Dai-kenchu-to Enhances Accelerated Small Intestinal Movement

Kazuko SATOH, Yoshio KASE,* Terumasa HAYAKAWA, Pin MURATA, Atsushi ISHIGE, and Hiroshi SASAKI

Kampo & Pharmacognosy Laboratory, R&D Division, Tsumura & Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300–1192, Japan. Received March 7, 2001; accepted July 4, 2001

The present study was conducted to clarify the effects of Dai-kenchu-to on accelerated small intestinal movement. We evaluated the effects of Dai-kenchu-to and its constituent herbs (dried ginger root, ginseng, zanthoxylum fruit, and malt sugar) on carbachol-accelerated mouse small intestinal transit, and contractions induced by low-frequency electrostimulation (ESC), KCl, or acetylcholine (ACh) using isolated guinea pig ileum. Dai-kenchu-to (10–300 mg/kg, p.o.) significantly improved carbachol-accelerated small intestinal transit in a dose-dependent manner. Using a concentration with the compounded rate for Dai-kenchu-to:300 mg/kg, carbachol-accelerated small intestinal transit was also significantly improved with a single dose of dried ginger root or ginseng. At a concentration of 3×10^{-5} g/ml or less, Dai-kenchu-to, dried ginger root, and ginseng all inhibited ESC but not KCl- or ACh-induced contractions. However, at a higher concentration of Dai-kenchu-to (10^{-4} g/ml) or zanthoxylum fruit (10^{-5} g/ml or more) the ESC were enhanced. Both Dai-kenchu-to and dried ginger root at 10^{-3} g/ml remarkably inhibited the KCl-induced contractions. These results indicate that Dai-kenchu-to improves accelerated small intestinal movement and that dried ginger root and ginseng may be involved in this effect. It is also thought that the mechanisms mainly involve the direct inhibition of smooth muscle but with a contribution from neural inhibition.

Key words Dai-kenchu-to; accelerated small intestinal movement; dried ginger root; ginseng

Dai-kenchu-to (Da-Jian-Zhong-Tang in Chinese) is a traditional Chinese herbal medicine, called kampo medicine in Japan, and is a mixture of dried ginger root, ginseng, zanthoxylum fruit, and malt sugar. This formula is known for its clinical effects on intestinal obstruction subsequent to laparotomy and on irritable bowel syndrome (IBS).1–4 Since intestinal motility is reduced following laparotomy, in many studies focusing on the mechanisms of enhanced intestinal motility have been reported.5–10 These reports indicated that zanthoxylum fruit was involved in the contraction induced by Dai-kenchu-to, and that part of the contractile mechanism was mediated by acetylcholine (ACh) release from the ends of cholinergic nerves and tachykinins from sensory nerves.8,10 However, intestinal obstruction following laparotomy is frequently accompanied by abdominal pain, which is thought to be associated with intestinal “cluster” contractions.11,12 Moreover, cramping abdominal pain is usually noted in IBS, which is characterized by hypercontractility.13 Although the relationship between abnormal gastrointestinal motility and the cause of abdominal pain remains controversial, muscarinic antagonists act as antispasmodics by reducing gastrointestinal hypermotility in patients.15 Thus it is thought that hypercontractility is due, at least in part, to stimulation of cholinergic neurotransmission.

In the present study, to clarify the effects of Dai-kenchu-to on accelerated small intestinal movement induced by the stimulation of cholinergic neurotransmission, we evaluated the effects of Dai-kenchu-to and its constituent herbs on carbachol-accelerated mouse small intestinal transit and on contractions induced by low-frequency electrostimulation (ESC), KCl, or ACh using isolated guinea pig ileum.

MATERIALS AND METHODS

Animals Male ICR mice weighing about 30 g (Japan SLC Inc., Shizuoka, Japan) were used for the in vivo studies, and male Hartley guinea pigs weighing 440–700 g (Japan SLC) were used for the in vitro studies. The animals were housed in an air-conditioned animal room kept at a temperature of 23–24 °C, a humidity of 50–65%, and a 12-h light-dark cycle, with free access to food and water.

Drugs Dai-kenchu-to was prepared by mixing Dai-kenchu-to extract powder and malt sugar at a ratio of 1:8. The Dai-kenchu-to extract powder (lot no. 25010020) (extracts in water from a 5:3:2 mixture of dried ginger root (Zingiber officinale Roscoe, rhizoma), ginseng (Panax ginseng C.A. Meyer, radix), and zanthoxylum fruit (Zanthoxylum piperitum De Candolle, pericarpium)) and malt sugar (candy produced from rice, wheat, and malt lot no. 14106950), and malt sugar (lot no. 1410064) were all manufactured by Tsumura & Co. (Tokyo, Japan). These extract powders were stored at room temperature prior to use. The yield of Dai-kenchu-to extract, dried ginger root extract, ginseng extract, and zanthoxylum fruit extract were 12.5%, 13.64%, 21.59%, and 17.76%, respectively. The quality of each constituent herb and their mixture was controlled using thin-layer chromatography analysis and maintaining the prescribed range of index components such as [6]-shogaol and ginsenoside Rb1. Other drugs used were: acetylcholine chloride (ACh; Ovisot Injection, Daiichi Pharmaceutical Co., Tokyo, Japan), and tetrodotoxin (TTX; Wako Pure Chemical Ind., Osaka, Japan). Carbachol, atropine sulfate, trimethubine maleate salt, and indomethacin were all purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Powder extracts were suspended in Krebs solution (in vitro) or distilled water (in vivo). Carbachol and atropine were dissolved in saline, and indomethacin was dissolved in a 1% Tween 80 solution. Other drugs were dissolved in distilled water.

Effects on Carbachol-Accelerated Mouse Small Intestinal Transit Mice were fasted for 24 h, and 8–11 allocated...
to each group. Carbachol 1 mg/kg, s.c. was administered to mice 15 min before administration of the test drugs. The test drugs were given orally to each mouse at a volume of 10 ml/kg, except for atropine (s.c.). Control animals were orally administered the same volume of distilled water. Thirty minutes after administration of the test drugs, 5% activated charcoal powder suspended in 10% gum arabic was orally administered to each mouse at a volume of 10 ml/kg. The animals were then killed 20 min later, and the small intestine completely removed. The small intestinal transit rate was obtained after dividing the migrating length of activated charcoal powder by the total length of the small intestine. The doses of Dai-kenchu-to given were shown to be effective in previous studies.3) Doses of the test drugs were as follows: Dai-kenchu-to, 10—300 mg/kg; dried ginger root, 150 mg/kg; ginseng, 90 mg/kg; zanthoxylum fruit, 60 mg/kg; malt sugar, 2400 mg/kg; atropine, 1 mg/kg; trimebutine, 30 mg/kg; and indomethacin 0.1—1 mg/kg. The doses of the powdered crude drug component extracts were the same as those in Dai-kenchu-to 300 mg/kg.

**Effect on Isolated Guinea Pig Ileum** Guinea pigs were sacrificed by decapitation and the ileum of each animal was immediately excised. After removing the mucosae, strips were suspended along the longitudinal muscle by 0.5 g loading in an organ bath with oxygenated (95% O2 and 5% CO2) Krebs solution at 37 °C. The composition of the Krebs solution was as follows (mmol/l): NaCl 118, KCl 4.8, MgSO4 1.2, NaH2PO4 1.2, CaCl2 2.5, NaHCO3 25, and glucose 11. Isotonic high KCl solution (4 × 10^{-2} mol/l) was prepared by replacing the NaCl with KCl. Experiments started after a 60-min equilibration. The preparations were exposed to ACh (5.5 × 10^{-7} mol/l) at the beginning of the experiment, and the contractile responses recorded isotonically.

Field stimulation was added to the suspended ileum via platinum electrodes placed parallel to the specimen using square waves (electric current 100 mA, duration 0.5 ms, frequency 0.1 Hz). The effects of a cumulative dose of Dai-kenchu-to, dried ginger root, ginseng, zanthoxylum fruit, and malt sugar (10^{-3}—10^{-2} g/ml) in the presence of 7.5 × 10^{-3} mol/l CaCl2 (final concentration 10^{-2} mol/l), and dried ginger root or ginseng (10^{-5}—10^{-3} g/ml, respectively) on ileal contraction were examined. The inhibition of the ESC by each dose of test drugs are presented as percent inhibitions compared to the control ESC when the animals received vehicle alone.

To evaluate whether the inhibitory effect of the test drugs was mediated by neural factors or factors directly influencing the smooth muscle, we examined the effects of a single dose of Dai-kenchu-to, dried ginger root, or ginseng (10^{-6}—10^{-4} g/ml, respectively) administered 10 min before ileal contraction was induced by cumulative administration of ACh (10^{-10}—10^{-8} mol/l). We also examined the effects of a cumulative dose of Dai-kenchu-to (10^{-5}—10^{-3} g/ml) in the presence of absence of 7.5 × 10^{-3} mol/l CaCl2 (final concentration 10^{-2} mol/l), and dried ginger root or ginseng (10^{-5}—10^{-3} g/ml, respectively) on ileal contraction induced by isotonic high KCl (4 × 10^{-2} mol/l). Dai-kenchu-to at concentrations of 3 × 10^{-4} g/ml or greater induced ACh release and ileal contraction. Therefore the doses of Dai-kenchu-to were 10^{-4} g/ml or less in ESC and ACh-induced contraction studies, while high doses were used in the KCl-induced contraction study. The results are expressed as a percentage of the maximal responses of ACh-induced precontractions, and as percent inhibitions compared with the precontractions induced by KCl.

**Statistical Analysis** All value are expressed as the mean±S.E. Statistical significance was assessed by Fisher’s PLSD test for *in vivo* results, or by the unpaired Student’s *t*-test for *in vitro* results.

**RESULTS**

**Effects on Carbachol-Accelerated Mouse Small Intestinal Transit** The small intestinal transit rate of activated charcoal powder was approximately 50% in the normal mouse. However, the small intestinal transit rate increased to approximately 80% after administration of carbachol 1 mg/kg, s.c. Dai-kenchu-to 10—300 mg/kg normalized the accelerated transit induced by carbachol in a dose-dependent manner (Fig. 1). Using a concentration with the compounded rate for Dai-kenchu-to 300 mg/kg, carbachol-accelerated small intestinal transit was significantly improved by dried ginger root 150 mg/kg and ginseng 90 mg/kg, but not by zanthoxylum fruit 60 mg/kg or malt sugar 2400 mg/kg (Fig. 2). The opioid receptor agonist trimebutine 30 mg/kg and atropine 1 mg/kg also improved the accelerated small intestinal transit (Figs. 1, 2), while indomethacin 0.1—1 mg/kg improved the accelerated transit in dose-dependent manner (Fig. 3).

---

**Statistical Analysis** All value are expressed as the mean±S.E. Statistical significance was assessed by Fisher’s PLSD test for *in vivo* results, or by the unpaired Student’s *t*-test for *in vitro* results.

**RESULTS**

**Effects on Carbachol-Accelerated Mouse Small Intestinal Transit** The small intestinal transit rate of activated charcoal powder was approximately 50% in the normal mouse. However, the small intestinal transit rate increased to approximately 80% after administration of carbachol 1 mg/kg, s.c. Dai-kenchu-to 10—300 mg/kg normalized the accelerated transit induced by carbachol in a dose-dependent manner (Fig. 1). Using a concentration with the compounded rate for Dai-kenchu-to 300 mg/kg, carbachol-accelerated small intestinal transit was significantly improved by dried ginger root 150 mg/kg and ginseng 90 mg/kg, but not by zanthoxylum fruit 60 mg/kg or malt sugar 2400 mg/kg (Fig. 2). The opioid receptor agonist trimebutine 30 mg/kg and atropine 1 mg/kg also improved the accelerated small intestinal transit (Figs. 1, 2), while indomethacin 0.1—1 mg/kg improved the accelerated transit in dose-dependent manner (Fig. 3).
Effect on Isolated Guinea Pig Ileum  Under the conditions of the present study, the ESC were completely inhibited by atropine $10^{-7}$ g/ml or TTX $10^{-6}$ g/ml. Dai-kenchu-to $10^{-7} - 3 \times 10^{-5}$ g/ml inhibited ESC with maximal inhibition compared with the control contraction of 16.6% obtained at a concentration of $10^{-5}$ g/ml. However, at $10^{-4}$ g/ml, Dai-kenchu-to enhanced the ESC (Fig. 4). At a concentration of $10^{-7} - 3 \times 10^{-5}$ g/ml, dried ginger root and ginseng also inhibited ESC, but zanthoxylum fruit enhanced the ESC in a dose-related manner. The concentration of malt sugar with the compounded rate for Dai-kenchu-to $10^{-5}$ g/ml was $8 \times 10^{-5}$ g/ml, but malt sugar $3 \times 10^{-5}$ g/ml had no effect on ESC (Fig. 5). Dai-kenchu-to $10^{-4}$ g/ml also inhibited ACh-induced contractions, while dried ginger root and ginseng had no effect (Fig. 6). Dai-kenchu-to or dried ginger root $10^{-3}$ g/ml, respectively, remarkably inhibited KCl-induced contractions (Fig. 7). Eighty percent inhibition of KCl-induced contractions was achieved with Dai-kenchu-to alone. However, this inhibitory effect was significantly reduced to 48% in the presence of CaCl$_2$ $7.5 \times 10^{-3}$ mol/l.

DISCUSSION

Low-frequency electrical stimulation (0.1 Hz) is known to evoke ileal contractions by increasing ACh release from cholinergic nerves, and this is inhibited by trimebutine or cy-
the Ca antagonistic effect of [6]-gingerol may be involved in the inhibitory effect of Dai-kenchu-to at high concentrations. In addition, it has been suggested that there is an inhibitory effect of [6]-gingerol or [6]-shogaol on COX in vivo and in vitro, and that PGs contribute to peristaltic activity via a direct action on the muscle cells and stimulating nerves. Since it was confirmed that PGs were involved in the present models, the inhibition of COX may contribute to the inhibitory effects of dried ginger root on accelerated small intestinal motility.

These results indicate that at low concentrations (3×10^{-5} g/ml or less), Dai-kenchu-to, dried ginger root, and ginseng inhibited ESC via neural factors, but at higher concentration (10^{-3} g/ml), Dai-kenchu-to and dried ginger root inhibited KCl-induced contractions via direct factors influencing the smooth muscle.

The concentrations of Dai-kenchu-to and zanthoxylum fruit that enhanced the ESC corresponded with those that induced ileal contractions in a previous report, confirming the ACh-releasing effect of Dai-kenchu-to and zanthoxylum fruit.

Clinical conditions such as ileus and IBS exhibit various symptoms depending on critical factors. For example, in relation to adhesive ileus, it is known that the intestinal motility is different on the proximal side compared with the distal side at the obstruction. Previous and the present results seem to indicate that Dai-kenchu-to has a dual effect on intestinal motility: enhancement in the normal condition; and normalization in the accelerated condition. The effect of Dai-kenchu-to when intestinal motility was enhanced in the present study, was mainly due to the effects of dried ginger root and ginseng. However, it has been shown that zanthoxylum fruit improves intestinal motility when it is inhibited. Hence the prescription of compounded herbal medicines with various effects is useful from the viewpoint of clinical treatment. Intestinal dysmotility due to dysfunction of the autonomic nervous system is considered one etiology of postoperative ileus and IBS.

In conclusion, Dai-kenchu-to improved the accelerated small intestinal movement induced by stimulation of cholinergic neurotransmission, and dried ginger root and ginseng may be involved in this effect. It is also thought that the mechanism mainly involves the direct inhibition of smooth muscle, but partially involves neural inhibition. Thus Dai-kenchu-to may modulate small intestinal motility by either enhancing or inhibiting intestinal muscle tonus.

REFERENCES

6) Kurosawa S., Nishikawa S., Kaneko M., Nakamura T., Gastroenterol-
1126 Vol. 24, No. 10


