The Influence of Glycyrrhiza and Antibiotics on the Purgative Action of Sennoside A from Daiokanzoto in Mice

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Daiokanzoto (DKT), a Kampo medicine that includes the combination of two crude drugs (rhubarb and glycyrrhiza), is clinically effective for constipation. The aim of this study is to clarify the influence of glycyrrhiza, three glycyrrhiza constituents (glycyrrhizin, liquiritin, and liquiritin apioside), and eight antibiotics on the purgative action of DKT, rhubarb, or sennoside A, a constituent of rhubarb, in mice. The purgative actions of rhubarb and sennoside A were significantly intensified when glycyrrhiza was co-administered orally to mice. Liquiritin and liquiritin apioside but not glycyrrhizin showed significant amplification of the purgative action in a dose-dependent manner. The purgative actions of DKT and sennoside A were significantly reduced by the pre-administration of ampicillin, cefcapene pivoxil, faropenem, fosfomycin, or kanamycin, but were not affected by the pre-administration of clarithromycin or levofloxacin. On the other hand, the purgative action of sennoside A was significantly reduced by the pre-administration of minocycline, whereas that of DKT was not affected. The effect of minocycline on the purgative action of sennoside A was lost when glycyrrhiza was co-administered. These results suggest that liquiritin and liquiritin apioside contribute as active substances for the purgative action of DKT, and some antibiotics reduce the purgative action of DKT and sennoside A. Furthermore, glycyrrhiza has the ability to recover the purgative action of sennoside A suppressed by minocycline *via* an unknown mechanism.

Key words interaction; antibiotic; daiokanzoto; sennoside A; glycyrrhiza; liquiritin

Kampo medicine is Japanese traditional medicine originating from ancient China. It is produced by combining multiple crude drugs and includes many constituents. This makes it extremely difficult to identify the active constituents and to reveal the mechanism of the pharmacological action. An exception to this is daiokanzoto (DKT), a simple formulation that consists of two kinds of crude drugs, Rhei Rhizoma (rhubarb) and Glycyrrhizae Radix (glycyrrhiza). It is the most well-known Kampo medicine and was demonstrated to be applicable for constipation in a clinical double-blind study.[1] The purgative effect of DKT is due to rhubarb, which is considered to promote the secretion of water and electrolytes into the colon and bowel movement.[2] Sennoside A, which is a well-known diarrheal constituent of rhubarb, is an inactive glycoside, and is transformed to an active metabolite, rheinanthrone, by intestinal bacteria.[3—5] Therefore, investigation of the purgative action and metabolism of sennoside A is important in order to understand the character of DKT.

Glycyrrhiza is a crude drug contained in more than 70% of Kampo medicines certified by the Japanese Ministry of Health and Welfare.[6] Unlike rhubarb, glycyrrhiza does not induce diarrhea directly. Glycyrrhiza has been considered to moderate the effects of other crude drugs in Kampo medicine. In DKT, glycyrrhizin and liquiritin, the main constituents of glycyrrhiza, cooperatively prevent the rhubarb-induced strong spiking activity of colonic circular muscle.[7] Interestingly, it has been reported that glycyrrhiza shows a significant potentiating effect on the purgative action of rhubarb in rats when DKT is prepared at a ratio (4 : 1) of rhubarb to glycyrrhiza that is used for the traditional formulation.[8] However, it is not clear how glycyrrhiza potentiates the purgative action of rhubarb. Recently, we found that the activity of sennoside A metabolism in intestinal bacteria was significantly accelerated when glycyrrhiza, liquiritin, or liquiritin apioside coexisted with sennoside A.[9] In this study, we investigated whether glycyrrhiza, liquiritin, or liquiritin apioside actually potentiates the purgative action of sennoside A in mice. Since the bioavailability of felodipine was shown to be improved with grapefruit juice in a clinical study,[10] a number of the studies have been published on interactions between Western drugs and herbal medicines as medicinal topics.[11,12] Kampo medicine is clinically used for the treatment of a wide variety of diseases in Japan. Since 86.3% of physicians have been reported to use Kampo medicines in Japan,[13] Kampo medicines are frequently prescribed with Western drugs for the treatment of various chronic diseases. The possibilities of interaction between Kampo medicines and Western drugs in experimental studies have been reported in recent years.[14—17]

Combinational use of Kampo medicines and antibiotics was observed in 7% of cases of Kampo prescription.[18] Because most glycosides are transformed by intestinal bacteria before being absorbed into the body,[19,20] the metabolism of available glycosides in Kampo medicine administered orally may be influenced by antibiotics.[21—23] Therefore, it is clinically important to evaluate whether Kampo medicine has drug interactions with antibiotics. In this study, we also investigated whether eight antibiotics (ampicillin, kanamycin, fosfomycin, cefcapene pivoxil, clarithromycin, levofloxacin, minocycline, and faropenem) actually affect the purgative action of sennoside A in mice.

MATERIALS AND METHODS

Materials The chopped crude drugs in DKT, rhubarb (kinmon-daio in Japanese) and glycyrrhiza (touhoku-kanzo in Japanese), were purchased from Tochinototenkaido (Osaka, Japan). Sennoside A and glycyrrhizin were purchased from Wako Pure Chemical Industries (Osaka, Japan).

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Liquiritin and liquiritin apioside were used as authentic samples. All other reagents were of analytical grade.

Ampicillin (ABPC, Viccillin Dry Syrup 10%), kanamycin (KM, Kanamycin Dry Syrup 20%), and fosfomycin (FOM, Fosmicin Syrup 400) were purchased from Meiji Seika Kaisha (Tokyo, Japan). Cefcapene pivoxil (CFPN-PI, Flomox), clarithromycin (CAM, Klaricid Syrup 10%), levofloxacin (LVFX, Cravit Fine Granules), minocycline (MINO, Minomycin Granules 2%), and faropenem (FRPM, Farom Dry Syrup for Pediatric) were purchased from Shionogi (Osaka, Japan), Abbott Japan (Tokyo, Japan), Daiichi Sankyo (Tokyo, Japan), Pfizer Japan (Tokyo, Japan), and Maruho (Osaka, Japan), respectively. Antibiotics were dissolved in distilled water before use.

Freeze-dried extracts of DKT, rhubarb, glycyrrhiza, sennoside A, liquiritin, and liquiritin apioside were dissolved in 0.01 M potassium phosphate buffer (pH 7.4) and all samples were orally administered to mice at a constant volume of 20 ml/kg body weight.

Preparation of Freeze-Dried Extract The freeze-dried extracts of DKT (4 g of rhubarb and 1 g of glycyrrhiza; yield: 2.25±0.04 g; sennoside A: 14.0±0.13 mg/g extract) and glycyrrhiza (1 g; yield: 0.33±0.03 g; glycyrrhizin: 186.7±8.11 mg; liquiritin: 61.2±1.82 mg; liquiritin apioside: 43.9±5.49 mg/g extract) were prepared as follows: chopped crude drugs were boiled with 500 ml of water on an electric heater for 40 min, filtered, and lyophilized. The freeze-dried extracts of rhubarb (4 g of rhubarb; yield: 1.94 g; sennoside A: 15.5 mg/g extract), DKT (4 : 2) (4 g of rhubarb and 2 g of glycyrrhiza; yield: 2.56 g; sennoside A: 12.7 mg/g extract), and DKT (4 : 4) (4 g of rhubarb and 4 g of glycyrrhiza; yield: 2.95 g; sennoside A: 10.5 mg/g extract) were prepared in the same manner and then dissolved in 73 ml of 0.01 M potassium phosphate buffer (pH 7.4) and given at a constant volume of 20 ml/kg body weight.

Animal Preparation Animal experiments were all carried out in accordance with the Guidelines for Animal Experimentation of Fukuyama University. Male ddY mice, weighing 30—40 g, were obtained from SHIMIZU Laboratory Supplies (Kyoto, Japan) and housed in a 12 h light–dark cycle at 21 to 24 °C for at least one week before the experiments. They were given free access to food and water before experiments.

The Purgative Action of DKT or Sennoside A Mice were isolated in a wire-bottomed cage covered with a beaker (11×15 cm), which was placed on blotting paper. The condition of feces was observed 1 h before administration of each sample, and only the mice that excreted normal feces were used. The dose of DKT and glycyrrhiza extract was estimated based on the prescription ratio (4 : 1) and the dose of rhubarb.[24] Sennoside A (15 mg/kg) and suspensions of glycyrrhiza extract at doses of 170, 340, and 680 mg/kg were prepared with 0.01 M potassium phosphate buffer, and these mixtures were orally administered to mice in a single dose. Other samples were prepared in the same manner. After the oral administration of sample, the condition of feces was observed at intervals of 1 h for 10 h. The feces with the worst condition were graded into three consistency levels as follows: 0: normal, 1: soft, 2: unformed.[25] The feces score was the mean value for the total consistency level of every hour in each mouse.

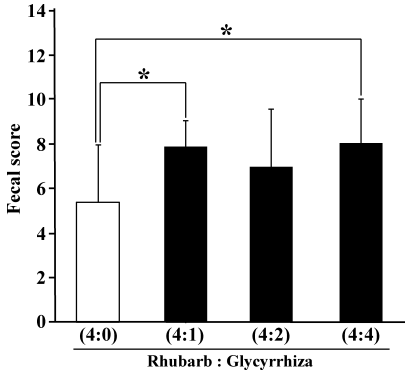

Fig. 1. Effects of Various Combination Ratios of Rhubarb and Glycyrrhiza on Purgative Action

The combination of rhubarb and glycyrrhiza, (4 : 0), (4 : 1), (4 : 2), or (4 : 4), was administered orally to mice; the dosages of these were 0.53 g/kg, 0.60 g/kg, 0.70 g/kg, and 0.81 g/kg, respectively. Each column represents the mean±S.D. of 12 mice. $*p<0.05$, significant difference from (4 : 0) by Steel's test.

Administration of Antibiotics Antibiotics were orally administered to mice in a single dose at 10 times the human daily dose (ABPC, 83 mg/kg; CFPN-PI, 50 mg/kg; KM, 660 mg/kg, CAM, 67 mg/kg; LVFX, 50 mg/kg; FOM, 500 mg/kg; MINO, 33.4 mg/kg: FRPM, 330 mg/kg). The control group received water instead of antibiotics. Only mice that excreted normal feces were used. Nineteen hours after administration of each antibiotic, DKT (1.21 g/kg) or sennoside A (40 mg/kg) was administered orally in a single dose. These dosages were selected to get comparable feces scores. The condition of feces was observed at intervals of 1 h for 8 h. After pretreatment with MINO, sennoside A (40 mg/kg) with glycyrrhiza extract (454 mg/kg), glycyrrhizin (90 mg/kg), or liquiritin (30 mg/kg) was prepared with 0.01 M potassium phosphate buffer and these mixtures were orally administered to mice in a single dose. These dosages were decided on the basis of the concentration ratio in DKT.

Statistical Analyses Data are shown as the mean±S.D. Statistical comparisons between two groups were made using Mann–Whitney's *U*-test (KyPlot, Kyence Inc.). To compare more than two groups, Steel's test (KyPlot, Kyence Inc.) was used. A probability value of $p<0.05$ was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Effect of Glycyrrhiza on Purgative Action of Rhubarb and Sennoside A DKT consists of two kinds of crude drugs, rhubarb and glycyrrhiza; it is clinically used for constipation, and rhubarb is well known to induce diarrhea. In contrast, glycyrrhiza has not been reported to have a purgative action, and a significant effect was not shown in our investigation involving oral administration to mice (680 mg/kg, data not shown), suggesting that glycyrrhiza, at least, does not induce diarrhea directly. To investigate the effect of glycyrrhiza in DKT, the condition of feces after oral administration of the freeze-dried extracts that were prepared at three combination ratios of rhubarb and glycyrrhiza was observed. As can be seen in Fig. 1, there was a significant difference in the feces score between freeze-dried extracts with and without glycyrrhiza. Our data are similar to a report that stated

that glycyrrhiza shows a significant potentiating effect on the purgative action of rhubarb in rats when DKT is prepared at a combination ratio (4 : 1) of rhubarb to glycyrrhiza that is used for the traditional formulation.[8] These results suggest that glycyrrhiza is effective in increasing purgative action of rhubarb.

In order to clarify which constituent of glycyrrhiza is associated with the purgative action of sennoside A, the feces score of sennoside A with glycyrrhizin, liquiritin, or liquiritin apioside is compared with that of sennoside A alone, as shown in Fig. 2. The feces score of sennoside A is significantly accelerated when glycyrrhiza extract is concomitantly given with sennoside A in a dose-dependent manner (Fig. 2A). Although glycyrrhizin is known as one of the active saponins of glycyrrhiza, it shows no influence on the feces score of sennoside A (Fig. 2B). On the other hand, the feces score of sennoside A is significantly accelerated by increasing the amount of liquiritin and liquiritin apioside, which are flavonoid glycosides, abundantly contained in glycyrrhiza (Figs. 2C, D). These results indicated that glycyrrhiza significantly enhanced the feces score of sennoside A, and liquiritin and liquiritin apioside contributed as active substances in glycyrrhiza, which promoted that of sennoside A. Sennoside A is transformed by intestinal bacteria in intestinal tract into an active metabolite rheinanthrone.[3—5] In a previous paper, we demonstrated that the activity of sennoside A metabolism in intestinal bacteria was significantly accelerated when glycyrrhiza, liquiritin, or liquiritin apioside coexisted with sennoside A, whereas glycyrrhizin showed no influence on the metabolic ratio of sennoside A.[9] The result of sennoside A metabolism experiment accords with the data of glycyrrhiza constituents on the purgative action of sennoside A. Therefore, it is presumed that the influence of these constituents on the fate of rheinanthrone transformed from sennoside A may promote the purgative action of sennoside A.

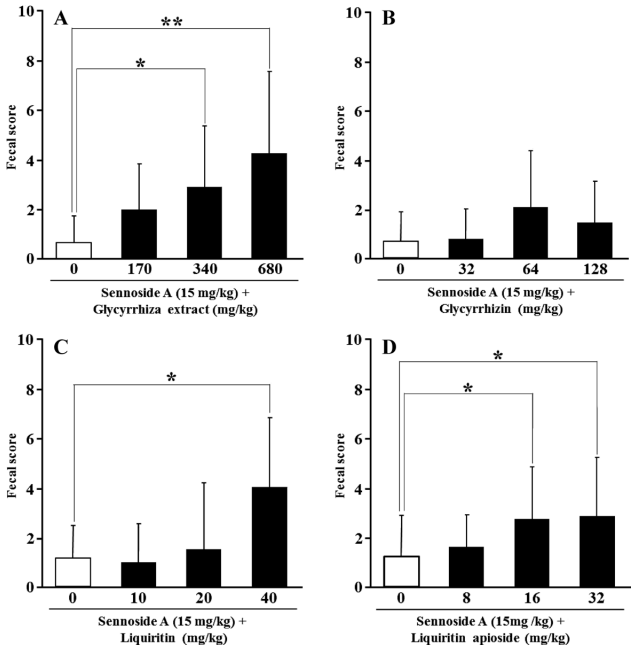

Fig. 2. Effects of Glycyrrhiza (A), Glycyrrhizin (B), Liquiritin (C), and Liquiritin Apioside (D) on Purgative Action of Sennoside A

Each column represents the mean±S.D. of 10—12 mice. $*p<0.05$, $**p<0.01$, significant difference from control (0 mg) by Steel's test.

Effect of Pretreatment of Antibiotics on Purgative Action of DKT and Sennoside A Kampo medicines are widely used together with Western drugs in Japan.[26] It has been reported that combinational use of Kampo medicines with antibiotics was observed in 7% of cases of Kampo prescription.[18] Because most glycosides are transformed by intestinal bacteria before being absorbed into the body,[19,20] the metabolism of available glycosides in Kampo medicine administered orally may be influenced by antibiotics.[21—23] Actually, DKT and a sennoside A preparation are sometimes used as the treatment of constipation for the patient who is taking an antibiotics. Figure 3 shows the mean feces scores of DKT (1.21 g/kg) and sennoside A (40 mg/kg) for 6 to 14 mice, which were orally administered with antibiotics at 10 times the human daily dosage 19 h before screening. The feces scores of DKT and sennoside A were significantly reduced by the pretreatment with ABPC, CFPN-PI, KM, FOM, or FRPM. Sennoside A is hydrolyzed to sennidin A by β-glucosidase of *Bifidobacterium* sp. SEN and then reduced to rheinanthrone by reductase of *Peptostreptococcus intermedius*.[27,28] Since the intestinal bacteria play an extremely key role in the bioavailability of sennoside A in DKT, this remarkable reduction on the feces scores of DKT and senno-

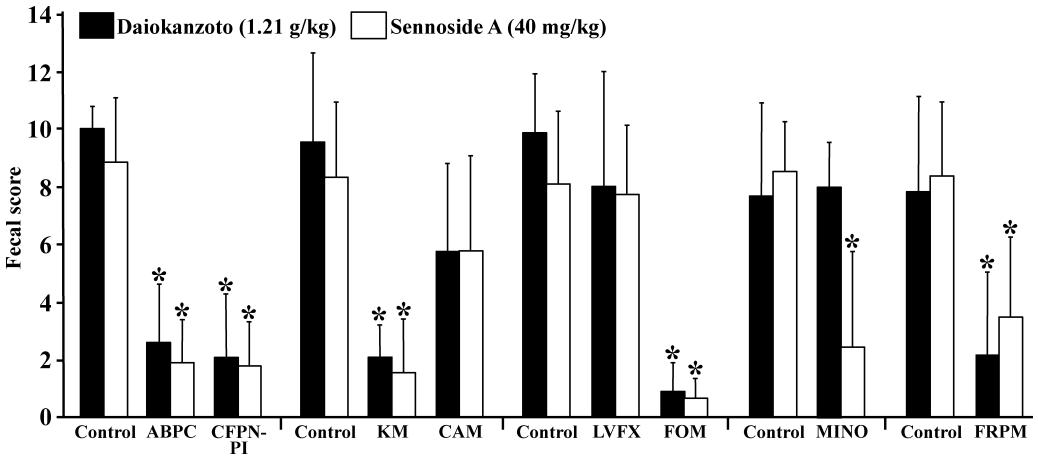

Fig. 3. Effects of Antibiotics on Purgative Action of Daiokanzoto and Sennoside A

ABPC: ampicillin, CFPN-PI: cefcapene pivoxil, KM: kanamycin, CAM: clarithromycin, LVFX: levofloxacin, FOM: fosfomycin, MINO: minocycline, FRPM: faropenem. Each column represents the mean±S.D. of 6—14 mice. $*p<0.01$, significant difference from control (without antibiotics) by Steel's test.

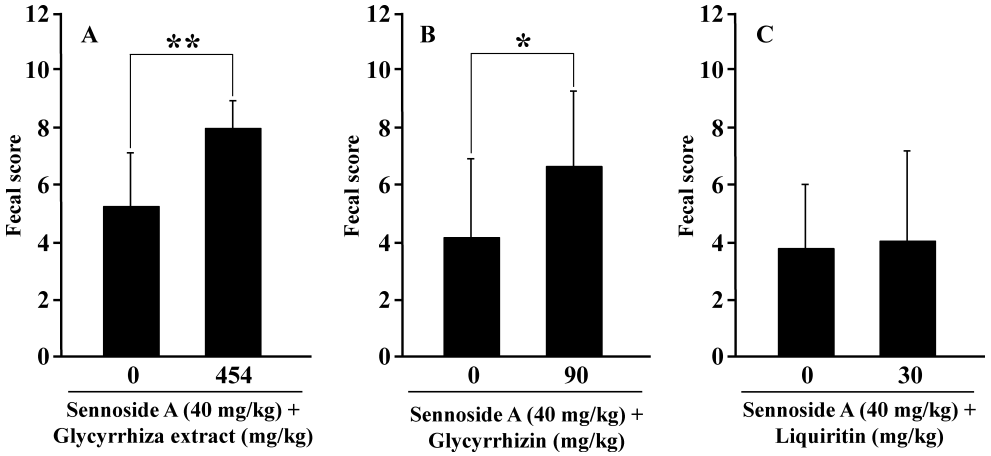

Fig. 4. Effects of Glycyrrhiza (A), Glycyrrhizin (B), and Liquiritin (C) on Purgative Action of Sennoside A after Pretreatment of Minocycline

Each column represents the mean±S.D. of 8 mice. $*p<0.05$, $**p<0.01$, significant difference from control by Mann–Whitney's *U*-test.

side A can be explained by the eradication of the intestinal bacteria that convert sennoside A to rheinanthrone as a result of the antibiotic treatment. ABPC, CFPN-PI, KM, CAM and FRPM had an antibacterial activity against *B.* sp. SEN and *P. intermedius*,[18] but CAM did not show a significantly influence on the feces scores of DKT and sennoside A. It can be presumed that CAM and LVFX did not affect the purgative action of DKT or sennoside A, since hydrophobic CAM and LVFX are readily absorbed from intestinal tract. On the other hand, it is supposed that ABPC, CFPN-PI, KM, FOM and FRPM, a hydrophilic bactericidal antibiotic, are poorly absorbed from intestinal tract and might act on intestinal bacteria capable of metabolizing sennoside A. The feces score of sennoside A was significantly reduced by the pretreatment with MINO, whereas that of DKT was not affected. In this respect, the detailed examination using the specific strain will be necessary from now on.

In order to clarify the interaction of glycyrrhiza and sennoside A, after treatment of MINO, the feces score for oral co-administration of sennoside A (40 mg/kg) with or without glycyrrhiza extract, glycyrrhizin, or liquiritin was examined as shown in Fig. 4. The feces score of sennoside A recovered by co-administration of glycyrrhiza extract when compared to that without glycyrrhiza extract (Fig. 4A). Although the co-administration of glycyrrhizin also recovered the feces score of sennoside A, concomitant liquiritin did not recover that of sennoside A (Figs. 4B, C), suggesting that glycyrrhiza and glycyrrhizin recovered the purgative action of sennoside A suppressed by MINO. The reason for the recovering effect of glycyrrhizin against MINO, however, remains unknown.

In this study, we revealed that liquiritin and liquiritin apioside contributes to the purgative action of sennoside A in DKT in mice and that the combination of rhubarb and glycyrrhiza is essential for inducing diarrhea. Furthermore, our data suggest that use of DKT and a sennoside A preparation for the treatment of constipation might necessitate attention being paid to the interaction between sennoside A and antibiotic. On the other hand, glycyrrhizin has various pharmacological effects and is frequently used for the treatment of hepatitis in Japan.[29] Therefore, we have to shed light on the interaction of glycyrrhizin and MINO in the future.

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