
EFFECT OF SHAKUYAKU-KANZOH-TOH, A PRESCRIPTION COMPOSED OF SHAKUYAKU (PAEONIAE RADIX) AND KANZOH (GLYCYRRHIZAE RADIX) ON GUINEA PIG ILEUM

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The actions of shakuyaku-kanzohtoh (SK), a prescription of the traditional Chinese medicine, on an isolated guinea pig ileum were studied by comparing those of shakuyaku (S) (peony root, Paeoniae Radix) and kanzoht (K) (licorice root, Glycyrrhizae Radix). SK, S and K suppressed the neurogenic contractions of ileum induced by electrical stimulation and ganglionic stimulating agents such as DMPP and nicotine. Although S did not influence acetylcholine (ACh)–induced contraction of ileum, K inhibited ACh-induced contraction to the same extent as the neurogenic contraction. Also SK inhibited ACh-induced contraction but its inhibition was smaller than that of the neurogenic contraction. SK and K inhibited 40 mM KCl-induced contraction of ileum and the specific binding of [3H]-QNB on muscarinic receptors in ileum, but S at $3 \times 10^{-4}$ and $10^{-3}$ g/ml, which were enough to suppress the neurogenic contraction of ileum, did not inhibit them. These results suggest that the inhibitory actions of S and K on the neurogenic contraction are due to an inhibition of ACh release from cholinergic nerve and an inhibition of ACh action on ileum smooth muscle, respectively, and that the inhibitory actions of SK are responsible for both inhibitions by S and K. The inhibitions of ACh action by SK and K are presumed to be due to inhibitions of ACh binding on muscarinic receptors and of contractile machinery of smooth muscle.

Keywords — shakuyaku-kanzohtoh; Paeoniae Radix; Glycyrrhizae Radix; guinea pig ileum; neurogenic contraction; [3H]-QNB; traditional Chinese medicine; antispasmodic action

INTRODUCTION

Shakuyaku-kanzohtoh (SK), composed of shakuyaku (S) (peony root, Paeoniae Radix) and kanzoht (K) (licorice root, Glycyrrhizae Radix), is one of prescriptions in the traditional Chinese medicine. According to ancient textbooks of the traditional Chinese medicine, the prescription should be applied to patients with convulsions, spasms and pains in the limb, abdomen, stomach, intestine, ureter, gallbladder, trachea, etc. Since it has been clinically used as an antispasmodic and an analgesic, certain action(s) of SK on skeletal and smooth muscle could be expected. Pharmacological study on actions of SK was carried out by Hosono et al. who observed depressant activities of SK on spontaneous motilities and agonist-induced contractions of rabbit stomach and jejunum. Since then, little report has been seen on antispasmodic actions of SK, and therefore the mechanism by which SK suppresses the digestive organs is not known.

In the present study, the effect of SK on

* The inhibitory action of shakuyaku-kanzohtoh which has been known as an antispasmodic in the traditional Chinese medicine, on the neurogenic contraction of an isolated ileum from guinea pig was investigated and mechanisms were discussed.
guinea pig ileum was investigated to document the action on smooth muscles. Since it has been found that SK and its constituents, S and K, suppressed the neurogenic contraction of ileum, inhibitory mechanisms on ileum contraction by SK will be discussed by comparing with those by S and K alone.

METHODS AND MATERIALS
Preparations of Extracts of Shakuyaku-Kanzoh-Toh, Shakuyaku and Kanzoh — Shakuyaku (Paeoniae Radix), kanzoh (Glycyrrhizae Radix) and a mixture of them (1:1) were boiled in 10 volumes of water for 30 min. After filtration through gauze, the filtrate was concentrated to approximately half the volume by heating and then was lyophilized. The lyophilized materials were stored at −20°C and were dissolved in water when they were used.

Preparation of Isolated Guinea Pig Ileum — Male guinea pigs (Hartley strain) weighing 300 to 500 g were killed by a sharp blow on the head and a 3 cm strip of the terminal ileum was excised after discarding the 5 cm strip nearest to the ileoecal junction. The ileum was used for experiments by setting in a Magnus tube (10 ml capacity) with Tyrode’s solution gassed with 5% CO₂ and 95% O₂.

Effect on Contraction of Ileum induced by Electrical Stimulation — Electrical stimulation was essentially carried out according to the method of Paton et al. Ileum was transmurally stimulated by a pair of parallel platinum electrodes, the cathode in the lumen of ileum and the anode outside of ileum. The stimulation parameters were 0.1 Hz, 0.4 ms and 10 V. The twitch responses of ileum were isometrically recorded by means of a force-displacement transducer (TB-611T, Nihon Koden) on a polygraph recorder (WI 641G, Nihon Koden). The inhibitions of the twitch contractions by the drugs are represented as percent inhibitions compared with the twitch height obtained just before the drugs were applied to the Magnus tubes.

Effect on Contraction of Ileum induced by Dimethylphenylpiperazinium (DMPP), Acetylcholine (ACH), Nicotine and Potassium Chloride — Contractions induced by 4 × 10⁻⁶ M DMPP, 5 × 10⁻⁶ M nicotine, 4 × 10⁻⁵ M potassium chloride and various concentrations of ACh were isotonically recorded by an isotonic transducer (TD-112S, Nihon Koden) on a polygraph recorder (WI 641G, Nihon Koden). The inhibitory actions of the drugs were measured by comparing the heights of contractions induced by the agonist before and after the pretreatments with the drugs for 1 min.

Effect on the Specific Binding of ³H-Quinclidinyl Benzoate (³H-QNB) on Ileum Muscarinic Receptors — Ileum was homogenized in 10 volumes of phosphate buffer (0.05 M, pH 7.4), and aliquots of homogenate were used to measure the binding of ³H-QNB on muscarinic receptors according to the method of Yamamura et al. The specific binding of ³H-QNB on muscarinic receptors was calculated as the difference between the bindings of ³H-QNB in the presence and the absence of atropine (10⁻⁷ M). In the typical experiment, an aliquot of ileum homogenate was incubated with about 16000 cpm of ³H-QNB, and 4996 cpm of total binding occurred. Nonspecific binding was 126 cpm which was less than 3% of the total activity. The inhibitory actions of the drugs were measured by comparing the specific binding of ³H-QNB in the presence and the absence of the drugs in the assay medium.

Materials — Shakuyaku (Paeoniae Radix) and kanzoh (Glycyrrhizae Radix) were purchased from Niiya (Shizuoka). The best quality of chemicals available were obtained from the following sources: ACh and potassium chloride, Wako; DMPP, Aldrich, atropine sulfate, Merk; ³H-QNB, New England Nuclear.

RESULTS
Effect on Contraction of Ileum induced by Electrical Stimulation and Ganglionic Stimulating Agents
The characteristic actions of SK, S and K on the twitch responses of ileum to electrical stimulation are shown in Fig. 1. SK gradually sup-
pressed the twitch responses to make the maximum inhibition after a few minutes and the inhibitory action was still observed after 30 min. K produced the same inhibitory profile of the twitch responses as that by SK. On the other hand, the inhibition of the twitch responses by S reached maximum within 1 min and, after that, weakened gradually until the inhibitory action disappeared completely after several minutes. The maximum inhibitions by SK, S and K were dependent on concentrations in a range of $10^{-4}$ g/ml to $3 \times 10^{-2}$ g/ml, and IC$_{50}$ of those were not significantly different from each another (Fig. 2A).

Also SK, S and K at concentrations of $10^{-4}$ g/ml to $3 \times 10^{-3}$ g/ml inhibited in a concentration-dependent way the contractions induced by ganglionic stimulating agents, DMPP (Fig. 2B) and nicotine (Fig. 4). IC$_{50}$ of g/ml.}

**FIG. 1.** Inhibitory Profiles of Shakuyaku-Kanzoh-Toh (A), Shakuyaku (B) and Kanzoh (C) on the Twitch Responses of Guinea Pig Ileum induced by Electrical Stimulation (0.1 Hz, 0.4 ms and 10 V)

The twitch responses of ileum were isometrically recorded by means of a force-displacement transducer.

**FIG. 2.** Dose-Response Curves for the Inhibitory Actions of Shakuyaku-Kanzoh-Toh (closed circles), Shakuyaku (open triangles) and Kanzoh (open circles) on Electrical Stimulation-induced Twitch Responses (A) and on DMPP ($4 \times 10^{-6}$ M)-induced Contraction (B) of Guinea Pig Ileum

Electrical stimulation was applied and twitch responses were recorded as described in Fig. 1. Contractions induced by DMPP was isotonically recorded by means of an isotonic transducer. Each point represents the mean of thirteen (A) and twelve (B) separate experiments and S.E.M. was not indicated to avoid complication of figures.
those on DMPP-induced contraction of ileum were not significantly different from each another.

**Effect on Contractions of Ileum induced by ACh and Potassium Chloride**

Fig. 3 depicts effects of SK, S and K at concentrations of \(3 \times 10^{-4}\) g/ml and \(10^{-3}\) g/ml on the dose-response curves for ACh-induced contraction of ileum. K at both concentrations markedly reduced the maximal contraction of ileum induced by ACh, and SK did to a lesser extent. At the concentration of \(10^{-3}\) g/ml, K shifted the dose-response curve to right with the reduced maximal response. But the inhibitory effect of S was not observed, even if at a concentration of \(3 \times 10^{-3}\) g/ml which completely inhibited the contractions of ileum induced by electrical stimulation or ganglionic stimulating agents (data not shown). When ACh was applied to ileum after pretreatments with SK, S and K which were markedly suppressing nicotine-induced contraction (Fig. 4A) and twitch responses to electric stimulation (Fig. 4B), ACh-induced contraction was markedly inhibited by K and partially by SK, but not by S.

Also the contraction induced by 40 mM potassium chloride was markedly inhibited by SK and K, but the inhibition by S was not observed (Fig. 5). The inhibition by K at \(10^{-3}\) g/ml and \(3 \times 10^{-3}\) g/ml was significantly greater than that caused by SK at the respective concentrations.

**Effect on the Specific Binding of \(^3\text{H}\)-QNB on the Muscarinic Receptors**

As shown in Fig. 6, the specific binding of \(^3\text{H}\)-QNB was markedly inhibited by K in a dose-dependent manner at the concentrations tested. However the inhibitory action of S at \(3 \times 10^{-4}\) g/ml and \(10^{-3}\) g/ml was not observed although the inhibition by S at \(3 \times 10^{-3}\) g/ml was found to be approximately 30%. On the other hand, the inhibition of the binding by SK was very weak and, even if the concentration of SK was increased to \(3 \times 10^{-3}\) g/ml, the inhibition was not proportionally increased. Influences of SK, S and K on the nonspecific binding of \(^3\text{H}\)-QNB were negligible. Since the nonspecific binding was so low, the binding experiment was carried out at 3-fold larger scale and the nonspecific binding

![Graphs A, B, C](image)

**FIG. 3. Dose-Response Curves for Acetylcholine-induced Contractions in the Presence (closed circles, \(3 \times 10^{-4}\) g/ml; closed triangles, \(10^{-3}\) g/ml) and Absence (open circles) of Shakuyaku-Kanzoh-Toh (A), Shakuyaku (B) and Kanzoh (C)**

Contraction induced by acetylcholine was isotonically recorded by means of an isotonic transducer. Each point represents the mean ± S.E.M. of seven separate experiments.
was found not to be affected by SK, S and K at concentrations of $3 \times 10^{-4}$ and $10^{-5}$ g/ml. Accordingly the inhibitory effects of SK, S and K on the $^3$H-QNB binding observed in Fig. 6 could not be due to the interaction between the drugs and $^3$H-QNB.

**Comparison of the Inhibitory Effect on Contractions of Ileum induced by Electrical Stimulation, ACh and Potassium Chloride and on the Specific Binding of $^3$H-QNB on the Muscarinic Receptors**

The inhibitory actions of SK, S and K on various stimulation-induced contractions of ileum are compared in Fig. 7. SK, S and K at the concentrations of $3 \times 10^{-4}$ g/ml and $10^{-5}$ g/ml suppressed the twitch responses induced by electrical stimulation to the same extent, but did not inhibit ACh-induced contraction. Namely, S did not inhibit ACh-induced contraction, and K in-

**FIG. 5. Inhibitory Effects of Shakuyaku-Kanzoh-Toh (closed circles), Shakuyaku (open triangles) and Kanzoh (open circles) on Contraction of Guinea Pig Ileum induced by 40 mM Potassium Chloride**

Each point represents the mean $\pm$ S.E.M. of seven separate experiments.

**FIG. 6. Inhibitory Effects of Shakuyaku-Kanzoh-Toh (closed circles), Shakuyaku (open triangles) and Kanzoh (open circles) on the Specific Bindings of $^3$H-QNB on Muscarinic Receptors of Guinea Pig Ileum**

Each point represents the mean $\pm$ S.E.M. of three separate experiments.
hibited it to the same extent as the electrical stimulation-induced contraction, and SK did it but to a lesser extent than the electrical stimulation-induced contraction.

Inhibitory actions of K on the contraction induced by 40 mM potassium chloride and the specific binding of \(^3\text{H}\)-QNB on the muscarinic receptors were weaker than that on ACh-induced contraction (Fig. 7). The same observation was made on the inhibitory actions of SK (Fig. 7).

![Graph](image)

**FIG. 7. Comparison of the Inhibitory Effects of Shakuyaku-Kanzoh-Toh, Shakuyaku and Kanzoh on Contractions induced by Electrical Stimulation (0.1 Hz, 0.4 ms and 10 V, closed columns), Acetylcholine (10\(^{-7}\) M, shaded columns) and Potassium Chloride (40 mM, dotted columns) of Guinea Pig Ileum, and on the Specific Bindings of \(^3\text{H}\)-QNB (open columns) on Muscarinic Receptors of Guinea Pig Ileum**

Each column represents percent inhibition of contraction induced by each stimulus or percent inhibition of \(^3\text{H}\)-QNB bindings, and values were referred from those in Fig. 2A, 3, 5 and 6.

**DISCUSSION**

In the traditional Chinese medicine, SK has been applied to patients with convulsions or spasms of skeletal and smooth muscles.\(^{13}\) But efforts have not been made to clarify the pharmacological actions of SK except Hosono et al., who carried out a pharmacological study of SK on rabbit stomach and jejunum.\(^{2-4}\) They found that SK blocked spontaneous motilities and agonist-induced contraction of the organs, and that S induced increases in tonus and motilities of the organs, and the action of SK was attributed to the action of K.

In the present work, SK was demonstrated to suppress the contractions of guinea pig ileum induced by electrical stimulation and ganglionic stimulating agents such as DMPP and nicotine (Fig. 1, 2 and 4). Also it was found that the constituents of SK, S and K, produced inhibitions of the contraction of ileum to the same extent as SK (Fig. 1, 2 and 4). The electrical stimulation (0.1 Hz, 0.4 ms and 10 V)\(^{5,7-10}\) and ganglionic stimulating agents\(^{11-13}\) have been known to evoke the contraction of ileum by increasing ACh release from cholinergic nerve endings in ileum. Although such neurogenic contraction of ileum was inhibited to the same extent by SK, S and K, the influences on ACh-induced contraction were not identical (Fig. 3 and 4), suggesting that these drugs inhibited the neurogenic contraction of ileum in different manners. The neurogenic contraction of ileum could be inhibited by suppressing ACh release from cholinergic nerves (a presynaptic event) and/or by suppressing ACh action on ileum smooth muscle (a postsynaptic event).

It was shown that S at concentrations enough to inhibit markedly the neurogenic contraction of ileum did not affect ACh-induced contraction of ileum (Fig. 3). In addition, ACh-induced contraction of ileum was not influenced by S which was inhibiting the neurogenic contraction of ileum (Fig. 4). These observations suggest that the inhibitory action of S on the neurogenic contraction of ileum could be caused by suppressing ACh release from cholinergic nerves but not by
suppressing an ACh action. This interpretation is supported by the results that neither 40 mM potassium chloride-induced contraction of ileum (Fig. 5) nor the specific binding of \(^3\)H-QNB on muscarinic receptors (Fig. 6), which were postsynaptic events, was affected by S. We also found that the inhibitory action of S was partially blocked by phentolamine, \(\alpha\)-blocker, but not by propranolol, \(\beta\)-blocker (unpublished data). In addition, the action of S was partially blocked by theophylline and enhanced by dipyridamole (unpublished data), suggesting an involvement of an adenosine-like action. However, the action of S was not completely blocked even in the presence of theophylline and phentolamine (unpublished data). Therefore, the action of S could be attributed to plural components including adenosine-like material(s) and \(\alpha\)-adrenergic material(s).

K produced an inhibition of ACh-induced contraction of ileum as much as that of the neurogenic contraction of ileum (Fig. 4 and 7). These results suggest that the inhibitory action of K should result from a suppression of an ACh action on smooth muscle. K also inhibited the specific binding of \(^3\)H-QNB on muscarinic receptors and potassium chloride-induced contraction of ileum, and those inhibitions were less than the inhibition of ACh-induced contraction (Fig. 7). Hosono et al. reported that K inhibited the contraction of digestive organs induced not only by ACh but also by other postsynaptic stimuli such as histamine and barium chloride.\(^{2 \sim 4}\) Therefore, the inhibition of ACh-induced contraction by K presumably results from inhibitions of ACh binding on muscarinic receptors and of contractile machinery of smooth muscle. Since the inhibition of ACh-induced contraction by K seems not to be competitive (Fig. 3), the inhibition of ACh binding on muscarinic receptors is presumed not to be an atropine-like action. The detail mechanism of the inhibition by K must wait to be clarified by using purified material(s).

Although SK suppressed ACh-induced contraction of ileum, the suppression of it was smaller than that of the neurogenic contraction of ileum (Fig. 7). In addition, when ACh was applied to ileum of which the neurogenic contraction was being markedly suppressed by SK, ACh-induced contraction of ileum was only partially suppressed (Fig. 4). On the bases of these findings, the actions of SK are presumed to be due not only to a suppression of ACh release from cholinergic nerve but also a suppression of ACh action on smooth muscle. Since the inhibition of the neurogenic contraction by SK was as strong as those caused by S and K, the actions of SK were presumably due to the actions of S and K. However, Kimura et al. have reported that the twitch contraction of mouse nerve-diaphragm muscle preparations indirectly stimulated was blocked by paoniflorin and glycyrrhizin which were isolated from S and K, respectively, and the blockade was potentiated by the simultaneous application of them.\(^{14}\) Potentiation on actions of S and K, in the present work, has not been observed although extracts of the herbs were used. In our preliminary experiments, plural components which suppressed the neurogenic contraction of guinea pig ileum has been fractionated from S and K (unpublished data). Also as described above, we found an adenosine-like action and an \(\alpha\)-adrenergic agonist-like action in the crude extract of S. Therefore, the actions of SK might not be able to be elucidated solely by paoniflorin and glycyrrhizin. So that it should be noted that further studies must be carried out to clarify the actions of SK.

In conclusion, in the present pharmacological study, it has been demonstrated on guinea pig ileum that anti-spasmodic actions of SK may be due to an inhibition of ACh release from cholinergic nerves by S and an inhibition of ACh action on smooth muscle of ileum.

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REFERENCES


