Oral Administration of SSC201, a Medicinal Herbal Formula, Suppresses Atopic Dermatitis-Like Skin Lesions

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ABSTRACT Atopic dermatitis (AD) is a chronic inflammatory skin disease, which requires safe and effective treatment. In this study, we evaluated the effects of SSC201, a herbal formulation consisting of *Stemonae Radix*, *Spirodelae Herba*, and *Cnidii Fructus*, on the development of AD induced by 2,4-dinitrochlorobenzene in the NC/Nga murine model. Oral administration of SSC201 significantly reduced the severity of dermatitis and the tendency of mice to scratch their lesions. SSC201 significantly reduced the thickening of the epidermis/dermis and the infiltration of T cells, eosinophils, and mast cells into the dermis. These results were supported by findings of reduced numbers of CD4⁺, CCR3⁺, and CD117⁺FcεRIα⁺ cells in the skin. Furthermore, SSC201 significantly decreased the number of CD4⁺, CD8⁺, and CD3⁺CD69⁺ T cells in lymph nodes. SSC201 not only decreased the plasma levels of immunoglobulin E (IgE) and the numbers of IgE-producing B cells (B220⁺CD23⁺), but also reduced the number of eosinophils and the levels of eotaxin as well as concentrations of thymus and activation-regulated chemokine in the periphery. Splenic levels of Th2 cytokines, including interleukin (IL)-4, IL-5, and IL-13, were reduced, whereas the levels of IL-12, a Th1 cytokine, were increased. Taken together, our data suggest that SSC201 may be an effective therapeutic agent for the treatment of AD.

KEY WORDS: • atopic dermatitis • *Cnidii Fructus* • NC/Nga atopic dermatitis murine model • *Spirodelae Herba* • SSC201 • *Stemonae Radix*

INTRODUCTION

ATOPIC DERMATITIS (AD) is a chronic inflammatory skin disease characterized by eczematous and pruritic lesions.¹ Its clinical signs and symptoms are associated with underlying immunological imbalances dominated by Th2 immune responses, leading to immunoglobulin E (IgE) production and the accumulation of eosinophils,^{2,3} and therefore resembles a Th1 deficiency.⁴ Indeed, the number of Th2 cells producing interleukin (IL)-4, IL-5, and IL-13 increases during the acute and chronic stages of AD in inflamed skin and contributes to epidermal hyperplasia, keratinization, and barrier dysfunctions.^{5,6} Furthermore, IL-4 and IL-13 mediate the IgE-isotype switch in B cells.⁷ The levels of IL-5, which promote survival and activation of eosinophils, are also increased in the sera of patients with AD, resulting in eosinophilia.⁸ Chemokines, such as eotaxin and thymus and activation-regulated chemokine (TARC)

produced by Th2 cells, increase the number of immune cells expressing CCR3, a C–C chemokine receptor, such as eosinophils and mononuclear cells,⁹ as well as mast cell precursors in the circulation and assist in their recruitment into dermal infiltration.¹⁰ Furthermore, the levels of IgE are associated with the severity of AD, and mast cells display FcεRI on their surfaces; crosslinking of FcεRI by surface IgE and antigen generates rapid release of various inflammatory mediators that contribute to clinical manifestations, such as itching, erythema, and edema.¹¹

NC/Nga (NC) mice develop clinical signs of AD that begin with scratching followed by the onset of eczematous skin lesions.¹² The AD-like skin lesions are caused by infiltration of various inflammatory cells, including T cells, mast cells, and eosinophils. NC mice also produce high concentrations of total IgE in plasma.¹³ Because the Th2 dominant pathophysiological characteristics of NC mice closely resemble those of human patients with AD, these mice provide a useful animal model to study the efficacy of medicine on human AD as well as the pathogenesis of AD.¹⁴

SSC201 is a herbal formulation containing *Stemonae Radix*, *Spirodelae Herba*, and *Cnidii Fructus*. SSC201 has

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been used as a medicinal formula to alleviate skin inflammation and itching in traditional herbal medicine.^{15–17} The biological activities of each herb have been reported as follows: *Stemona* Radix has been used to treat eczema.¹⁸ *Cnidii Fructus* has been reported to have antipruritic effects in substance P-induced itching ICR mice,¹⁹ and suppress chemical-induced contact dermatitis.²⁰ An extract of *Spirodelae Herba* inhibited lipopoly-saccharide-induced inflammation in macrophages by suppressing NO production and nuclear factor-kappa B activation.^{21,22} However, the therapeutic potential of SSC201 for AD has not been investigated. In this study, we tested whether oral administration of SSC201 inhibits AD in 2,4-dinitrochlorobenzene (DNCB)-induced atopy model of NC/Nga mice.

MATERIALS AND METHODS

Preparation of SSC201

The herbs, *Stemona* Radix (*Stemona japonica*, *Stemona*aceae), *Spirodelae Herba* (*Spirodela polyrhiza*, *Lemnaceae*), and *Cnidii Fructus* (*Torilis japonica* Dc., *Umbelliferae*) were purchased from Nanum Pharm Co., Ltd. (Yeongcheon, Gyeongbuk, Korea). SSC201 was prepared by boiling the three herbs (1:1:1) in distilled water (DW) at 100°C for 2 h. The boiled herbs were then filtered through a Whatman no. 2 filter (Maidstone, United Kingdom), concentrated under vacuum conditions, and freeze dried. The yield of the dried extract was ~10.5%.

Standardization of SSC210

Coumaroylquinic acid (*Stemona* Radix), vitexin (*Spirodelae Herba*), and torilin (*Cnidii Fructus*) were used as surrogates^{23,24} and quantified using high-performance liquid chromatography (Waters Alliance 2695 system; Waters Co., Milford, MA, USA) coupled with a 2996 photodiode array detector as follows: Phenomenex Luna C18 column (250 mm×4.6 mm; particle size 5 µm; Phenomenex, Torrance, CA, USA) was used as stationary phase and the mobile phase was composed of 0.1% (v/v) trifluoroacetic aqueous solution (A) and acetonitrile (B). The elution conditions were as follows: at $t=0$ min, the mobile phase consisted of 90% A/10% B and was held for 10 min. From 10 to 70 min, a gradient was applied to 30% A/70% B, which was followed by a wash with 100% B for 5 min and a 15-min equilibration period at 90% A/10% B. The separation temperature was kept at 40°C throughout the analysis, with a flow rate of 1.0 mL/min and injection volume of 20 µL. Identification was based on retention time and UV spectra by comparison with standards. The components were quantified based on peak areas at the maximal wavelength. The quantification was determined by comparison with standard curves for authentic coumaroylquinic acid (identified from *Stemona* Radix by electrospray ionization-MS and ¹H-NMR, ¹³C-NMR) (KOC Biotec Corp., Daejeon, Korea), vitexin (Sigma-Aldrich, St. Louis, MO, USA), and torilin (Hankook-shinyak Corp., Chungnam, Korea).

Animal experiments

Specific pathogen-free (SPF) 6-week-old male NC/Nga (NC) mice were purchased from Central Lab Animal, Inc. (Seoul, Korea). Animal experiments complied with the guidelines of the Daejeon University Animal Care and Use Committee (Written approval number DJUAR2012-008). NC mice were divided into five groups ($n=10$ –12 per group) and were orally administered DW, dexamethasone (Dex; 3 mg/kg/day; Sigma-Aldrich), or SSC201 (250 or 500 mg/kg/day) for 5 weeks. The shaved dorsal skin of four of these groups was treated with 0.2% DNCB diluted in an acetone and olive oil solution (3:1) once each week to induce dermatitis (Sigma-Aldrich). The Naïve group was not treated with DNCB and was administered oral doses of DW. The Normal group was maintained in SPF condition without DNCB treatment. The severity of dermatitis was assessed once each week by three persons unaware of the identities of the groups, according to the method described by Leung *et al.*²⁵ The total clinical index of dermatitis severity was defined as the sum of the individual scores graded as follows: 0 (none), 1 (mild), 2 (moderate), and 3 (severe) for each of the five signs and symptoms (erythema/hemorrhage, edema/hematoma, excoriation/erosion, itching/dryness, and lichenification (thickness of the skin)). The frequency of using the hind paws to scratch the body was measured during a 20-min period.

Enzyme-linked immunosorbent assay

Splenocytes (2×10^6) were stimulated with a plate-bound anti-CD3 antibody (5 µg/mL; BD Biosciences, San Diego, CA, USA) for 72 h. The levels of mouse IL-4, IL-5, IL-12p40, IL-13, and interferon (IFN)- γ in the supernatants of the suspensions were determined using enzyme-linked immunosorbent assay (ELISA) kits (BD Biosciences or Life Technologies, Camarillo, CA, USA). The plasma levels of mouse IgE (Shibayagi, Gunma, Japan), eotaxin, and TARC (R&D Systems, Minneapolis, MN, USA) were measured using ELISA kits.

Analysis of total leukocytes and eosinophils in peripheral blood

Blood was collected from individual groups of mice, 11 weeks of age, through heart puncture. The number of total leukocytes and eosinophils in the heparinized blood was counted using a CELL-DYN® 3200 (Abbott Laboratories, Santa Clara, CA, USA).

Flow cytometry (fluorescence-activated cell sorting)

Dorsal skin and axillary lymph node were minced and incubated in phosphate-buffered saline (PBS) containing 1 mg/mL collagenase IV (Sigma-Aldrich) and 2 mg/mL dispase (BD Biosciences) for 40 min at 37°C. Cells were stained with antibodies specific for mouse CD4-FITC, CD8-PE, CD3-PerCP, CD69-PE, B220-PE, CD23-FITC, CCR3-PE, CD117-PE, and Fc ϵ RI α -FITC (BD Biosciences) in a staining buffer (PBS containing 1% fetal bovine serum and

0.01% sodium azide) for 30 min on ice and analyzed by fluorescence-activated cell sorting (FACS) on a FACScan analyzer, using Cell-Quest software (both from BD Biosciences).

Histological examination of AD-like skin lesions

Small biopsies were obtained from the dorsal skin of the mice ($n=8-10/\text{group}$). Skin sections were fixed and embedded in paraffin, cut to a thickness of $4\ \mu\text{m}$, stained with hematoxylin and eosin or Toluidine blue for detection of various inflammatory cells and mast cells, respectively. Cells in the dermis were observed using a visible light microscope at a magnification of $200\times$.

Statistical analysis

All data are expressed as mean $\pm$ standard deviation and represent one of three independent experiments. One-way analysis of variance (ANOVA using SPSS Software) was used to analyze the differences between the Control and experimental groups. Duncan's multiple comparison tests were used to compare the mean values of the treatments.

P values $< .05$ were considered statistically significant. A repeated measures ANOVA was performed to determine the effects of the treatments during the oral administration period. One-way ANOVA was used to analyze the interaction effect between the groups for which the parameters were found to be significantly different at the indicated week (Figs. 2 and 5).

RESULTS

Oral administration of SSC201 inhibits the development of DNCB-induced dermatitis in NC mice

As shown in Figure 1, we standardized SSC201 using coumaroylquinic acid ($0.253 \pm 0.007\ \text{mg/g}$), vitexin ($0.725 \pm 0.013\ \text{mg/g}$), and torilin ($0.061 \pm 0.003\ \text{mg/g}$). To evaluate whether SSC201 suppressed AD, NC mice were fed SSC201 (250 or 500 mg/kg/day), Dex (3 mg/kg/day), or DW (Control, $100\ \mu\text{L}/\text{mouse}/\text{day}$) daily for 5 weeks. The increase in the dermatitis severity index of the Control group indicated the development of dermatitis with time (Fig. 2A). However, oral administration of SSC201 for 3 weeks significantly decreased the dermatitis index,

FIG. 1. Standardization of SSC201. **(A)** Chemical structures of three standard compounds in SSC201. High-performance liquid chromatography chromatogram of **(B)** three standard mixtures and **(C)** a three-herb combinational extract SSC210 at 235 nm. (a) Coumaroylquinic acid, (b) vitexin, and (c) torilin appeared at a retention time of ~ 10.52 min, 24.16 min, and 65.40 min, respectively. **(D)** Identification of useful components from SSC201.

FIG. 2. Effects of SSC201 on the development of atopic dermatitis in NC mice. **(A)** Dermatitis index, **(B)** scratching frequency, and **(C)** clinical features were assessed by the criteria described in **Materials and Methods**. ($n=10$; one-way analysis of variance [ANOVA] test $*P<.05$, $**P<.01$, $***P<.001$ vs. control; $\#P<.05$ vs. dexamethasone [Dex]). Color images available online at www.liebertpub.com/jmf

resulting in less hemorrhage, edema, dryness, and erythema in a dose-dependent manner, which was accompanied by less frequent scratching. SSC201 significantly lowered the scratching frequency almost to that of the Dex group (Fig. 2B). Although scratching frequency in the Control group at 11 weeks was slightly less compared with the values at 10 weeks, the treatment with this herbal formula maintained the scratching frequency lower than Control. Moreover, these outcomes were consistent with the analysis of the overall clinical appearance of the mice (Fig. 2C). Treatment with Dex also lessened the severity of dermatitis and scratching frequency. These data indicate that SSC201 suppressed DNCB-induced dermatitis in NC mice.

SSC201 not only inhibits the thickening of the epidermis/dermis, but also T-cell activation and infiltration of inflammatory cells

Control NC mice exhibited a marked thickening of the epidermis and dermis, prominent hyperkeratosis, hemor-

rhage, and infiltration of inflammatory cells (Fig. 3A-ii, B-ii). However, SSC201 inhibited the pathologic changes (Fig. 3A-iv, v) resulting in a histological environment very similar to that of the Naïve group. Toluidine blue staining indicated that SSC201 inhibited the infiltration of mast cells (Fig. 3B-iv, v). Dex also produced suppressive effects (Fig. 3A-iii, B-iii). Furthermore, in dorsal skin, there was a prominent increase in the percentage of $CD4^+$ T cells (~ 3 -fold) and $CCR3^+$ cells (~ 4.6 -fold) in the Control group, respectively (Table 1). SSC201 treatment resulted in significantly fewer numbers of $CD4^+$ T cells and $CCR3^+$ cells, to almost normal levels. There was also a significant increase up to 1.8-fold of mast cells by detection of CD117 (a mast cell marker) and the IgE receptor ($Fc\epsilon RI\alpha^+$) in the Control group; however, SSC201 reduced their levels to that of the Dex group. Furthermore, in axillary lymph nodes, the Control group showed a three- to fourfold increase in T-cell populations, including $CD4^+$ and $CD8^+$ cells as well as $CD3^+CD69^+$ cells, which express a very early inducible activation marker (CD69) of T lymphocytes.²⁶ However, there were significant decreases with SSC201 treatment,

FIG. 3. Effects of SSC201 on skin lesions in NC mice. Histological examination of dorsal skin in SSC201-treated NC mice. Dorsal skin of NC mice was stained with (A) hematoxylin and eosin or (B) Toluidine blue for detection of various inflammatory cells and mast cells, respectively. Cells in the dermis were observed using a visible light microscope at a magnification of 200 $\times$. Color images available online at www.liebertpub.com/jmf

resulting in a 50% decrease in the numbers of all T-cell populations analyzed (Fig. 4). These data suggest that SSC201 treatment suppressed AD-like skin lesions, possibly by mediating the suppression of T-cell activation and inhibiting infiltration of inflammatory immune cells into skin.

TABLE 1. EFFECTS OF SSC201 ON LYMPHOCYTE POPULATION IN THE SKIN OF NC MICE

Sample	Treatment		CD4 ⁺ (%)	CCR3 ⁺ (%)	CD117 ⁺ FceRI α ⁺ (%)
	Concentration (mg/kg body weight)				
Naïve	—		1.3 $\pm$ 0.1***	1.7 $\pm$ 0.1**	4.3 $\pm$ 0.6*
Control	—		4.5 $\pm$ 0.3	7.9 $\pm$ 1.6	7.8 $\pm$ 1.0
Dex	3		1.2 $\pm$ 0.7***	2.5 $\pm$ 1.2**	3.4 $\pm$ 0.6**
SSC201	250		2.2 $\pm$ 0.3***#	4.8 $\pm$ 0.2#	4.5 $\pm$ 0.9
	500		1.4 $\pm$ 0.4***	2.0 $\pm$ 0.5**	3.5 $\pm$ 0.5**

The isolated cells were stained with cell-type-specific antibodies and detected using FACS.

The data represent the mean $\pm$ SD of triplicate determinations. $n=10$.

* $P<.05$, ** $P<.01$, *** $P<.001$ versus control; # $P<.05$ versus Dex (one-way ANOVA).

Dex, dexamethasone; SD, standard deviation; ANOVA, analysis of variance; FACS, fluorescence-activated cell sorting.

SSC201 not only reduces the number of eosinophil, but also decreases the levels of eotaxin and TARC

As shown in Table 2, the number of total leukocytes of the Control group increased greatly compared with the Naïve group. In particular, the number of eosinophils increased considerably in the Control group, although it could not reach eosinophilia (>4.5). However, SSC201 reduced the numbers of total leukocytes and eosinophils, presumably by contributing to the suppression of increasing eosinophils. In addition, Dex significantly reduced the number of eosinophils. The levels of eotaxin and TARC decreased in SSC201-treated mice by 50% and 65%, respectively, compared with those in the Control group (Table 2). These data indicate that SSC201 inhibited the production of eotaxin and TARC, which prevented Th2-mediated increase in the number of eosinophils.

SSC201 reduces the level of IgE in plasma and the number of IgE-producing B cells

The concentration of IgE in the plasma of Naïve group was ~ 370 ng/mL. In contrast, IgE levels in the Control group gradually increased with age to almost 16 mg/mL at 10 weeks. Administration of SSC201 and Dex lowered the IgE concentration, resulting in a 51–59% and 84% lower level of IgE, respectively (Fig. 5A). Further, the percentage of B cells expressing FceRII (identified by surface expression of B220, a B cell marker, and CD23, an FceRII marker) increased in the Control group by threefold compared with the Naïve group. However, in the SSC201 group, the percentage of B cells decreased almost to the same extent as the Naïve group (Fig. 5B). These data indicate that SSC201 lowered the production of IgE and the number of IgE-producing B cells.

SSC201 regulates the balance of Th1/Th2 cytokine production in splenocytes

To understand the molecular basis of the effects of SSC201, the levels of cytokines produced by Th1 and Th2

FIG. 4. Effects of SSC201 on the T-cell population of axillary lymph nodes of NC mice. Fluorescence-activated cell sorting (FACS) analysis of (A) CD4⁺, (B) CD8⁺, and (C) CD69⁺ T cells from axillary lymph nodes of NC mice. ($n=10$; one-way ANOVA test * $P<.05$, ** $P<.01$, *** $P<.001$ vs. control; # $P<.05$ vs. Dex). Color images available online at www.liebertpub.com/jmf

cells were measured in splenocytes. In the presence of anti-CD3 antibodies, the cells from the Control group produced IL-4, IL-15, and IL-13 at levels up to 5.3-, 5.5-, and 7.5-fold, respectively, compared with those of the Naïve group. However, treatment with SSC201 (500 mg/kg/day) decreased production of IL-4, IL-5, and IL-13 by 19%, 50%,

and 32%, respectively (Table 3). The levels of IL-12p40 increased by CD3 stimulation in the Control group, and SSC201 further increased the levels up to 132–149% compared with the Control group. In contrast, SSC201 showed no significant effects on IFN- γ production. The levels of IFN- γ increased by anti-CD3 up to 1.6-fold in the

TABLE 2. EFFECTS OF SSC201 ON THE NUMBER OF EOSINOPHILS AND THE CONCENTRATIONS OF EOTAXIN AND THYMUS AND ACTIVATION-REGULATED CHEMOKINE IN PERIPHERAL BLOOD

Treatment		Cell ($\times 10^5$ cells/mL)		Chemokine (pg/mL)	
Sample	Concentration (mg/kg of body weight)	Total leukocyte	Eosinophils	Eotaxin	TARC
Naïve	—	32.0 ± 5.1**	0.2 ± 0.1**	503.7 ± 36.7	9.4 ± 1.6
Control	—	46.2 ± 1.5	3.2 ± 0.2	774.6 ± 46.9	21.2 ± 2.9
Dex	3	28.7 ± 5.9**	0.9 ± 0.2*	447.1 ± 42.0*	16.5 ± 5.2
SSC201	250	35.9 ± 4.4*	1.4 ± 0.3*#	543.5 ± 133.5	12.3 ± 4.4*
	500	32.6 ± 5.4**	0.5 ± 0.1***#	390.6 ± 8.5*	7.5 ± 3.9*

Blood samples were isolated from each group of mice at the age of 11 weeks. Levels of eotaxin and TARC were determined by ELISA. The data represent the mean ± SD of triplicate determinations. $n=10$.

* $P<.05$, ** $P<.01$ versus control; # $P<.05$ versus Dex (one-way ANOVA).

TARC, thymus and activation regulated; ELISA, enzyme-linked immunosorbent assay.

FIG. 5. Effects of SSC201 on the plasma levels of immunoglobulin G (IgE) and IgE-producing B cells in NC mice. **(A)** The levels of IgE measured in plasma obtained from NC mice at the ages of 7, 8, 9, 10, and 11 weeks. **(B)** FACS analysis of B220 and CD23 double-positive cells from axillary lymph nodes of NC mice ($n=10$; one-way ANOVA test * $P<.05$, ** $P<.01$, *** $P<.001$ vs. control; # $P<.05$, ## $P<.01$, ### $P<.001$ vs. Dex).

Control group, but SSC201 treatment had no detectable effects (Table 3). Dex potently suppressed the levels of Th2-cytokines, but increased IL-12p40 levels as previously reported.²⁷ These results suggest that SSC201 regulates the production of cytokines by splenic Th1/Th2 cells, resulting in decreased or increased levels of Th2 or Th1 cytokines, respectively.

DISCUSSION

Systemic corticoids are effective for treating AD by mediating a wide range of immunomodulatory effects, such

as suppressing cytokine and IgE production, and inhibiting leukocyte chemotaxis.²⁸ However, they can cause adverse effects, including altering metabolism, suppressing the hypothalamic–pituitary–adrenal axis,²⁹ inhibiting growth and increasing susceptibility to infection and rebound flare-ups. The diminishing effectiveness of corticosteroids over time, along with these side effects, significantly limits their use.³⁰ Oral antihistamines are relatively safe, but seem to be ineffective; however, they prevent the interruption of sleep caused by pruritus.³¹ Therefore, medicinal herbs have attracted considerable attention as beneficial therapeutics for treating AD. However, because only a few herbal medicines

TABLE 3. EFFECTS OF SSC201 ON THE PRODUCTION OF TH1 AND TH2 CYTOKINES BY CULTURED SPLENOCYTES

Sample	Treatment Concentration (mg/kg of body weight)	Th2 cytokines			Th1 cytokines	
		IL-4 (pg/mL)	IL-5 (pg/mL)	IL-13 (pg/mL)	IFN- γ (pg/mL)	IL-12p40 (pg/mL)
Normal	—	1.5 ± 0.4	0.8 ± 0.2	3.5 ± 0.8	4.9 ± 0.3	9.5 ± 0.5
Naïve	CD3 (5 μ g/mL)	13.0 ± 2.3**	42.0 ± 8.4**	218.4 ± 25.2**	306.3 ± 26.8*	559.6 ± 40.8
Control		70.4 ± 8.5	230.9 ± 10.5	1646.4 ± 31.5	510.8 ± 24.8	520.9 ± 53.7
Dex	3	25.4 ± 2.7**	163.0 ± 5.8*	1296.3 ± 17.4*	512.7 ± 25.6	731.5 ± 98.8*
SSC201	250	64.2 ± 8.1##	153.7 ± 5.1*	1307.9 ± 15.3*	535.5 ± 21.6	687.6 ± 59.1*
	500	57.8 ± 3.2*#	117.1 ± 8.2**	1121.6 ± 14.6**	512.4 ± 29.2	773.6 ± 54.0*

The isolated cells were stimulated with anti-CD3 antibody (5 μ g/mL) for 72 h. Levels of IL-4, IL-5, IL-13, IFN- γ , and IL-12p40 were determined using ELISA. The data represent the mean $\pm$ SD of three independent experiments.

* $P<.05$, ** $P<.01$ versus control; # $P<.05$, ## $P<.01$ versus Dex (one-way ANOVA).

IL, interleukin; IFN, interferon.

have been demonstrated to be effective treatments for AD,³² further research for developing safe and effective orally applicable alternative therapies is required. The results of the present study indicate that this will be a worthy endeavor.

SSC201 consists of three medicinal herbs having well-recognized safety for long history of use and effectiveness for inflammatory skin disease as alternative therapy; however, their efficacy and cellular and molecular mechanisms for AD have been rarely studied.

Our present results indicate that oral administration with SSC201 suppresses the development of DNCB-induced dermatitis, possibly by controlling processes associated with Th1 and Th2 cell function, specifically, downregulation of the expression of IL-4, IL-5, IL-13, chemokines, and IgE as well as the upregulation of the expression of IL-12. The biological consequences of treatment with SSC201 in this model included the suppression of the thickening of the epidermis and dermis, the highly decreased number of eosinophils, and early activated T cells (CD4⁺CD69⁺) and inhibition of the infiltration of various inflammatory cells, including T cells, eosinophils (CCR3⁺), and mast cells (CD117⁺/FcεRIα⁺) into skin. There was a significant decrease in the levels of eotaxin, IL-5, IL-13, and TARC (Tables 1 and 3). A particularly interesting aspect of our present findings is the upregulation of the expression of IL-12. IL-12 suppresses the production of cytokines by Th2 cells, such as IL-4 and IL-5 as well as IgE. IL-12 also plays a role in IgE-mediated-mast cell activation through the augmented expression of IFN-γ.³³ Based on our data (Table 3), we assume that the increased expression of IL-12, induced by SSC201 treatment, likely contributed to the suppression of the production of Th2-cytokines and IgE as well as the activation of mast cells.³³ Taken together, our results suggest that by balancing the immune responses mediated by Th1 and Th2 cells, SSC201 may serve as an effective treatment for AD through targeting multiple pathologic factors.

Osthol from *Cnidii Fructus* has been identified as an active compound for itching¹⁹ and exerts antiallergic effects.²⁰ Intake of stemonine found in *Stemonae Radix* has been known to suppress immune activation by reducing the number of macrophages and lymphocytes,³⁴ and stemofoline is supposed to have anti-inflammatory activity, inhibiting the production of NO in macrophages.³⁵ *Spirodelae Herba* has several compounds such as apigenin, luteolin, and vitexin,²³ which suppress dermatitis as well as secretion of IgE and Th2 cytokines in basophils and animals.^{36,37}

Although several compounds from the three herbs used in this study are known to prevent dermatitis and allergies, this study did not include data for their active ingredients. Furthermore, most of herbal medicines are administrated by the oral route and multiple active ingredients can undergo chemical modification and are metabolized in the absorption process; studies on bioavailability of active compounds in herbal mixtures are very important for quality control and safety.³⁸ Finding active compounds and bioavailability study are definitely needed for further development of SSC201 as an anti-AD agent. Considering that current orally available therapeutics for AD not only are ineffective, but

also have harmful side effects, further investigations of SSC201 are clearly warranted.

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AUTHOR DISCLOSURE STATEMENT

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REFERENCES

1. Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA: New insights into atopic dermatitis. *J Clin Invest* 2004;113:651–657.
2. Sabin BR, Peters N, Peters AT: Chapter 20: Atopic dermatitis. *Allergy Asthma Proc* 2012;33:S67–S69.
3. Berke R, Singh A, Guralnick M: Atopic dermatitis: an overview. *Am Fam Physician* 2012;86:35–42.
4. Ferran M, Santamaria-Babi LF: Pathological mechanisms of skin homing T cells in atopic dermatitis. *World Allergy Organ J* 2010;3:44–47.
5. Novak N, Bieber T, Leung DY: Immune mechanisms leading to atopic dermatitis. *J Allergy Clin Immunol* 2003;112:S128–S139.
6. Ong PY, Leung DY: Immune dysregulation in atopic dermatitis. *Curr Allergy Asthma Rep* 2006;6:384–389.
7. Ryzhov S, Goldstein AE, Matafonov A, Zeng D, Biaggioni I, Feoktistov I: Adenosine-activated mast cells induce IgE synthesis by B lymphocytes: an A2B-mediated process involving Th2 cytokines IL-4 and IL-13 with implications for asthma. *J Immunol* 2004;172:7726–7733.
8. Sanderson CJ: Interleukin-5, eosinophils, and disease. *Blood* 1992;79:3101–3109.
9. Ma W, Bryce PJ, Humbles AA, *et al.*: CCR3 is essential for skin eosinophilia and airway hyperresponsiveness in a murine model of allergic skin inflammation. *J Clin Invest* 2002;109:621–628.
10. Kawakami T, Ando T, Kimura M, Wilson BS, Kawakami Y: Mast cells in atopic dermatitis. *Curr Opin Immunol* 2009;21:666–678.
11. Poulsen LK, Hummelshoj L: Triggers of IgE class switching and allergy development. *Ann Med* 2007;39:440–456.
12. Vestergaard C, Yoneyama H, Matsushima K: The NC/Nga mouse: a model for atopic dermatitis. *Mol Med Today* 2000;6:209–210.
13. Park EJ, Park KC, Eo H, *et al.*: Suppression of spontaneous dermatitis in NC/Nga murine model by PG102 isolated from *Actinidia arguta*. *J Invest Dermatol* 2007;127:1154–1160.
14. Tanaka A, Amagai Y, Oida K, Matsuda H: Recent findings in mouse models for human atopic dermatitis. *Exp Anim* 2012;61:77–84.

15. Chen S: [*Compendium on External Diseases in Traditional Chinese Medicine*]. Rénmín wèishēng chūbǎn shè [People's Health Publishing House], Shanghai, China, 1983. (In Chinese.)
16. Heo J: [*Mirror of Eastern Medicine*]. (Yoon SH, Kim HJ, eds.) Dong ui bo gam chul pan sa, Gyeongnam, Korea, 2006. (In Korean.)
17. Li S: [*Compendium of Materia Medica*]. Zhōngguó yīyào kējì chūbǎn shè [Chinese Medical Science and Technology Press], Beijing, China, 2011, pp. 713–715. (In Chinese.)
18. Kang BS, Kim YP: [*Clinical Herbal Pharmacology*]. Younglimsa, Seoul, Korea, 1994, pp. 605–606. (In Korean.)
19. Basnet P, Yasuda I, Kumagai N, *et al.*: Inhibition of itch-scratch response by fruits of *Cnidium monnieri* in mice. *Biol Pharm Bull* 2001;24:1012–1015.
20. Matsuda H, Tomohiro N, Ido Y, Kubo M: Anti-allergic effects of *Cnidii Monnieri Fructus* (dried fruits of *Cnidium monnieri*) and its major component, osthol. *Biol Pharmaceut Bull* 2002;25:809–812.
21. Ko WS, Kim YH, Yoon JW, Yoon SW, Kim HD: Inhibitory effect of *Spirodela polyrhiza* on the secretion of NO in LPS-stimulated macrophages. *Am J Chin Med* 2004;32:65–73.
22. Seo CS, Lee MY, Shin IS, Lee JA, Ha H, Shin HK: *Spirodela polyrhiza* (L.) Sch. ethanolic extract inhibits LPS-induced inflammation in RAW264.7 cells. *Immunopharmacol Immunotoxicol* 2012;34:794–802.
23. Kim J, Lee I, Seo J, *et al.*: Vitexin, orientin and other flavonoids from *Spirodela polyrhiza* inhibit adipogenesis in 3T3-L1 cells. *Phytother Res* 2010;24:1543–1548.
24. Oh H, Kim JS, Song EK, *et al.*: Sesquiterpenes with hepatoprotective activity from *Cnidium monnieri* on tacrine-induced cytotoxicity in Hep G2 cells. *Planta Med* 2002;68:748–749.
25. Leung DY, Hirsch RL, Schneider L, *et al.*: Thymopentin therapy reduces the clinical severity of atopic dermatitis. *J Allergy Clin Immunol* 1990;85:927–933.
26. Borges O, Borchard G, de Sousa A, Junginger HE, Cordeiro-da-Silva A: Induction of lymphocytes activated marker CD69 following exposure to chitosan and alginate biopolymers. *Int J Pharm* 2007;337:254–264.
27. Kim MS, Hur YG, Kim WG, *et al.*: Inhibitory effect of *Platycodon grandiflorum* on T(H)1 and T(H)2 immune responses in a murine model of 2,4-dinitrofluorobenzene-induced atopic dermatitis-like skin lesions. *Ann Allergy Asthma Immunol* 2011;106:54–61.
28. Pucci N, Lombardi E, Novembre E, *et al.*: Urinary eosinophil protein X and serum eosinophil cationic protein in infants and young children with atopic dermatitis: Correlation with disease activity. *J Allergy Clin Immunol*. 2000;105:353–357.
29. Ellison JA, Patel L, Ray DW, David TJ, Clayton PE: Hypothalamic-pituitary-adrenal function and glucocorticoid sensitivity in atopic dermatitis. *Pediatrics* 2000;105:794–799.
30. Sidbury R, Hanifin JM: Systemic therapy of atopic dermatitis. *Clin Exp Dermatol* 2000;25:559–566.
31. Klein PA, Clark RA: An evidence-based review of the efficacy of antihistamines in relieving pruritus in atopic dermatitis. *Arch Dermatol* 1999;135:1522–1525.
32. Hon KL, Chan BC, Leung PC: Chinese herbal medicine research in eczema treatment. *Chin Med* 2011;6:17.
33. Finkelman FD, Madden KB, Cheever AW, *et al.*: Effects of interleukin 12 on immune responses and host protection in mice infected with intestinal nematode parasites. *J Exp Med* 1994;179:1563–1572.
34. Lee H, Kim Y, Kim HJ, *et al.*: Herbal formula, PM014, attenuates lung inflammation in a murine model of chronic obstructive pulmonary disease. *Evid Based Complement Alternat Med* 2012;2012:769830.
35. Hosoya T, Yamasaki F, Nakata A, *et al.*: Inhibitors of nitric oxide production from *Stemona javanica*. *Planta Med* 2011;77:256–258.
36. Yano S, Umeda D, Yamashita S, Yamada K, Tachibana H: Dietary apigenin attenuates the development of atopic dermatitis-like skin lesions in NC/Nga mice. *J Nutr Biochem* 2009;20:876–881.
37. Hirano T, Higa S, Arimitsu J, *et al.*: Flavonoids such as luteolin, fisetin and apigenin are inhibitors of interleukin-4 and interleukin-13 production by activated human basophils. *Int Arch Allergy Immunol* 2004;134:135–140.
38. Liu JY, Lee KF, Sze CW, *et al.*: Intestinal absorption and bioavailability of traditional Chinese medicines: a review of recent experimental progress and implication for quality control. *J Pharm Pharmacol* 2013;65:621–633.