Effects of Daio-kanzo-to(Da-Huang-Gan-Cao-Tang) on colonic circular muscle motility in conscious rat: Role of glycyrrhiza in the prescription

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Abstract

Rhubarb, a laxative, is known to affect large intestinal motility and sometimes cause diarrhoea accompanied by abdominal pain which can be decreased by adding glycyrrhiza for its antispasmodic and moderating effects.

This study was performed to clarify the effect of Daio-kanzo-to on rat colonic circular muscle motility by means of chronically implanted force transducers in conscious rats. Also examined was the significance of combining rhubarb and glycyrrhiza as a preparation.

Daio-kanzo-to significantly depressed the increase in the high amplitude contractions after the onset of diarrhoea compared with rhubarb. Combined administration of glycyrrhizic acid, liquiritin and rhubarb reproduced the effect of Daio-kanzo-to with respect to suppression of high amplitude contractions.

We concluded that in Daio-kanzo-to, glycyrrhiza prevents the rhubarb-induced strong spiking activity of the colonic circular muscle and consequently may moderate abdominal pain at the onset of diarrhoea. Glycyrrhizic acid and liquiritin cooperatively contribute to the effect.

Key words Daio-kanzo-to (Da-Huang-Gan-Cao-Tang), glycyrrhiza, rhubarb, rat, colonic circular muscle motility.

Abbreviations Daio-kanzo-to (Da-Huang-Gan-Cao-Tang), 大黄甘草湯; HAC, high amplitude contraction; HPLC, high-performance liquid chromatography; LAC, low amplitude contraction.

Introduction

Daio-kanzo-to (Da-Huang-Gan-Cao-Tang) is a Kampo medicine, which has been in practical use for thousands of years to treat patients with disorders of the stomach and bowel movement. It consists simply of two kinds of crude drugs, rhubarb and glycyrrhiza. Rhubarb, a laxative, is known to affect large intestinal motility and sometimes cause diarrhoea accompanied by abdominal pain. Glycyrrhiza has been most frequently prescribed in Chinese traditional medicine and is considered to moderate the effects of other crude drugs in a preparation.[1] This moderating effect has been described in many traditional herbal medicine textbooks, for example "Yakucho". It is also used as a sweetening agent. In Daio-kanzo-to, glycyrrhiza is thought to prevent severe diarrhoea and abdominal pain by its moderating and antispasmodic effects.[2]

Previously, we found that glycyrrhiza had a significant potentiating effect on the purgative action of rhubarb in rats when the prescription Daio-kanzo-to was prepared as a proportion of 4:1 of rhubarb and glycyrrhiza. This potentiating effect is mainly due to enhanced secretion of water in the colon, and not to accelerated transit through the large intestine or stimulated colonic mucus secretion.[3]

Staumont *et al.*[4] described the effects of sennosides, the main purgative constituents of rhubarb, on colonic motility in humans by electromyographic method. Sennosides increased propulsive activity expressed as an increased number of migrating long-spike bursts in the left

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and sigmoid human colon. The action of laxatives and sennosides on canine colonic motility has been investigated by recording mechanical activity using strain gauge transducers sutured to the serosa.[5,6] These findings have shown that sennosides induce inhibition of basal colonic motility and cause the great contractions associated with defecation.

The present study was undertaken to examine the effects of Daio-kanzo-to on rat colonic circular muscle motility in situ by means of chronically implanted force transducers in conscious rats and to elucidate the significance of combining rhubarb and glycyrrhiza as a prescription.

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) and glycyrrhiza (Glycyrrhizae Radix, Kanzo) root of *Glycyrrhiza uralensis* FISHER (Northeast; China), were both gifts from Takeda Health Care Co., Ltd. (Fukuchiyama, Japan).

Prescription: Daio-kanzo-to, a mixture of rhubarb and glycyrrhiza (4: 1).

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 at a dose corresponding to 500 mg/kg body weight of rhubarb which produced a constant diarrhoeal effect in most of the rats.[3] Glycyrrhizic acid, liquiritin and their mixture at a dose corresponding to 125 mg/kg body weight of glycyrrhiza did not influence the purgative effect of rhubarb (500 mg/kg).

Glycyrrhizic acid, sennoside A and sennoside B were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Liquiritin was purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

Animals : Rats (Jcl: Wistar ♀ 150 ~ 200 g, Clea Japan Inc., Tokyo) were kept at an ambient temperature of 22-25°C and allowed free access to diet of CE-2 (Clea Japan Inc.) and tap water during the experiments similar to the series of purgative experiments described in a previous paper.[7]

Measurement of colonic motility: Rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.). 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. Recording axis of the transducer was parallel to the transverse axis of the colon.[4] The free end of the force transducer was led subcutaneously to a incision made in the back of the animal's neck where it was drawn out and covered with a protective jacket (FPJ-32, Star Medical). The rats were housed in individual experimental cages with a wire meshed floor raised 2.5 cm above blotting paper to allow observation of faeces excretion.

The experiments were started on the day following the surgery at 9:00 a.m. The free end of the force transducer was connected to a colonic motility measuring system (PA-001, Star Medical). 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.*[8] When amplitude of the contraction was larger than 0.5mmHg, each contraction was considered as a contractile wave. 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 ± SD). On the next day at 9:00 a.m., the test solution was administered and then colonic motility was recorded. Excretion of wet or unformed faeces with staining on the blotting paper was judged to be diarrhoea.[9] 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 percentages of the number of HAC and LAC to the total number of waves in the respective period. In the control period, the amplitude of the contractions was usually normally distributed, and 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.

Determination of sennoside A and B in rhubarb preparation by high-performance liquid chromatography (HPLC) : Sennosides A and B were determined by an-ion-exchange solid phase extraction[10] and HPLC. HPLC was performed using a Shimadzu LC-10A, a stainless-steel column (250 mm x 4.6 mm i.d., Wakosil-II 5C18-HG, Wako Pure Chemical Industries), and a photo diode array detector (SPD-M10A VP, Shimadzu Corporation, Kyoto, Japan). The mobile phase was 1.25% aqueous acetic acid-acetonitrile (4:1) and the flow rate was 1.0 ml/min at 40 ℃. The eluted substances were detected by Shimadzu Class VP at 340 nm.

Determination of glycyrrhizic acid and liquiritin in glycyrrhiza preparation by high-performance liquid chromatography : Glycyrrhizic acid and liquiritin were determined by the HPLC method of Kitagawa *et al.*[11] with slight modification. HPLC was performed using a Shimadzu LC-10A, a stainless-steel column (150 mm x 4.6 mm i.d., Wakosil- II 5C18-100, Wako Pure Chemical Industries), and a photo diode array detector (SPD-M10A VP, Shimadzu Corporation). A mixture of 1% aqueous acetic acid and acetonitrile was used as the mobile phase in a gradient system and the flow rate was 1.0 ml/min at 40 ℃. Spectrometric determination was done at 254 nm for glycyrrhizic acid and 257 nm for liquiritin using a Shimadzu Class VP.

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

Effects of rhubarb on rat colonic motility

Figure 1a shows the typical contractile waves of conscious rat colon in the control period. Constant contractile waves with a stable base line were observed during the control period. Figure 1b shows typical rat colonic muscle contractions induced by rhubarb at the dose of 500 mg/kg. Rhubarb produced diarrhoea 4～4.5 hrs after administration to all test animals at this dose. From about 30 min before the onset of diarrhoea, the phase of regular spiking activity such as control period disappeared, being replaced by continuous irregular spiking activity. Rhubarb significantly decreased the number of contractile waves compared with the control period. Thus, immediately before the onset of diarrhoea, high amplitude contractions (HAC) accompanied by low amplitude contractions (LAC) were observed and the percentage of HAC and of LAC to the total waves were significantly higher than those of the respective control period values (Table I).

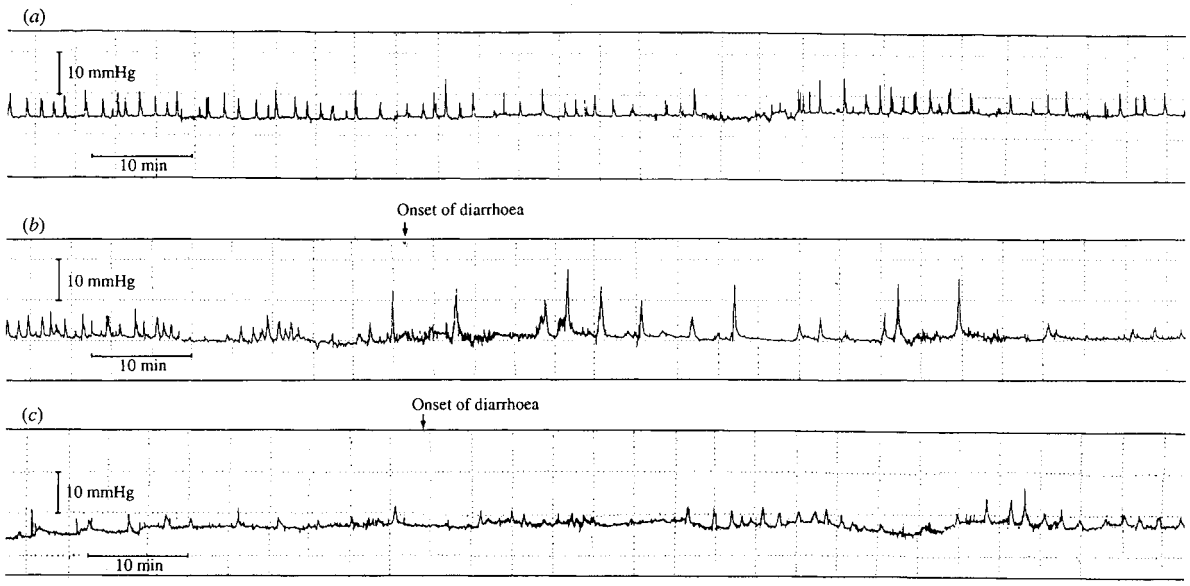

Fig. 1 Contractile waves of rat colon in the control period (*a*), induced by rhubarb (500 mg/kg,p.o.) (*b*) and induced by Daio-kanzo-to (mixture of rhubarb 500 mg/kg and glycyrrhiza 125 mg/kg,p.o.) (*c*) measured with strain gauge force transducer.

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

Dose(mg/Kg)	n	Number of contractile waves		HAC (%)		LAC (%)	
		Control	Test	Control	Test	Control	Test
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*
Daio-kanzo-to (Rhubarb 500+ glycyrrhiza 125)	10	69.80 ± 5.09	53.80 ± 5.88*	3.03 ± 0.65	4.06 ± 0.94++	7.70 ± 1.69	29.28 ± 4.17**++
Glycyrrhiza(125)	9	83.44 ± 4.31	87.10 ± 6.47	3.80 ± 0.67	4.76 ± 1.70	7.80 ± 2.28	19.33 ± 6.07*

Results are means ± s.e.m.
*$p < 0.05$ and **$p < 0.01$ compared with the respective control group.
++$p < 0.01$ compared with the respective rhubarb group.

Table II Effects of glycyrrhizic acid and liquiritin on rhubarb-induced colonic motility in rats

Dose(mg/Kg)	n	Number of contractile waves		HAC (%)		LAC (%)	
		Control	Test	Control	Test	Control	Test
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*
Rhubarb(500) + glycyrrhizic acid(5)	14	85.79 ± 4.00	68.71 ± 8.19*	4.47 ± 0.64	11.48 ± 2.33*	4.87 ± 1.19	7.27 ± 2.43
Rhubarb(500) + liquiritin(2.5)	9	83.11 ± 10.09	68.57 ± 6.99*	3.45 ± 0.50	7.10 ± 1.81*	5.63 ± 1.34	11.23 ± 3.72*
Rhubarb(500) + glycyrrhizic acid(5) liquiritin(2.5)	13	71.92 ± 3.58	50.16 ± 3.67***	3.68 ± 0.76	6.33 ± 1.81+	7.30 ± 1.83	14.46 ± 4.24

Results are means ± s.e.m.
Rhubarb, glycyrrhizic acid and liquiritin were administered orally.
*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared with the respective control group by paired Student's t-test.
+$p < 0.01$ compared with the rhubarb group.

Sennoside A and B contents in 500 mg of rhubarb were 4.95 mg and 4.60 mg, respectively. Sennoside A at a dose of 10 mg/kg induced diarrhoea, a decrease in the number of contractile waves and increases in HAC and LAC like rhubarb at 500 mg/kg (data not shown).

Effects of Daio-kanzo-to on rat colonic motility

Figure 1c shows the typical rat colonic muscle contraction induced by Daio-kanzo-to (mixture of 500 mg/kg rhubarb and 125 mg/kg glycyrrhiza). It significantly decreased the number of contractile waves but did not increase the percentage of HAC and significantly increased the percentage of LAC compared with the control period. It significantly decreased the percentage of HAC and increased the percentage of LAC compared with the rhubarb-administered group (Table I). Glycyrrhiza alone at a dose of 125mg/kg did not influence the number of contractile waves and the percentage of HAC but significantly increased the percentage of LAC compared with the control period (Table I).

Effects of glycyrrhizic acid and liquiritin in Daio-kanzo-to on rat colonic motility

Glycyrrhizic acid and liquiritin in glycyrrhiza were determined by HPLC. Glycyrrhizic acid and liquiritin contents in 125 mg of glycyrrhiza were 5 mg and 2.5 mg, respectively.

Glycyrrhizic acid (5 mg/kg) administered with rhubarb (500 mg/kg) significantly decreased the number of contractile waves and significantly increased the percentage of HAC but did not influence the percentage of LAC compared with the control periods (Table II).

Liquiritin (2.5 mg/kg) administered with rhubarb (500 mg/kg) significantly decreased the number of contractile waves and significantly increased the percentage of HAC and of LAC compared with the control period (Table II).

Combined administration of glycyrrhizic acid (5 mg/kg) and liquiritin (2.5 mg/kg) with rhubarb (500 mg/kg) significantly decreased the number of contractile waves and did not increase the percentage of HAC and LAC compared with the control period (Table II). The percentage of HAC was significantly decreased compared with the rhubarb-administered group (Table II).

Table III Effects of glycyrrhizic acid and liquiritin on rat colonic motility

Dose(mg/Kg)	n	Number of contractile waves		HAC (%)		LAC (%)	
		Control	Test	Control	Test	Control	Test
Glycyrrhizic acid(5)	12	89.17 ± 5.78	71.11 ± 5.48	3.35 ± 0.43	4.48 ± 1.80	5.71 ± 1.79	7.30 ± 1.97
Liquiritin(2.5)	11	82.36 ± 5.03	75.50 ± 6.11	4.68 ± 0.38	6.11 ± 2.69	6.43 ± 1.17	11.07 ± 3.20
Glycyrrhizic acid(5)+ Liquiritin(2.5)	11	71.08 ± 3.94	65.61 ± 2.96	3.73 ± 0.80	3.55 ± 1.96	8.25 ± 1.75	14.40 ± 4.87

Results are means ± s.e.m.
Glycyrrhizic acid and liquiritin were administered orally.

Single administration of glycyrrhizic acid or liquiritin and combination of both without rhubarb influenced neither the number of contractile waves nor percentage of HAC and of LAC compared with the respective control periods (Table III).

Discussion

In this study, we measured colonic motility in conscious rats with chronically implanted force transducers to investigate the effect of glycyrrhiza in Daio-kanzo-to, particularly the significance of combination with glycyrrhiza.

The results showed that in the control period, the amplitude of the contractions was usually normally distributed.

About 4 hrs after administration of rhubarb at a dose of 500 mg/kg which induced diarrhoea to all test animals, the phase of regular spiking activity disappeared and strong spiking activity appeared at the onset of diarrhoea. Rhubarb significantly increased the percentages of HAC and LAC compared with those in the control period. HAC and LAC increased with a corresponding onset of diarrhoea. These effects seem to be mainly due to the effect of sennosides. Rhubarb significantly decreased the number of contractions and seemed to disturb colonic regular motility. This disturbance of the normal spiking activity may have been the cause of abdominal pain.[12] This effect was similar to the results with sennosides in dogs, which indicates that sennosides induced inhibition of the basal colonic motility and the appearance of high amplitude contractions associated with diarrhoea.[5,6] Staumont *et al.*[4] provided evidence for a strong increase in the number of migrating long-spike bursts as the main effect of sennosides on human colonic motility by electromyographic method.

Daio-kanzo-to did not increase the percentage of HAC compared with the control period in contrast with rhubarb. When Daio-kanzo-to was administered to rats, the strong spiking activity, which is considered to be one reason for abdominal pain, did not occur at the onset of diarrhoea. So, HAC like the strong spiking activity is not necessary for the purgative effect, because Daio-kanzo-to has same purgative activity as rhubarb at this dose. Glycyrrhiza weakened the enhanced colonic contractions and moderated the colonic motility suggesting that it could prevent abdominal pains induced by rhubarb.

In prior experiments, we measured the colonic motility of longitudinal muscle. Administration of rhubarb or Daio-kanzo-to induced similar results. We used circular muscles which present clearer contractions than those of longitudinal muscles.

Daio-kanzo-to significantly increased the percentage of LAC compared with the control period. The increasing effect on LAC was stronger than that of rhubarb ($p<0.01$). Glycyrrhiza did not influence the number of contractile waves but induced an increase in LAC compared with the control period by its moderating effect. The increase in the percentage of LAC in rhubarb may be partly due to the decrease in the number of contractile waves. These findings suggest that rhubarb and glycyrrhiza additively increased the percentage of LAC in Daio-kanzo-to and the increase in LAC may not be a reason for abdominal pain at the onset of diarrhoea because it has been known that clinical administration of glycyrrhiza does not cause abdominal pain.

We tried to identify the constituents of glycyrrhiza contributing to its effect in Daio-kanzo-to. Rhubarb, 500 mg/kg was administered with either glycyrrhizic acid or

liquiritin at a dose corresponding to 125 mg/kg of glycyrrhiza. These preparations significantly increased the percentage of HAC compared with the respective control periods, such as the administration of rhubarb. Glycyrrhizic acid or liquiritin at a dose corresponding to 250 mg/kg of glycyrrhiza also significantly increased the percentage of HAC (data not shown). These findings did not suggest that the effect of the addition of glycyrrhiza is due to singly glycyrrhizic acid or liquiritin. However, when both, at the same dose, were administered simultaneously with rhubarb, no significant increase in HAC was observed compared with the control period. The percentage of HAC was significantly decreased compared with the rhubarb-administered group. This was a reproduction of the effect of Daio-kanzo-to related to a suppression of strong spiking activity.

We concluded that in Daio-kanzo-to, glycyrrhiza prevents the rhubarb-induced appearance of spiking activity and may lead to moderate abdominal pain at the onset of diarrhoea. Our findings showed that both glycyrrhizic acid and liquiritin seem to cooperatively contribute to the effect. The present results alone do not clarify the mechanism of this cooperating effect, additive or synergistic, and further studies are needed.

和文抄録

大黄甘草湯は大黄と甘草で構成される漢方方剤で，甘草の鎮痙緩和作用で大黄の瀉下作用に伴う激しい腹痛を軽減するとされている。本研究では大黄甘草湯のラット結腸輪状筋収縮運動に及ぼす影響を，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，大黄に甘草を配合する意義について検討することを目的とした。

大黄甘草湯は大黄と比較して瀉下作用発現時の強い収縮の増加を有意に抑制した。大黄に甘草の成分であるグリチルリチンとリクイリチンを加えると，大黄甘草湯の瀉下作用発現時の強い収縮を抑制する作用が復元された。

よって，大黄甘草湯において甘草は大黄によってひきおこされる結腸輪状筋の強い収縮を抑制し，その結果瀉下作用発現時の腹痛を緩和すること，並びにグリチルリチンとリクイリチンが協力してこの作用に関与することが示唆された。

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