Pharmacological Studies of the Effect of Dai-kenchu-to on Spontaneous Contraction of Isolated Rabbit Jejunum

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Abstract

We studied the effects of Dai-kenchu-to on the spontaneous contraction in isolated rabbit jejunum. Dai-kenchu-to (10^-3 g/ml) increased jejunal contraction, such as phasic-like contraction and contractile amplitude. Zanthoxyli Fructus (2×10^-4 g/ml) exhibited an action identical to that of Dai-kenchu-to. While Zingiberis Siccata Rhizoma (5×10^-4 g/ml) continuously decreased the amplitude of contraction. Ginseng Radix (3×10^-4 g/ml) and Saccharum Granorum (8×10^-3 g/ml) had no effect on spontaneous contraction. Dai-kenchu-to and Zanthoxyli Fructus reversed the decrease of contraction produced by atropine. However, phasic-like contraction induced in the absence of atropine was antagonized by atropine. Dai-kenchu-to and Zingiberis Siccata Rhizoma further decreased spontaneous contraction in the presence of tetrodotoxin.

It was clarified that Dai-kenchu-to possesses gastrokinetic effect, and Zanthoxyli Fructus mainly contributed to this effect. It was suggested that the cholinergic and non-cholinergic nervous systems were involved in increasing intestinal motility. It was also suggested that Dai-kenchu-to acted on multiple points of the intestine, and actions at these points might intensify to improve ileus.

Key words: Dai-kenchu-to, ileus, jejunum, spontaneous contraction

Introduction

Intestinal obstruction subsequent to laparotomy (postoperative ileus) still remains the most common postoperative complication. 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). Our previous report described the usefulness of Dai-kenchu-to on an animal model with intestinal adhesion (Hayakawa et al., 1999). Concerning the pharmacological action of Dai-kenchu-to, several interesting reports confirm that Dai-kenchu-to has been found to significantly increase gastrointestinal motility in conscious dogs (Furukawa et al., 1995; Shibata et al., 1998) and contract distal colon in the...
guinea pig (Kurosawa, 1997). Thus, the clinical usefulness of Dai-kenchu-to has been supported by multiple aspects. However, many issues regarding improvement of intestinal obstruction using Dai-kenchu-to remain unclear. Moreover, since the results of our previous study were not sufficient to explain the adhesive ileus-improving action of Dai-kenchu-to, further detailed evaluation of the mode of action is required.

In this study, we seek to clarify the mechanism of gastroprokinetic effect of Dai-kenchu-to and the action of crude drug component on the spontaneous contraction in isolated rabbit jejunum.

**Materials and Methods**

**Animals**

Male J.W. rabbits (Nippon SLC Inc., Shizuoka, Japan) were used. The animals were housed at 23±2°C, 55±10% humidity, 12 hr light–dark cycle (7:00-19:00 light 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 Chinese preparations and crude drug components (Tsumura & Co., Tokyo, Japan) were used: Dai-kenchu-to (Lot No. 250100020), Zanthoxyl Fructus (Lot No. 14100240), Ginseng Radix (Lot No. 911015001P), and Zingiberis Siccatum Rhizoma (Lot No. 241067010). Saccharum Granorum (Lot No. 1410064) (Tsumura & Co., Tokyo, Japan) was also used. The powder of Dai-kenchu-to was extracted from a mixture of Zanthoxyl 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. Concentrations of powdered crude drug component extracts were the same as those in the Dai-kenchu-to preparation. Each extract was dissolved in distilled water. Indomethacin, atropine sulfate and tetrodotoxin (SIGMA, St. Louis, MO, USA) were used. Indomethacin was dissolved in dimethyl sulfoxide (DMSO). Atropine and tetrodotoxin were dissolved in distilled water.

**Effects of Dai-kenchu-to on the spontaneous contraction in isolated rabbit jejunum.**

The rabbits were fasted overnight and sacrificed by a blow to the head. After laparotomy, strips were removed from the jejunum and placed in oxygenated (95% O2-5% CO2) tyrode solution (NaCl: 137.9 mM, KCl: 2.7 mM, CaCl2: 1.8 mM, MgCl2: 0.5 mM, NaHPO4: 1.1 mM, NaHCO3: 11.9 mM, glucose 5.6 mM) at 37°C. They (approximately 2 cm) were then mounted in a 20 ml organ bath filled with tyrode solution and set up for isotonic tension recording under 1 g applied tension. During an equilibration period of 30 minutes the preparations showed regular spontaneous contraction. Test drugs were added to the organ bath and the spontaneous contraction was recorded by an isotonic transducer for 15 minutes. Mean contraction amplitude for 5 minutes before drug administration was taken as 100% contraction. Variation in contraction amplitude was determined at 5-minute intervals. Atropine and tetrodotoxin
were administered 5 minutes before administration the test drugs.

Statistical analysis

All data were expressed as means±S.E.M. Statistical significance was assessed by Fisher's PLSD. A p<0.05 was considered significant.

Results

Effects of Dai-kenchu-to on rabbit jejunum.

1. Effects of Dai-kenchu-to on spontaneous contraction in isolated rabbit jejunum

Fig. 1 shows typical spontaneous contraction.

Dai-kenchu-to (10^-3 g/ml) induced phasic-like contraction. Subsequently, the compound continuously increased the amplitude of contraction (Fig.1A). Thus, contraction induced by Dai-kenchu-to suggested the involvement of two distinct components. Zanthoxyli Fructus (2×10^-4 g/ml) induced weak phasic-like contraction and subsequently continuously increased the amplitude of contraction (Fig.1B). Ginseng Radix (3×10^-4 g/ml) induced weak transient contraction (Fig.1C). Zingiberis Siccatum Rhizoma (5×10^-4 g/ml) continuously suppressed the amplitude of contraction (Fig.1D). Saccharum Granorum (8×10^-3 g/ml) had no effect on spontaneous contraction (Fig.1E). Dai-kenchu-to produced a dose-dependent increase of

Fig. 1. A typical effect of Dai-kenchu-to and its crude drug components on the spontaneous contraction of isolated jejunum in the rabbit.
Fig. 2. Effect of Dai-kenchu-to (A) and its crude drug components (B) on the spontaneous contraction of isolated jejunum in the rabbit. Each point is expressed as mean±S.E.M. of 4 to 6 experiments. (*) and (**) significantly different from the vehicle at p<0.05 and p<0.01, respectively.

(A) ○: vehicle, ●: Dai-kenchu-to 10^-3 g/ml, □: Dai-kenchu-to 3×10^-4 g/ml, ■: Dai-kenchu-to 10^-4 g/ml, △: Dai-kenchu-to 10^-5 g/ml, ♦: Dai-kenchu-to 10^-6 g/ml

(B) ○: vehicle, ●: Dai-kenchu-to 10^-3 g/ml, □: Zanthoxyli Fructus 2×10^-4 g/ml, ■: Ginseng Radix 3×10^-1 g/ml, △: Zingiberis Siccatum Rhizoma 5×10^-1 g/ml, ♦: Saccharum Granorum 2.4 g/ml

spontaneous contraction (Fig. 2A). Dai-kenchu-to and Zanthoxyli Fructus increased the spontaneous contraction whereas Zingiberis Siccatum Rhizoma suppressed spontaneous contraction (Fig. 2B).

2. Effects of Dai-kenchu-to on spontaneous contraction following atropine administration

Atropine (10^-7 g/ml) continuously decreased the amplitude of contraction, thus showing its inhibitory effect on spontaneous contractions. Dai-kenchu-to and Zanthoxyli Fructus reversed the decrease of atropine-induced contraction. But these drugs also demonstrated that phasic-like contraction induced in the absence of atropine was antagonized by atropine. Zingiberis Siccatum Rhizoma (5×10^-4 g/ml) and Saccharum Granorum (8×10^-3 g/ml) continuously further decreased the amplitude of contraction in the presence of atropine (Fig. 3A).

3. Effects of Dai-kenchu-to on spontaneous contraction following tetrodotoxin administration

Tetrodotoxin (10^-6 g/ml) slightly decreased the amplitude of contraction. Dai-kenchu-to (10^-3 g/ml) and Zingiberis Siccatum Rhizoma (5×10^-4 g/ml) further decreased intestinal motor activity. The other crude drug components had no effect on spontaneous contraction in the presence of tetrodotoxin (Fig. 3B). Indomethacin also suppressed spontaneous contraction and it further decreased contraction in the presence of atropine or tetrodotoxin (Fig. 4).

Discussion

Postoperative intestinal obstruction (postoperative ileus) is considered to be partly caused by incomplete intestinal motility resulting from hyperactivity of the sympathetic nervous system after laparotomy (Jacques et al., 1997; Sagrada et al., 1987). Therefore, gastropro-
Dai-kenchu-to and spontaneous contraction of isolated rabbit jejunum

Fig. 3. Effect of Dai-kenchu-to and its crude drug components on the spontaneous contraction of isolated jejunum in the rabbit in the presence of atropine (A) and tetrodotoxin (B). Each point is expressed as mean±S.E.M. of 4 experiments.

- ●: Dai-kenchu-to 10⁻³ g/ml
- □: Zanthoxyli Fructus 2×10⁻⁴ g/ml
- ■: Ginseng Radix 3×10⁻⁴ g/ml
- △: Zingiberis Siccatum Rhizoma 5×10⁻⁴ g/ml
- ▲: Saccharum Granorum 2.4 g/ml

Fig. 4. Effect of indomethacin (10⁻⁷ g/ml) on the spontaneous contraction of isolated jejunum in the rabbit in the presence of atropine and tetrodotoxin. Each point is expressed as mean±S.E.M. of 4 experiments.

- ○: atropine
- ●: tetrodotoxin

kinetics are used to treat postoperative intestinal obstruction. However, the therapeutic results are not always satisfactory. Since the clinical usefulness of Dai-kenchu-to has been reported, this drug has received attention from numerous investigators. However, the gastroprokinetic effect alone has been reported as the pharmacological action of Dai-kenchu-to, and many issues involved in improvement of ileus still remain unclear. Therefore, to evaluate the ileus-improving effect of Dai-kenchu-to, we evaluated the effect of Dai-kenchu-to on the spontaneous contraction in isolated rabbit jejunum. Furthermore, we described the respective
roles of various crude drug components.

Dai-kenchu-to induced phasic-like contraction and continuously increased contractile amplitude. Zanthoxyli Fructus showed the effect similar to that of Dai-kenchu-to. Therefore, it was thought that Zanthoxyli Fructus was mainly responsible for the effect of Dai-kenchu-to. Zingiberis Siccatum Rhizoma on the other hand continuously decreased contractile amplitude. How the inhibitory effect of Zingiberis Siccatum Rhizoma in improvement of ileus still remains unclear. Improvement of ileus after administration of gastroprokinetics alone was slight in the animal model and clinical trials. Therefore, it was considered that the inhibitory effect of Zingiberis Siccatum Rhizoma might contribute to ileus improvement.

Spontaneous contraction is myogenic, yet it is partially regulated by the cholinergic nervous system of the myenteric plexus (Gonzalez and Morales-Aguilera, 1985). In the present study, atropine decreased contractile amplitude. Dai-kenchu-to and Zanthoxyli Fructus reversed decreased amplitude of contraction by atropine. However, these drugs induced phasic-like contraction were antagonized by atropine. In the presence of tetrodotoxin, Dai-kenchu-to further decreased spontaneous contraction, while Zanthoxyli Fructus did not influence spontaneous contraction. These results suggest that the phasic-like contraction might be induced via the cholinergic nervous system, while increased contractile amplitude might be induced via the atropine-resistant nervous system. Dai-kenchu-to and Zanthoxyli Fructus augment acetylcholine release from the myenteric plexus of guinea pig ileum and colon (Kurosawa, 1998). A similar effect is thought to be involved in the induced phasic-like contraction of Dai-kenchu-to. Since it was reported that non-adrenergic and non-cholinergic neurons seem to play an important role in regulation of intestinal motility (Leander et al., 1981; Abrahamsson, 1986), increase of contraction on amplitude by Dai-kenchu-to might be related to these nervous systems. Dai-kenchu-to further decreased spontaneous contraction in the presence of tetrodotoxin. The inhibitory effect of Zingiberis Siccatum Rhizoma might be involved in this process.

The inhibitory effect of Zingiberis Siccatum Rhizoma on contraction was shown in the presence of atropine or tetrodotoxin, and thus may derive from some action other than that of the nervous system. Spontaneous contraction was suppressed with inhibition of COX activity. Indomethacin suppresses contraction by inhibiting COX activity (Northover, 1971; Burleigh, 1977). Indomethacin decreased spontaneous contraction and it further decreased contraction in the presence of atropine or tetrodotoxin in this study. Furthermore, preliminary evaluations demonstrated that spontaneous contraction was inhibited by selective COX-2 inhibitors. Our previous report clarified that Dai-kenchu-to inhibited COX-2 activity. Therefore, it was speculated that the inhibitory effect was due partly to the COX activity.

Although it remained unclear how the inhibitory effect of Zingiberis Siccatum Rhizoma was related to the ileus improvement action, the possibility of a correlation cannot be ruled out.

In conclusion, Dai-kenchu-to has shown gastroprokinetic effect, and Zanthoxyli Fructus contributed to this action. The gastroprokinetic effect of Dai-kenchu-to and Zanthoxyli Fructus was partly induced via the cholinergic nervous system. However, it was clarified that Zingiberis Siccatum Rhizoma inhibited intestinal motility. Meanwhile, inhibitory effect of Zingiberis Siccatum Rhizoma may be involved in COX-2 inhibition. Therefore, Dai-kenchu-to
acts on multiple points in the intestine, and actions at these points might intensify to improve ileus.

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**References**


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