

Anti-allergic effects of So-Cheong-Ryong-Tang, a traditional Korean herbal medicine, in an allergic rhinitis mouse model

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Abstract The herbal medicine, So-Cheong-Ryong-Tang (SCRT) has been empirically used for the treatment of allergic rhinitis for hundreds of years; however, its *in vivo* effects on allergic rhinitis have been rarely elucidated. We aimed to evaluate the anti-allergic effects of SCRT in an allergic rhinitis mouse model and to examine the underlying mechanism(s) of its anti-allergic effects. BALB/c mice were sensitized with ovalbumin (OVA) and alum and then challenged intranasally with OVA. SCRT (1 g/kg) was given to the treatment group, and multiple parameters of allergic responses were evaluated to determine the effects of SCRT on allergic rhinitis. SCRT reduced allergic symptoms, such as rubbing and sneezing, and eosinophil infiltration into the nasal mucosa. It also suppressed serum total IgE, OVA-specific IgE, and OVA-specific IgG1 levels and increased OVA-specific IgG2a level. SCRT significantly reduced expression of the Th2 cytokine, IL-4; however, the expression of IL-5, IFN- γ , and IL-10 was unchanged in the nasal mucosa of the treatment group (by real-time

RT-PCR). In splenocyte culture, levels of both IL-4 and IL-5 decreased, and IFN- γ level increased in the treatment group; however, levels of IL-10 and TGF- β were unaffected by administration of SCRT. This study shows that SCRT induced anti-allergic effects by decreasing, locally and systemically, the Th2 cytokine IL-4, isotype switching to IgE, and eosinophilic infiltration into the nasal mucosa in an allergic rhinitis mouse model. Thus, SCRT might be considered a potential therapeutic agent in treating allergic rhinitis.

Keywords Allergic rhinitis · So-Cheong-Ryong-Tang · Mouse model

Abbreviations

SCRT So-Cheong-Ryong-Tang
OVA Ovalbumin
IFN- γ Interferon-gamma

Introduction

The traditional herbal medicine “So-Cheong-Ryong-Tang” (SCRT) has been used clinically for the treatment of allergic rhinitis, bronchitis, bronchial asthma, and cold symptoms for hundreds of years in Asian countries; it is called “Sho-seiryu-to” in Japan and “Xiao-Qing-Long-Tang” in China. Its *in vitro* effects have been studied by several groups, and various effects have been identified including inhibition of histamine release and degranulation of mast cells, inhibition of proliferation of eosinophils and basophils, inhibition of cholinergic effects for nasal gland acinar cells, inhibition of synthesis of TNF- α by peripheral blood mononuclear cells, and inhibition of contraction of bronchial smooth muscle cells [1].

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Several other studies have evaluated the anti-allergic effects of SCRT *in vivo*. Most studies have evaluated its anti-allergic effects in a bronchial asthma model or in a passive cutaneous anaphylactic reaction model [1–4]. Kao et al. [2] evaluated its anti-allergic effects in a mite-sensitized bronchial asthma mouse model and in an OVA-sensitized bronchial asthma guinea pig model [3]. Nagai et al. [1] also evaluated its effect in OVA-sensitized mouse asthma model, and Sakaguchi et al. [4] reported its anti-anaphylactic effects in the PCA reaction in rats.

In the present study, we evaluated the effects of SCRT on allergic rhinitis and examined the underlying mechanisms of its anti-allergic effects in a mouse model.

Materials and methods

Animals

Four-week-old female BALB/c mice were obtained from Korea Biolink Co. (Eumsung, Korea). All animal experiments in this study followed the guidelines for Institutional Animal Care at the Clinical Research Institute of Seoul National University Hospital.

Preparation of SCRT extract

SCRT, which is derived from eight species of medicinal plants and they were purchased from the Korean Association of Crude Medicinal Herbs. Every herb was grounded to fine powder and mixed with the others with the ration as follows; *Pinelliae Tuber* (6.0 g, Pinelliac acid, tuber of *Pinellia ternata*), *Ephedrae Herba* (6.0 g, stem of *Ephedra sinica*), *Schizandrae Fructus* (6.0 g, fruit of *Schisandra chinensis*), *Paeoniae Radix* (6.0 g, root of *Paeonia lactiflora*), *Glycyrrhizae Radix* (4.0 g, root of *Glycyrrhiza uralensis*), *Zingiberis Siccata Rhizoma* (4.0 g scalded rhizoma of *Zingiber officinale*), *Asiasari Radix* (6.0 g root of *Asiasarum sieboldii*), and *Cinnamomi Cortex* (4.0 g, bark of *Cinnamomum cassia*). The SCRT was dissolved in distilled water for administration to mice.

Murine allergic rhinitis model and treatment

Mice were divided into three groups, each consisting of 10 mice (group A: negative control group, group B: positive control group, group C: treatment group; Fig. 1; Table 1). The procedures for allergen sensitization and treatment are summarized in Fig. 1. Briefly, mice were sensitized with an intraperitoneal injection of 25 µg of ovalbumin (OVA; grade V; Sigma, St. Louis, MO, USA) and 1 mg of aluminum hydroxide gel on days 0, 7, and 14. After general sensitization, mice were locally challenged with 500 µg of

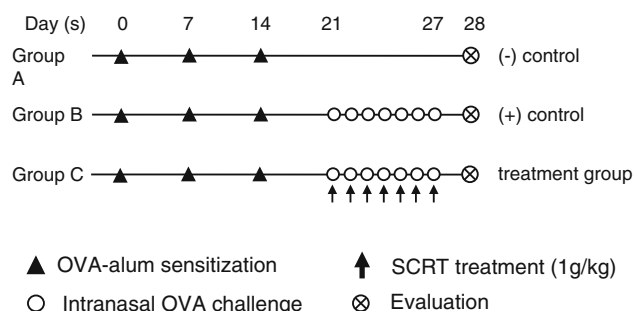


Fig. 1 Experimental protocol. BALB/c mice were sensitized with ovalbumin (OVA) and 1 mg of aluminum hydroxide gel (alum) on days 0, 7, and 14 (general sensitization). All groups except for group A, the negative control, received intranasal OVA from day 21 to 27. In addition to sensitization and challenge, group C mice were fed with So-Cheong-Ryong-Tang (1 g/kg/day for 1 week) 3 h before intranasal OVA challenge

Table 1 Experimental groups and characteristics

Group (n)	Systemic sensitization	Local sensitization	Treatment
A (10) (–) control	PBS	PBS	50 µL of PBS
B (10) (+) control	25 µg OVA	500 µg OVA	50 µL of PBS
C (10) SCRT-treatment group	25 µg OVA	500 µg OVA	1 g/kg p.o. for 1 week

SCRT So-Cheong-Ryong-Tang, PBS phosphate-buffered saline

OVA into their nostrils from days 21 to 27. Along with sensitization and challenge, the mice in the treatment group were fed the SCRT (1 g/kg for 1 week) 3 h before intranasal OVA challenge.

Symptom score and tissue preparation

At 15 min after the final OVA challenge on day 27, a blinded observer recorded the frequencies of sneezing and nasal rubbing over a 15-min interval. Mice were then sacrificed 24 h after the last OVA challenge. After perfusion with 4 % paraformaldehyde, the heads of five mice from each group were removed en bloc and then fixed in 4 % paraformaldehyde. After removing the nasal cavity from the head of the other five mice, the nasal mucosa was meticulously removed using a small curette. Samples in the same group were pooled because the nasal mucosa in each mouse was so small. Nasal mucosa was immediately immersed in liquid nitrogen and stored at –70 °C until use for reverse transcription-polymerase chain reaction (RT-PCR).

Eosinophils in the nasal septal mucosa

For evaluation of nasal histology, nasal tissues were decalcified, embedded in paraffin, and sectioned coronally (4 μ m thick) approximately 5 mm from the nasal vestibule. Each section was stained with hematoxylin and eosin, and the number of eosinophils on both sides of the septal mucosa was counted. The number of eosinophils in the submucosal area of the whole nasal septum was counted under a light microscope (400 $\times$ magnification).

Real-time RT-PCR for IL-4, IL-5, IL-10, and IFN- γ in the nasal mucosa

Total RNA was prepared from the nasal mucosa using the TriZol reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) was synthesized using superscript reverse transcriptase (Invitrogen) and oligo(dT) primers (Fermentas, Burlington, ON, Canada). For analysis of IL-4 (Mm00445258_g1), IL-5 (Mm01290072_g1), IL-10 (Mm00439616_m1), IFN- γ (Mm99999071_m1), and GAPDH (Mm03302249_g1), PDAR (pre-developed assay reagent) kits of primers and probes were purchased from Applied Biosystems (Foster City, CA, USA). Amplification of IL-4, IL-5, IL-10, IFN- γ , and GAPDH cDNA was carried out in MicroAmp optical 96-well reaction plates (Applied Biosystems). The reaction was performed using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). The average transcript levels of genes were then normalized to GAPDH.

Measurement of cytokines (IL-4, IL-5, IL-10, IFN- γ , TGF- β) in spleen cell culture

Spleen single-cell suspensions were plated in 24-well cell culture plates at a final concentration of 10^5 cells/mL using RPMI 1640 media (Gibco, Grand Island, NY, USA). The cells were incubated in a CO₂ incubator at 37 °C for 72 h and stimulated with OVA for 72 h. The culture supernatant was collected and stored at –70 °C until cytokines were measured. Cytokines were assayed in culture supernatant using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (R&D

Systems, Minneapolis, MN, USA), according to the manufacturer's protocol. After measuring the optical density (OD) at 450 nm, the concentrations of IL-4, IL-5, IL-10, IFN- γ , and TGF- β were determined by interpolation from a standard curve, and all data were expressed in nanograms per milliliter.

Serum levels of total immunoglobulin (Ig) E, OVA-specific IgE, IgG1, and IgG2a

Serum levels of total and OVA-specific IgE were measured by solid-phase ELISA. The detailed procedure was described in our previous article [5].

For the analysis of OVA-specific IgG1 and IgG2a, Nunc 96-well immuno plates were coated with 100 μ g/mL OVA in coating buffer (carbonate–bicarbonate) overnight at 4 °C. To detect OVA-specific IgG1 and IgG2a, serially diluted serum samples were incubated with biotinylated rat anti-mouse IgG1 (BD Pharmingen, San Jose, CA, USA) and IgG2a (BD Pharmingen), respectively, and then streptavidin-HRP was added. SureBlue TMB Microwell Substrate (KPL, Gaithersburg, MD, USA) was used for peroxidase detection and color development for 30 min. After stopping the reaction by adding 1 M HCL, the OD was measured using a microplate reader (Molecular Devices, Silicon Valley, CA, USA). The endpoint titers of OVA-specific IgGs are expressed as the reciprocal log₂ titer.

Statistical analysis

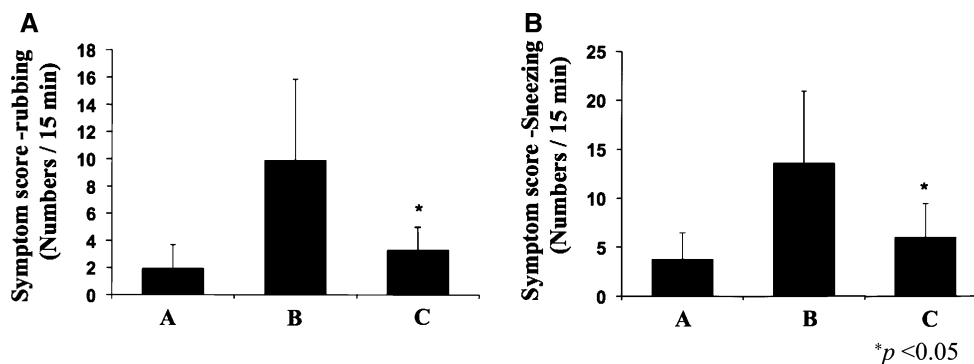
Results are shown as the mean $\pm$ SD. A Mann–Whitney *U* test was used to compare results between negative and positive controls and between treatment group and positive control. A *P* value less than 0.05 was deemed to indicate statistical significance.

Results

Symptom score (Fig. 2)

The nasal rubbing and sneezing symptom scores in the positive control group (group B) were 9.86 ± 5.87 and

Fig. 2 Systemic treatment with So-Cheong-Ryong-Tang suppressed allergic symptoms. **a** Rubbing symptom score. **b** Sneezing symptom score



13.6 ± 7.36 , respectively, and were significantly elevated versus the negative control group (group A). The nasal rubbing and sneezing symptom scores were 3.25 ± 1.69 and 6.0 ± 3.46 , respectively, in the SCRT-treatment group (group C), significantly lower than those in the positive control group (both $P < 0.05$).

Eosinophil infiltration into the nasal mucosa (Fig. 3)

Eosinophil number in the positive control group was 42.1 ± 9.87 , which was significantly different from that in the negative control group (1.8 ± 1.4 ; $P = 0.002$). The eosinophil number in group C was 22.3 ± 7.8 , which was significantly reduced versus the positive control ($P = 0.015$;

Fig. 3a). These results indicate that SCRT significantly decreased eosinophil migration in the nasal mucosa. Figure 3b shows the histology from each group. These figures demonstrate decreased eosinophil infiltration and epithelial layer disruption in group C compared with those in group B.

Serum total IgE and OVA-specific IgE, IgG1, and IgG2a levels (Fig. 4)

We also evaluated the effects of the SCRT on humoral immunity. Serum total and OVA-specific IgE levels were significantly decreased in treatment group C compared with the positive control group (both $P < 0.05$). These results

Fig. 3 Systemic treatment with So-Cheong-Ryong-Tang suppressed eosinophil infiltration in the nasal mucosa. **a** Eosinophil counts in the nasal mucosa. Eosinophil count was significantly reduced in treatment group C compared with the positive control (group B). **b** Histological findings of the nasal mucosa in each group ($\times 400$ magnification). Eosinophil infiltration markedly decreased in treatment group C compared with the positive control (group B)

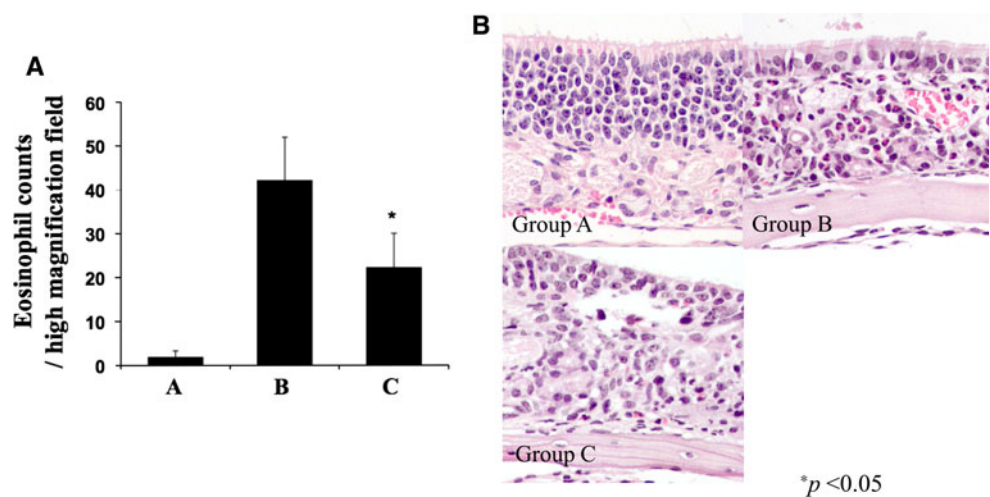
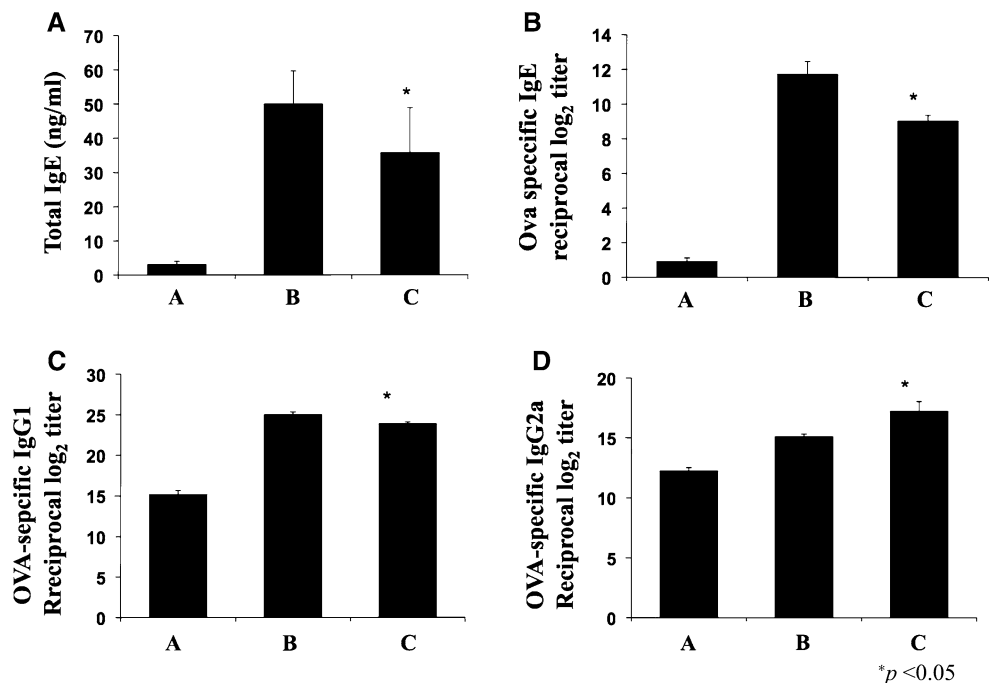


Fig. 4 Systemic treatment with So-Cheong-Ryong-Tang decreased serum total and OVA-specific immunoglobulin (Ig) E and OVA-specific IgG1 levels, and increased serum OVA-specific IgG2a levels. **a** Serum total IgE level. **b** Serum OVA-specific IgE level. **c** Serum OVA-specific IgG1 level. **d** Serum OVA-specific IgG2a level



suggested that SCRT reduced the isotype switch to IgE in an allergic rhinitis mouse model. The level of serum OVA-specific IgG1, a Th2-related immunoglobulin, also decreased significantly in the treatment group compared with the positive control group ($P = 0.0079$). Additionally, the level of serum OVA-specific IgG2a, a Th1-related immunoglobulin, increased significantly in the treatment group compared with the positive control group ($P = 0.0079$).

Fig. 5 Local Th2 cytokine decreased after So-Cheong-Ryong-Tang administration. IL-4 (a), IL-5 (b), IFN- γ (c), and IL-10 (d) mRNA expression levels in the nasal mucosa by real-time RT-PCR. Transcriptional activity of IL-4 decreased significantly; however, that of IL-5, IFN- γ , and IL-10 did not significantly change in the treatment group C versus the positive control (group B)

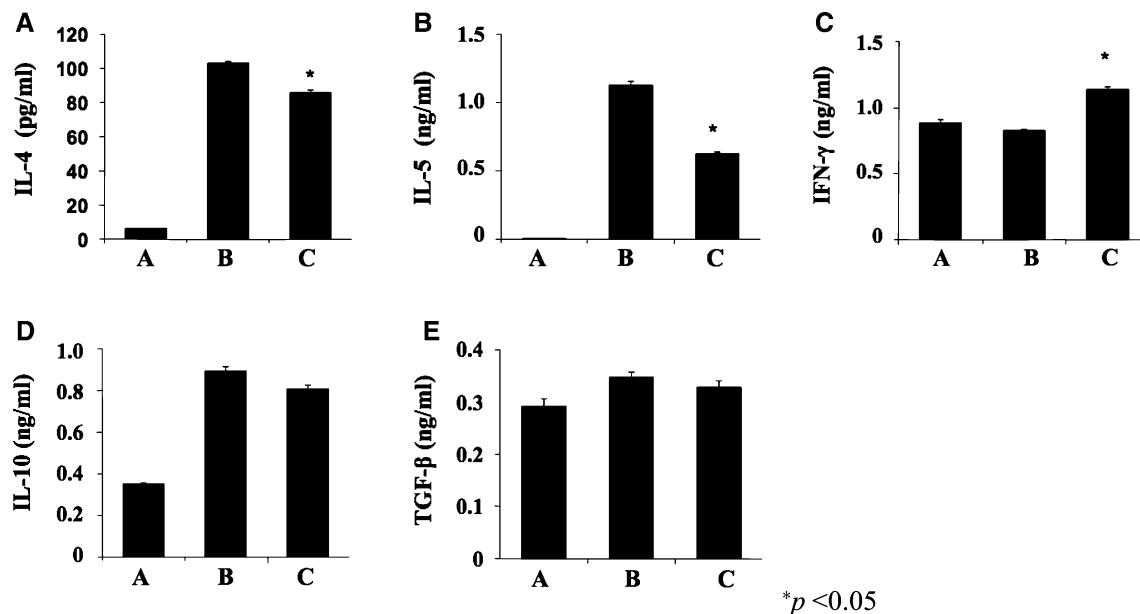
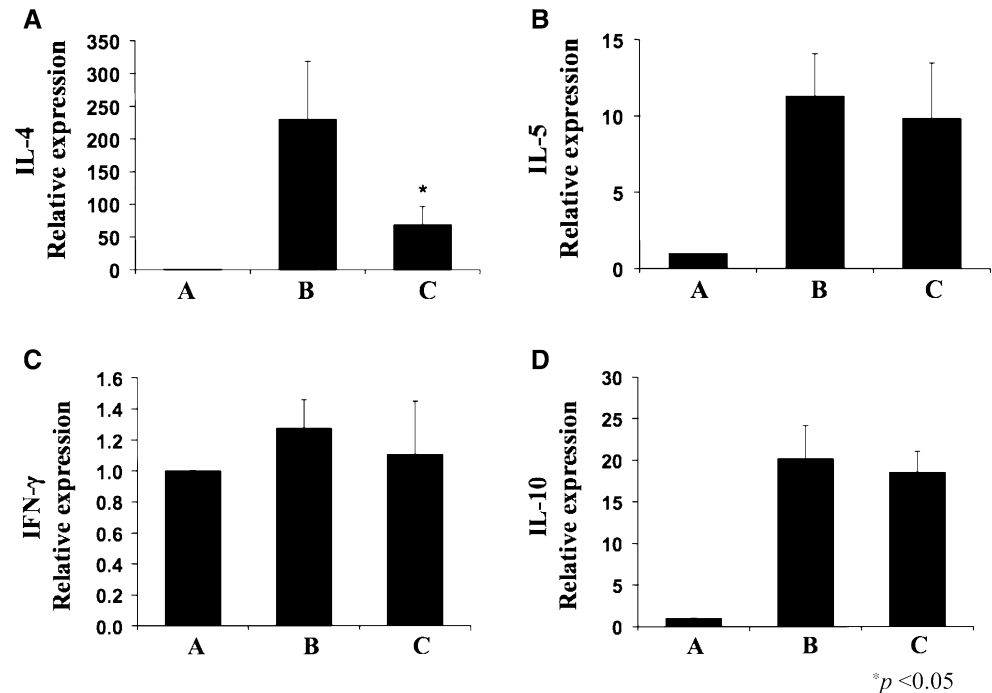


Fig. 6 Systemic Th2 cytokines decreased after So-Cheong-Ryong-Tang administration. In splenocyte culture, IL-4 (a) and IL-5 (b) concentrations were suppressed significantly, and the IFN- γ

Cytokines in nasal mucosa and splenocyte culture (Figs. 5, 6)

mRNA expression of the Th2 cytokine, IL-4, decreased significantly in the nasal mucosa of treatment group C (Fig. 5a), whereas that of IL-5 decreased slightly, but not significantly, in treatment group C (Fig. 5b) compared with the positive control group B. Expression of the Th1

(c) concentration increased significantly after SCRT treatment. However, IL-10 (d) and TGF- β (e) levels did not significantly change after treatment

cytokine, IFN- γ , did not change significantly in any group (Fig. 5c). For IL-10, the treatment group did not show a significant change compared with the positive control group (Fig. 5d).

Systemic Th2 cytokines assessed in splenocyte culture showed similar results, again showing anti-allergic effects. Both IL-4 and IL-5 concentrations significantly decreased in the treatment group (both $P < 0.05$; Fig. 6a, b). The level of the Th1 cytokine, IFN- γ , increased significantly in treatment group C compared with the positive control group ($P < 0.05$; Fig. 6c). However, the concentrations of the inhibitory cytokines, IL-10 and TGF- β , did not change significantly in the treatment group versus the positive control group (Fig. 6d, e).

Discussion

The Korean herbal medicine SCRT is a complex drug mix, consisting of eight herbal plants, and there have been some efforts to identify its active compound(s) [6]. Pinelliac acid from the tuber of *Pinellia ternata* was suggested as an active herbal component of SCRT and showed oral adjuvant activity. However, pinelliac acid alone was not enough to induce all the anti-allergic activities of SCRT, and some other active constituents were suggested to play a combinatorial role in the medicine [1]. Other ingredients such as paeoniflorin [7], α -asarone [8], Schizandrin [9], 6-gingerol [10], and glycyrrhizic acid [11, 12] also have been reported to have anti-allergic effects. Hence, they might contribute to a combinational or synergistic anti-allergic effect of SCRT.

The present study clarified the effects of SCRT on allergic rhinitis in a mouse model, demonstrating effects on Th2 responses in allergic rhinitis. SCRT has been used widely in traditional medicine in Asian countries for centuries. Its efficacy and mechanism of action in several allergic diseases have been investigated since the 1980s. A few human studies have shown its anti-allergic effects. A double-blind clinical trial in Japan demonstrated its clinical efficacy in allergic rhinitis, and another study claimed that PBMCs obtained from patients with allergic rhinitis showed decreased TNF- α production after SCRT treatment [6].

SCRT's in vitro effects have been widely reported in the literature. It decreased the expression of IL-4 mRNA and increased that of IFN- γ in mice CD4⁺ T cells [7], and it suppressed Th2 lineage development under Th2-polarized conditions [8]. It also suppressed mast cell degranulation [9], inhibited the increase in vascular permeability induced by histamine [10], and suppressed growth and differentiation of basophils [13]. These findings are consistent with our data with respect to its anti-allergic effects.

However, in vivo studies are rare, and to our knowledge, there are only two English-language reports on anti-allergic effects of SCRT in an allergic rhinitis model, one in a guinea pig model using an OVA antigen [4] and the other in a rat model using a TDI antigen [14]. Sakaguchi et al. [4] showed that SCRT decreased symptom scores and eosinophil infiltration in the nasal mucosa and inhibited the antigen-induced decrease in volume of the nasal cavity, but did not evaluate local or systemic changes in cytokine profiles or immunoglobulin levels. Das et al. [14] demonstrated that SCRT alleviated nasal symptoms and suppressed histamine gene up-regulation and IL-4 and IL-5 gene expression in the nasal mucosa, although TDI-mediated rhinitis is a non-IgE-mediated disease. Our study is the first in the English-language literature to demonstrate decreased local and systemic Th2 cytokine profiles and Th2 immunoglobulin levels as anti-allergic effects of SCRT in allergic rhinitis using an OVA allergen in a mouse model of allergic rhinitis.

Our study evaluated both systemic and local cytokine profiles. Th2 cells are known to play important roles in allergic diseases, including allergic rhinitis [15]. Although the Th17 subset has been included in T cell subsets recently, allergic diseases are still typically evaluated in terms of the Th1/Th2 paradigm, and Th2 predominance is a characteristic of allergic diseases. In the systemic cytokine profile, both Th2 cytokines, IL-4 and IL-5, decreased significantly, and the Th1 cytokine, IFN- γ , increased significantly in the treatment group versus the positive control, showing the anti-allergic effects of SCRT. However, in the cytokine profile of the nasal mucosa, only IL-4 decreased significantly, whereas IL-5 did not. The reason for this apparent difference between systemic and local cytokine profiles is unclear; however, it might be due to different actions of SCRT on systemic and local immune responses. This needs to be clarified further.

Only IL-4 showed a consistent decrease, both systemically and locally. IL-4 is well known to be an important cytokine not only for the Th2 phenotype, but also for IgE production [16, 17]. These findings are consistent with our data in that SCRT inhibited IL-4 secretion, resulting in decreased levels of serum total and OVA-specific IgE (Fig. 4a, b).

IL-10 is an inhibitory cytokine of inflammation. It was first identified as a Th2 cytokine and was later revealed to be produced by Th1, Th2, Th17, and regulatory T cells [18]. Local and systemic IL-10 decreased slightly, but not significantly, demonstrating that SCRT had little effect on the induction of regulatory T cells and the production of IL-10.

Immunoglobulin isotype switching and the induction of antibody production are complex processes induced by IL-4- or IL-13-triggered binding of phosphorylated STAT6

to the promoter regions of IgE [19] and IgG1 [20]. OVA-specific IgG1 has been used as a Th2 marker for a long time due to its relative ease of measurement, versus the less abundant IgE, and it has a strong correlation with total and OVA-specific IgE levels [21]. Isotype switching to IgG2a is driven by IFN- γ , a Th1 cytokine; thus, IgG2a levels represent Th1 activity [22]. In our study, SCRT significantly suppressed OVA-specific IgG1 and increased OVA-specific IgG2a (both $P = 0.0079$), showing decreased Th2 immunity and increased Th1 immunity as a result of SCRT. These data are consistent with previous reports showing a suppressed Th2 response and an enhanced Th1 response as a result of SCRT.

Overall, in allergic situation, increased Th2 cytokine (IL-4, IL-5) helped to induce local infiltration of effector cells (including eosinophils), isotype switching to IgE and induce mast cell degranulation synergistically. Mast cell degranulation can trigger symptoms such as itching, sneezing and rhinorrhea, and infiltration of effector cells can aggravate allergic inflammatory process to induce nasal obstruction. All of these processes were inhibited by applying SCRT and allergic symptoms could be alleviated in murine model of allergic rhinitis in our study.

Conclusion

The herbal medicine SCRT showed inhibitory effects on symptoms and other allergic parameters in a mouse model of allergic rhinitis. SCRT inhibited Th2 cytokine release from T cells and total and OVA-specific IgE secretions from B cells. Additionally, SCRT decreased eosinophil migration into the nasal mucosa. These multiple effects might synergize to reduce allergic symptoms. This study provided evidence of efficacy of SCRT in an in vivo model of allergic rhinitis and demonstrated some aspects of the underlying mechanism of its anti-allergic effects. Thus, SCRT might be considered a potential therapeutic agent in treating allergic rhinitis.

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Conflict of interest All authors have no conflict of interest and do not have any financial relationship with the organization that sponsored the research.

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