In vivo anti-influenza virus activity of Kampo (Japanese herbal) medicine "Ryo-kan-kyo-mi-shin-ge-nin-to" on allergic pulmonary inflammation model mice

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Abstract

When BALB/c mice were treated with a Kampo (Japanese herbal) medicine "Ryo-kan-kyo-mi-shin-ge-nin-to (苓甘姜味辛夏仁湯 ; RKST)" (1 g/kg, 10 times) orally from 7 days before to 4 days after the infection with mouse-adapted influenza virus A/PR/8/34 by nasal-site restricted infection, replication of the virus was effectively inhibited at 5 days after infection in comparison with water-treated mice in the broncho-alveolar cavity but not in the nasal cavity. RKST caused increment of the influenza virus-specific IgG antibody but not IgA antibody in broncho-alveolar wash at 5 days after infection in comparison with water-treated mice. When ovalbumin-sensitized allergic pulmonary inflammation model mice were infected with mouse-adapted influenza virus A/PR/8/34 and administered orally with RKST (1 g/kg, 4 times) from 2 h to 3 days after the infection, replication of the virus was significantly inhibited at 5 days after infection in comparison with water-treated control in the broncho-alveolar cavity by augmenting antiviral IgG antibody but not in the nasal cavity. These results indicate that RKST shows anti-influenza virus activity only in the broncho-alveolar cavity by augmentation of antiviral IgG antibody and RKST may be useful for treatment of influenza virus infection on patients with bronchial asthma.

Key words antiviral activity, bronchial asthma, influenza virus, Ling-Gan-Jiang-Wei-Xin-Xia-Ren-Tang, Ryo-kan-kyo-mi-shin-ge-nin-to.

Abbreviations BSA, bovine serum albumin ; CT, cholera toxin ; EMEM, Eagle's minimum essential medium ; FBS, fetal bovine serum ; MDCK, Madin-Darby canine kidney ; OVA, ovalbumin ; PBS, phosphate-buffered saline ; RKST, Ryo-kan-kyo-mi-shin-ge-nin-to (Ling-Gan-Jiang-Wei-Xin-Xia-Ren-Tang), 苓甘姜味辛夏仁湯 ; SRW, short ragweed pollen extracts ; Th1, T-helper type 1 ; Th2, T-helper type 2.

Introduction

Several Kampo (Japanese herbal) medicines have traditionally been used for the treatment of the respiratory diseases, such as "cold" syndrome, bronchial asthma and so on. Influenza virus infection is known as one of the causes of the "cold" syndrome.[1] We previously reported that Sho-seiryu-to (小青竜湯 ; Xiao-Qing-Long-Tang in Chinese) showed potent antiviral activity in the nasal and broncho-alveolar cavities of BALB/c mice against H1N1 and H3N2 subtypes of influenza A virus and influenza B virus.[2,3,4] We also reported that the antiviral IgA antibody in the nasal and broncho-alveolar washes of the Sho-seiryu-to treated mice increased in comparison with that of water-treated control.[2,3,4] Oral administration of Sho-seiryu-to caused increment of the cells secreting the influenza virus hemagglutinin-specific IgA antibody in nasal lymphocyte and augmented IL-2 receptor β chain$^+$ (activated) T-cell in intestinal Peyer's patch lymphocyte in comparison

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with water-treated mice.[4,5] These results suggest that oral administration of Sho-seiryu-to shows anti-influenza virus activity in the nasal cavity by activation of T-cell in intestinal Peyer's patch lymphocyte and stimulation of production of anti-influenza virus IgA antibody in nasal lymphocyte. However, anti-influenza virus activity of Ryo-kan-kyo-mi-shin-ge-nin-to (苓甘姜味辛夏仁湯; RKST, Ling-Gan-Jiang-Wei-Xin-Xia-Ren-Tang in Chinese) which is used for similar application as Sho-seiryu-to has not been studied.

Patients of bronchial asthma are known as one of the high risk groups of influenza virus infection. We have reported that prophylactic plus therapeutic administration of Sho-seiryu-to inhibits replications of the virus in the both nasal and broncho-alveolar cavities of allergic pulmonary inflammation (bronchial asthma) model mice by augmenting antiviral IgA antibody, but Sho-seiryu-to inhibits replication of the virus only in the nasal cavity of allergic pulmonary inflammation model mice by augmenting antiviral IgA antibody when it was administered therapeutically.[4,5] These results suggest that Sho-seiryu-to is useful for both prophylaxis and treatment of influenza virus infection on patients of bronchial asthma. Therefore it is important to study anti-influenza virus activity of RKST on the allergic pulmonary inflammation model mice.

Sho-seiryu-to and RKST have traditionally been used for the treatment of the respiratory diseases with tendency of unusual the body's water metabolism. However, these two Kampo medicines have been clinically used distinguishably. Sho-seiryu-to is prescribed for the patients with ectodermal symptoms (so called 'Hyo-Sho' in Kampo medicine), whereas RKST is administered to the patients with endodermal symptoms (so called 'Ri-Sho'). But differences of pharmacological activities between these two Kampo medicines have never been evaluated scientifically.

The present paper describes the *in vivo* effects of RKST against influenza virus infection and its mode of action in normal and allergic pulmonary inflammation model mice in order to compare with that of Sho-seiryu-to under the same experimental conditions. It is known that the same Kampo medicine is effective in some cases but ineffective in other cases, clinically. We also studied to elucidate the reasons through the anti-influenza virus activity of RKST with different administration schedule in normal and allergic pulmonary inflammation model mice.

Materials and Methods

Materials : Medicinal plants used for preparation of a Kampo medicine, Ryo-kan-kyo-mi-shin-ge-nin-to (RKST), were purchased from Uchida Wakan yaku Co. Ltd. (Tokyo, Japan). RKST was prepared as follows : mixture of crude drugs consisting of Poria (4 g), Pinelliae Tuber (4 g), Armeniacae Semen (4 g), Schizandrae Fructus (3 g), Glycyrrhizae Radix (2 g), Asari Herba Cum Radice (2 g) and Zingiberis Siccatum Rhizoma (1 g) was decocted with 600 ml of water to half volume. After the extract was centrifuged at 7500 rpm for 30 min, the supernatant was lyophilized (3.8 g, yield 19 %). Protein G Sepharose® 4 Fast Flow was obtained from Pharmacia Biotech Inc. (Uppsala, Sweden). Alkaline phosphatase-coupled goat anti-mouse IgA (α-chain specific) and IgG were purchased from Zymed Laboratories, Inc. (San Francisco, CA, U.S.A.) and Cappel Research Products, Organon Teknika Corp. (Durham, NC, U.S.A.), respectively.

Cell, virus and vaccine: Madin-Darby canine kidney (MDCK) cells were grown in Eagle's minimum essential medium (EMEM) containing 10 % inactivated fetal bovine serum (FBS), penicillin G (100 units/ml), streptomycin (100 μg/ml) and amphotericin B (2.5 μg/ml) (growth medium). The cells were maintained in a humidified atmosphere containing 5 % CO_2 at 37°C. Mouse-adapted influenza virus A/PR/8/34 (H1N1 subtype) was kept at the Kitasato Institute (Tokyo, Japan). The virus was grown in allantoic cavity of 10-day-old embryonated eggs for 48 h at 34°C. The allantoic fluid was harvested and centrifuged at 1000×g for 20 min, and then the resulting supernatant was stored in small portions at -80°C. Influenza HA vaccine was prepared from mouse-adapted influenza virus A/PR/8/34 by the method of Davenport *et al.*.[6]

In vivo anti-influenza virus experiments : Female BALB/c mice (Japan SLC Co., Ltd., Shizuoka, Japan) were used in all experiments. The animals were kept

in plastic cages with wood shavings, 5 mice each, maintained in an animal room at an ambient temperature of 24-25°C and humidity of about 50 % under a 12 h dark-light cycle. They were fed on a commercial feed (type CE-2, CLEA Japan Inc., Tokyo, Japan) and tap water ad libitum. Four to 10 mice were used in each experimental group. Influenza virus was infected by a nasal site-restricted infection as described previously.[2,3,5,7] Mice were anesthetized by an intraperitoneal injection of sodium amobarbital (0.2 ml of a saline solution of 11 mg/ml), and then infected intranasally by dropping 1 μl of mouse-adapted influenza virus A/PR/8/34 suspension ($2 \times 10^{6.5}$ 50 % egg-infecting dose/mouse) in phosphate-buffered saline (PBS), pH 7.2, containing 0.1 % bovine serum albumin (BSA) into each nostril. This procedure caused an infection that was initiated in the nasal mucosa and spread to the lung during a 3- to 7-day period, but was not lethal.[3,7] RKST lyophilizate was suspended in water (40 mg/ml), and then the suspension was administered orally by gavage to the mouse at 1 g/kg one time daily at -7, -5, -4, -3, -2, -1, 0, 2, 3 and 4 days (total 10 times) or at 2 h, 1, 2 and 3 days (total 4 times) after infection of the virus. Five days after infection, a nasal wash was obtained by washing the nasal cavity of the head with a 2 ml of PBS containing 0.1 % BSA and antibiotics. A broncho-alveolar wash was obtained by injecting a 2 ml of washing solution, twice, into the trachea and lungs which were separated from the body. Serial 4-fold or 10-fold dilutions of the nasal and broncho-alveolar washes were prepared in EMEM containing 1 % BSA and antibiotics and 0.1 ml of each dilution was added to the confluent monolayers of MDCK cells in the wells of a 96 well culture plate, and then incubated at 37°C for 3 days under a 5 % CO_2 atmosphere. The virus titers of the nasal and broncho-alveolar washes were expressed as the lowest dilution of the wash which was capable of infection in the MDCK cells by cytopathic effect. At 5 days after infection, serum specimens were prepared from mice by drawing blood from the heart with a syringe under ether anesthesia.

ELISA for anti-influenza viral IgA and IgG antibodies : The amount of IgA or IgG antibodies against mouse-adapted influenza virus A/PR/8/34 was measured for nasal and broncho-alveolar washes and serum by ELISA, as described previously,[2,3,5,8] with modification. The wells of a 96-well ELISA plate (H-type, Sumitomo Bakelite) were coated with 100 μl of the influenza HA vaccine (5 μg/ml) in 10 mM carbonate/bicarbonate buffer, pH 9.6. The plate was incubated for 2 h at room temperature and then washed three times with 200 μl/well of PBS containing 0.05 % Tween 20 and 0.1 % NaN_3 (PBS-Tween). The blocking solution, containing 1 % BSA and 0.1 % NaN_3 in PBS, was placed in the wells (200 μl) and incubated overnight at 4°C. After washing with PBS-Tween, each sample (100 μl) was added to a set of three wells. Samples for ELISA were prepared as follows. Protein G Sepharose packed into Ultrafree®-MC centrifugal filter units with low binding Durapore® membrane (pore size 0.45 μm ; Millipore) was equilibrated with 20 mM sodium phosphate buffer, pH 7.0 (binding buffer). The nasal and broncho-alveolar washes or serum diluted with the binding buffer was applied to the Protein G Sepharose column, and then the column was washed with the same buffer by centrifugation at 1000 rpm for 5 min. IgA antibody was obtained in the unabsorbed fraction, and then the IgG antibody was eluted from the column with 0.1 M glycine-HCl buffer, pH 2.7. The eluate was neutralized with 1 M Tris-HCl buffer, pH 9.0, immediately. The plates were incubated for 2 h at room temperature and washed with PBS-Tween. Alkaline-phosphatase-coupled goat anti-mouse IgA or IgG diluted with the blocking solution was added to each well (100 μl). The plates were incubated overnight at room temperature and then washed with PBS-Tween. Finally, *p*-nitrophenylphosphate (1 mg/ml) in 10 % diethanolamine buffer at pH 9.8 (150 μl) was added to each well. After the incubation at 37°C, the absorbance of the wells was read at 405 nm in a Microplate Reader.

Preparation of allergic pulmonary inflammation model mice : Allergic pulmonary inflammation model mice were prepared according to the method described previously.[5,9,10] Female BALB/c mice, 6 weeks old, were sensitized by an intraperitoneal injection of 0.5 ml alum-precipitated antigen containing 8 μg of ovalbumin (OVA, grade VI, Sigma) absorbed to 2 mg of alum (aluminum hydroxide hydrate gel, LSL Co. Ltd.) in saline vehicle. A booster injection of this

alum-OVA mixture was given 5 days later. Nonsensitized control animals received alum only. At 7 days after sensitization, the mice were exposed to antigen bronchoprovocation. For the antigen challenge, the mice were placed in a chamber and exposed to aerosolized OVA (0.5 %) for 1 h with 4 h interval. The aerosolized OVA was produced by an ultrasonic nebulizer (Model NE-U12, OMRON).

Measurements of serum IgE and broncho-alveolar lavage eosinophils : Serum IgE was determined with mouse IgE EIA kit Yamasa. Briefly, serum samples and standards (100 μl) were added to polystyrene microtiter wells coated anti-mouse IgE monoclonal antibody and incubated for 30 min at room temperature. Plates were washed 5 times with 0.02 % Tween 20 in PBS (pH 7.4) (300 μl). Horseradish peroxidase-conjugated anti-mouse IgE monoclonal antibody (100 μl) was added and incubated for 30 min at room temperature. After washing, color development solution (200 μl) containing hydrogen peroxide and tetramethylbenzidine was added and incubated for 15 min at room temperature. The plates were added 2 N HCl (50 μl) and read absorbance at 450 nm with Microplate Reader. Serum IgE levels were quantified by comparison with standard. The broncho-alveolar washes were smeared on slide glasses. The smears were fixed and stained with Wright-Giemsa stain. The number of eosinophils were counted under microscope.

Statistics : All data are presented as the mean±S.E. The significance of differences between experimental groups was analyzed with ANOVA followed by Fisher's PLSD procedure. The probability (p) values< 0.05 were considered significant.

Results

Effect of prophylactic plus therapeutic administration of Ryo-kan-kyo-mi-shin-ge-nin-to (RKST) against influenza virus infection in mice

When mice were administered with RKST (1 g/kg, 10 times) orally from 7 days before to 4 days after the infection of mouse-adapted influenza virus A/PR/8/34, virus titer of the broncho-alveolar wash at 5 days after infection was significantly reduced in comparison with that of water-treated control ($p<0.05$, Fig. 1A). However, the reduction of virus titer of the nasal wash was not significant in comparison with that of water-treated mice (Fig. 1B). These results indicate that prophylactic plus therapeutic administration of RKST has anti-influenza virus activity in the broncho-alveolar cavity of mice.

We previously reported that Sho-seiryu-to significantly augmented antiviral IgA antibody titers in the nasal and broncho-alveolar washes in mice inoculated with influenza virus.[2,3] Therefore effects of RKST on the antiviral IgA and IgG antibody titers in the respiratory tracts of mice infected with influenza virus were evaluated. When the mice were administered with RKST and inoculated with influenza virus as described above, antiviral IgG antibody titer of the broncho-alveolar wash significantly increased in com-

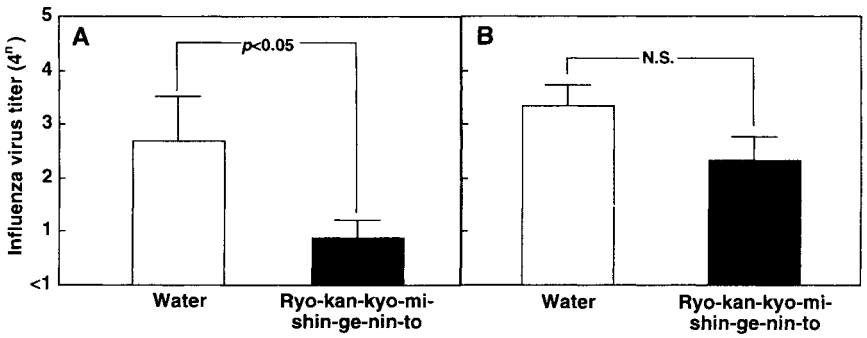

Fig. 1 Effect of prophylactic plus therapeutic administration of Ryo-kan-kyo-mi-shin-ge-nin-to (RKST) against influenza virus infection in mice. BALB/c mice were treated *p.o.* with RKST (1 g/kg) or water 10 times from 7 days before to 4 days after mouse-adapted influenza virus A/PR/8/34 exposure. At 5 days after infection, virus titers of broncho-alveolar wash (A) and nasal wash (B) were determined. Values represent mean±S.E. (n=8-10). N.S.=not significant.

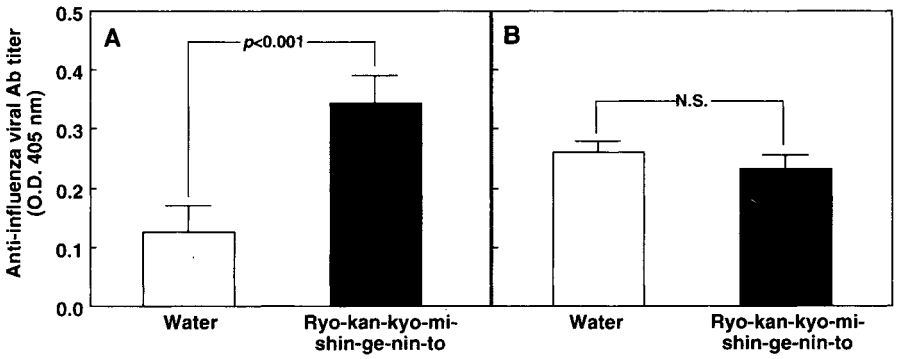

Fig. 2 Effect of RKST on augmentation of antiviral antibody in mice. BALB/c mice were treated with RKST or water and infected as described in legend of Fig.1. Anti-influenza viral IgG (A) and IgA antibody titers (B) of broncho-alveolar wash were determined as described in Materials and Methods. Values represent mean±S.E.(n=10). N.S.=not significant.

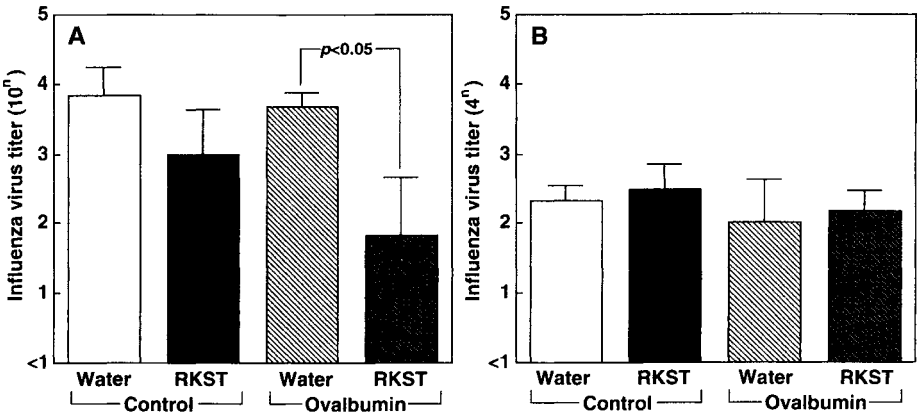

Fig. 3 Therapeutic effect of RKST against influenza virus infection in allergic pulmonary inflammation model mice. Ovalbumin-sensitized BALB/c mice were treated *p.o.* with RKST (1 g/kg) or water 4 times from 2 h to 3 days after mouse-adapted influenza virus A/PR/8/34 exposure. At 5 days after infection, virus titers of broncho-alveolar wash (A) and nasal wash (B) were determined. Values represent mean±S.E.(n=6).

parison with that of water-treated mice at 5 days after infection ($p<0.001$, Fig. 2A). However, antiviral IgA antibody titers of the broncho-alveolar wash did not increase compared with the control (Fig. 2B). The antiviral IgA and IgG antibody titers in the nasal wash were almost at the same level as those of water-treated controls (data not shown).

Therapeutic effect of RKST against influenza virus infection in allergic pulmonary inflammation model mice

RKST has traditionally been used for treatment of bronchial asthma.[11,12] Patients of bronchial asthma are one of the high risk groups for influenza virus infection. Therefore anti-influenza virus activity of RKST in allergic pulmonary inflammation model mice was studied. On OVA-sensitization, serum IgE level was increased extremely and proportion of eosinophils in broncho-alveolar lavage cells was also increased (data not shown).[5] It is known that pulmonary inflammation with eosinophil infiltration is a prominent feature of allergic respiratory diseases, such as bronchial asthma.[9] Therefore this mouse is useful as a bronchial asthma model. When OVA-sensitized mice were infected with mouse-adapted influenza virus A/PR/8/34 and administered with RKST (1 g/kg, 4 times) orally from 2 h to 3 days after the infection, virus titer of the broncho-alveolar wash at 5 days after infection was significantly reduced in

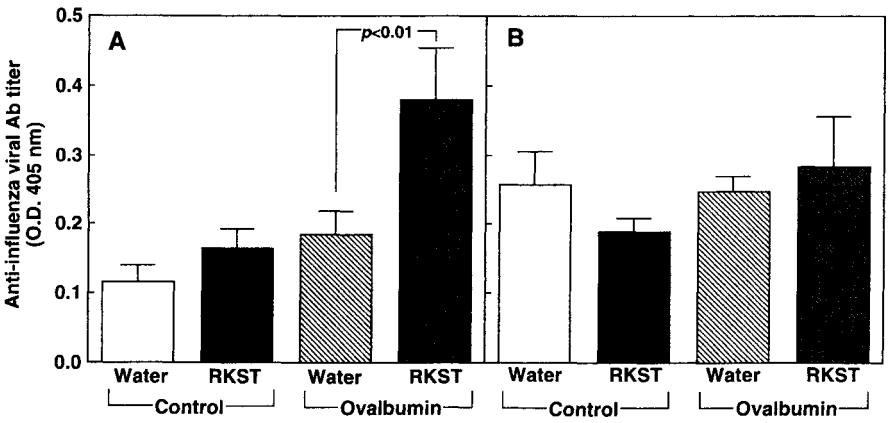

Fig. 4 Effect of RKST on augmentation of antiviral antibody in allergic pulmonary inflammation model mice. Ovalbumin-sensitized BALB/c mice were treated with RKST or water and infected as described in legend of Fig. 3. Anti-influenza viral IgG (A) and IgA antibody titers (B) of broncho-alveolar wash were determined as described in Materials and Methods. Values represent mean± S.E. (n=4-8).

comparison with water-treated control ($p<0.05$, Fig. 3A). However, virus titer of the nasal wash was almost same in comparison with that of water-treated OVA-sensitized mice (Fig. 3B). These results indicate that RKST has therapeutic anti-influenza virus activity in the broncho-alveolar cavity of allergic pulmonary inflammation model mice. Whereas, RKST did not show any significant antiviral activity in the nasal (Fig. 3B) and broncho-alveolar cavities (Fig. 3A) of OVA non-sensitized control mice, respectively, when it was administered (1 g/kg, 4 times) from 2 h to 3 days after the infection of influenza virus. When the OVA-sensitized mice were administered with RKST and infected as described above, antiviral IgG antibody titer in the broncho-alveolar wash significantly increased in comparison with that of water-treated mice at 5 days after infection ($p<0.01$, Fig. 4A). However, antiviral IgA antibody titer of broncho-alveolar wash did not increase compared with the control (Fig. 4B). The antiviral IgA and IgG antibody titers in the nasal wash and antiviral IgG antibody titers in the serum were almost at the same level as those of water-treated controls (data not shown). When OVA non-sensitized control mice were administered with RKST after the infection of influenza virus, the antiviral IgG antibody titer in the broncho-alveolar wash did not increase significantly in comparison with that of water-treated mice (Fig. 3A), and the antiviral IgA antibody titer also did not increase (Fig. 3B).

Discussion

In the present experiments, anti-influenza virus activity of RKST, a kind of Kampo formula which has been used for treatment of the respiratory diseases clinically, was determined with mice and it was shown that proliferation of the influenza virus was inhibited in the broncho-alveolar cavity by augmentation of antiviral IgG antibody, but RKST did not show any antiviral activity in the nasal cavity. In the previous study, *in vivo* anti-influenza virus activity of Sho-seiryu-to, another Kampo medicine which has been used for treatment of the respiratory diseases, was studied and it was clearly demonstrated that oral administration of Sho-seiryu-to decreased virus replication in the nasal cavity and also inhibited virus spread to the broncho-alveolar cavity, augmenting nasal and broncho-alveolar antiviral IgA antibody titers in the virus-infected mice.[2,3,4] These results indicate that both RKST and Sho-seiryu-to show anti-influenza virus activity in mice but effective site in respiratory tracts and mode of action of these Kampo medicines are from different each other (Table I).

Because bronchial asthmatic patients are one of the groups at high risk for influenza virus infection, it

Table I Anti-influenza virus activities and clinical effects of Ryo-kan-kyo-mi-shin-ge-nin-to (RKST) and Sho-seiryu-to.

Kampo medicine	Normal mouse				Bronchial asthma model mouse				Clinical effects
	Antiviral activity		Antiviral antibody		Antiviral activity		Antiviral antibody		
	Nasal cavity	Broncho-alveolar cavity	Nasal cavity	Broncho-alveolar cavity	Nasal cavity	Broncho-alveolar cavity	Nasal cavity	Broncho-alveolar cavity	
RKST	×	○	→	IgG ↑	×	○	→	IgG ↑	Respiratory diseases (bronchitis, bronchial asthma, cold, *etc.*) accompanying with 'Ri-Sho (a cold constitution, gastrointestinal disorder, *etc.*)' Respiratory diseases of elderly people
Sho-seiryu-to	○	○	IgA ↑	IgA ↑	○	×	IgA ↑	→	Respiratory diseases (pollinosis, bronchitis, bronchial asthma, cold, rhinitis, *etc.*) accompanying with 'Hyo-Sho (fever, chill, *etc.*)'

○ : positive ; × : negative ; ↑ : increase : → : no change compared with Kampo medicine untreated group.

is very important to repair the influenza virus infection of bronchial asthmatic patients. In the present study, anti-influenza virus activity of RKST was studied on OVA-sensitized allergic pulmonary inflammation model mice, and the present results indicate that RKST shows the antiviral activity only in lower respiratory tract, the broncho-alveolar cavity, by enhancement of augmentation of influenza virus-specific IgG antibody by therapeutic administration. We previously reported that Sho-seiryu-to showed potent anti-influenza virus activity in the upper respiratory tract, the nasal cavity, of allergic pulmonary inflammation model mice through augmentation of the antiviral IgA antibody titer by therapeutic administration.[4,5] These results clearly demonstrated that therapeutic administrations of RKST and Sho-seiryu-to conferred better protection against influenza virus infection on the allergic pulmonary inflammation model mice but effective site in respiratory tracts and mode of action of these Kampo medicines are different from each other (Table I). These differences of anti-influenza virus activity between RKST and Sho-seiryu-to on normal and allergic pulmonary inflammation model mice may be related to the differences of clinical application of these Kampo medicines. Sho-seiryu-to is prescribed for the patients of respiratory diseases with ectodermal symptoms (so called 'Hyo-Sho'), whereas RKST is administered to the patients with endodermal symptoms (so called 'Ri-Sho'), clinically (Table I). 'Hyo-Sho' may be related to the nasal symptoms and 'Ri-Sho' may be related to the broncho-alveolar symptoms on respiratory diseases, such as influenza virus infection.

Sho-seiryu-to contains Ephedrae Herba (terrestrial stem of *Ephedra sinica* Stapf (Ephedraceae)) as one of its component herbs, and it is well known that E. Herba contains (-)-ephedrine which is a sympathomimetic agent[13,14] and a stimulant of central nervous system.[15,16] Therefore Kampo formulas which contain E. Herba must be prescribed carefully for the patients with cardiovascular diseases, such as angina pectoris, myocardial infarction and hypertension, and elderly people. RKST has similar indications with Sho-seiryu-to but does not contain E. Herba as a component herb, therefore RKST has been prescribed for treatments of respiratory diseases of these patients. These results suggest that RKST is useful for the treatment of influenza viral infection on bronchial asthmatic patients with cardiovascular diseases and elderly people.

In the present paper, therapeutic administration of RKST inhibited proliferation of the influenza virus and enhanced augmentation of antiviral IgG antibody in the broncho-alveolar cavity of OVA-sensitized pulmonary inflammation model mice. Whereas, RKST did not show significant effect on the OVA non-sensitized mice, when it was administered after the influenza virus infection. Serum IgE antibody level and proliferation of eosinophils in broncho-alveolar lavage were enhanced in allergic pulmonary inflammation model mice.[9,10] It has been reported that Th2 cell encourages production of systemic IgE anti-

body[17,18,19,20] and enhances proliferation of eosinophils.[19,21] It has been also reported that Th2 cell activates production of systemic IgG1 subclass antibody in mice.[17,18,20,22] These results suggest that RKST enhances the production of IgG effectively and shows potent anti-influenza virus activity in the Th2-dominant condition, such as allergic pulmonary inflammation. Assessment of IgG subclass in the broncho-alveolar cavity enhanced by administration of RKST and mechanism of augmentation of antiviral IgG antibody in the broncho-alveolar cavity by RKST are now in progress. In the present paper, prophylactic plus therapeutic administration of RKST (10 times) showed potent anti-influenza virus activity in normal mice, but therapeutic administration of RKST (4 times) did not show significant antiviral activity in OVA non-sensitized mice. These results indicate that administration schedule and/or administration time were also related to the effectiveness of RKST adding to OVA-sensitization or non-sensitization of mice.

RKST has been used clinically for the treatments of allergic diseases in the respiratory system, such as bronchial asthma.[11,12] Marinaro *et al.* reported that when mice were orally immunized with OVA and cholera toxin (CT) as the adjuvant, this regimen induced antigen-specific IgE and IgG1 responses in the serum.[18] They also reported that when these mice were systemically challenged with homologous antigen at the time of peak IgE response, none of the mice demonstrated signs of anaphylaxis.[18] Moreover, it is known that mice orally immunized with tetanus toxoid and CT, that were challenged *s.c.* with tetanus toxin did not exhibit anaphylaxis.[23] Litwin *et al.* reported that when short ragweed pollen extracts (SRW)-sensitive pollinosis patients were treated with microcapsulated SRW orally, the patients had high titers of serum SRW-specific IgG antibodies and lowered SRW-induced hay fever symptoms, a kind of allergic rhinitis.[24] In the present study, we showed that RKST enhanced antigen-specific IgG augmentation in the respiratory tract, broncho-alveolar cavities. Since high level of antigen-specific IgG antibody was induced in broncho-alveolar cavities of these mice, the IgG antibody may block binding of the antigen to IgE on sensitized mast cells. This mechanism may be related to the anti-allergic activity of RKST. Investigation of the relationship between anti-influenza virus activity and anti-allergic activity of RKST on bronchial asthmatic model mice is now in progress.

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和文抄録

苓甘姜味辛夏仁湯の抗インフルエンザウイルス活性についてマウスを用いて検討した。ウイルス感染7日前から4日後まで苓甘姜味辛夏仁湯の熱水抽出エキス（1 g/kg, 10回）を経口投与したBALB/cマウスに，マウス馴化インフルエンザウイルスA/PR/8/34を上気道感染させたところ，水投与（コントロール）群に比べ苓甘姜味辛夏仁湯群ではウイルス感染5日後において肺洗液のウイルス価を有意に低下させた。これに対し，鼻腔洗液中のウイルス価は低下させなかった。また，苓甘姜味辛夏仁湯は肺洗液のインフルエンザウイルス特異的IgG抗体価を有意に上昇させたが，IgA抗体価は上昇させなかった。卵白アルブミンを吸入させて作製した気道炎症（気管支喘息）モデルマウスにおける苓甘姜味辛夏仁湯の抗インフルエンザウイルス活性を検討した。気道炎症モデルマウスにマウス馴化インフルエンザウイルスA/PR/8/34を上気道感染させ，2時間後から4日後まで苓甘姜味辛夏仁湯の熱水抽出エキス（1 g/kg, 4回）を経口投与したところ，同漢方方剤は肺においてのみインフルエンザウイルスに特異的なIgG抗体を上昇させることにより，抗インフルエンザウイルス活性を示した。以上の結果より，苓甘姜味辛夏仁湯は正常マウスおよび気道炎症モデルマウスの肺でウイルス特異的IgG抗体産生を増強することにより抗インフルエンザウイルス活性を示すことが明らかとなった。また，苓甘姜味辛夏仁湯は気管支喘息患者におけるインフルエンザウイルス感染の治療に有効である可能性が示唆された。

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