Effects of Dai-kenchu-to on Intestinal Obstruction Following Laparotomy

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Abstract

To confirm the usefulness of Dai-kenchu-to for intestinal obstruction, investigation of the effects of Dai-kenchu-to on postoperative intestinal adhesion was conducted. Repeated administrations of Dai-kenchu-to (100 or 300 mg/kg) significantly inhibited the formation of intestinal obstruction. Motor disturbance and inflammation are thought to be involved in the etiology of intestinal adhesion. A single treatment of Dai-kenchu-to (300 mg/kg) significantly reduce intestinal transit time in postoperative ileus and chemically-induced ileus. Dai-kenchu-to (10^-4 g/ml) significantly inhibited COX-2 activity.

These results suggest that Dai-kenchu-to prevents postoperative intestinal adhesion by gastroprokinetic and anti-inflammatic effects. Dai-kenchu-to thus demonstrates positive effect on postoperative ileus.

Key words: Dai-kenchu-to, ileus, intestinal adhesion, COX activity

Introduction

Intestinal obstruction subsequent to laparotomy (postoperative ileus) still remains the most common postoperative complication. Postoperative ileus is treated by decompression using an ileus tube, transfusion or gastroprokinetics. The therapeutical effects of these methods are not always satisfactory and other complementary treatment should be developed. Various pharmacotherapies are available (Davidson et al., 1979; Edward and Edward, 1990; Jepsen et al., 1986), and Dai-kenchu-to has been shown useful for treatment of postoperative ileus (Seki, 1987). Dai-kenchu-to is a herbal medicine used to treat patients suffering from reduced strength, frigidity in the extremities, soma flatulence and peristalsis anxiety. It was thus considered that this drug might possibly be useful for treating postoperative ileus. In this study, evaluation of the drug for intestinal obstruction was made based on its capacity to prevent intestinal adhesion with intestinal obstruction.
Methods

Animals
Intestinal obstruction was induced in 6 to 7-week-old male SD rats (Charles River Japan Inc., Tsukuba, Japan) and a 4-week-old male ICR mice (Charles River Japan, Atsugi, Japan). The animals were housed at 23±2°C, 55±10% humidity, 12 hr light-dark cycle (7:00-19:00 lights on). They were deprived of food for 18 hr prior to the experiments but had free access to tap water.

Drugs
Powdered extracts of the following preparations (Tsumura & Co., Tokyo, Japan) were used: Dai-kenchu-to (Lot No. 250100020). The powder of Dai-kenchu-to was extracted from a mixture of Zanthoxyli Fructus (component ratio=2), Ginseng Radix (3) and Zingiberis Siccatum Rhizoma (5). Dai-kenchu-to was obtained by mixing the powdered extract with Saccharum Granorum at 1:8 ratio. Dai-kenchu-to at concentration identical to that of the powdered extract of Dai-kenchu-to was used. Dai-kenchu-to was dissolved in distilled water. Cisapride was prepared by purifying Acenalin® Fine Granules (Kyowa Hakko Kogyo Co., ltd., Tokyo, Japan) at Tsumura Central Institute. Indomethacin and carbachol (SIGMA, St. Louis, MO, USA) were used. Indomethacin was suspended in 1% Tween 80 solution or dimethyl sulfoxide (DMSO), cisapride in 0.5% carboxymethyl cellulose sodium solution and carbachol was dissolved in distilled water.

(I) Effects of Dai-kenchu-to on the ileus

1. Intestinal adhesion induced by talc
   This parameter was determined using a modified method of Kamffer et al. (1992). Laparotomy was conducted on 6-week-old SD rats under ether anesthesia, exposing the small intestine, and 50 mg talc was uniformly dispersed over the area of exposure. After replacing small intestine, the wound was closed. Four days later, the abdomen was reopened and adhesion was evaluated based on the standard presented in Table 1. Test drugs were orally administered twice a day for 3 days before and after surgery. On the day of surgery, the drugs were intragastically administered once immediately following the surgery.

2. Postoperative ileus
Seven-week-old SD rats were anesthetized by intravenous injections of sodium thiopental (30 mg/kg) following the method of Pairet and Ruckebusch (1989). Surgical trauma was

| Score | Adhesive area | Strength |
|-------|---------------|----------|
| 0     | none          | —        |
| 1     | small         | slight   |
| 2     | 1/3           | slight   |
| 3     | 1/3           | moderate |
| 4     | 2/3           | moderate |
| 5     | 2/3           | severe   |

Table 1. Adhesion scoring system
caused by laparotomy and mobilization of the caecum from the retroperitoneum for 10 min. 
After its replacement, the wound was closed. Fifteen minutes later, the charcoal marker (an 
aqueous suspension of 5% gum arabic and 10% carbon black) was intragastrically administered 
at 1 ml per animal. The gastrointestinal transit, measured 20 min later, was expressed as the 
percentage of length of the small intestine covered by the front of the charcoal marker. 
Control animals were treated in the same way without abdominal surgery, and received test 
meal at the same time following anesthesia. The test drugs or vehicles were given orally 15 
min prior to anesthesia.

3. Chemically-induced ileus

Four-week-old male ICR mice were used at 15 h-fast. Using a modified method of Pairet 
and Ruckebusch (1989), peritoneal irritation was obtained by the intraperitoneal administration 
of 0.45% acetic acid. The charcoal marker was given orally (0.4 ml per mouse) 30 min after the 
intraperitoneal injection of acetic acid (0.2 ml per mouse). The mice were killed by cervical 
dislocation 20 min after the charcoal marker. Gastrointestinal transit was expressed as above. 
Tested drugs or vehicles were administered orally 15 min before peritoneal irritation.

(II) Effects of Dai-kenchu-to on cyclooxygenase (COX) activity

The effect of Dai-kenchu-to on COX activity was assessed using a slightly modified method 
of Futaki et al. (1994). One unit COX-1 or COX-2 was dissolved in 0.3 ml of 100 mM Tris–HCl 
buffer (pH 8.0) containing hematin (final concentration : 1 μM) and phenol (final concentration : 
2 mM). The mixture was preincubated with drug for 2 min at 37°C, and then arachidonic acid 
(at a final concentration of 100 μM) was added to a total volume of 0.5 ml. The mixture was 
incubated again for 2 min at 37°C. Two milliliters of n-hexane/ethyl acetate (2:1, v/v) was 
added to terminate the reactions. PGE2 was then extracted by centrifuging the preparation at 
3,000 rpm for 10 min. The aqueous phase was frozen, and the organic solvent phase was 
discarded. The aqueous phase was extracted again, 1 ml of ethanol was added, and the 
preparation was centrifuged for additional 10 min at 3,000 rpm. The supernatant was collected 
and subjected to vacuum distillation. PGE2 in the residue was quantified using an EIA kit 
(Cayman Chemical Company, Ann Arbor, MI, USA). Absorbance at 405 nm was measured 
using a thermomax microplate reader (Molecular Device Corporation, USA). Each test drug 
was dissolved in dimethyl sulfoxide (final concentration : 1%).

Statistical analysis

All data were expressed as means±S.E.M. Statistical significance was assessed by Mann– 
Whitney’s U test in the animal model with intestinal adhesion induced by talc. Fisher’s PLSD 
was used to evaluate statistical significance in all other experiments. A p<0.05 was consid 
ered significant.

Results

(I) Effects of Dai-kenchu-to on the ileus
1. Effects of Dai-kenchu-to on intestinal adhesion induced by talc
Fig. 1. Photographs showing intestinal adhesion of rats in the control (A) and Dai-kenchu-to 300 mg/kg (B), groups.

Fig. 2. Preventive effect of Dai-kenchu-to on talc-induced intestinal adhesion in rats. Test drugs were orally administered twice a day for 3 days before and after surgery. Each column represents the mean±S.E.M. of 6 to 7 rats. (*) significantly different from the control at $p<0.05$.

Fig. 1 shows representative effects of Dai-kenchu-to on intestinal adhesion.
Dai-kenchu-to (100 or 300 mg/kg) significantly improved adhesion dose-dependently (Fig. 2). Indomethacin (0.1 or 0.3 mg/kg) significantly improved adhesion, but indomethacin (1 mg/
kg) showed no significant effect (Fig. 3A). Continuous pretreatment with indomethacin (3 mg/kg) itself induced intestinal adhesion, and thus its effects (3 mg/kg) could not be evaluated at this level (data not shown). Cisapride (0.3 to 3.0 mg/kg) did not improve adhesion (Fig. 3B).

2. Effects of Dai-kenchu-to on postoperative ileus

Intestinal transit time was significantly reduced in the control group. Dai-kenchu-to (300 mg/kg) as well as carbachol (0.1 mg/kg) significantly improved intestinal transit (Fig. 4).

3. Effects of Dai-kenchu-to on chemically-induced ileus

Intestinal transit was significantly reduced by inflammation induced by 0.45% acetic acid.

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**Fig. 3.** Preventive effect of indomethacin and cisapride on talc-induced intestinal adhesion in rats. Test drugs were orally administered twice a day for 3 days before and after surgery. Each column represents the mean ± S.E.M. of 6 to 8 rats. (*) significantly different from the control at p < 0.05.

**Fig. 4.** Protective effects of Dai-kenchu-to on the inhibition of gastrointestinal transit due to laparotomy in rats. Transit is expressed as the percentage of the length of the small intestine covered by the charcoal maker within 20 min following its oral administration. Each column represents the mean ± S.E.M. of 10 rats. (*) and (**) significantly different from the control at p < 0.05 and p < 0.01, respectively.

IND: indomethacin, CCh: carbachol.
Fig. 5. Protective effects of Dai-kenchu-to on the inhibition of gastrointestinal transit due to acetic acid in mouse. Transit is expressed as the percentage of the length of the small intestine covered by the charcoal maker within 20 min following its oral administration. Each column represents the mean±S.E.M. of 6 to 21 mice. (*) and (**) significantly different from the control at p<0.05 and p<0.01, respectively. IND: indomethacin, CCh: carbachol.

Dai-kenchu-to (300 mg/kg) significantly improved intestinal transit time as also noted for indomethacin (1 mg/kg) and carbachol (0.1 mg/kg) (Fig. 5).

(II) Effects of Dai-kenchu-to on COX activity

Dai-kenchu-to did not inhibit COX-1 activity while COX-2 activity was inhibited at 10^{-4} g/ml. Indomethacin (10^{-6} g/ml) significantly inhibited both COX-1 and COX-2 activity (Fig. 6).

Discussion

Diminished spontaneous contraction brought on by hyperactivity of the sympathetic
nervous system due to laparotomy or intestinal displacement and dryness (Sagrada et al., 1987; Tanila et al., 1993) and inflammatory reaction in tissue induced by laparotomy are considered aspects of the etiology of intestinal adhesion. Dai-kenchu-to dose-dependently improved adhesion in animals. Even though indomethacin (0.1 and 0.3 mg/kg) shows preventive effect, this drug (3 mg/kg) induced intestinal mucosal disorders with consequent intestinal adhesion. Thus, although this drug prevents general adhesion, it may possibly exacerbate intestinal adhesion. Cisapride showed no significant preventive effect in this study.

To clarify the mechanism of Dai-kenchu-to for preventing intestinal adhesion, its effects on animals with different etiologies of intestinal adhesion such as motor disturbance and inflammatory intestinal obstruction were studied. The drug was found to improve intestinal transit in all cases. The inducement of spontaneous contraction and the anti-inflammatory action by this drug should thus follow from its preventive effect on intestinal adhesion. In intestinal adhesion, indomethacin demonstrated preventive effect in this study, therefore we examined the effect of Dai-kenchu-to on COX activity. Dai-kenchu-to did not inhibit COX-1 activity, while COX-2 activity was inhibited at $10^{-4}$ g/ml dose. From these results, Dai-kenchu-to may have the anti-inflammatory effect on intestinal abstraction. Dai-kenchu-to significantly stimulates COX-1 activity, and Futaki et al. (1994) reported that NS-398, selective COX-2 inhibitor, also stimulates COX-1 activity. Although further investigation is necessary, it is likely that COX-2 inhibitor stimulates COX-1 activity in the present study.

Dai-kenchu-to has been shown to significantly increase spontaneous contraction in fasted dogs and in dogs immediately after feeding (Furukawa et al., 1995; Shibata et al., 1998) and cause resected colon to contract in guinea pig (Kurosawa et al., 1997).

The present study confirms the therapeutic effect of Dai-kenchu-to on intestinal obstruction. Stimulation of spontaneous contraction and anti-inflammatory action may thus be considered effects of this drug.

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