receptors.[28,29] On the other hand, *Zanthoxylum* fruit elicited contractions mainly in duodenum and jejunum, whereas dried ginger rhizome induced contractions in the antrum only and *Ginseng* root had no effect.[28,29] Furthermore, the effects of DKT have been reported to be dependent on anatomic site and timing of its administration.[30] During the fasting state, DKT had evident prokinetic effects.[30] In addition, in isolated guinea pig ileum, DKT was found to elicit contractile responses via muscarinic and 5-HT_4Rs[31] and to ameliorate morphine-induced GI transit disorder in mice as determined by colon transit times.[32] In one study, it was suggested that small intestine motility improvement by DKT was due to its ginger root and

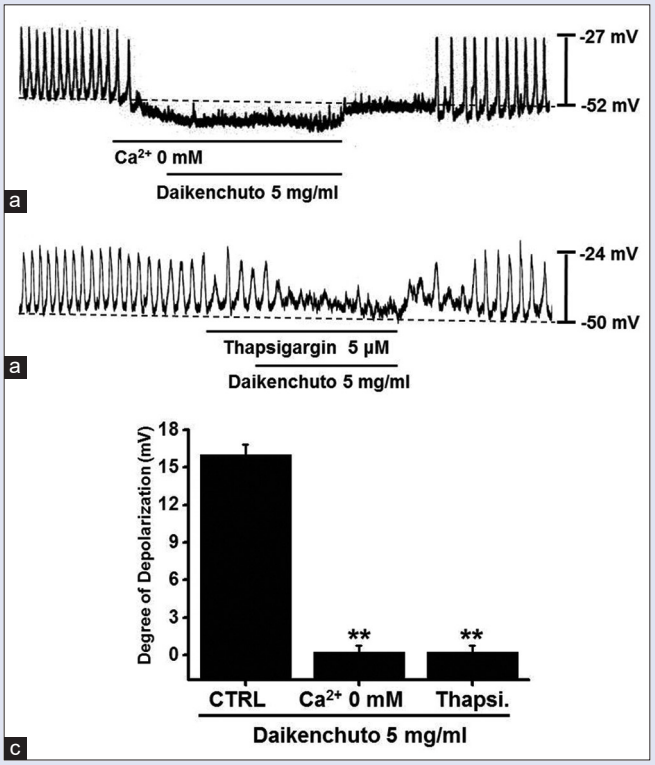

Figure 4: Effects of an external Ca^{2+}-free solution and of thapsigargin on daikenchuto-induced pacemaker potential depolarization in cultured interstitial cells of Cajal from mouse small intestine. (a) External Ca^{2+}-free solution and (b) thapsigargin (5 μM) abolished the generation of pacemaker potentials and blocked daikenchuto-induced pacemaker potential depolarization. (c) Responses to daikenchuto in external Ca^{2+}-free solution and in the presence of thapsigargin are summarized. Bars represent mean ± standard error of the means. $^{**}P < 0.01$. Significantly different from nontreated controls. CTRL: Control, Thapsi: Thapsigargin

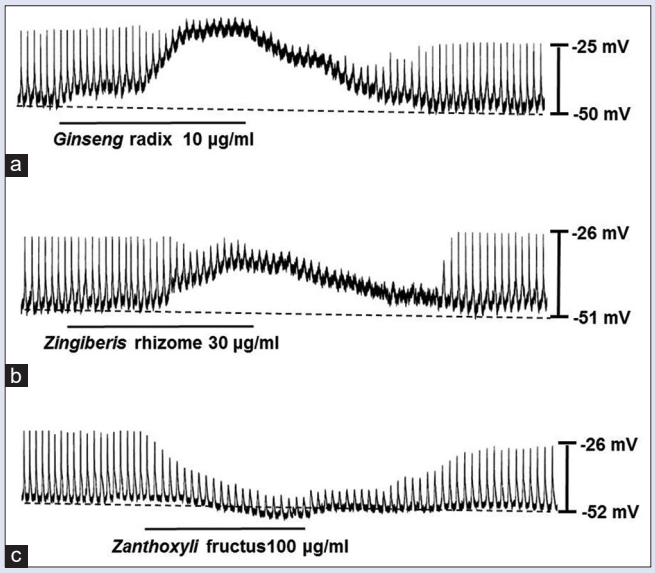

Figure 5: Effects of *Ginseng*, dried ginger, and *Zanthoxylum* fruit on pacemaker potentials in cultured interstitial cells of Cajal from mouse small intestine. (a and b) *Ginseng* and dried ginger depolarized pacemaker potentials, whereas (c) *Zanthoxylum* fruit had a hyperpolarizing effect

Ginseng components via smooth muscle and neural inhibition.[33] In addition, Kito *et al.*[34] suggested that DKT had no effect on pacemaker mechanisms and electrical coupling between ICCs and smooth muscle cells in mouse small intestine, and that therefore, it may contract smooth muscles by depolarizing membranes directly.[34]

Most 5-HT are found in the GI tract, and abnormalities in 5-HT signaling or metabolism are associated with several GI tract disorders, such as, dyspepsia, nausea, vomiting, coeliac disease, inflammatory bowel disease, and irritable bowel syndrome (IBS).[35-37] 5-HT_4R is expressed in several different cell types in intestine, where it stimulates intestinal activity and is a target for the treatment of constipation-predominant IBS and chronic constipation.[38,39] 5-HT_7 receptors are often located near 5-HT_4Rs where they augment 5-HT induced responses.[38,40] Furthermore, 5-HT_3 receptor antagonists are known to decrease colonic motility, secretion, and nociception, and are currently targeted to treat diarrhea-predominant IBS.[35,36] Acetylcholine is involved in the control of almost all the functions of GI organ systems, and muscarinic receptors, which are commonly expressed in the GI tract, are important for organ function.[41,42] The tissues and cell types that express receptors are numerous and include ICCs and smooth muscle and mucosal cells in the stomach and intestine. Muscarinic receptors are classified into five subtypes, that is, muscarinic M_1, M_2, M_3, M_4, and M_5 receptors,[43,44] and belong to the family of G protein-coupled receptors – heterotrimeric guanine nucleotide-binding proteins that regulate second messengers and ion channels.[45] Three 5-HT receptors (5-HT_3, 5-HT_4, and 5-HT_7) and two muscarinic receptors (M_2 and M_3) are present on ICCs.[13,20-22] In the present study, pretreatment with Y25130 (a 5-HT_3 receptor antagonist) or SB269970 (a 5-HT_7 receptor antagonist) facilitated DKT-induced membrane depolarization [Figure 2a and c], but pretreatment with RS39604 (a 5-HT_4R antagonist) blocked the effect of DKT [Figure 2b]. On the other hand, methoctramine (a muscarinic M_2 receptor antagonist) did not block DKT-induced PP depolarization [Figure 3a], but pretreatment with 4-DAMP (a muscarinic M_3 receptor antagonist) blocked DKT-induced PP depolarization [Figure 3b]. In addition, in GI tract, generally both M_2 and M_3 were involved in GI motility. However, GI tract is composed of smooth muscle, enteric nervous system and ICCs, and so on. Therefore, we think that DKT may act on only M_3 receptor in ICCs. In addition, So *et al.*[46] suggested that the modulation of pacemaker currents by carbachol in ICCs is mediated by only muscarinic M_3 receptors not M_2 receptors. Therefore, we think that DKT could modulate the PPs through only muscarinic M_3 receptors in ICCs such as carbachol. These findings indicate that DKT affects ICCs through 5-HT_4 and M_3 receptors and that these receptors have important roles in the modulation of GI motility.

ICCs are the pacemaker cells of the GI tract and generate and propagate the slow waves that regulate GI motility,[9] and networks of ICCs are never static even during physiological conditions. Apoptosis and transdifferentiation cause loss of ICCs, and these losses can be restored by the proliferation and differentiation of stem cells or ameliorated by increasing the survival of ICCs.[47,48] Losses of or deficiencies in ICCs have been observed in intestines of animal models of GI dysfunction and are believed to contribute to the development of motility disorders.[49,50] Therefore, ICCs play a critical physiological role in the coordination of intestinal contractile activity and constitute an important aspect of intestinal motility.

DKT is composed of *Ginseng* radix, *Zingiberis* siccatum rhizome, and *Zanthoxyli* fructus,[2] and they have influences on the regulation of GI tract motility. *Ginseng* radix affects the GI tract. *Ginseng* increases mouse intestinal movement and promotes the relaxation of circular muscles

in the gastric body.[51] In isolated guinea pig GI tract tissues, *Ginseng* increases longitudinal muscle contraction in the ileum and distal colon[52] and in the rabbit intestine, *Ginseng* stimulates intestinal motility.[53] Furthermore, ginsenoside Re regulates the pacemaking activity of ICCs in mouse small intestine.[54] *Zingiberis* siccatum rhizome continuously decreased the amplitude of contraction and *Zanthoxyli* fructus increased jejunal contraction in isolated rabbit jejunum.[55]

In the present study, DKT depolarized PPs in ICCs, and *Ginseng* and dried ginger also had depolarizing effects [Figure 5a and b], whereas *Zanthoxylum* fructus hyperpolarized PPs [Figure 5c], which suggests that the main depolarizing component in DKT is probably a common component of *Ginseng* radix and *Zingiberis* rhizome.

CONCLUSION

The present study shows that in ICCs, DKT depolarizes and decreases the amplitudes of PPs in a concentration-dependent manner via internal or external Ca^{2+}. RS39604 (a 5-HT_4R antagonist) and 4-DAMP (a muscarinic M_3 receptor antagonist) blocked DKT-induced PP depolarization. *Ginseng* radix and *Zingiberis* rhizome depolarized ICC PPs, whereas *Zanthoxyli* fructus fruit had a hyperpolarizing effect. We suggest that the main depolarizing component in DKT is probably an ingredient in *Ginseng* radix and *Zingiberis* rhizomes. These findings suggest that DKT is a good candidate for the development of a prokinetic agent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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