Pharmacological Characteristics of Ryokan-kyo-mi-shinge-nin-to, an Antiallergic Kampo Medicine

Masaru SAKAGUCHI,* Yoshiki IKEDA, Toshitaka KIDO, Mitsutoshi YUZURIHARA, Yoshio KASE, Masahiro YAMAMOTO, Atsushi ISHIGE, and Hiroshi SASAKI

Kampo & Pharmacognosy Laboratory, Tsumura & Co.; 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300–1192, Japan. Received April 1, 2002; accepted August 27, 2002

The pharmacological characteristics of Ryokan-kyo-mi-shinge-nin-to (RKS), a traditional oriental herbal (Kampo) medicine which has been used for the treatment of allergic asthma and rhinitis, were investigated. The number of sneezes by actively sensitized mice after a topical antigen challenge was significantly reduced by pretreatment with RKS (300 and 1000 mg/kg, p.o.). Although RKS did not inhibit the antigen-induced histamine release from rat peritoneal exudate cells (PEC), it significantly inhibited an increase in vascular permeability induced by histamine and serotonin. These results suggest that RKS has antiallergic activity in animals, and the functional antagonism of a histamine response may be one of the mechanisms of its effect. In addition, RKS prevented histamine hypersensitivity in actively sensitized mice. Because RKS did not affect sleeping time induced by pentobarbital in mice and did not inhibit gastric emptying in rats, the drug appears to be useful for treating allergic patients suffering from classical antihistamines side effects such as stomach discomfort or relative drowsiness.

Key words Ryokan-kyo-mi-shinge-nin-to; antiallergy; stomachic; ketotifen

Allergic asthma and rhinitis are the most common forms of atopic disease. However, many of the antiallergics used to treat these illnesses can cause gastrointestinal disorders. The traditional herbal (Kampo) medicine Ryokan-kyo-mi-shinge-nin-to (RKS) has long been used for the treatment of allergic diseases in Japan where Kampo medicines are manufactured with a standardized quality and quantity of ingredients. However, few experimental data are available on the antiallergic properties of RKS, except for reports about studies on the release of histamine from mast cells. In the present study, we investigated RKS for possible antiallergic effects in experimental models, with a special emphasis on possible side effects.

MATERIALS AND METHODS

Animals Male BALB/c mice (5 weeks old), male ICR mice (5 weeks old) and male SD (IGS) rats (5 weeks old) were purchased from Charles River, Inc. (Tsukuba, Japan), and housed at constant temperature (23 ± 2 °C) and humidity (55 ± 10%) until use. All experiments were conducted according to the institution's guidelines for care and use of laboratory animals in research.

Drugs Ryokan-kyo-mi-shinge-nin-to (RKS; Tsumura & Co., Tokyo, Japan) is a powdered extract from a mixture of 7 medicinal plants: Hoelen (4 g), Glycyrrhizae Radix (2 g), Zingiberis Siccatum Rhizoma (2 g), Schizandraceae Fructus (3 g), Asiasari Radix (2 g), Pinelliae Tuber (4 g) and Armeniaca Semein (4 g). RKS, seratrodast (Takeda, Osaka, Japan), ketotifen fumarate and metoclopramide monohydrochloride (Sigma Chemical, St. Louis, MO, U.S.A.) were administered p.o.

The other drugs were ovalbumin (OA; Fraction V), anti-dinitrophenil (DNP) IgE, histamine dihydrochloride, serotonin creatinine sulfate complex, Evans blue (Sigma Chemical), aluminum hydroxide (Al(OH)₃; Wako Pure Chemical Industries, Ltd., Osaka, Japan), DNP-BSA (SLS, Tokyo, Japan) and sodium pentobarbital (Dinabol Laboratoris, North Chicago, IL, U.S.A.).

Antigen-Induced Sneezing in Mice Male BALB/c mice were actively sensitized with an intraperitoneal injection of 1 µg of OA containing 0.5 mg of Al(OH)₃ in 0.2 ml of saline at 0, 1, 2 and 3 weeks. One week after the last sensitization, 20 µl of OA in saline (40 mg/ml) was administered intranasally for 10 d. The next day, a topical antigen challenge was performed by dropping 20 µl of OA solution (40 mg/ml) intranasally, and the number of sneezes was counted for 10 min. Mice were pretreated with 300 or 1000 mg/kg of RKS, 5 mg/kg of ketotifen or distilled water (solvent) 30 min before the antigen challenge.

Antigen-Induced Histamine Release from Rat Peritoneal Exudate Cells This test was conducted according to Abe et al.[5] In brief, male SD rats were administered 1 ml of monoclonal anti-DNP IgE (titer: 1000; i.p.). At 24 h, the animals were bled to death, and their bodies were injected i.p. with 10 ml of Tyrode’s solution (pH 7.4) containing 0.3% BSA (Sigma Chemical) and 5 units/ml of heparin (Mochida Pharmaceutical, Tokyo, Japan). After gently massaging the abdomen for 2 to 3 min, peritoneal cells were obtained and washed 3 times with Tyrode’s solution. The mast cells in a cell suspension were counted by staining with 0.05% toluidine blue (Kanto Chemicals), resuspended in Tyrode’s solution containing 0.1% BSA and 10 mM HEPES buffer (Sigma Chemical), and adjusted to a concentration of 1 × 10⁶ cells/ml. The cell suspension was preheated for 5 min to 37 °C, and incubated with the test drugs for 10 min, challenged with DNP-BSA at a final concentration of 10 µg/ml in the presence of 10 µg/ml of phosphatidyl serine (Sigma Chemical) and incubated for an additional 10 min. The concentration of histamine in the supernatant was determined according to the method of Shore et al.[6] The percentage of histamine released was calculated from the total cellular content of histamine in each experiment.

Histamine- and Serotonin-Induced Skin Reactions in Rats This test was conducted according to Inagaki et al.[7] In brief, histamine (1 µg/0.1 ml), serotonin (30 ng/0.1 ml) and saline (solvent; 0.1 ml) were administered i.d. at 3 sites on
the shaved backs of rats, and 3 different reactions were elicited in the same animal. Saline containing 1% Evans blue was immediately administered i.v. After 30 min, the rats were bled to death, the blue area on the dorsal skin was cut out, and Evans blue was extracted for measurement of absorbance at 620 nm. Test drugs were administered p.o. 1 h prior to the injection of the mediator.

**Histamine-Hypersensitivity in Mice** This test was conducted according to Watanabe et al. Male BALB/c mice were actively sensitized with an intraperitoneal injection of 1 µg of OA containing 0.1 mg of Al(OH)₃ in 0.2 ml of saline at 0, 4 and 20 d. One week after the last sensitization, 20 µl of OA in saline (40 mg/ml) was administered intranasally, and the drug was administered p.o. for 5 d. The next day, histamine (1 ng/ml—10 mg/ml) was administered intranasally at a low dose without drug administration, and the number of sneezes was counted for 10 min. The threshold of the number was determined according to Watanabe et al. using un-sensitized mice. Test drugs were RKS at 30, 100 or 300 mg/kg, ketotifen at 5 mg/kg, seratrodast at 0.5 mg/kg or 5% arabic gum water (solvent).

**Sleeping Time Induced by Pentobarbital in Mice** RKS (300 and 2000 mg/kg), ketotifen (5 and 30 mg/kg) or distilled water (solvent) was administered p.o. to ICR mice, and 1 h later, 50 mg/kg of pentobarbital was administered i.p. The time between the disappearance and recovery of the righting reflex was measured with a stopwatch.

**Gastric Emptying in Rats** Rats, fasted for 24 h, received an oral dose of the test drugs, RKS (300 and 600 mg/kg), ketotifen (5 and 10 mg/kg), metoclopramide (3 mg/kg) and distilled water (solvent). Thirty minutes later (RKS, ketotifen and distilled water) or 15 min later (metoclopramide), phenol red at 100 µg/ml was orally administered, and 15 min thereafter, the animals were sacrificed and the amount of phenol red retained in their stomachs was measured according to Yokochi et al.

**Statistics** Each result is shown as the mean±S.E. The significance of difference was assessed by the Fisher method for gastric emptying test and the Dunnett method for other examinations.

## RESULTS

**Antigen-Induced Sneezing in Mice** The number of sneezes was counted for 10 min after the antigen challenge to sensitized mice. The number after the topical OA challenge was significantly reduced in the group pretreated with RKS (300 and 1000 mg/kg; p.o.) as compared to the group pretreated with distilled water p.o. (5.0±1.6 and 2.6±0.5 vs. 16.6±1.8) (Fig. 1).

**Antigen-Induced Histamine Release from Rat Peritoneal Exudate Cells** The effects of RKS in this test are shown in Fig. 2. The anaphylactic histamine release was not affected by RKS (0.1—1 mg/ml). Tranilast (1 mg/ml) markedly inhibited the anaphylactic histamine release (p<0.05).

**Histamine- and Serotonin-Induced Skin Reactions in Rats** Table 1 shows the effects of RKS and ketotifen on the exudation of dye in the dorsal skin of rats treated with histamine and serotonin. RKS (300 and 1000 mg/kg) significantly inhibited histamine- and serotonin-induced dye exudation compared with the control. Ketotifen at 5 mg/kg reduced dye exudation caused by histamine and serotonin.

**Histamine-Hypersensitivity in Mice** Sneezing was induced by a 1000-times lower concentration of histamine in the control group than in the unsensitized (normal) group (Fig. 3). RKS (100 and 300 mg/kg, p.o.) and seratrodast (0.5 mg/kg) caused a significant improvement in terms of the concentration of histamine that brings about sneezing. Ketotifen (5 mg/kg) had no effect.

**Sleeping Induced by Pentobarbital in Mice** These effects of RKS are shown in Fig. 4. No effect was observed for...
RKS (300 and 2000 mg/kg), however, ketotifen caused a significant prolongation of sleep at 5 and 20 mg/kg (p<0.01).

**Gastric Emptying in Rats**  RKS (300 mg/kg) as well as metoclopramide (3 mg/kg) significantly accelerated gastric emptying in rats (Fig. 5). RSK (600 mg/kg) and ketotifen (5 mg/kg) had no effect.

**DISCUSSION**

The drugs used most widely to treat allergic disorders are anti-histaminics, because histamine is considered to play an important role in type I allergies. In this study, RKS ameliorated allergic sneezing and histamine-induced inflammation without inhibiting the release of histamine. Nyunt et al. also reported that RKS did not inhibit histamine release from rat peritoneal exudate cells. Therefore, we considered that RKS exerts an antiallergic effect, not by inhibiting the release of histamine from mast cells, but by suppressing the response to histamine. Because RKS (0.3 mg/ml and 1 mg/ml) showed an inhibitory effect on the contraction in guinea pig ileum induced by $10^{-6} \text{M}$ histamine (data not shown), RKS may contain histamine H1 receptor antagonists as active ingredients.

Many antiallergic medicines with antihistaminic activity cause drowsiness. It is suspected that this effect is attributable to a blockade of the central histamine H1 receptor. As shown in Fig. 4, ketotifen, a histamine H1 receptor antagonist as well as a mast cell stabilizing drug, prolonged sleep induced by pentobarbital in mice. In contrast, RKS showed no effect on pentobarbital-induced sleeping time. Therefore, we want to identify which active ingredients in RKS might lead to the development of blood-brain barrier impermeable histamine H1 receptor antagonists.

RKS prevented an acceleration of hypersensitivity to histamine in mice. In recent studies, many allergic patients experienced histamine hypersensitivity. The rise in serum IgE levels, eosinophil infiltration, mast cell penetration, phospholipase A2 (PLA2) and 5-lipoxygenase (5LO) are important factors in the hypersensitive response. PLA2 and 5LO are suggested to be involved in the establishment of hypersensitivity, because a thromboxane A2 antagonist or a leukotriene D4 antagonist inhibited airway hypersensitivity. Glycyrrhizin, a triterpenoid saponin contained in a constituent herb of RKS, Glycyrrhizae Radix, selectively inhibits the activities of arachidonate cascade-related enzymes, such as 5LO and PLA2. Therefore, it is possible that glycyrrhizin contributes to the prevention of hypersensitivity by RKS. However, further study will be necessary to identify the active ingredient and the mechanism of action in RKS.

About 1—3% of all patients treated with antiallergic medicines have been reported to develop gastrointestinal disorders. In this study, RKS (300 mg/kg p.o.) accelerated gastric emptying in rats, as well as metoclopramide as a positive control. Because RKS showed no effect at the higher dose (600 mg/kg), it remains to be determined whether RKS has beneficial effects on the gastrointestinal system. However, the result suggests that RKS, at least, has no inhibitory effect on gastric emptying.

In conclusion, RKS may be beneficial for the treatment of allergic disorders, especially when patients suffer from drowsiness and gastrointestinal disorders, the most frequent adverse effects of antiallergics.

**REFERENCES AND NOTES**


