

RESEARCH ARTICLE

Inhibitory effects of *Schizandra chinensis* extract on atopic dermatitis in NC/Nga mice

Yun Hwan Kang and Heung Mook Shin

Department of Physiology, College of Oriental Medicine, Dongguk University, Republic of Korea

Abstract

Context: *Schizandra chinensis* Baillon (SC) is traditionally used as a medicinal plant in the Orient. Recently, SC has become recognized as an adaptogen by the mainstream medical community. Phytoadaptogens influence respiratory, cardiovascular, uterus myotonic, and immune activities. Atopic dermatitis (AD) is an allergic inflammatory skin disease caused by aberrant and over-reactive immune responses.

Objective: This study assessed the suppressive effect of SC extract (SCE) on 1-chloro-2,4-dinitrobenzene (DNCB)-induced AD in a NC/Nga mouse model.

Materials and methods: AD was induced by topically applying 0.2% DNCB to the hairless-back of NC/Nga mice for 4 weeks. Treated mice received SCE or dexamethasone after AD induction.

Results: SCE markedly suppressed DNCB-induced dermatitis, as determined by a count of scratching frequency; measurement of IgE, IgM, and histamine levels in serum; and histological observation of epidermal hyperplasia and mast-cell infiltration. Additionally, SCE lessened DNCB-induced histamine receptor mRNA expression in skin tissue and the splenic expressions of interleukin (IL)-4, IL-5, and high-affinity IgE receptor B protein.

Conclusion: SCE appears useful for suppression of AD, even though the active pathway(s) remain unknown.

Keywords: *Schizandra chinensis* extract (SCE), atopic dermatitis (AD), NC/Nga mouse, immunoglobulin E (IgE), Histamine

Introduction

Atopic dermatitis (AD) is a highly pruritic and chronic inflammatory skin disease, whose pathogenesis is believed to be mediated by T-helper 2-type cell (Th2)-driven inflammatory responses, and which is also associated with high IgE levels.⁽¹⁾ Because AD is a common pediatric disease for which there is no satisfactory therapy, better knowledge of AD through research using animal models is necessary.⁽²⁾ Mouse models are used mainly because of their ease of handling. Among mice, the NC/Nga mouse is the first spontaneously occurring AD model reported.^(2,3) Skin changes that closely mimic human AD are induced spontaneously in NC/Nga mice by exposure to various environmental aeroallergens.⁽²⁾ It was reported that 1-chloro-2,4-dinitrobenzene (DNCB) induces atopic dermatitis-like skin lesions in NC/Nga mice.^(4,5) The sign of the skin changes is scratching behavior, which is followed by the rapid development of

erythema, lichenification with edema, and hemorrhage on the ear, neck, and back.^(2,6) Also, histological examinations have revealed dermal infiltration and hyperplasia in the skin lesions.^(2,4,7) Along with the skin changes, NC/Nga mice display increased Th2 cell differentiation in the spleen, dense accumulation of eosinophils and mast cells in the skin lesions, and elevation of total serum IgE.^(2,3,6) Mast cells can serve as effector cells in the immune system and can be activated by high-affinity IgE receptor (FcεRI) to produce the various cytokines and histamine.^(8,9,10) Histamine is a well-established potent itch mediator in humans.⁽⁶⁾ Cross-linking of allergen-specific IgE leads to the release of histamine from mast cells.^(9,10) However, the neural pathway for histamine-induced itch is not clear.

Allergic hypersensitivity is divided into IgE-mediated and non-IgE-mediated.⁽¹¹⁾ Of the two, IgM plays an important role in non-IgE-mediated allergic disease and is involved in eliciting contact sensitivity.^(12,13) According

Address for Correspondence: Heung Mook Shin, Department of Physiology, College of Oriental Medicine, Dongguk University, Gyeongju, 780-714, Republic of Korea. Tel: +82-54-770-2372. Fax: +82-54-742-5441. E-mail: heungmuk@dongguk.ac.kr

(Received 19 February 2011; revised 04 May 2011; accepted 24 May 2011)

to these results, IgM may be related to pruritus in the skin lesions.

Although topical steroid therapy is a common remedy for AD, many patients suffer from serious steroid-side effects.^(4,14) Recognizing this shortcoming, studies have sought to identify plant-based medicinal compounds for AD relief that are safer.

Schizandra chinensis (SC) has traditionally been used in Asia to treat pollution, impaired body fluids, body weakness, cough, asthma, and exhaustion.⁽¹⁵⁾ More recently, the benefits of SC to liver detoxifying activity, antioxidant activity, blood sugar balance, reduction of blood pressure, local anti-inflammatory effect, and wound healing have been reported.^(15,16,17) However, the anti-allergic dermatitis effects of SC extract (SCE) have not been demonstrated. This study examined the use of SCE to protect from topically applied DNCB-induced dermatitis in NC/Nga mice.

Materials and methods

Animals

Male, 6-week old, 21–26 g NC/Nga mice (Central Lab. Animal, Seoul, Korea) were used for all experiments. All animals were provided with food and water *ad libitum* and allowed to adapt to the experimental conditions (temperature, 21 ± 2°C; humidity, 50–60%) for 1 week.

Preparation of SCE

Dried SC (200 g) was extracted with 2 l of water by the established dipping method at room temperature for 36 h. The extract was filtered through No. 2 filter paper (Whatman International, Maidstone, Kent, UK) to remove insoluble debris. After filtration, the extracts were concentrated by rotary evaporation using a model VV2000 apparatus (Heidolph, Schwabach, Bavaria, Germany) at a temperature of 70°C and then dried using a model FD8508S freeze dryer (ILSHIN, Busan, Korea). The yields of dry matter from the extracts were approximately 6.3%. The dried extracts were stored at -20°C until use.

Induction of AD and drug treatment on back skin of NC/Nga mice

Controlled dermatitis was induced by topically applying 0.2% DNCB dissolved in acetone/olive oil (1:3), to the hair-removed back of mice three times per week for 4 weeks. In the case of the DNCB-SCE (male, *n*=6) or DNCB-dexamethasone (Dex; male, *n*=5) group, SCE (1%) or Dex (1 µg/ml) dissolved in water was spread over the bare back skin three times per day for 4 weeks as a remedy after DNCB-induced AD. DNCB-treated control animals (DNCB-Control group; male, *n*=6) received water at the remedy step. Naïve mice (male, *n*=5) treated acetone/olive oil (1:3) at the AD induction step and water at the remedy step. Mice were sacrificed 43 days after first applying DNCB and blood was collected from the heart. Also, skin from the affected region of the back was excised and subjected to histological examination.

All procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee of Dongguk University.

Evaluation of skin severity

To reduce experimental variation and induce stable clinical dermatitis over a short period, 0.2% DNCB was applied for 4 weeks to the backs of NC/Nga mice. The development of erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). Dermatitis was scored by tallying up the points of each assessment item. The dermatitis scores increased rapidly by 4 weeks after the first DNCB application and the skin had become thick. When the average sum score equilibrated to score 8, the remedy treatment involving SCE or Dex commenced. Over the study period, the severity of dermatitis was evaluated once a week, 3 h after the application of DNCB, CRE or Dex.

Behavioral observation

Behaviors of mice were monitored as previously described.⁽¹⁸⁾ The frequency of scratching on facial or dorsal skin was counted with a 30-min visual observation on the first day and on day 14 after SCE application.

Enzyme-linked immunosorbent assay (ELISA)

Serum levels of IgE, IgM, and histamine were measured in the mice using a commercial kit (KOMABIOTECH, Seoul, Korea) according to manufacturer's instructions. Absorbance was measured at a wavelength of 450 nm (IgE and IgM) and 650 nm (histamine), and the concentration was calculated from a standard curve using serial dilutions of each protein.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from NC/Nga mouse back skin and was reverse-transcribed using an oligo(dT) hexamer as a primer and Moloney murine leukemia virus reverse transcriptase (Fermentas, Glen Burnie, MD) to produce the cDNAs. PCR was performed using the selective primers for the mouse histamine H1 receptor (H1R) (sense primer: 5'-CCGAAGACAAGATGTGTGAGG-3', antisense primer: 5'-TGATGCCAGCCAAGTATAGG-3'), histamine H2 receptor (H2R) (sense primer: 5'-TTCCTTACTTCACTGCCTTCG-3', antisense primer: 5'-TTGTGAGAGTTGTGGCTTGC-3'), histamine H3 receptor (H3R) (sense primer: 5'-TTCAACATCGTGCTGATCAG-3', antisense primer: 5'-TGTTCAAGGTAGATGCTGAGG-3'), histamine H4 receptor (H4R) (sense primer: 5'-TGTGGTGACAGAAACCTTAGACA-3', antisense primer: 5'-AAGAATCTGAAGCCAGAATCATCG-3'), and GAPDH (sense primer: 5'-CCATGGAGAAGGCTGGGG-3', antisense primer: 5'-CAAAGTTGTCATGGATGACC-3'). PCR was carried out for 30 cycles using the following conditions: denaturation at 95°C for 10 s; annealing at 54.2,

53.5, 50.0, 57.2 and 56°C for 30 s; and elongation at 72°C for 1 min. The band intensities of the amplified DNAs were compared after visualization on an ultraviolet transilluminator.

Histopathological examination

After sacrifice, the back skin of each NC/Nga mouse was fixed in 10% formalin for 24 h at 4°C. The tissues were embedded in paraffin and serially sectioned at a thickness of 4 µm. Sections were stained with hematoxylin and eosin (Sigma-Aldrich, St. Louis, MO). Photographs were taken using a C-4040ZOOM digital camera (Olympus America, New York, NY), and montages were assembled using Adobe Photoshop software. To measure mast-cell infiltration, skin sections were stained with toluidine blue.

Dot blot assay

Total protein extract was isolated from spleens of NC/Nga mice. The amount of protein was measured using the Bradford protein assay solution (Bio-Rad Laboratories, Hercules, CA). Ten micrograms total protein of each spleen sample was spotted onto a nitrocellulose membrane (Bio-Rad Laboratories). Each membrane was incubated with goat polyclonal antibody to interleukin (IL)-4, IL-5, or high-affinity IgE receptor B (FcεRIβ) (1:200 dilution for each), followed by a 1:1000 dilution of horseradish peroxidase (HRP)-conjugated secondary antibody. Spots were detected using an enhanced chemiluminescence system (Amersham Biosciences, Buckinghamshire, UK).

Agents

SC was purchased from Humanherb (Gyeongsan, Gyeongbuk, Korea). Goat polyclonal antibodies against IL-4, IL-5, and FcεRIβ were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). DNCB, Dex, and most of the reagents used for molecular studies were obtained from Sigma-Aldrich.

Statistical analysis

Data are presented as mean ± SD. Differences between treatment groups were examined using a paired Student's *t*-test. *p* < 0.05 was considered significant.

Results

SCE inhibits AD symptoms in NC/Nga mice

In order to induce stronger and more controlled dermatitis, 200 µl of 0.2% DNCB was topically applied to the backs of NC/Nga mice. DNCB-treated mice displayed progressively diffuse erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness on the treated region of the back. Subsequent application of SCE decreased the intensity of dermatitis and, in particular, decreased the extent of incrustation (Figure 1A). A statistical difference in the clinical skin severity score was observed between the DNCB-Control group and DNCB-SCE group on day 14 after SCE application. The DNCB-SCE group displayed

a significantly lower clinical skin severity score, similar to the DNCB-Dex group, compared with the DNCB-Control group (Figure 1B).

Decreased scratching frequency in SCE-applied mice

The frequency of scratching of facial and back skin was observed for 30 min on day 14 after SCE application. The frequency of scratching by the DNCB-SCE group (53 times) and DNCB-Dex group (52 times) was significantly less (approximately 49% and 48%, respectively) than that of the vehicle treated DNCB-Control group (108 times) (Figure 2).

SCE treatment decreases serum IgE and IgM

The concentration of IgE, a diagnostic marker of AD, was measured in the serum, because increased IgE production is a hallmark of Th2 immune responses.^(1,2,19) Figure 3A shows the concentration of IgE in the serum of the untreated and SCE-treated mice. The IgE concentration was 146–861 ng/ml, 779–1568 ng/ml, 306–786 ng/ml and 341–1110 ng/ml in the naïve group, DNCB-Control group, DNCB-SCE group, and DNCB-Dex group, respectively. The increased serum IgE levels by DNCB-treatment were significantly inhibited in the SCE- and Dex-treated groups. In addition, we measured the serum concentration of IgM, which plays an important role not only in the regulation of drug-induced chronic urticaria and exogenous allergic alveolitis or immune complex-associated vasculitis, but also in contact sensitivity.^(11,12,13) The concentration of IgM in the serum displayed a similar pattern as IgE (Figure 3B). The IgM concentration was 66–139 µg/ml, 134–241 µg/ml, 72–161 µg/ml, and 104–177 µg/ml in the naïve group, DNCB-Control group, DNCB-SCE group, and DNCB-Dex group, respectively.

Effect of SCE on modulation of serum histamine level and histamine receptor expression in skin

Histamine levels were significantly higher in the DNCB-Control group because of exposure to DNCB compared to the naïve group, indicating a successful induction of AD-like alteration of the immune system. Two weeks after SCE application, the DNCB-SCE group showed significantly lower serum histamine level (mean value: 12.7 ng/ml) than the DNCB-Control group (mean value: 21.4 ng/ml), and the DNCB-SCE group displayed a statistically insignificant difference from the naïve group (mean value: 16.8 ng/ml) and DNCB-Dex group (mean value: 16.5 ng/ml) (Figure 4A). RNA extracted from the back skin of NC/Nga mice was transcribed, amplified, and then subjected to agarose gel electrophoresis. RT-PCR analysis clearly identified histamine H1 (H1R), H2 (H2R), H3 (H3R), and H4 (H4R) receptors. Similar analyses showed that randomly-selected back skin of the DNCB-Control group hyperexpressed the histamine receptor gene, compared to naïve mice, and that the induced histamine receptor genes were down-regulated by SCE or Dex application, except for H2R (Figure 4B).

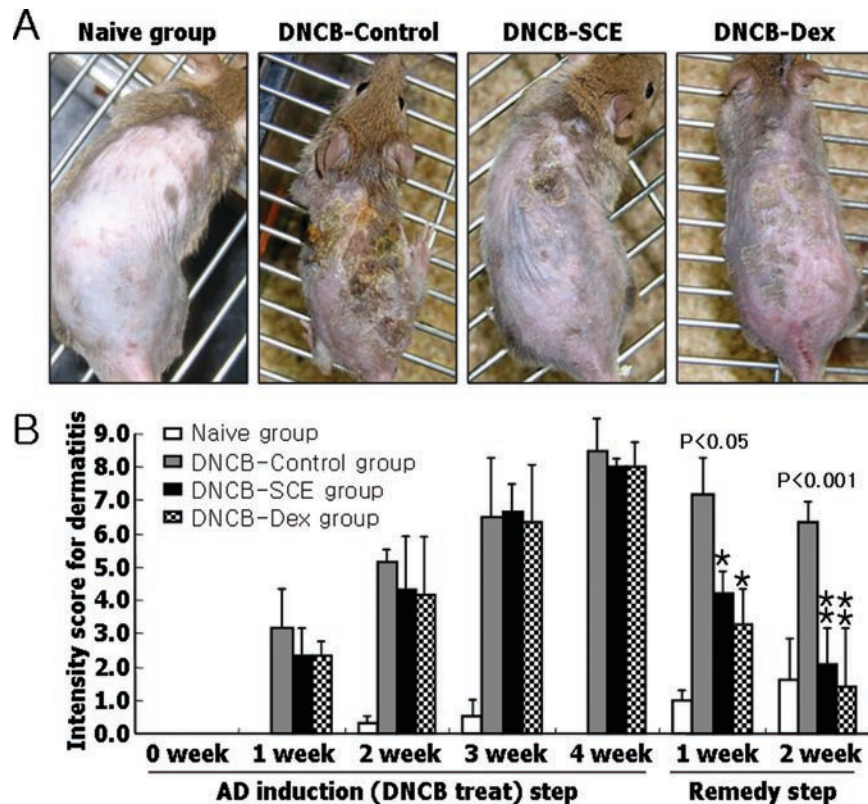


Figure 1. Inhibitory effect of SCE on dermatitis progression in NC/Nga mice following the topical application of DNCB. A. Representative clinical skin features, visually demonstrating a marked reduction in dermatitis severity. B. Temporal changes in mean clinical skin severity score. Clinical skin score of dermatitis was defined as the average sum of the individual scores graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe) for each of five signs and symptoms (itch, erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness). Each value is the average of three-way repeated-measures. Statistically significant values are calculated by comparing with DNCB-Control group by t-test (* $p < 0.05$, ** $p < 0.001$).

Histological examination

Figure 5 shows the H&E- and toluidine blue-stained sections of the back skins of NC/Nga mice at day 15 after SCE application. Hyperplasia of the epidermis was clearly evident in the DNCB-Control group. These characteristic features of inflamed skin tissues were not observed in the skin sections from the DNCB-SCE group, which were indistinguishable from the skin sections from naïve and DNCB-Dex mice. The numbers of mast cells or mast cell degranulation in skin sections were significantly less in the DNCB-SCE or Dex groups compared with the DNCB-Control group.

SCE inhibits IL-4, IL-5, and FcεRIβ expression in the spleen of AD-induced NC/Nga mice

Total proteins extracted from spleens of NC/Nga mice were used in dot blot analysis. SCE significantly lowered the elevated protein expression of IL-4 and IL-5 in NC/Nga mice exposed to DNCB (Figure 6), consistent with the idea that SCE affects Th2-specific cytokine response at the protein expression level. The results with the Dex group of mice were similar to the SCE group. The expression pattern displayed by FcεRIβ was similar to those of IL-4 and IL-5 expression; DNCB-induced increased IgE levels stimulated FcεRIβ expression,

and SCE and DEX treatment significantly lowered the expression.

Discussion

The anti-allergic effect of SC has not been reported, despite its useful application against cough and asthma.⁽¹⁵⁾ The present data reveal the inhibitory effects of SCE on DNCB-induced AD-like symptoms in NC/Nga mice.

The release of histamine from activated mast cells is a typical sign of development of allergic disease and pruritus.^(6,9,10) Pruritus is a symptom of skin disorders like AD and allergic contact dermatitis.^(6,10,14) In AD, scratching of the itchy skin is damaging and induces an inflammatory response, which can lead to severe pruritus.^(6,20,21) Reduction of itchy sensations and scratching frequency is very important for prevention of the development of skin lesions due to pruritic disorders and for an improved quality of life. The present results demonstrated the significant reduction in the scratching frequency of facial and back skin by 1% SCE application, similar to Dex treatment (Figure 2).

NC/Nga mice typically spontaneously develop dermatitis by IgE elevation with age.^(2,3) An elevated level of IgE is important in the development of AD-like skin

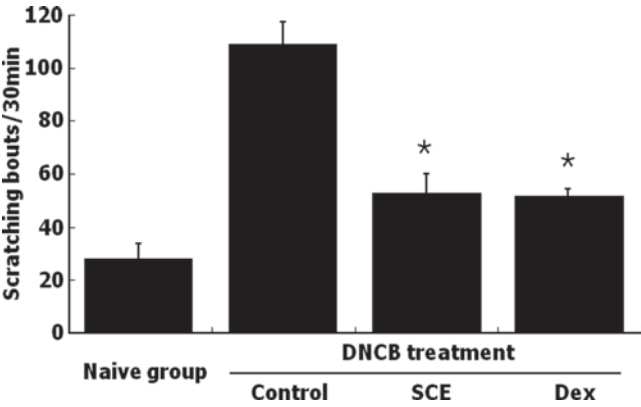


Figure 2. Effects of SCE on DNCB-induced scratching in NC/Nga mice. Scratching was counted for 30 min at 60-min intervals after spraying of SCE and Dex. Each value represents the mean $\pm$ SD for 5 or 6 animals. * p < 0.05 when compared with DNCB-treated Control group by t-test.

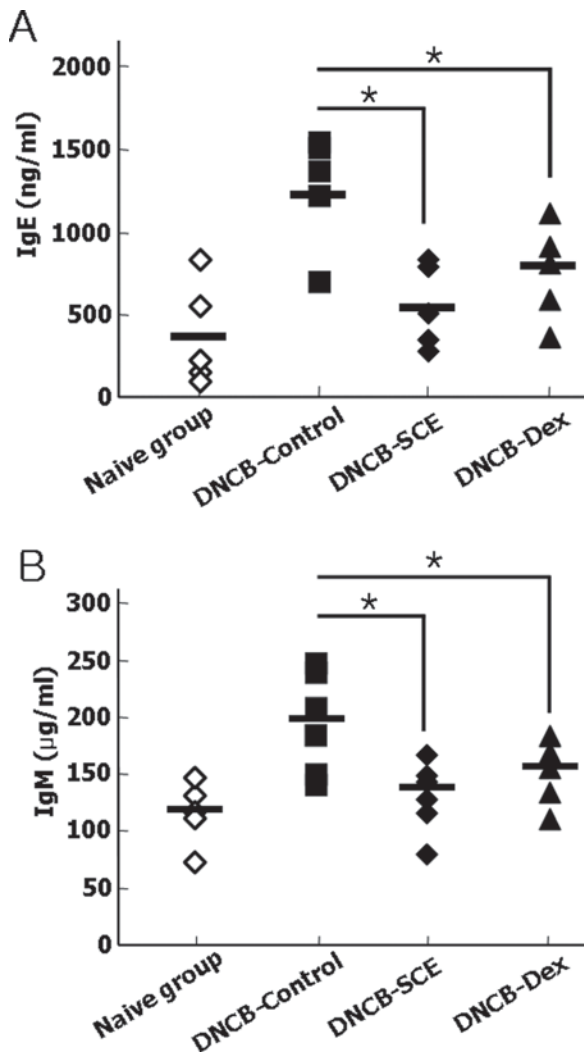


Figure 3. Effects of SCE on the serum IgE (A) and IgM (B) levels in NC/Nga mice. DNCB (0.2%) was applied topically to the backs of the NC/Nga mice three times per week for 4 weeks. SCE (1%) was sprayed to the backs three times per day during the experiment period. The result values of five or six different samples are presented as spots and average values are marked with a black bar. * p < 0.05 when compared with DNCB-Control group by t-test.

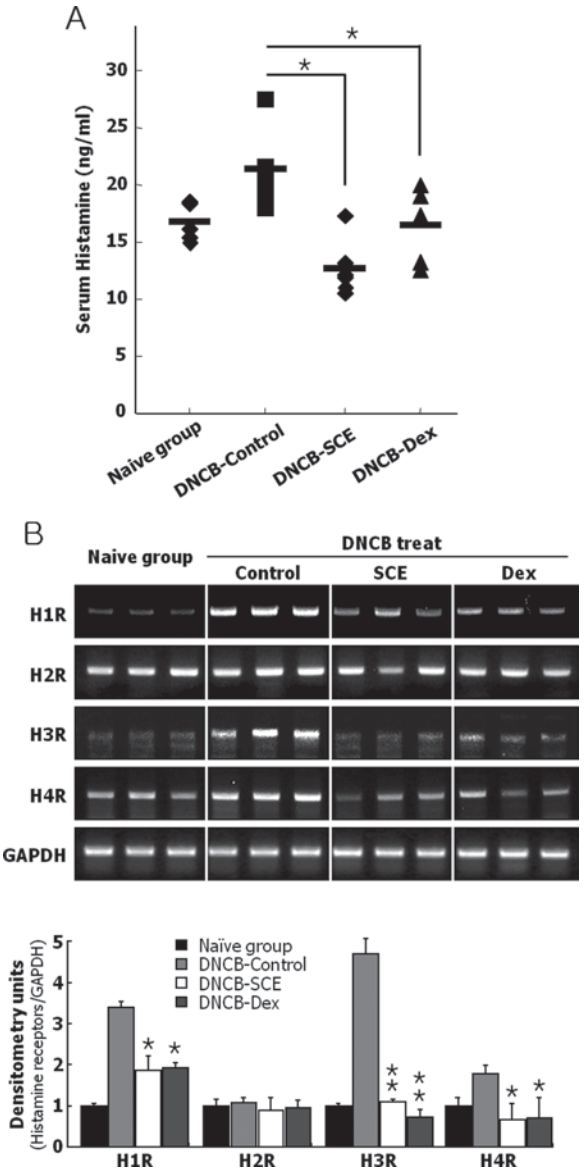


Figure 4. Effects of SCE on serum histamine level and expression of histamine receptor. A. The changes in serum histamine levels of the NC/Nga mice. After induction of AD with DNCB, mice were treated by SCE or DEX application for 2 weeks. The result values of five or six different samples are presented as spots and the average values are marked with a black bar. B. Expression of mRNAs for histamine H1 (H1R), H2 (H2R), H3 (H3R), and H4 (H4R) receptors by SCE treatment. Total RNA was extracted from the back skins of NC/Nga mice and reverse-transcribed. After amplification, PCR products were subjected to electrophoresis and visualized by ethidium bromide staining. * p < 0.05, ** p < 0.001 when compared with DNCB-Control group by t-test.

lesions.^(3,11,22) Furthermore, elevation of serum IgE level has been correlated with the degranulation of mast cells.^(23,24,25) Presently, the DNCB-induced serum IgE level was reduced by SCE application, as occurred using Dex (Figure 3A).

In allergic disease, humoral cytotoxic immune responses such as in drug-induced chronic urticaria are mediated by IgM.⁽¹¹⁾ Also, recent studies of spleen B-1 cells have revealed their exuberant expression of IgM to elicit contact sensitivity.^(13,26) These results suggest that IgM

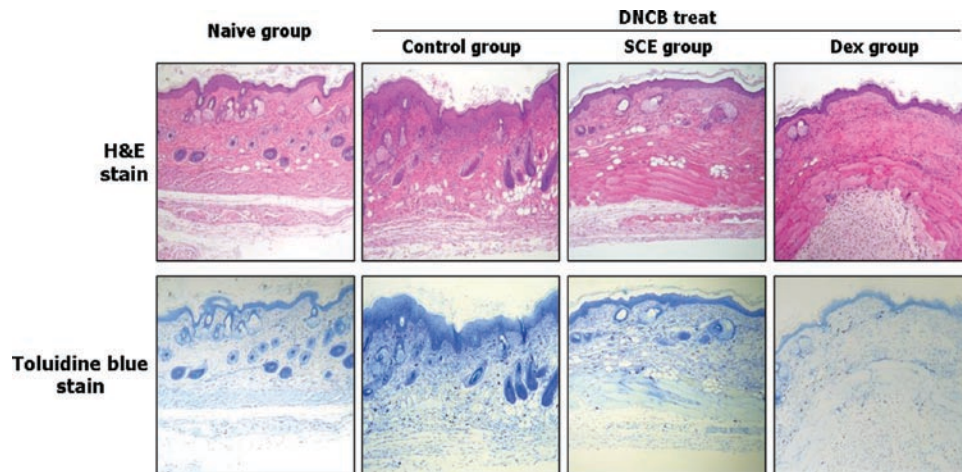


Figure 5. Histological features of back skin after SCE application in NC/Nga mice. The skin was stained with H&E or toluidine blue. H&E-stained sections showed thickening of the epidermal tissue by DNCB-induced dermatitis and toluidine blue-stained sections provided information about dermal mast cell infiltration in skin. The microscope original magnification was $\times 100$.

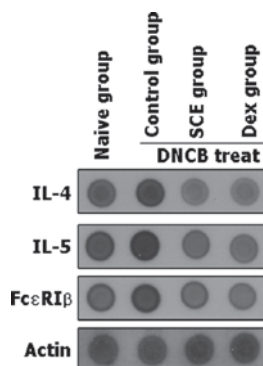


Figure 6. Effects of SCE on IL-4, IL-5, and Fc ϵ RI β expression level in spleens of NC/Nga mice. The IL-4, IL-5, and Fc ϵ RI β levels were measured 2 weeks after SCE application. Dot blot analysis compared the expression of the naive group, DNCB-treated Control group, SCE group, and Dex group. These are representative data of two independent experiments.

may be implicated in pruritus of skin lesions. Therefore, it was presently appropriate to measure the serum IgM level in relation to scratching frequency. DNCB-induced serum IgM level was reduced by SCE application, similar to the pattern of IgE (Figure 3B).

Histamine is the best-known potent itch mediator, and histamine receptors are activated by endogenous histamine for initiation of the itching signal. However, the pathway for histamine-induced itch has been unclear and antihistamine (i.e., histamine receptor blockers), are not sufficient to inhibit pruritus of dermatitis.⁽⁶⁾ Therefore, it is very important to discover new potential antihistamine or antipruritus herbs. The present results demonstrate the down-regulation of serum histamine level by SCE application following AD induction (Figure 4A).

Histamine-induced pruritus is mediated by histamine receptors. Four subtypes of histamine receptors (H1R, H2R, H3R, and H4R) have been characterized to date. H1R has been the most extensively studied, and it is partially involved in treatment of pruritus and allergic

inflammation.⁽²⁷⁾ H2R is not related to pruritus because H2R agonist does not induce scratching.⁽²⁸⁾ However, systemically administered H3R and H4R antagonist thioperamide dose-dependently reduced histamine-induced scratching behavior in mice.⁽²⁹⁾ The results of Figure 4B demonstrate that SCE significantly reduced the gene expression of H1R ($p < 0.05$), H3R ($p < 0.001$), and H4R ($p < 0.05$) in the affected region of back skin for 2 weeks after the start of SCE application following AD induction. But, H2R did not respond to DNCB, SCE, and Dex application. These results were consistent with previous experiments.

Epicutaneous sensitizes mice develop lesions with epidermal and dermal thickening, infiltration of mast cells, and up-regulates the expression of the Th2 cytokines IL-4 and IL-5.^(9,10,30) IL-4 secreted from mast cells provides signals for IgE synthesis or amplification.⁽⁹⁾ The IgE receptor (Fc ϵ RI) is a high-affinity receptor for the Fc region of IgE, and the antibody isotype involved in allergy and parasite resistance.^(8,31,32) Cross-linking of the Fc ϵ RI via IgE-antigen complexes leads to the degranulation of mast cells and release of inflammatory mediators.^(8,9,33) In addition, because binding of IgE to Fc ϵ RI stabilizes the receptor at the cell surface, the density of Fc ϵ RI correlates with serum IgE levels.^(9,34) Also, Fc ϵ RI-IgE interactions may induce mast cell survival.⁽⁹⁾ Presently, H&E- and toluidine blue-stained sections of the back skin of NC/Nga mice revealed the decrease of epidermal and dermal thickening, and decreased mast cell number and degranulation by SCE application (Figure 5). Furthermore, the protein levels of IL-4, IL-5, and Fc ϵ RI β were inhibited by 1% SCE application. The efficacies of SCE were similar to that of Dex, and these results clearly demonstrate that SCE treatment can inhibit AD by suppressing the immune response and pruritus.

Conclusions

SCE regulates DNCB-induced AD pathways. SCE application on the skin of AD can alter the production of IL-4

and IL-5 from Th2 cells, and can also alter IgE, IgM, and histamine production. Thus, SCE may perform a series of immunoregulatory functions to alleviate the occurrence or progression of AD. Hence, we believe that the application of SCE on AD will be significantly meaningful for public health and alternative medicine.

Acknowledgements

This work was supported by Research Grants of Mungyeong Agricultural Technology Center and the Dongguk University Research Fund of 2011

Declaration of interest

The authors declare that they have no potential conflicts of interest.

References

- De Benedetto, A., Agnihothri, R., McGirt, L.Y., Bankova, L.G., Beck, L.A. Atopic dermatitis: a disease caused by innate immune defects? *J Invest Dermatol* 2009, 129, 14–30.
- Jin, H., He, R., Oyoshi, M., Geha, R.S. Animal models of atopic dermatitis. *J Invest Dermatol* 2009, 129, 31–40.
- Takeda, K., Gelfand, E.W. Mouse models of allergic diseases. *Curr Opin Immunol* 2009, 21, 660–665.
- Pokharel, Y.R., Lim, S.C., Kim, S.C., Heo, T.H., Choi, H.K., Kang, K.W. Sopungyangjae-Tang Inhibits Development of Dermatitis in NC/Nga Mice. *Evid Based Complement Alternat Med* 2008, 5, 173–180.
- Jung, B.G., Cho, S.J., Koh, H.B., Han, D.U., Lee, B.J. Fermented Maesil (*Prunus mume*) with probiotics inhibits development of atopic dermatitis-like skin lesions in NC/Nga mice. *Vet Dermatol* 2010, 21, 184–191.
- Ikoma, A. Analysis of the mechanism for the development of allergic skin inflammation and the application for its treatment: mechanisms and management of itch in atopic dermatitis. *J Pharmacol Sci* 2009, 110, 265–269.
- Hattori, K., Nishikawa, M., Watcharanurak, K., Ikoma, A., Kabashima, K., Toyota, H., Takahashi, Y., Takahashi, R., Watanabe, Y., Takakura, Y. Sustained exogenous expression of therapeutic levels of IFN- γ ameliorates atopic dermatitis in NC/Nga mice via Th1 polarization. *J Immunol* 2010, 184, 2729–2735.
- Siraganian, R.P. Mast cell signal transduction from the high-affinity IgE receptor. *Curr Opin Immunol* 2003, 15, 639–646.
- Prussin, C., Metcalfe, D.D. 4. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 2003, 111, S486–S494.
- Navi, D., Saegusa, J., Liu, F.T. Mast cells and immunological skin diseases. *Clin Rev Allergy Immunol* 2007, 33, 144–155.
- Averbeck, M., Gebhardt, C., Emmrich, F., Treudler, R., Simon, J.C. Immunologic principles of allergic disease. *J Dtsch Dermatol Ges* 2007, 5, 1015–1028.
- Kerfoot, S.M., Szczepanik, M., Tung, J.W., Askenase, P.W. Identification of initiator B cells, a novel subset of activation-induced deaminase-dependent B-1-like cells that mediate initiation of contact sensitivity. *J Immunol* 2008, 181, 1717–1727.
- Tsuji, R.F., Szczepanik, M., Kawikova, I., Paliwal, V., Campos, R.A., Itakura, A., Akahira-Azuma, M., Baumgarth, N., Herzenberg, L.A., Askenase, P.W. B cell-dependent T cell responses: IgM antibodies are required to elicit contact sensitivity. *J Exp Med* 2002, 196, 1277–1290.
- Anderson, P.C., Dinulos, J.G. Atopic dermatitis and alternative management strategies. *Curr Opin Pediatr* 2009, 21, 131–138.
- Panossian, A., Wikman, G. Pharmacology of *Schisandra chinensis* Baill.: an overview of Russian research and uses in medicine. *J Ethnopharmacol* 2008, 118, 183–212.
- Liu, C., Zhang, S., Wu, H. Non-thermal extraction of effective ingredients from *Schisandra chinensis* Baill and the antioxidant activity of its extract. *Nat Prod Res* 2009, 23, 1390–1401.
- Guo, L.Y., Hung, T.M., Bae, K.H., Shin, E.M., Zhou, H.Y., Hong, Y.N., Kang, S.S., Kim, H.P., Kim, Y.S. Anti-inflammatory effects of schisandrin isolated from the fruit of *Schisandra chinensis* Baill. *Eur J Pharmacol* 2008, 591, 293–299.
- Kobayashi, Y., Takahashi, R., Ogino, F. Antipruritic effect of the single oral administration of German chamomile flower extract and its combined effect with antiallergic agents in ddY mice. *J Ethnopharmacol* 2005, 101, 308–312.
- Bos, J.D., Van Leent, E.J., Sillevius Smitt, J.H. The millennium criteria for the diagnosis of atopic dermatitis. *Exp Dermatol* 1998, 7, 132–138.
- Koblenzer, C.S. Itching and the atopic skin. *J Allergy Clin Immunol* 1999, 104, S109–S113.
- Lee, S.H., Heo, Y., Kim, Y.C. Effect of German chamomile oil application on alleviating atopic dermatitis-like immune alterations in mice. *J Vet Sci* 2010, 11, 35–41.
- Beltrani, V.S. The clinical spectrum of atopic dermatitis. *J Allergy Clin Immunol* 1999, 104, S87–S98.
- Costa-Pinto, F.A., Basso, A.S., Russo, M. Role of mast cell degranulation in the neural correlates of the immediate allergic reaction in a murine model of asthma. *Brain Behav Immun* 2007, 21, 783–790.
- Hakim-Rad, K., Metz, M., Maurer, M. Mast cells: makers and breakers of allergic inflammation. *Curr Opin Allergy Clin Immunol* 2009, 9, 427–430.
- Matsuda, H., Watanabe, N., Geba, G.P., Sperl, J., Tsudzuki, M., Hiroi, J., Matsumoto, M., Ushio, H., Saito, S., Askenase, P.W., Ra, C. Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. *Int Immunol* 1997, 9, 461–466.
- Wortis, H.H., Berland, R. Cutting edge commentary: origins of B-1 cells. *J Immunol* 2001, 166, 2163–2166.
- Akdis, C.A., Akdis, M., Bieber, T., Bindslev-Jensen, C., Boguniewicz, M., Eigenmann, P., Hamid, Q., Kapp, A., Leung, D.Y., Lipozencic, J., Luger, T.A., Muraro, A., Novak, N., Platts-Mills, T.A., Rosenwasser, L., Scheynius, A., Simons, F.E., Spergel, J., Turjanmaa, K., Wahn, U., Weidinger, S., Werfel, T., Zuberbier, T.; European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology. Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Report. *J Allergy Clin Immunol* 2006, 118, 152–169.
- Bell, J.K., McQueen, D.S., Rees, J.L. Involvement of histamine H4 and H1 receptors in scratching induced by histamine receptor agonists in Balb C mice. *Br J Pharmacol* 2004, 142, 374–380.
- Rosbach, K., Wendorff, S., Sander, K., Stark, H., Gutzmer, R., Werfel, T., Kietzmann, M., Bäumner, W. Histamine H4 receptor antagonism reduces hapten-induced scratching behaviour but not inflammation. *Exp Dermatol* 2009, 18, 57–63.
- Spergel, J.M., Mizoguchi, E., Oettgen, H., Bhan, A.K., Geha, R.S. Roles of TH1 and TH2 cytokines in a murine model of allergic dermatitis. *J Clin Invest* 1999, 103, 1103–1111.
- Fridman, W.H. Fc receptors and immunoglobulin binding factors. *FASEB J* 1991, 5, 2684–2690.
- Erb, K.J. Helminths, allergic disorders and IgE-mediated immune responses: where do we stand? *Eur J Immunol* 2007, 37, 1170–1173.
- Pawankar, R. Mast cells as orchestrators of the allergic reaction: the IgE-IgE receptor mast cell network. *Curr Opin Allergy Clin Immunol* 2001, 1, 3–6.
- Saini, S.S., Klion, A.D., Holland, S.M., Hamilton, R.G., Bochner, B.S., Macglashan, D.W. Jr. The relationship between serum IgE and surface levels of Fc ϵ RI on human leukocytes in various diseases: correlation of expression with Fc ϵ RI on basophils but not on monocytes or eosinophils. *J Allergy Clin Immunol* 2000, 106, 514–520.

Copyright of Immunopharmacology & Immunotoxicology is the property of Taylor & Francis Ltd and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.