Short Communication

Pharmacological analysis for the optimal combination ratio of Shakuyaku and Kanzo in Shakuyakukanzoto

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Shakuyakukanzoto (SKT) consists of two kinds of crude drugs, Shakuyaku and Kanzo. SKT is used for leg cramps, stomachache, and menstrual colic pain. Clinically, different combination ratios of Shakuyaku and Kanzo exists, including the use of Kagenho (modified formulations) of an other Kampo formulation. But it remains un clear which ratio is best to apply.

We investigated the best combination ratio of Shakuyaku and Kanzo using mouse for the estimation of intestinal tract movement. SKT composed of Shakuyaku 50% and Kanzo 50% significantly decreased intestinal tract movement, comparable to atropin. These data suggest that these combination ratios of Shakuyaku and Kanzo work best for suppressing peristaltic motion.

In this study, we demonstrated that the combination ratio of Shakuyaku and Kanzo used as the formulation is reasonable from the standpoint of pharmacological background.

Key words Shakuyakukanzoto, combination ratio, intestinal motility.
Abbreviations SKT, Shakuyakukanzoto; HKT, Hangekobokuto.

Introduction

SKT consists of two kinds of crude drugs, Shakuyaku and Kanzo. SKT is used for leg cramps, stomachache, menstrual colic pain. Recently, SKT is also applied to premedication for endoscopy or barium enema to suppress peristaltic motion and its effectiveness have been reported.123) SKT is effective for pain primarily related to muscle contraction, and it works immediately after oral intake.

Clinically, a 1:1 combination ratio of Shakuyaku and Kanzo is used based on Shokanron (Shang han lun: a classic textbook for acute infectious disease of traditional Chinese medicine), but different combination ratios of Shakuyaku and Kanzo exist, including the usage of Kagenho (modified formulations) of other Kampo formulations.

By previous reports, it was known that Paeoniflorin, the main component of Shakuyaku, promotes peristalsis of the intestinal tract; on the other hand, Kanzo or a combination of Shakuyaku and Kanzo suppresses it.45) However, there have been no reports of studies investigating the optimal combination ratio of Shakuyaku and Kanzo in SKT.

Pharmacological evidence regarding the effective combination ratios of crude drugs is important. We therefore attempted to evaluate the most effective combination ratios of Shakuyaku and Kanzo in SKT using the method of intestinal transit study, as it can clearly reflect the effects of muscle contraction.

Materials and Methods

Drugs and reagents. SKT was prepared as freeze-dried powder from hot-water extract composed of two crude drugs in fixed proportion: 6 g Shakuyaku (Paeonia Radix: the root of Paeonia lactiflora Pall.) and 6 g Kanzo (Glycyrrhiza Radix: the root of Glycyrrhiza glabra L.). The three-dimensional HPLC profile of the H2O solution of SKT is shown in Fig.1. Hangekobokuto (HKT) was prepared as freeze-dried powder from hot-water extract composed of 5 crude drugs in fixed proportion: 6 g Hange (Pinelliae Tuber: the tuber of Pinellia ternata Brit.), 5 g Bukuryo (Hoelen: the sclerotium of Poria cocos Wolf), 3 g Koboku (Magnoliae Cortex: the bark of Magnolia obovata Thumb.), 2 g Soyo (Perillae Herba: the leaf of Perilla frutescens Britton var. acuta Kudo), and 0.5 g Shokyo (Zingiberis Rhizoma: the rhizome of Zingiber officinale Rosc.). Atropin sulfate injection was purchased from Tanabe Co., Ltd. (Osaka, Japan). Blue Dextran 2000 was purchased from Amersham Pharmacia Biotech (Little Chalfont, UK).

Extract preparation. SKT consisted of 100% Shakuyaku and 0% Kanzo, 75% Shakuyaku and 25% Kanzo, 50% Shakuyaku and 50% Kanzo, 25% Shakuyaku and 75% Kanzo, 0% Shakuyaku, and 100% Kanzo or HKT were boiled in 600 ml of water for 60 min to 300 ml, and then we collected 3.76g freeze-dried powder for SKT and 3.15 g...
Fig. 1 A: HPLC profile of SKT consisted of different ratio of Shakuyaku and Kanzo.
Shakuyaku 100% • Kanzo 0%: SKT consisted of 100% Shakuyaku and 0% Kanzo, Shakuyaku 75% • Kanzo 25%: SKT consisted of 75% Shakuyaku and 25% Kanzo, Shakuyaku 50% • Kanzo 50%: SKT consisted of 50% Shakuyaku and 50% Kanzo, Shakuyaku 25% • Kanzo 75%: SKT consisted of 25% Shakuyaku and 75% Kanzo, Shakuyaku 0% • Kanzo 100%: SKT consisted of 0% Shakuyaku and 100% Kanzo.
B: Three-dimensional HPLC profile of SKT consisted of 50% Shakuyaku and 50% Kanzo. [a: Paeoniflorin, b: Liquiritin, c: Glycyrrhizin ]
freeze-dried powder for HKT. The powder was dissolved in distilled water just before use. As shown in Fig.1, a peak of Paeoniflorin derived from Shakuyaku rose as the ratio of Shakuyaku increased. According to the ratio of Kanzo decreased, peaks of Liquiritin and Glycyrrhizin derived from Kanzo lowered.

**HPLC analysis.** SKT was dissolved with H$_2$O, filtered and analyzed by HPLC (HP-1090,Series II, Hewlett-Packard) under the following conditions: column, TSK gel ODS-80Ts (4.60 X 250mm); mobile phase, 10mM phosphoric acid : CH$_3$CN(linear gradient, 95:5~40:60, for 1h); flow rate,0.8ml/min; oven temperature, 40°C ; injection volume, 5μl. HPLC pattern was analyzed by absorbance at 220nm.

**Dose and combination ratio of SKT.** In first experiment, doses of SKT composed of 50% Shakuyaku and 50% Kanzo extracts was adjusted to 1, 5, 10, 50 - fold higher than the common human daily dose , i.e. 75.2 mg/kg, 376 mg/kg, 752 mg/kg, 3.76 g/kg.

In the second experiment, Shakuyaku and Kanzo were mixed at different ratios. The grams (ratio) of Shakuyaku in SKT were changed as follows: 12 g (100%), 9 g (75%), 7.2 g (60%), 6 g (50%), 3 g (25%), and 0 g (0%). These were used at 10-fold higher than the common human daily dose.

**Animals.** Male ICR mice at the age of 6 weeks (SLC Hamamatsu, Japan) were used. The animals were housed under a 12-hour light/dark cycle at a room temperature of 24 ± 1°C with a relative humidity of 55 ± 5%. Food and water were supplied ad libitum.

**Effects of Shakuyakukanzo on intestinal tract movement.** Intestinal tract movement was performed following the procedure reported previously with some modification. Briefly, after overnight starvation, water was orally administered. Thirty minutes after, Kampo medicine was administered. Atropin (100 μg/kg) was used as a positive control. To confirm this method can also evaluate prokinetic effect of drugs, HKT (630 mg/kg) and Metoclopramide (6 g/kg) were used. Atropin was administered intraperitoneally. Thirty minutes after administration, pigment was orally administered. Blue dextran 2000 was used as the pigment. Thirty minutes after pigment administration, resection was performed. The length of pigment movement was directly measured and compared with the full length of the intestine.

**Statistical analysis.** The data were expressed as the mean ± S.E. of the indicated number (n) of experiments. Data were analyzed by the paired t-tests. A p value < 0.05 was considered statistically significant.

### Results and Discussion

At first, we investigated the optimal concentrations of SKT including Shakuyaku 6 g and Kanzo 6 g for mouse, as there have been no previous reports of these values. Doses of SKT, including Shakuyaku 50% and Kanzo 50%, were adjusted to 1, 5, 10, and 50 - fold higher than the common human daily dose and used for the experiments of intestinal tract movement. As shown in Fig. 2, SKT extracts 10 - fold higher than human daily dose showed the strongest anti-peristaltic effects among the tested doses and they were comparable with atropin. SKT extracts 50-fold higher than the human daily dose did not work dose-dependently. The

**Fig. 2** Effects of SKT on intestinal tract movement among four different doses.

The dosage of SKT extracts was adjusted 1 (X 1), 5 (X 5), 10 (X10), 50, (X50) - fold higher than the common human daily dose i.e. 37.6 mg/kg, 376 mg/kg, 752 mg/kg, 3.76 g/kg. Atropin (100μg/kg) was used as positive control. n=10 *P<0.05 , **P<0.01 v.s. control.

**Fig. 3** Various combination ratios of Shakuyaku and Kanzo in SKT and their effects on intestinal tract movement. The ratio of Shakuyaku in SKT were changed 0, 25, 50, 75, 100%. Hangekobokuto (HKT), Metoclopramide were used to confirm their prokinetic effects, and atropin was used as positive control. n=10 *P<0.05, **P<0.005 v.s. control.
exact reasons remain unclear, but it is possible that the higher dose of SKT did not thoroughly dissolve in water. Therefore, the 10-fold higher concentrations were used in the later experiments. In contrast, we showed that HKT extracts increase intestinal tract movement stronger than metoclopramide (121% of control for SKT, 108% of control for Metoclopramide) (Fig. 3). According to these results, we confirmed that this method can also detect the prokinetic effects of Kampo medicine.

Next, we evaluated the optimal combination ratio of SKT. As shown in Fig. 3, SKT composed of 50% Shakuyaku tended to show the strongest anti-peristaltic effect (79% of control) among the five tested combination ratios. Our data might indicate that the most popular combination ratio of Shakuyaku and Kanzo is reasonable from the standpoint of pharmacological background. In addition, we tested SKT composed of 60% Shakuyaku because it is the combination ratio that we usually use in our Kampo clinic. SKT including 60% Shakuyaku suppressed peristaltic motion almost the same as SKT composed of 50% Shakuyaku (80% of control, data not shown in Fig. 3). Kanzo (SKT composed of 0% Shakuyaku) also decreased the movement of pigment, but its effect seemed to be weaker than that of SKT composed of 50% Shakuyaku. In contrast, Shakuyaku (SKT composed of 100% Shakuyaku) had no apparent effect on the peristaltic motion of mice in this study, suggesting that the combination of Shakuyaku and Kanzo is an essential condition for suppressing peristaltic motion of mice.

In this study, we demonstrated the optimal combination ratio of SKT for the first time. All of the Kampo formulations were created based on the experience of many ancient Kampo physicians. Therefore, we must also re-evaluate other important combinations of crude drugs and find their optimal combination by scientific method in the future. Furthermore, there have been many reports of the side effects of glycyrrhizin and Kanzo, including edema, high blood pressure, quadriplegia, and pseudoaldosteronism. Evaluation of optimal combinations for minimal side effects will also be needed.

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References


Japanese abstract

芍薬と甘草の2種類の生薬から構成される芍薬甘草湯は、その鎮痛・鎮痙効果より平滑筋収縮によっておこる腸痛や月経痛、骨格筋の緊張によっておこるこけ返りなどの治療薬として篤用される漢方薬である。臨床では、腸挙留に基づく芍薬50%・甘草50%の配合比が主に用いられているが、生薬で処方される場合には加味方も含め、異なる配合比が用いられることがある。しかしながら、これまで、最も有効な芍薬と甘草の配合についての検討は行われていなかった。そこで、芍薬甘草湯の芍薬及び甘草の最適な配合比を、マウスの腸管運動に対する作用で検討したところ、芍薬50%・甘草50%より構成される芍薬甘草湯が腸管運動を有意に抑制し、その効果はアトロビンに拮抗するものであることが明らかとなった。このことから、これまで経験に基づき思案と使われてきた芍薬甘草湯の配合比が腸管蠕動運動抑制に対して薬理的にも最適な配合比であることが示された。

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