Effects of Shojoki-to (Xiao-Cheng-Qi-Tang) on colonic circular muscle motility in conscious rat: Role of immature orange and magnolia bark in the prescription

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Abstract

This study was performed to clarify the purgative activity and effect of Shojoki-to on rat colonic circular muscle motility by means of chronically implanted force transducers in conscious rats. Also examined was the composition of Shojoki-to, i.e., the significance of combining rhubarb, immature orange and magnolia bark for its preparation. Rhubarb, a laxative, is known to affect large intestinal motility. It significantly decreased the number of contractile waves and increased the percentages of high-amplitude contractions and low-amplitude contractions to the total contractions of rat colonic circular muscle at the onset of diarrhoea.

In Shojoki-to, the purgative activity of rhubarb was significantly potentiated. The preparation also significantly prevented some rhubarb-induced effects on the colonic motility such as a decrease in the number of contractile waves, and increased the high-amplitude contractions in comparison with rhubarb alone after the onset of diarrhoea. Combined administration of either immature orange or magnolia bark with rhubarb did not reproduce the effect of Shojoki-to on colonic circular muscle motility.

We concluded that in Shojoki-to, immature orange and magnolia bark activate colonic muscle motility, consequently preventing the rhubarb-induced disturbance of the regular spiking activity of colonic circular muscle, such as a decrease in the number of contractile waves. These effects may contribute to its potentiating effect on the purgative activity of rhubarb.

Key words Shojoki-to (Xiao-Cheng-Qi-Tang), immature orange, magnolia bark, rhubarb, rat, colonic circular muscle motility.

Abbreviations HAC, high-amplitude contraction; LAC, low-amplitude contraction; Shojoki-to (Xiao-Cheng-Qi-Tang), 小承気湯.

Introduction

The Chinese traditional prescription Shojoki-to (Xiao-Cheng-Qi-Tang), composed of rhubarb, immature orange and magnolia bark, has been used for thousands of years for the treatment of constipation with a sensation of fullness in the abdomen.

Rhubarb, described in many traditional herbal medicine textbooks, is one of the most popular laxatives. It is known to affect large intestinal motility and water movement. Miyawaki *et al.*[1] showed that rhubarb accelerated large intestinal propulsion in rats. Recently we found that rhubarb at a dose of 500 mg/kg disturbed colonic regular spiking activity and significantly increased the percentages of high-amplitude contraction (HAC) and low-amplitude contraction (LAC) to the total contractions at the onset of diarrhoea; we used chronically implanted force transducers in conscious rats. In Daio-kanzo-to (a combination of rhubarb and glycyrrhiza 4:1), glycyrrhiza prevents the rhubarb-induced appearance of the irregular spiking activity of the colonic circular muscle and consequently may moderate abdominal pain at the onset of diarrhoea.[2]

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Immature orange has been used for the treatment of stomach disorders and is frequently combined in prescriptions regarded as laxatives in traditional medicine. One of the constituents of immature orange, naringin, was found to significantly inhibit small intestinal transit in mice.[3] Marmin and nobiletin have been shown to significantly inhibit gastric motor activity in conscious rats and to exhibit concentration-dependent relaxations of contractions induced by acetylcholine, transmural electrical stimulation and histamine in isolated guinea pig ileum.[4]

Magnolia bark is an important component of crude drug prescriptions in traditional medicine. It has been used for treatment of gastrointestinal disturbance, acute pain, diarrhoea, coughs and anxiety. Magnolol and honokiol, the constituents of magnolia bark, possess antimicrobial and antifungial activity,[5] a centrally acting muscle relaxant effect[6] and central depressant effects.[7]

In the present investigation, we examined Shojoki-to for its purgative activity and effects on colonic circular muscle motility in situ by means of chronically implanted force transducers in conscious rat to empirically justify the significance of combining rhubarb, immature orange and magnolia bark in the prescription of Shojoki-to.

Materials and Methods

Materials: Rhubarb (Rhei Rhizoma, Shinshu-daio), a rhizome of the hybrid of *Rheum palmatum* LINNÉ and *Rheum coreanum* NAKAI c.v. (Hokkai-do; Japan) was a gift from Takeda Health Care Co., Ltd. (Fukuchiyama, Japan). Immature orange (Aurantii Fructus Immaturus), immature fruit of *Citrus aurantium* LINNÉ (Zhe-jiang, China) and magnolia bark (Magnoliae Cortex), bark of *Magnolia obovata* THUNBERG (Akita Pref., Nagano Pref.; Japan) were purchased from Tochimoto-tenkai-do Co., Ltd. (Osaka, Japan).

Preparation of extract: Powdered crude drugs were extracted for 30 min with a 20-fold amount of water in a boiling water bath under refluxing and centrifuged for 15 min at 3300 rpm. The supernatant solution was administered orally to rats.

Prescription: Shojoki-to is a mixture of rhubarb, immature orange and magnolia bark (1: 1: 1).

Dose: Samples of Shojoki-to were administered orally at a dose corresponding to 250 mg/kg or 500 mg/kg body weight of rhubarb.

Animals: Female rats (Jcl: Wistar 150~200 g, Clea Japan Inc., Tokyo) were kept at an ambient temperature of 22-25℃ and allowed free access to a diet of CE-2 (Clea Japan Inc.) and tap water during experiments similar to the series of purgative experiments described in a previous paper.[8]

Purgative test: The purgative test was carried out as described previously[9] except for a slight modification. The test solutions were administered orally. The rats were observed over 8 h for diarrhoea (excretion of wet or shapeless faeces with staining on the blotting paper). Diarrhoea was scored as follows: 1 = normal faeces, 2 = moist faeces with faint staining on the under surface of blotting paper, 3 = soft faeces with staining on the blotting paper, 4 = shapeless sludge faeces, 5 = shapeless mucoid faeces. The purgative activity was expressed as the cumulative number and score of faeces. The cumulative score was expressed as the product of the score and the number of excreted faeces per rat.

Measurement of colonic motility: The contractile activity of the colonic circular muscle was measured as described previously.[2] To measure circular muscle contractions, a strain gauge force transducer (F-04SS, Star Medical, Tokyo) was sewn onto the serosa of the colon 4 cm distal to the ileocaecal junction. The recording axis of the transducer was parallel to the transverse axis of the colon.[10]

The experiments were started on the day following surgery at 9:00 am. The contractile activity of the colonic circular muscle was recorded with a thermal array recorder (RTA-1100M, Nihon Kohden Co., Tokyo) according to the method of Nagakura *et al.*[11] The number and amplitude of contractile waves were measured for each rat for 2 hrs and the average contraction amplitude and its standard deviation were assigned as the standard value (SV $\pm$ SD). On the next day at 9:00 am, the test solution was administered and then colonic motility was recorded. The number and amplitude of contractile waves were measured for 2 hrs from 30 min before the onset of diarrhoea or from 4 hrs after administration of the test solution without rhubarb. Each contraction was classified as follows:

High-amplitude contraction (HAC), over (SV + 1.96 x SD)

Low-amplitude contraction (LAC), below (SV - 1.28 x SD)

Others, between LAC and HAC.

The colonic motility was expressed as the total number of contractile waves and the percentages of the number of HAC and LAC to the total number of contractions, assigned as the percentages of the number of HAC and LAC, in the respective period. In the control period, the amplitude of the contractions was usually normally distributed, in which case, HAC, which rarely occurs in the control period, is expected to account for 2.5 percent of all contractions and LAC is expected to account for 10 percent of all contractions.

Statistical evaluation: The results were expressed as mean values ± s.e.m. Statistical evaluation was assessed using Student's *t*-test for paired data and Student's *t*-test or Welch test for unpaired data; a p value < 0.05 was considered statistically significant.

Results

Purgative activities of rhubarb and Shojiki-to

Fig.1 shows that at a dose of 250 mg/kg, rhubarb and Shojoki-to caused diarrhoea. The cumulative number and score of excreted faeces induced by Shojoki-to were significantly higher than those of rhubarb.

At a dose of 500/mg kg, rhubarb and Shojoki-to caused severe diarrhoea, with the cumulative number and score being significantly higher than those of the respective 250 mg/kg groups. They displayed nearly equal purgative activity with regard to the cumulative number and score of excreted faeces at this dose (Fig. 1).

Effects of rhubarb on rat colonic motility

Fig. 2a shows the typical contractile waves of conscious rat colon in the control period. Constant contractile waves with a stable baseline were observed during the control period. Fig. 2b shows typical rat colonic muscle contractions induced by rhubarb at the dose of 250 mg/kg. About 30 min before the onset of diarrhoea, the phase of regular spiking activity disappeared, being replaced by continuous irregular spiking activity. Rhubarb significantly decreased the number of contractile waves compared with the control period. Immediately before the onset of diarrhoea, high-amplitude contractions (HAC) accompanied by low-amplitude contractions (LAC) were observed. Rhubarb significantly increased

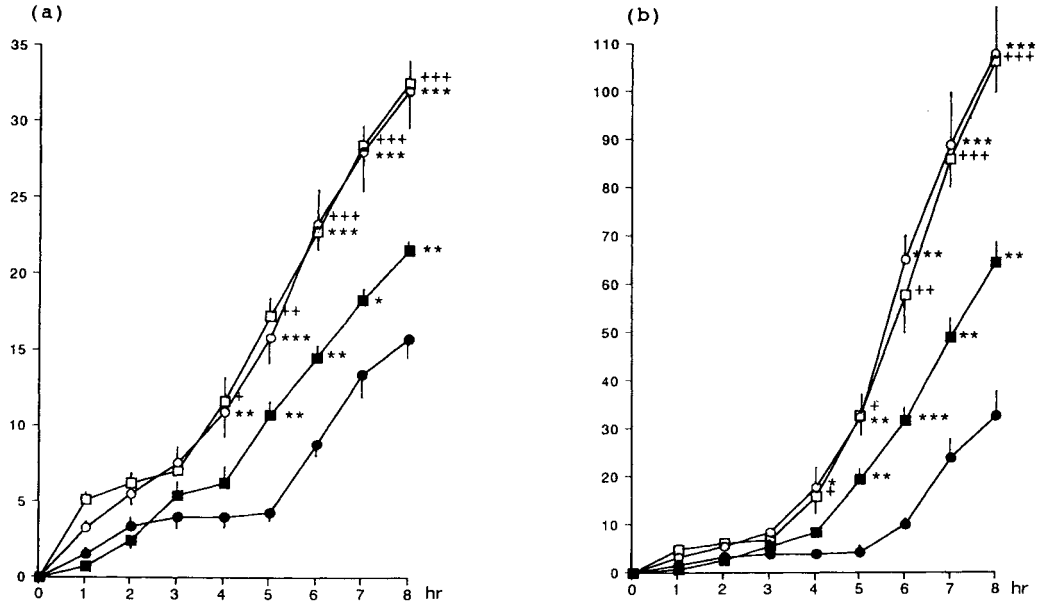

Fig. 1 Time course of purgative activities of rhubarb and Shojoki-to administered orally to rats. Cumulative number of faeces (a) and cumulative score of faeces (b).
Results are means ± s.e.m.
●, rhubarb (250 mg/kg, n=9) ; ○, rhubarb (500 mg/kg, n=10) ; ■, Shojoki-to (250 mg/kg, n=9) ; □, Shojoki-to (500 mg/kg, n=10).
*p<0.05, **p<0.01 and ***p<0.001 compared with rhubarb (250 mg/kg) group.
+p<0.05, ++p<0.01 and +++p<0.001 compared with Shojoki-to (250 mg/kg) group.

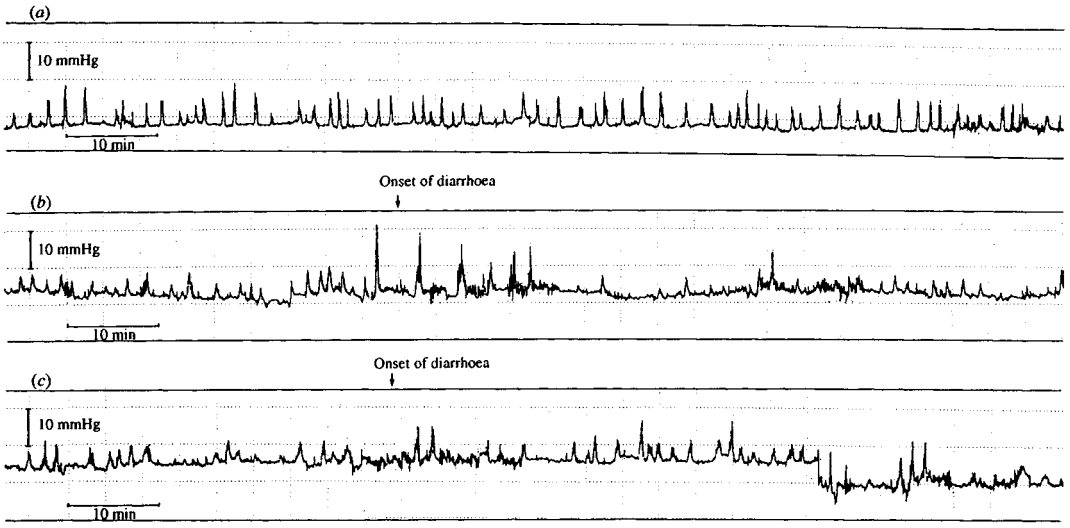

Fig. 2 Contractile waves of rat colon in the control period (*a*), induced by rhubarb (250 mg/kg,p.o.) (*b*), and induced by Shojoki-to (mixture of rhubarb 250 mg/kg, immature orange 250 mg/kg and magnolia bark 250 mg/kg,p.o.) (*c*) measured with strain gauge force transducer.

Table I Effects of rhubarb and Shojoki-to on rat colonic motility

Dose (mg kg^{-1})	n	Number of contractile waves		HAC (%)		LAC (%)	
		Control	Test	Control	Test	Control	Test
Rhubarb (250)	12	81.33 ± 4.02	59.27 ± 5.06***	5.33 ± 0.41	7.88 ± 1.80	3.70 ± 1.11	9.43 ± 3.43*
Shojoki-to (250)	11	77.79 ± 5.45	76.13 ± 5.39+	4.04 ± 0.78	15.67 ± 3.28***+	3.69 ± 1.18	9.45 ± 3.25*
Rhubarb (250) + Immature orange (250)	13	84.92 ± 4.02	65.92 ± 5.41**	3.95 ± 0.45	7.98 ± 1.39*†	5.25 ± 1.07	15.49 ± 3.67**
Rhubarb (250) + Magnolia bark (250)	10	86.20 ±3.31	72.62 ± 4.66*	4.43 ± 0.76	11.13 ± 3.36*	5.13 ± 2.28	19.33 ± 6.07*
Rhubarb (500)	10	64.50 ± 4.70	49.20 ± 6.06*	4.10 ± 0.56	14.98 ± 2.70**+	4.85 ± 1.17	12.58 ± 4.08*
Shojoki-to (500)	9	71.10 ± 5.54	50.09 ± 4.77**	3.52 ± 0.21	12.48 ± 2.82**	10.67 ± 1.46	29.73 ± 4.99**※

Results are means ± s.e.m.
HAC: contraction amplitude is over (average contraction amplitude + 1.96 X SD)
LAC: contraction amplitude is below (average contraction amplitude - 1.28 X SD)
*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared with respective control group by paired Student's t-test.
+$p < 0.05$ compared with the rhubarb (250) group by unpaired Student's t-test.
※$p < 0.05$ compared with the rhubarb (500) group by unpaired Student's t-test.
†$p < 0.05$ compared with the Shojoki-to (250) group by unpaired Student's t-test.

the percentage of LAC to the total contractions, but did not increase the percentage of HAC to the total contractions compared with the control period. At the dose of 500 mg/kg, rhubarb significantly decreased the number of contractile waves compared with the control period. The percentages of HAC and LAC to the total contractions were significantly higher than those of the respective control period values (Table I).

Effects of Shojoki-to on rat colonic motility

Figure 2c shows the typical rat colonic muscle contraction induced by Shojoki-to (mixture of 250 mg/kg each of rhubarb, immature orange and magnolia bark). It did not decrease the number of contractile waves, but significantly increased the percentages of HAC and LAC compared with the control period. It significantly increased the percentage of HAC compared with the rhubarb (250 mg/kg)-administered group (Table I). Immature orange or magnolia bark at a dose of 250 mg/kg administered with rhubarb (250 mg/kg) significantly decreased the number of contractile waves, and significantly increased the percentages of HAC and LAC compared with the respective control period (Table I).

Table II Effects of immature orange and magnolia bark on rat colonic motility

Dose (mg/kg)	n	Number of contractile waves		HAC (%)		LAC (%)	
		Control	Test	Control	Test	Control	Test
Immature orange (250)	11	93.20 ± 5.31	82.42 ± 4.40	5.16 ± 0.45	12.42 ± 3.75	4.37 ± 1.26	8.56 ± 4.43
Magnolia bark (250)	11	96.09 ± 7.58	87.20 ± 5.47	4.22 ± 0.47	4.75 ± 1.88	3.74 ± 1.35	5.55 ± 1.91
Immature orange (250)+ Magnolia bark (250)	11	83.27 ± 5.31	76.45 ± 4.10	4.64 ± 0.48	5.62 ± 1.89	5.15 ± 1.46	5.78 ± 3.84

Results are means ± s.e.m.
HAC: contraction amplitude is over (average contraction amplitude + 1.96 X SD)
LAC: contraction amplitude is below (average contraction amplitude - 1.28 X SD)

Shojojki-to at the dose corresponding to 500 mg/kg rhubarb significantly decreased the number of contractile waves and increased the percentages of HAC and LAC compared with the control period, but did not prevent the rhubarb-induced decrease in the number of contractile waves.

Effects of immature orange and magnolia bark on rat colonic motility

Single administration of immature orange (250 mg/kg) or magnolia bark (250 mg/kg) or the combination of both without rhubarb did not influence the number of contractile waves and the percentages of HAC and LAC compared with the respective control periods (Table II).

Discussion

We studied the purgative activity of Shojoki-to and its effect on colonic muscle motility in comparison with those of rhubarb alone in order to clarify the significance of adding immature orange and magnolia bark to rhubarb as a prescription. At a dose corresponding to 250 mg/kg rhubarb, Shojoki-to significantly increased the cumulative score of excreted faeces compared with rhubarb alone, but not at a dose equivalent to 500 mg/kg rhubarb. In Daio-kanzo-to, the purgative action of rhubarb at a dose of 500 mg/kg in rats was significantly potentiated by glycyrrhiza. This potentiating effect is mainly due to enhanced secretion of water in the colon and is not due to accelerated transit through the large intestine.[1] Our present findings suggest that immature orange and magnolia bark can induce a potentiating effect on the purgative activity of rhubarb only at its lower dose. Their potentiating effects at a dose corresponding to 250 mg/kg rhubarb may be due to stimulation of colonic motility. The accelerating effect of rhubarb on rat colonic transit may be maximal at the dose of 500 mg/kg, thus ruling out further potentiation.

We examined the effects of immature orange and magnolia bark in Shojoki-to on colonic motility induced by rhubarb in conscious rats with chronically implanted force transducers. After administration of rhubarb at doses of 250 mg/kg and 500 mg/kg, strong spiking activity and small contractions appeared at the onset of diarrhoea. Both doses of rhubarb significantly decreased the number of contractions and seemed to disturb colonic regular motility. This disturbance of the normal spiking activity may be the cause of abdominal pain.[12] At 250 mg/kg, rhubarb did not induce a significant increase in the percentage of HAC compared with the control period, in contrast with 500 mg/kg, which significantly increased in the percentages of HAC and LAC at the onset of diarrhoea.

Shojoki-to at a dose corresponding to 250 mg/kg rhubarb did not decrease the number of contractile waves compared with the control period, in contrast with rhubarb at the same dose. It significantly increased the percentages of HAC and LAC compared with the control period and significantly increased the percentage of HAC compared with the group administered 250 mg/kg of rhubarb. These findings suggest that immature orange and magnolia bark activate colonic motility, consequently preventing rhubarb-induced disturbance of normal spiking activity, namely a decrease in the number of contractile waves. The activation of colonic contraction and maintaining of regular spiking activity may contribute to the potentiating effects of Shojoki-to on purgative activity.

In prior experiments, we measured the colonic motility of longitudinal muscle. Administration of rhubarb or Shojoki-to induced similar results. We used circular

muscles which present clearer contractions than those of longitudinal muscles.

Recently, we showed that in Daio-kanzo-to, glycyrrhiza prevents the rhubarb-induced appearance of spiking activity and may moderate abdominal pain at the onset of diarrhoea.[2] Unlike glycyrrhiza, immature orange and magnolia bark seemed to stimulate rhubarb-induced rat colonic muscle motility and thus potentiate the purgative effect of rhubarb at a dose of 250 mg/kg. This potentiation was not observed at 500 mg/kg. Our findings support the hypothesis that the potentiating effect of immature orange and magnolia bark in Shojoki-to on purgative activity is due to activation of colonic muscle motility, namely recovery from a decrease in the number of contractile waves, and an increase in the percentage of HAC. Both Daio-kanzo-to and Shojoki-to possess potentiating effects on the purgative action of rhubarb, but with different potentiating mechanisms.

Addition of only immature orange or magnolia bark to rhubarb did not influence the rhubarb-induced decrease in the number of contractile waves and increase in the percentages of HAC and LAC. Thus, addition of either one of these items alone would not be sufficient for an effective preparation. Constituent of immature orange, naringin, has been found to inhibit intestinal transit.[3] It seems to be the combination of the constituents of immature orange and magnolia bark that induces stimulatory effects on the purgative activity and the colonic motility of rhubarb in rats.

We conclude that the combination of immature orange and magnolia bark in Shojoki-to activates rat colonic motility and prevents the rhubarb-induced decrease in the number of contractions, and may potentiate the purgative activity of rhubarb. The present study could not clarify the specific constituents of immature orange and magnolia bark contributing to their effects in Shojoki-to and further studies are needed.

和文抄録

小承気湯は大黄，枳実，厚朴で構成される漢方処方で，腹部膨満感を伴う便秘に用いられる。本研究は小承気湯の瀉下作用と結腸輪状筋収縮運動に及ぼす影響をラット結腸に縫着したフォーストランスジューサーを用いて調べ，枳実，厚朴を大黄に配合する意義を検討した。

小承気湯はラットの瀉下作用を大黄投与群と比較して有意に増強した。また，瀉下作用発現時に大黄による結腸の収縮数の減少を抑制し，強い収縮と弱い収縮の割合を大黄投与群と比較して有意に増加させた。枳実又は厚朴を大黄に添加しても小承気湯の作用は復元されなかった。

したがって，小承気湯において，枳実と厚朴は結腸の運動を促進し，大黄投与時にみられる結腸の収縮数の減少のような規則的な運動の阻害を抑制し，その結果，大黄の瀉下作用を増強することが示唆された。

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