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Short communication

Effect of Shin'iseihaito on murine allergic reaction induced by nasal sensitization

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ABSTRACT

Shin'iseihaito (Magnolia Flower Lung-Clearing Decoction; 辛夷清肺湯 xīn yí qīng fèi tāng), a formula of traditional Japanese kampo medicine (日本漢醫 rì běn hàn yī) and traditional Chinese medicine (TCM; 中醫 zhōng yī), has been used for the treatment of chronic sinusitis. The objective of this study was to evaluate the anti-allergic effect of shin'iseihaito on murine allergic reaction induced by nasal sensitization using ovalbumin (OVA) as an antigen. Extract of shin'iseihaito (SSHT) could reduce the eosinophil, serum IgE and interleukin (IL)-4 levels, while increased the interferon (IFN)-γ levels in allergic mouse. Furthermore, allergic-murine serum treated with SSHT could not activate passive cutaneous anaphylaxis (PCA) reaction in murine model. Thus, our study showed that SSHT may possess anti-allergic activity. We suggested that SSHT may contribute to inhibit the exacerbation of allergic reaction induced by nasal sensitization.

1. Introduction

Many people, approximately 20% of the population, suffer from allergic rhinitis worldwide.[1] From the clinical point of view, allergic rhinitis causes sneezing, nasal discharge, and nasal obstruction. From the immunological point of view, allergic rhinitis is a typical Th2 immune disorder characterized by a high level of antigen specific IgE production.[2] Because the enhanced IgE production and inflammatory response in rhinitis are due to predominant production of Th2 cytokines such as IL-4, the allergic symptoms can be alleviated by inhibition of Th2 cytokine responses.[2] Many researchers have investigated allergic rhinitis using an animal model.[1–3] The model of allergic mice made by OVA sensitization and challenge had increased serum IgE and eosinophil.[3]

Shin'iseihaito (SSHT; Magnolia Flower Lung-Clearing Decoction; 辛夷清肺湯 xīn yí qīng fèi tāng), a formula consisting of nine crude drugs, has been used for the treatment of nasal diseases such as chronic sinusitis in traditional Japanese kampo medicine (日本漢醫 rì běn hàn yī) and traditional Chinese medicine (TCM; 中醫 zhōng yī).[4,5] More than 50% of individuals with allergic rhinitis have clinical or radiographic evidence of chronic sinusitis and 25–58% of individuals with sinusitis have aeroallergen sensitization.[6] Elevated total IgE is a risk factor for the presence of severe chronic sinusitis.[6] Previous researches suggest that chronic sinusitis could be an atopic disease driven by IgE sensitization to aeroallergens.[6] From these studies, shin'iseihaito may be potential useful for allergic disease. However, it has not been clarified for its role in anti-allergy therapy before.

Thus, in the present study, we investigated whether SSHT is able to suppress the murine allergic reaction induced by nasal sensitization.

2. Methods

2.1. Animals

Female Balb/c mice (Japan SLC Ltd, Hamamatsu, Japan) were used. All animals were 6 week old at the start of the experiments.

2.2. Shin'iseihaito (SSHT; Magnolia Flower Lung-Clearing Decoction; 辛夷清肺湯 xīn yí qīng fèi tāng)

Shin'iseihaito consists of 3.0 g (daily dose for human) of *Gypsum fibrosum* (石膏 shí gāo), 3.0 g of tuber of *Ophiopogon japonicus* (麥門冬 mài mén dōng), 1.5 g of root of *Scutellaria baicalensis* (黃芩 huáng

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qín), 1.5 g of rhizome of *Anemarrhena asphodeloides* (知母 zhī mǔ), 0.75 g of fruit of *Gardenia jasminoides* (梔子 zhī zǐ), 1.5 g of bulb of *Lilium lancifolium* (百合 bǎi hé), 1.5 g of flower of *Magnolia salicifolia* (辛夷 xīn yí), 0.5 g of leaf of *Eriobotrya japonica* (枇杷葉 pí pá yè), 0.75 g of rhizome of *Cimicifuga heracleifolia* (升麻 shēng má). These crude drugs were boiled, filtered, and the decoction was dried to yield powdered extract (SSHT, 2.5 g for daily human dose). SSHT (Lot: 14B019) was provided as a generous gift from the Kobayashi Pharmaceutical Co., LTD (Osaka, Japan). SSHT was suspended in distilled water to prepare the stock solution at a concentration of 0.1 g/mL and kept in −20 °C until use.

2.3. Establishment of allergic murine model

Allergic murine models were established based on the previously described methods with minor modifications.[7,8] The experimental timetable is provided in Fig. 1. Mice were intraperitoneally administered 0.1 mg/mL OVA (Sigma–Aldrich, St. louis, MO, USA) and 40 mg/ml $Al(OH_3)$ in saline at a dosage of 100 μL/mouse. Sensitization was repeated twice (days 0 and 7), followed by daily injections of OVA solution (15 mg/mL in saline, 10 μL/each nostril) into nostrils from day 14–28 (challenge). In the SSHT-treated group, mice were force-fed SSHT (10 mg/0.1 mL/10 g body weight (bw)/day, 20-fold of human dosage) from day 13–28. Mice in the control group were given an equal volume of saline and were infected using the same method.[9] At the end of animal experiment, the mice were anesthetized and blood samples were collected by cardiac puncture. All animal procedures were approved by the Institutional Animal Care and Use Committee at Nagoya City University, Japan.

2.4. Leukocyte, eosinophil, IgE, interleukin (IL)-4, and interferon (IFN)-γ in blood sample

Blood samples from the allergic murine model were collected after 2 h of the last challenge on day 28. The number of leukocyte and eosinophilia were measured in Tohkai cytopathology institute (Gifu, Japan).

Concentrations of serum total IgE, IL-4, and IFN-γ were evaluated using Mouse IgE ELISA, Mouse IL-4, and Mouse IFN-γ ELISA kit (Biolegend Inc. San Diego, CA, USA) according to the manufacturer's instructions, respectively. Cytokine levels were calculated using standard murine recombinant cytokine curves run on the same immunoplate.

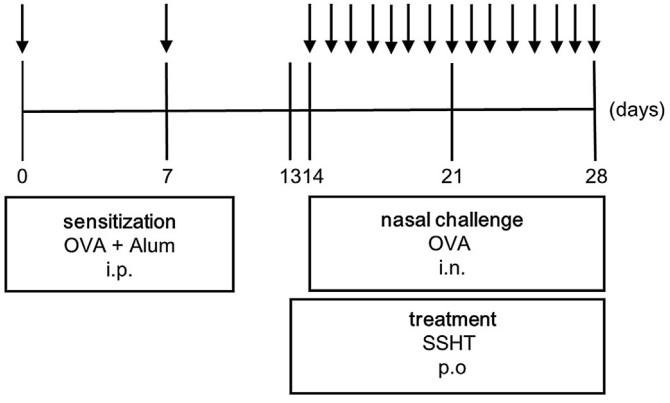

Fig. 1. Sensitization and challenge protocols for the experimental allergic murine model. Mice were sensitized by intraperitoneally (i.p.) injected OVA plus adjuvant Alum [$Al(OH)_3$] on days 0 and 7, followed by daily intranasal (i.n.) challenge with OVA solution on days 14–28. Allergic response was monitored on day 28.

2.5. Passive cutaneous anaphylaxis reaction (PCA)

Serum IgE antibodies specific to OVA were also determined using PCA tests. Anti-OVA serum was collected from previous allergic rhinitis murine model. The untreated female 6 week-old Balb/c mice were injected intradermally with 10 μL aliquot of 50 fold diluted anti-OVA of serum in saline into shaved dorsal skin sites. After two days, OVA (0.1 mg) with 0.5% Evans blue (Wako Pure Chemicals, Osaka, Japan) in saline was injected intravenously into the tail vein. One hour after antigen challenge, mice were euthanized and the dorsal skin of the mouse was removed to measure the pigment area. The area of blue spots on the internal surface of the skin was measured.[10,11]

2.6. Statistical analysis

Statistical analysis was performed by repeated one-way analysis of variance (ANOVA) and the Tukey/Bonferroni/Dunnett's multiple comparison test. A probability value ($P < 0.05$) was considered to be statistically significant.

3. Results

3.1. Shin'iseihaito (SSHT; Magnolia Flower Lung-Clearing Decoction; 辛夷清肺湯 xīn yí qīng fèi tāng) inhibit the eosinophil level of allergic mice

The leukocyte levels were significantly higher in allergic mice than those in untreated mice, but those levels were not significantly changed by SSHT-treatment [Fig. 2a]. The eosinophilia levels were significantly higher in allergic mice than those in untreated mice. Compared to allergic mice, the allergic rhinitis mice treated with SSHT had significantly decreased eosinophil levels ($P < 0.01$) [Fig. 2b].

3.2. SSHT inhibit the IgE and IL-4 levels of allergic mice

The IgE levels were higher in allergic mice than those in untreated mice. Compared to allergic mice, the allergic mice treated with SSHT had significantly decreased IgE levels [Fig. 3a]. The IL-4 levels were significantly higher in allergic mice than those in untreated mice. Compared to allergic mice, the allergic mice treated with SSHT had also significantly decreased IL-4 levels ($P < 0.01$) [Fig. 3b].

3.3. SSHT activate the IFN-γ levels of allergic mice

The IFN-γ levels were significantly lower in allergic mice than those in untreated mice. Compared to allergic mice, the allergic mice treated with SSHT had also significantly increased IFN-γ levels ($P < 0.01$) [Fig. 3c].

3.4. SSHT inhibits PCA reaction in allergic mouse

As we found that the eosinophilia and cytokine level of allergic mouse treated with SSHT were remarkably decreased, we also confirmed the effect of SSHT on the PCA reaction. The sera containing OVA-specific anaphylactic antibodies were intradermally injected and the PCA reaction was measured 1 h after the injection of the Evans blue solution containing OVA. We observed that PCA reactions using the sera of allergic mice were exhibited as blue spots. However, the average sizes of blue spots were significantly decreased in the sera of SSHT-treated allergic mice compared to those in the sera of allergic mice. No blue spot was observed in the

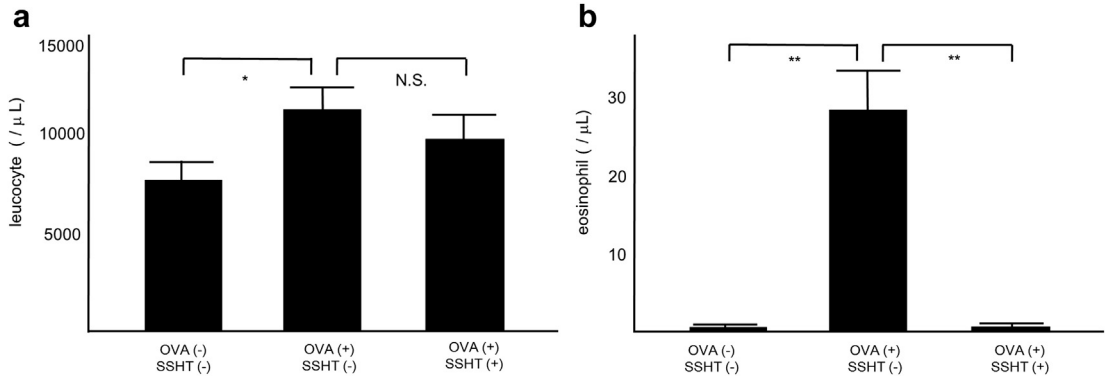

Fig. 2. Effect of SSHT on (a) leukocyte and (b) eosinophil level in allergic murine model. Data are mean ± S.D. ($n = 3$). $^{**}P < 0.01$.

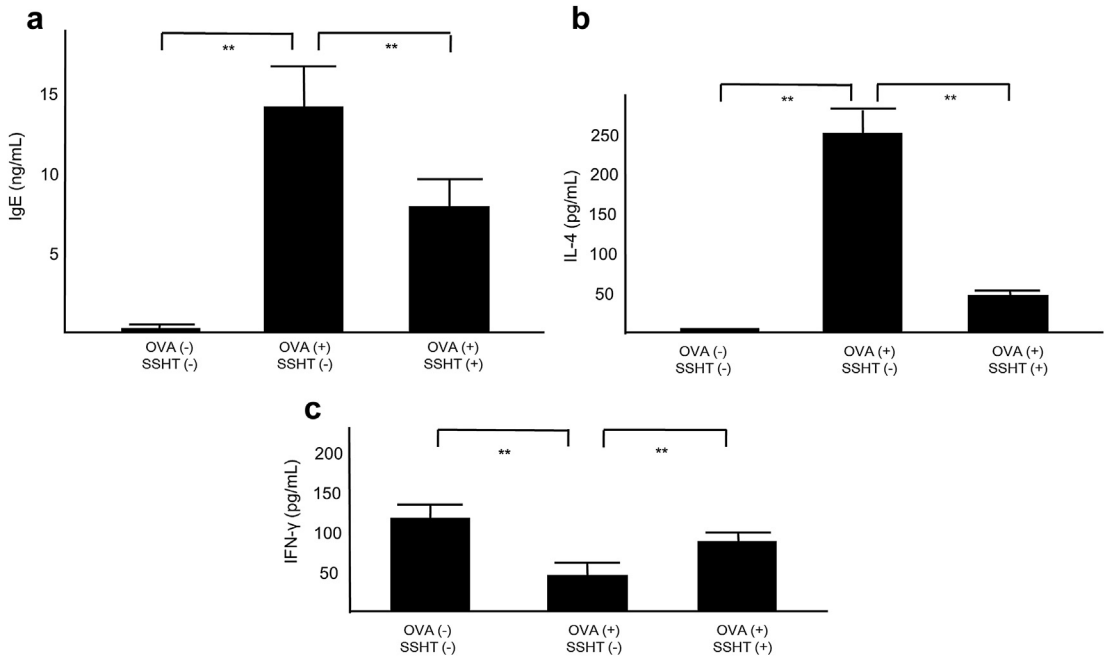

Fig. 3. Effect of SSHT on (a) IgE, (b) IL-4, and (c) IFN-γ in allergic murine model. Data are mean ± S.D. ($n = 6$). $^{**}P < 0.01$.

negative control [Fig. 4]. Our results revealed that SSHT-treatment dramatically inhibited the PCA reaction.

4. Discussion

In the present study, we showed that the administration of Shin'iseihaito (SSHT; Magnolia Flower Lung-Clearing Decoction; 辛夷清肺湯 xīn yí qīng fèi tāng) is capable of suppressing the IgE and IL-4 levels in murine allergic reaction induced by nasal sensitization. SSHT also activated the IFN-γ level in same allergic model. Thus, our result showed that SSHT may return the decreased IFN-γ/IL-4 (Th1/Th2) ratio of the allergic group to the normal range.

This result may also explain the reasons why the administration of SSHT suppressed the production of anti-OVA IgE antibody without affecting the development of $CD4^+$ T cells, since IL-4 is the cytokine known to induce immunoglobulin class switching to IgE.[12] IL-4 is known to serve not only as a mast cell growth factor but also as the major mast cell chemoattachment.[13] Therefore, it is possible that the decrease of Th2 responses in nasal mucosa modulated the mast cell (IgE)-mediated nasal symptoms, in concert with the decreased production of anti-OVA IgE antibody.[13]

Our result of PCA reaction also demonstrated that SSHT inhibited the anaphylaxic factors such as IgE and other antigens. SSHT may be effective for Type-I allergic reaction. The precise mechanism of the effect of SSHT on PCA reaction is to be determined in a future study.

Although our results demonstrate that SSHT had anti-inflammatory effect, each individual component of SSHT also shows anti-inflammatory effect. Especially, the flower of *M. salicifolia* (辛夷 xīn yí) has several anti-inflammatory effects including the inhibitory effects of mast cell-derived histamine release and PCA reaction.[14–17] The fruit of *G. jasminoides* (梔子 zhī zǐ) inhibits the histamine release from mast cells and lowered the serum level of IgE and histamine in allergic murine model.[18] The rhizome of *A. asphodeloides* (知母 zhī mǔ) inhibits NF-κB transcription activity via the p38 MAPK and ERK pathway.[19] The leaf of *E. japonica* (枇杷葉 pí pá yè) had anti-inflammatory effect with attenuation of p38MAP kinase and ERK.[20] Some researcher reported that the root of *S. baicalensis* (黃芩 huáng qín), the bulb of *L. lancifolium* (百合 bǎi hé), and the tuber of *O. japonicus* (麥門冬 mài mén dōng) had anti-inflammatory activity.[21–23] Although each component has anti-inflammation effect, this mechanism in detail has been unknown.

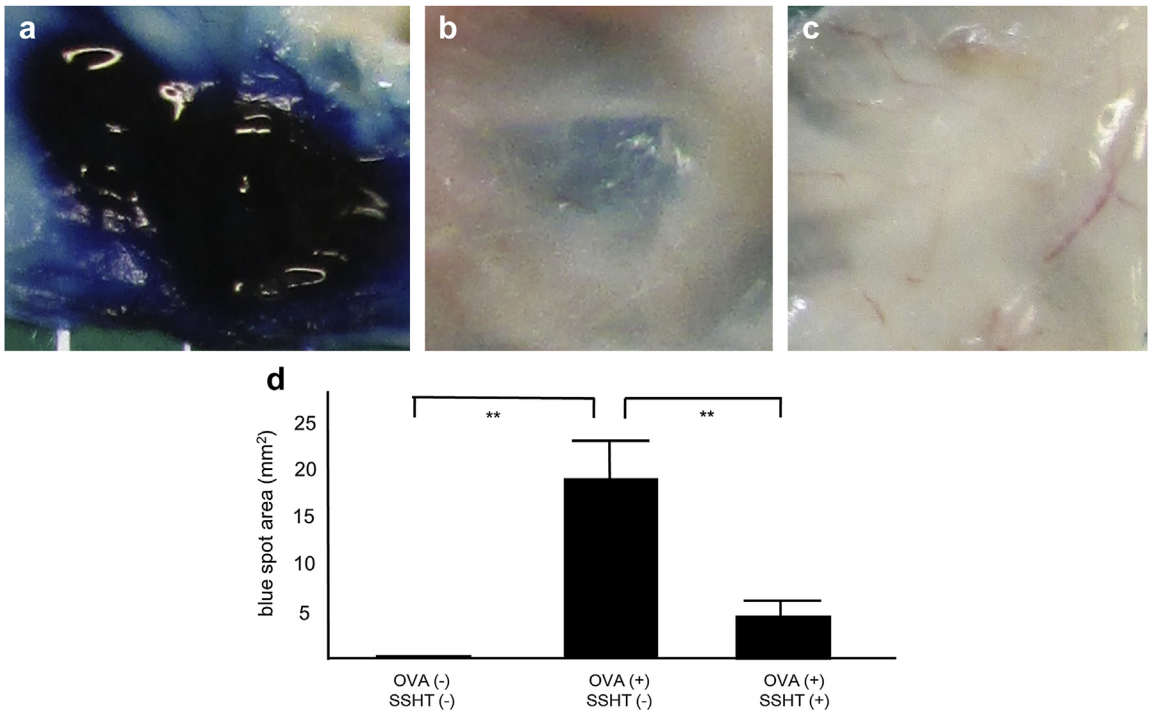

Fig. 4. PCA reaction induced by allergic murine serum. The serum with OVA-specific IgE from the mice was injected intradermally into the mouse. After two days, OVA was injected with Evans blue into the tail vein. The positive reactions were shown as blue spots. (a) OVA-induced allergic group, (b) SSHT-treated OVA-induced allergic group, (c) negative control. (d) The areas of blue spot on the internal surface of the skin were measured. Data are mean ± S.D. ($n = 6$). $^{**}P < 0.01$.

Japanese traditional kampo medicine (日本漢醫 rì běn hàn yī) is generally composed of several components and the interaction of them may enhance the effect of drugs. As further investigation from this perspective is needed, SSHT may have pronounced anti-inflammatory effects.

5. Conclusion

Administration of Shin'iseihaito (SSHT; Magnolia Flower Lung-Clearing Decoction; 辛夷清肺湯 xīn yí qīng fèi tāng) is capable of improving allergic status in OVA-induced allergic murine model. Further studies are required to confirm the anti-allergy effects and elucidate the mechanisms of anti-allergy action of SSHT in allergic murine model.

Conflict of interest

Zhixia Jiang and Tetsuya Arai are employees of Kobayashi Pharmaceutical Co. Ltd. Other authors have no conflict of interest.

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