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Abatement of Morphine-Induced Slowing in Gastrointestinal Transit by *Dai-kenchu-to*, a Traditional Japanese Herbal Medicine

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ABSTRACT—As a way of alleviating severe constipation in cancer patients taking morphine to relieve pain, effects of *Dai-kenchu-to* (DKT), a traditional Japanese herbal medicine (*Kampo* medicine), on gastrointestinal transit in mice or on the isolated guinea pig ileum were studied in special reference to morphine. Without altering the anti-nociceptive effect of morphine, DKT was significantly effective against morphine-induced disorder of gastrointestinal transit in mice as assessed by the charcoal meal test for the intestine and measurement of transit time for the colon tract. The results of in vitro studies with guinea pig ileum suggest that abatement of morphine-induced disorder of transit by DKT is caused by both moderate contraction of morphine-treated longitudinal muscle and relaxation of morphine-induced tonic contraction of circular muscle.

Keywords: *Dai-kenchu-to*, Morphine, Constipation

Morphine is the most effective antinociceptive agent known and is used to manage pain experienced by terminal cancer patients. However, it induces severe constipation, causing an obvious reduction in quality of life (QOL) (1). In a previous examination of records of cancer patients at a hospital (Chiba Rosai Hospital, Chiba), we found that 64.9% of patients who take morphine-containing drugs suffer from severe constipation (2). Other side effects were nausea and vomiting (43.9%), impairment of consciousness (less than 20%) and dysuria (less than 3%). Magnesium oxide or sennoside-containing drugs are typically administered for treatment of constipation, but it is difficult to control the dose of these drugs, and these therapies often become intolerable to the patients. Our survey also revealed that 5% of patients who take morphine-containing drugs stopped taking them due to severe constipation and that 54.0% of patients with constipation had to change their laxative because of ineffectiveness (2).

In the present paper, we demonstrate that the Japanese herbal medicine *Dai-kenchu-to* (DKT), which is frequently used to treat gastrointestinal disorders or post-operative ileum, lessens morphine-induced gastrointestinal disorders. There have been many studies of the effect of DKT on gastrointestinal disorders, but no pharmacological studies were performed with regard to morphine-induced constipation (3, 4); herein, we present pharmacological evidence that DKT is applicable for such a disorder.

All animal experiments were carried out according to the Principles of Laboratory Animal Care (NIH publication number 85-23, revised 1985) and Guidelines of the Animal Investigation Committee, Chiba University. Experiments were performed on male ddY mice (5 – 6-week-old; Takasugi Experimental Animals, Saitama) and male Hartley guinea pigs (300 – 450 g, Takasugi Experimental Animals). Animals were maintained on a 12 h light/dark cycle in a temperature-controlled animal colony and had ad libitum access to food and water prior to any procedure. Data were analyzed by the paired *t*-test and multiple-comparison (Dunnett's test) tests. Differences at $P<0.05$ were considered statistically significant.

DKT (gift from Tsumura & Co. (Tokyo), Lot No. 2990100010) is a *Kampo* medicine that is composed of four crude drugs, dried ginger rhizome, ginseng root, rice gluten and Zanthoxylum fruit. In order to evaluate the effect of DKT on morphine-induced gastrointestinal (GI) transit, three other *Kampo* medicines were used as reference medicines: *Sho-kenchu-to* (SKT) (gift from Tsumura & Co., Lot No. 290099010), which has similar indications to DKT, and the ingredients of SKT are ginger rhizome, rice gluten, jujube fruit, cinnamon bark, glycyrrhiza root and peony root; *Keishi-ka-shakuyaku-daio-to* (KSDT)

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(purchased from Tsumura & Co., Lot No. 18020001) which has the same composition as SKT except for rhubarb rhizome; and *Mashi-nin-gan* (MNG) (purchased from Tsumura & Co., Lot No. 18019362), which is composed of peony root, rhubarb rhizome, immature orange, apricot kernel, hemp seed and magnolia bark. Intestinal transit studies were performed following the method of Niijima et al. (5). Mice were starved for 16 h before oral administration of the samples or control. In in vivo experiments, these *Kampo* medicines were suspended in saline and orally administered, and morphine was dissolved in saline and subcutaneously injected at a dose of 2 mg/kg. The dose of morphine used was reported to elicit antinociception and retard GI transit in mice (6). Thirty minutes after oral administration of *Kampo* medicines (500 mg/kg, 0.1 ml /10 g of body weight), the mice were subcutaneously injected with morphine hydrochloride (2 mg/kg). Saline (0.1 ml/10 g of body weight) was administered as the control. One hour after administration of *Kampo* medicines, 5% (w/v) charcoal suspended in water containing 10% (w/v) gum arabic (charcoal meal) was orally administered (0.1 ml/10 g of body weight). Thirty minutes after ingestion of charcoal meal, mice were euthanized by CO_2 asphyxiation and the GI tract from the pylorus to the ileocecum was quickly removed. The transit rate (%) of the length from the pylorus to the tip of the charcoal against the total length of the removed GI tract was measured and expressed as the mean ± S.E.M. of each group (n = 5 – 6). In order to unify the data, transit rate of the vehicle control (transit (cm) / total length (cm) = 17.4 / 42.4) was referred to as 100%. The transit rate of charcoal meal after morphine injection was significantly decreased as compared with the vehicle control. DKT (300 mg/kg, p.o.) markedly recovered intestinal transit from morphine-induced inhibition (from 37.9% to 76.2% vs morphine control group). SKT significantly improved morphine-induced inhibition, but the effect was not as potent as DKT. The effects of MNG and KSDT, which contain rhubarb rhizome, were equivalent to that of DKT. Administration of DKT or MNG alone was also shown to significantly promote transit (Fig. 1A). The preventive effect of DKT against morphine-induced inhibition was seen in a dose-dependent manner (100, 300 and 1000 mg/kg), and was significantly different at 300 and 1000 mg/kg from the morphine control group (Fig. 1B).

Colonic transit was tested following the method of Broccardo et al. (7). Mice were fasted 16 h before administration of *Kampo* medicines. Morphine (2 mg/kg) was subcutaneously injected 30 min before administration of *Kampo* medicines. One hour after administration of *Kampo* medicines, glass beads (3 mm in diameter) were pushed 3 cm into the rectum, and the time to bead excretion was measured. In the colonic transit test, administration of each *Kampo* medicine showed no significant difference from the vehicle control. Morphine (2 mg/kg, s.c.) delayed glass bead excretion 4.9-fold (2373 s) slower than the vehicle control (482 s). In morphine-treated mice, excretion was 2.3-fold (1091 s) slower than vehicle control group after administration of DKT. The excretion time after DKT treatment was significantly different from the morphine-treated control and occurred in a dose-dependent manner (100, 300, 1000 mg/kg, p.o., Fig. 1D). In contrast, SKT, MNG and KSDT showed no significant effect against morphine-induced inhibition of excretion rate (Fig. 1C).

The effect of DKT on morphine-induced anti-nociception was studied by the formalin test and acetic acid-induced writhing test. In the writhing test, DKT did not significantly alter the anti-nociceptive effects of morphine (data not shown). Likewise, DKT did not change the anti-nociceptive effect of morphine in the first and second phases of the formalin test (Fig. 2).

To determine the effect of DKT on the basal or morphine-treated tone of the intestinal smooth muscle, measurement of contractile force was performed on guinea pig ileum by the Magnus method. Guinea pigs were euthanized with CO_2 and after laparotomy, the ileum was excised. Ileal segments (approximately 10 – 15 mm) were mounted longitudinally in an organ bath containing 32°C Krebs-Henseleit solution (112 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, 2.0 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 25 mM $NaHCO_3$ and 11.5 mM glucose) gassed with O_2/CO_2 (19:1). Resting tension on the segments was adjusted to a load of 1.0 g, and then the segments were allowed to equilibrate for at least 60 min. Isotonic contraction was measured with an isotonic transducer connected to an amplifier. Ileal longitudinal muscle responded to DKT (10^{-3} g/ml) with moderate transient contractions (Fig. 3A) and the effects were observed in a dose-dependent manner from 10^{-4} to 10^{-3} g/ml DKT (Fig. 3B). Contractions induced by 10^{-3} g/ml DKT were inhibited by addition of 1 μM morphine; however, application of 1 μM morphine alone did not influence longitudinal muscle contraction

Circular muscle (approximately 5-mm width) was excised from the same area as longitudinal muscle. Isometric contraction of circular muscle was measured with an isometric transducer connected to an amplifier under 0.2-g tension in an organ bath filled with oxygenated (O_2/CO_2 (19/1)) 32°C-Krebs-Henseleit solution. After equilibration for at least 60 min, contraction tests were performed. DKT alone did not affect the basal tone of circular muscle, while 1 μM morphine alone raised the basal tone, indicating a lasting tonic contraction. The morphine-induced tonic contraction was completely attenuated by addition of 10^{-3} g/ml of DKT (Fig. 3C).

The present study has shown that DKT relieves morphine-induced delay of GI transit without affecting the

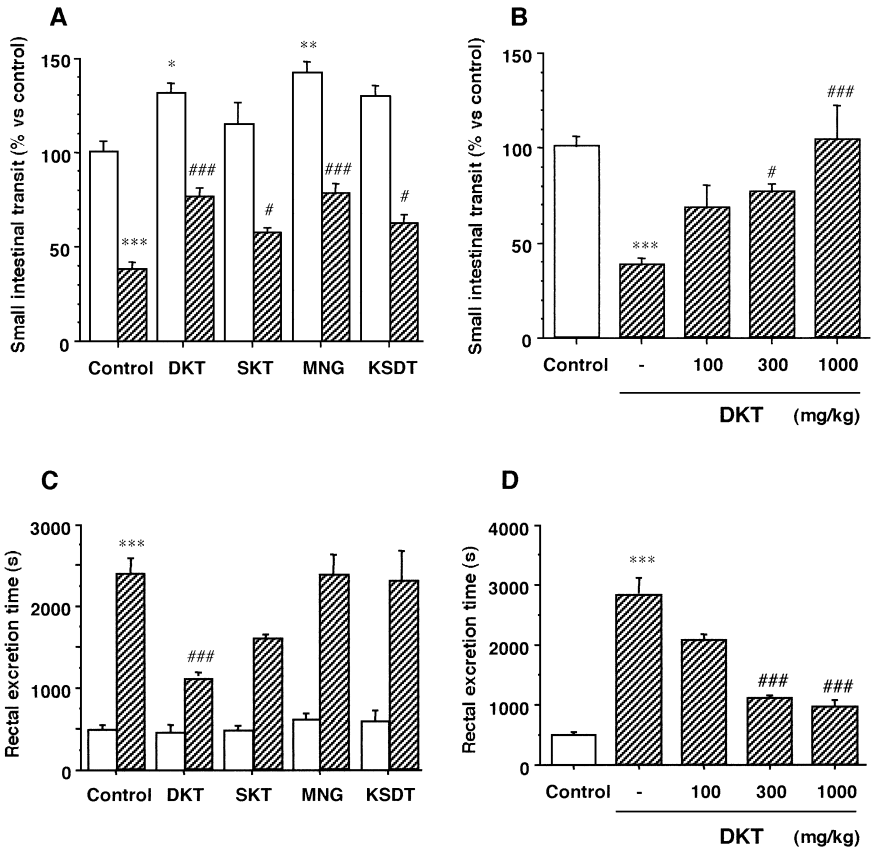

Fig. 1. Effects of *Kampo* medicines on small intestinal transit of charcoal meal and rectal excretion of glass beads in mice. A and C: Comparison between *Kampo* medicines (500 mg/kg, p.o.), *Dai-kenchu-to* (DKT), *Sho-kenchu-to* (SKT), *Mashi-nin-gan* (MNG) and *Keishi-ka-shakuyaku-daio-to* (KSDT). B and D: Dose-dependency of DKT. Each value (open bar, vehicle or *Kampo* medicines; striped bar, addition of morphine) represents the mean ± S.E.M. (n = 6). **P*<0.05, ***P*<0.01 and ****P*<0.001, significantly different from the vehicle control group. $^{\#}P$<0.05, $^{\#\#\#}P$<0.001, significantly different from the morphine control group.

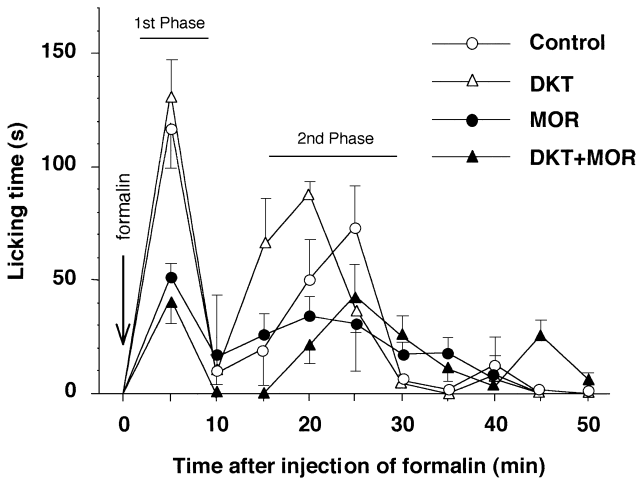

Fig. 2. Effect of *Dai-kenchu-to* (DKT) on anti-nociception of morphine in the formalin test in mice. DKT (500 mg/kg, p.o.) and morphine (2 mg/kg, i.p.) were administered 40 and 20 min before injection of 1%-formalin (20 μl), respectively.

antinociceptive effects of morphine. Furthermore, DKT causes moderate contraction of morphine-treated ileal longitudinal muscle and relaxation of morphine-induced contractions of ileal circular muscle. These effects of DKT on isolated intestine provide a pharmacological basis for recovery from morphine-induced disorder of GI transit. It has been recently demonstrated that GI transit may be coordinated by relaxation of the circular muscle and constriction of the longitudinal muscle through several neuronal networks including serotonin receptors (8, 9). This supports the previous work of Grider and Makhlouf who showed that morphine slows GI transit by inhibiting relaxation of the circular muscle and contraction of the longitudinal muscle (10).

DKT obviously showed improving effects against morphine-induced inhibition of intestinal and colonic transits, differently from three *Kampo* medicines of SKT, MNG and KSDT. The differential effects of DKT may be explained by crude drugs contained in DKT. Zanthoxylum fruit and ginseng root were contained in the three *Kampo* medicines SKT, MNG and KSDT. In the literature, however, there

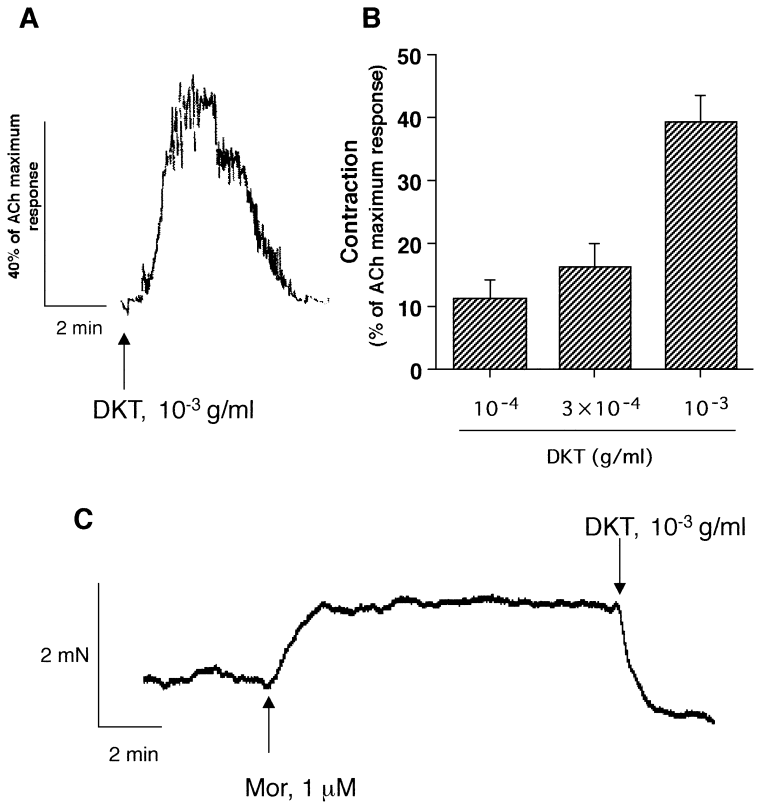

Fig. 3. Effects of *Dai-kenchu-to* (DKT) on morphine-induced contractile activity in guinea pig ileum. A: Typical recording of DKT-induced contraction of longitudinal muscle and B: the dose-dependency. C: Typical recording of tonic contraction of circular muscle by morphine and the complete relaxation by DKT.

was no report that showed the acceleration of GI transit by ginseng root. In contrast, zanthoxylum fruit (*Zanthoxylum piperitum*) and its components such as hydroxy-β-sanshool and γ-sanshool have been shown to induce contraction of GI tract in guinea pig probably through mechanisms causing acetylcholine release from intrinsic cholinergic nerves and tachykinin release from sensory neurons. Accordingly, it is postulated that the accelerating effects of DKT on the rates of intestinal and colonic transits in morphine-treated mice would largely result from those of zanthoxylum fruit. The effect of hydroxy-β-sanshool was significantly inhibited by the capsaicin receptor antagonist, capsazepine (11). Another DKT component, (6)-shogaol, isolated from ginger, is reported to act on gastrointestinal motor neurons and facilitate an intestinal transit of charcoal after oral administration (12). Moreover, Onogi et al. have suggested that (6)-shogaol exhibits a capsaicin-like effect on the terminals of primary afferent nerves containing substance P, causing the initial release of neuropeptides and finally depleting the contents of neuropeptides, by subsequent stimulation of the primary afferents (13). Furthermore, Shibata et al. have reported that intragastric capsaicin stimulates colonic motility via a neural reflex (14).

Our results, together with findings from the above reports, indicate that the neuronal mechanism by which DKT relieves morphine-induced constipation is at least partly associated with stimulation of serotonin receptors (9) and vanilloid receptors (14) by components of DKT such as (6)-shogaol and hydroxy-β-sanshool. However, further studies are necessary to elucidate the relaxatory mechanism of DKT in morphine-induced tonic contraction.

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