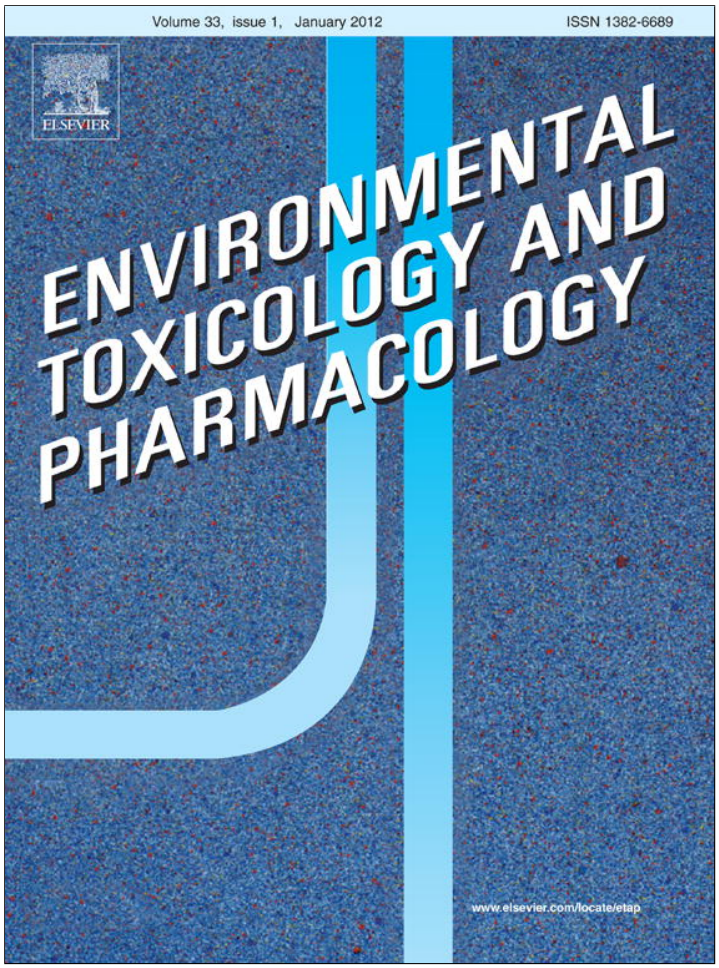

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Platycodi Radix suppresses development of atopic dermatitis-like skin lesions

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ABSTRACT

Platycodi Radix has been used to treat chronic diseases, such as bronchitis, asthma, and hyperlipidemia. In this study, we examined the effect of an aqueous extract, Changkil (CK), from the root of Platycodi Radix on 2,4-dinitrochlorobenzene (DNCB)-induced atopic dermatitis (AD)-like skin lesions. Administration of CK onto DNCB-induced AD-like skin lesions in NC/Nga mice ameliorated lesion intensity scores, levels of IgE, thymus and activation-regulated chemokine (TARC), TNF-α, and IL-4 in serum and ears. In contrast, CK increased level of the immunosuppressive cytokine IL-10. Histopathological examination showed reduced thickness of the epidermis/dermis and dermal infiltration of inflammatory cells in the ears. CK also suppressed TNF-α/IFN-γ-induced mRNA expression and production of TARC in HaCaT cells. CK exerts beneficial effects on AD symptoms, suggesting that CK is an effective potential therapeutic agent for AD.

1. Introduction

Atopic dermatitis (AD) is a chronic, relapsing, and inflammatory skin disease associated with eczematous symptoms and IgE hyperproduction (Gao et al., 2004). Itching is a serious problem in AD patients because scratching worsens the dermatitis (Jiang et al., 2009). Reduction of itching-associated scratching is the most effective therapeutic strategy for improving the quality of life for AD patients. Various allergens including the immunological and non-immunological abnormalities contribute to the pathogenesis and development of AD. AD incidence is increasing throughout the world, and its onset typically occurs during early infancy or childhood, but can also occur in adulthood. For the last four decades, topical steroids and immunosuppressive agents have been standard treatments for severe cases of AD. However, these treatments are often associated with severe adverse effects and are not sufficiently effective in a substantial number of patients with AD (Ingber, 2002). Therefore, there is a great need for the development of new and effective therapies for AD.

T helper (Th) cells are classified into two types, types 1 and 2, depending on the profile of cytokines secreted by the cell. The Th1/Th2 balance is important for the response to a variety of immunological diseases. The pathogenesis of AD is known to be the result of local and systemic immunologic dysfunction that leads to a Th1/Th2 cell imbalance, as well as susceptibility genes and environmental factors (Leung et al., 2004; Beltrani, 2005). Many studies report that Th2 cells are

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predominantly acute phase reactors, whereas Th1 reactions are predominantly chronic in AD skin lesions (Di Cesare et al., 2008). IL-4 induces the differentiation of T lymphocytes into Th2 cells and the switching of B lymphocytes into IgE producers (Di Cesare et al., 2008; Kawakami et al., 2009). TNF-α is also reported to be a contributor to the initiation of AD (Homey et al., 2006).

Platycodi Radix, the aqueous extract from the root of *Platycodon grandiflorum* A.DC. (Campanulaceae family), has been used as a food and traditional oriental medicine to treat chronic adult diseases (e.g., bronchitis, asthma, pulmonary tuberculosis, hyperlipidemia, and hypercholesterolemia) and inflammatory diseases (Lee, 1973). Changkil (CK) is the aqueous extract from the root of Platycodi Radix cultivated for more than 21-year-old plants. Recent studies have shown that CK has antioxidant effects (Lee and Jeong, 2002), anti-metastatic activities (Lee et al., 2006a,b), hepatoprotective and anti-fibrotic effects (Lee et al., 2001; Lee and Jeong, 2002; Lee et al., 2004), anti-inflammatory effect (Choi et al., 2009), and immune stimulation (Choi et al., 2001). Nonetheless, the effect of CK as an anti-atopic agent for AD remains unclear. In this study, we investigated the inhibitory effect of CK on the development of AD in NC/Nga mice and the human keratinocyte cell line HaCaT.

2. Materials and methods

2.1. Chemicals

DNCB (2,4-dinitrochlorobenzene), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide), TNF-α, and IFN-γ were all obtained from Sigma–Aldrich (Milwaukee, WI, USA). Enzyme-linked immunosorbent assay (ELISA) kits for IgE, TARC, and IL-10 were obtained from R&D Systems (Minneapolis, MN, USA) and BD Biosciences (San Diego, CA, USA). Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were obtained from Life Technologies, Inc. (Carlsbad, CA, USA). Polymerase chain reaction (PCR) oligonucleotide primers were custom synthesized by Bioneer Co. (Daejeon, Korea). All chemicals and solvents were of the highest grade commercially available.

2.2. Preparation of CK

CK, the aqueous extract from a 21-year-old Platycodi Radix root, was supplied by Jangsaeng Doraji Co., Ltd. (Jinju, South Korea) and prepared as described previously (Lee and Jeong, 2002). Briefly, distilled water at 90 °C was added to powered root (5 mL/g) and the temperature was maintained for 10 h. The mixture was allowed to cool to room temperature, filtered, and lyophilized. The yield of lyophilized residue corresponded to 33.5% (33.5 g of residue for each 100 g of original dry roots). The pale-yellow extract was dissolved directly in sterilized saline. The composition of CK has been previously published (Kim et al., 1995) and CK consisted of saponin (~2.5%), inulin (~60%) and oligosaccharide (~25%).

2.3. Animals and treatment

Specific pathogen-free male 6-week-old NC/Nga mice were obtained from SLC, Inc. (Shizuoka, Japan). Animals were acclimatized to the temperature (22 ± 2 °C) and humidity (55 ± 5%) of controlled rooms with a 12 h light/dark cycle for at least two weeks prior to experiments. Animals were allowed free access to Purina rodent chow (Seoul, Korea) and tap water. All experimental protocols for animal care were performed according to the rules and regulations of the Animal Ethics Committee, Chungnam National University. The mice were divided into three groups ($n = 5$ per group). To induce AD-like immunologic and skin lesions, DNCB was applied onto the dorsal skin and ears. After complete removal of dorsal hairs within an area approximately 8 cm^2, 200 μL of 1% DNCB solution (dissolved in a 3:1 mixture of acetone and olive oil) was applied for three consecutive days for sensitization. Four days after sensitization, the dorsal skin and ears were challenged with 200 μL of 0.2% DNCB solution three times per week for eight weeks. As soon as the challenge was completed, 100 mg/kg CK, dissolved in saline solution, was administered orally six times per week for four weeks. Control and DNCB-treated mice were administered saline intragastrically. Animals were sacrificed 64 days after the first application of DNCB (Fig. 1). Blood was collected from the vena cava, and the right ear was removed and used for histopathological examination.

2.4. Measurement of ear thickness

Ear thickness was measured with a micrometer (Mitutoyo, Kawasaki, Japan) on the day of sacrifice.

2.5. Histopathological studies

The right ear was removed from each mouse and fixed in 10% formalin solution. It was subsequently embedded in paraffin, sectioned (4 μm), and stained with hematoxylin and eosin. Histological changes were examined by light microscopy.

2.6. Evaluation of skin dermatitis severity

The severity of dermatitis in the ear and back lesions was evaluated twice a week. The development of (1) erythema/hemorrhage, (2) scarring/dryness, (3) edema, and (4) excoriation/erosion was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). The sum of the individual scores was defined as the dermatitis score (Matsuda et al., 1997).

2.7. Cell culture

The spontaneously immortalized human keratinocyte cell line HaCaT (a kind gift from N.E. Fusenig, German Cancer Research, Germany) (Boukamp et al., 1998) was grown in DMEM supplemented with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. Stock solutions of CK dissolved in dimethyl sulfoxide (DMSO) were directly applied to the culture medium 1 h before the addition of 10 ng/mL TNF-α and 10 ng/mL IFN-γ.

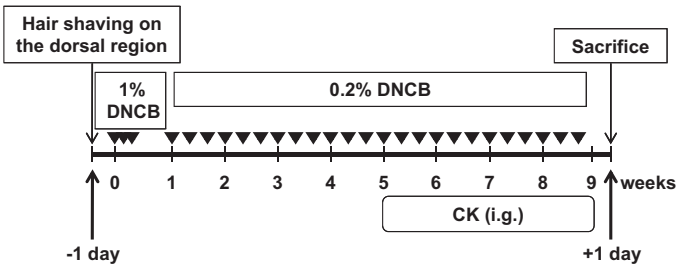

Fig. 1 – Schematic diagram of the experimental protocol in mice. Mice were divided into three groups ($n = 5$ per group). To induce AD-like immunologic and skin lesions, DNCB was applied to the dorsal skin and ears. After complete removal of dorsal hairs within an area approximately 8 cm^2, 200 μL of 1% DNCB was applied on the dorsal skin and ears for three consecutive days for sensitization. Four days after sensitization, the dorsal skin and ears were challenged with 200 μL of 0.2% DNCB solution three times per week for eight weeks. After challenge, mice were orally administered 100 mg/kg CK, dissolved in saline solution, six times per week for four weeks. Control and DNCB-treated mice were administered saline intragastrically. Animals were sacrificed 64 days after the first application of DNCB.

2.8. *Measurement of cell viability*

Cell viability was assessed by the MTT reduction assay, according to the manufacturer's instructions. Cells were plated at a density of 4×10^4 cells/500 μL in 48-well plates. After incubation, cells were treated with MTT solution (final concentration, 1 mg/mL) for 1 h. Dark blue formazan crystals that formed within intact cells were solubilized with DMSO, and the absorbance at 570 nm was measured with a microplate reader (Varioskan, Thermo Electron Co., Vantaa, Finland). This wavelength was determined not to interfere with CK.

2.9. *Semi-quantitative reverse transcription (RT)-PCR analysis*

Total RNA was extracted from left ear or cells using the RNAiso reagent (Takara, Kyoto, Japan), according to the manufacturer's protocol, and stored at −80 °C until use. Then, 0.5 μg RNA was used for reverse transcription and amplified by polymerase chain reaction (PCR) using the access RT-PCR system Takara thermal cycler (Takara, Seoul, Korea). The primer sets used in the PCR amplification were as follows: TNF-α (forward: 5′-CCTGTAGCCCACGTC GTAGC-3′, reverse: 5′-TTGACCTCAGCGCTGAGTTG-3′); IL-4 (forward: 5′-GAATGTACCAGGAGCCATATC-3′, reverse: 5′-CTCAGTACTACGAGTAATCCA-3′); GAPDH (forward: 5′-ACCACAGTCCATGCCATCAC-3′, reverse: 5′-TCCACCACCTGTTGCTGT-3′); and TARC (forward: 5′-ACTGCTCCAGGGATGCCATCGTTTTT-3′, reverse: 5′-ACAAGGGGATGGGATCTCCCTCACTG-3′). The PCR conditions for each target gene were as follows: TNF-α and IL-4: 30 cycles at 94 °C for 30 s, 56 °C for 30 s and 72 °C for 45 s; GAPDH: 28 cycles at 94 °C for 60 s, 54 °C for 60 s and 72 °C for 60 s; TARC: 35 cycles at 98 °C for 10 s, 60 °C for 30 s and 72 °C for 60 s. The termination cycle included a prolonged extension at 72 °C for 7 min. The PCR products were separated on a 2% agarose gel containing ethidium bromide, visualized, and photographed using a gel documentation system (UVP, Cambridge, UK). Band intensity was quantified by densitometry using Image J software (Image Processing and Analysis in Java, NIH, USA). Quantification of mRNA expression levels was expressed as the relative fold change, compared to the control group.

2.10. *ELISA assay*

Cytokines levels in serum or cell culture supernatants were measured by sandwich ELISA using the OptEIA Set mouse IgE kit from BD Biosciences, the DuoSet mouse IL-10, and the mouse and human CCL17/TARC kits from R&D Systems, according to the manufacturer's instructions. Cytokine concentrations were calculated using a linear regression equation obtained from standard absorbance values.

2.11. *Statistical analysis*

All experiments were repeated at least three times. Results are reported as means ± standard error of the mean (S.E.M.). Statistical significance was determined by a one-way analysis of variance (ANOVA), followed by the Tukey–Kramer multiple comparisons test. A significant value was defined as $p < 0.05$.

3. Results

3.1. *Effect of CK on DNCB-induced AD-like skin lesions in NC/Nga mice*

We chose the DNCB-induced AD murine model due to its reproducibility and repeatability (Kwon et al., 2010) and investigated the effect of CK on AD-like skin lesions in NC/Nga mice (Fig. 1). The skin lesions were evaluated twice a week for nine weeks using the dermatitis severity score. As shown in Fig. 2, repeated topical application of DNCB significantly increased dermatitis symptoms in NC/Nga mice as compared with control mice. The CK-administrated mice had lower dermatitis scores after eight weeks as compared with DNCB-treated mice (Fig. 2A). The AD induced by repeated application of DNCB resulted in dermatitis symptoms of severe erythema, hemorrhage, and edema on the dorsal skin and ears. These symptoms of AD were reduced by CK administration (Fig. 2B). Ear swelling was also increased by repeated application of

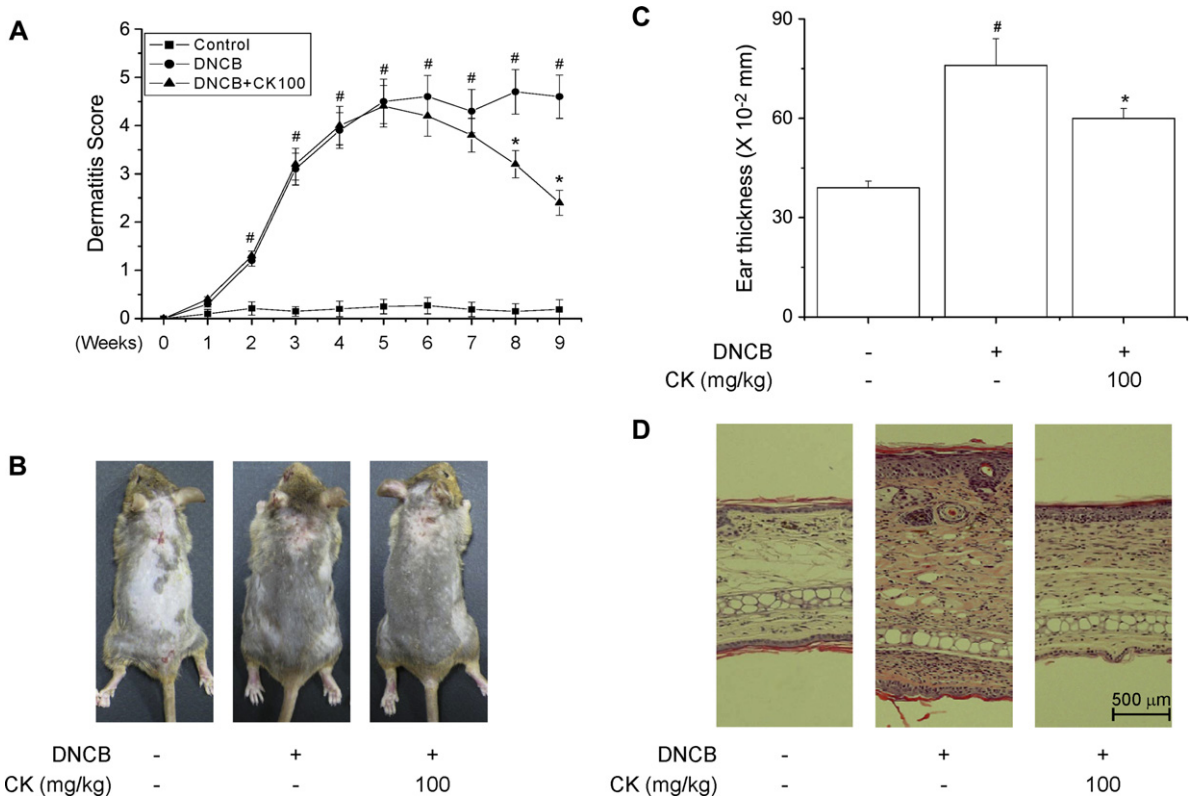

Fig. 2 – Effect of CK on DNCB-induced AD development in NC/Nga mice. (A) The dermatitis score was defined as the sum of scores for seven clinical criteria: erythema, hemorrhage, edema, excoriation, erosion, scarring, and dryness. (B) Example of the clinical severity of AD-like skin lesions. (C) Ear thickness was measured with a micrometer. (D) Ears were removed and fixed with 10% formaldehyde solution. Thin sections (4 μm) were cut and stained with hematoxylin and eosin, magnification, ×100. Results shown are representatives of five observations. Results are presented as mean ± S.E.M. ($n = 5$). $^{\#}p < 0.05$, compared to the control group; $^{*}p < 0.05$, compared to the DNCB-treated group.

DNCB, but ear thickness was reduced by CK administration (Fig. 2C).

3.2. *Effect of CK on DNCB-induced AD-like histopathological changes in NC/Nga mice*

Nine weeks after the first application of DNCB, ear lesions showed thickening of the dermis and epidermis, as well as an accumulation of inflammatory cells. These observations were all inhibited by CK administration (Fig. 2D). These results indicate that CK suppresses spontaneously induced AD in NC/Nga mice.

3.3. *Effect of CK on DNCB-induced cytokine expression in NC/Nga mice*

Many studies have reported that serum levels of IgE and TARC are elevated in patients with AD (Kakinuma et al., 2001; Fujisawa et al., 2002). Therefore, we investigated the effect of CK on serum levels of IgE and TARC in DNCB-treated mice. As shown in Fig. 3A and B, serum levels of IgE and TARC in DNCB-treated mice were significantly higher than those in the control mice. CK reduced the serum levels of IgE and TARC. IL-10 is a known anti-inflammatory cytokine (Wilson et al., 2007), so we investigated the effect of CK on serum level of IL-10 in DNCB-treated mice. As shown in Fig. 3C, the serum level of IL-10 in the DNCB-treated mice was significantly lower than that in the control mice. CK treatment increased the serum level of IL-10. We also investigated the effect of CK on the mRNA expression of cytokines within the ears. The levels of TNF-α and IL-4 mRNA in the DNCB-treated mice were markedly increased as compared with those in the control mice. The CK-administrated mice had lower TNF-α and IL-4 mRNA levels as compared with those in the DNCB-treated mice (Fig. 3D). These results indicate that CK treatment down-regulated cytokine expression, leading to the inhibition of skin inflammation caused by the infiltration of inflammatory cells.

3.4. *Effect of CK on TNF-α-/INF-γ-induced TARC expression in HaCaT cells*

To evaluate its effect on the viability of HaCaT cells, cells were treated with different concentrations of CK. CK was found to have no cytotoxic effects on HaCaT cells at concentrations of 0–200 μg/mL (Fig. 4A). Various chemokines are involved in the development of Th2-mediated inflammation, including AD (Kakinuma et al., 2002; Vestergaard et al., 2004). Thus, we investigated the effect of CK on the mRNA expression and production of TARC by HaCaT cells after stimulation with TNF-α and IFN-γ. The mRNA expression and production of

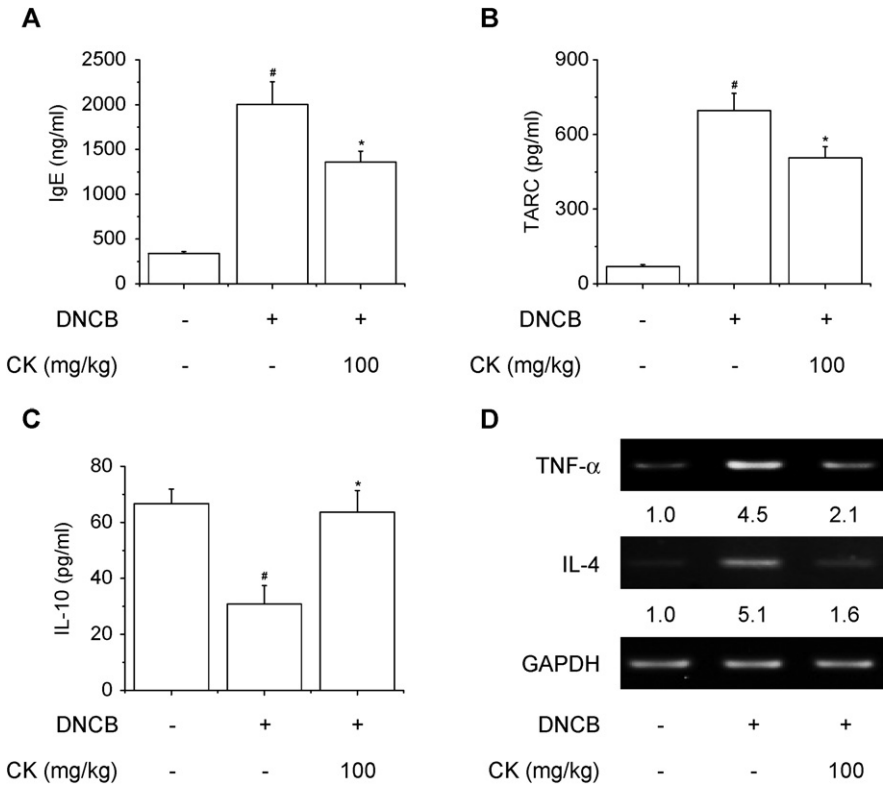

Fig. 3 – Effect of CK on DNCB-induced cytokine levels in NC/Nga mice. Serum was collected 24 h after the last CK treatment. Serum levels of (A) IgE, (B) TARC, and (C) IL-10 were analyzed by ELISA. (D) Total RNA was extracted from ear tissue and mRNA levels of IL-4, TNF-α, and GAPDH were determined by semi-quantitative RT-PCR. Results shown are representatives of five observations. Results are presented as mean ± S.E.M. ($n = 5$). $^{\#}p < 0.05$, compared to the control group; $^{*}p < 0.05$, compared to the DNCB-treated group.

TARC were markedly increased after stimulation with TNF-α and IFN-γ for 24 h. However, TNF-α- and INF-γ-induced mRNA expression and production of TARC were decreased by CK treatment in a concentration-dependent manner (Fig. 4B and C). These results indicate that CK treatment prevents AD by inhibiting the infiltration of inflammatory cells, including Th2 cells.

4. Discussion

We previously reported that CK has anti-allergic effect. CK significantly inhibited ovalbumin-induced leukocyte recruitment in lung tissues and IgE level in bronchoalveolar lavage (BAL) fluid (Choi et al., 2009). However, the effect of CK on AD-like skin inflammation remained unclear. Therefore, this study investigated the inhibitory effect of CK on AD-like skin lesions induced by DNCB in NC/Nga mice. NC/Nga mice spontaneously develop AD-like skin lesions under conventional conditions (Matsuda et al., 1997). Experimental animal models of AD use DNCB treatment to simulate the clinical features of human AD-like skin symptoms, such as erythema, hemorrhage, and edema (Pokharel et al., 2008). CK treatment suppresses the AD-like skin symptoms induced by DNCB, indicating its therapeutic potential for human AD like skin symptoms.

Mast cells, high-affinity receptors for IgE (FcεRI), are significantly increased in AD skin lesions as compared with healthy skin (Metzger, 1992). Activated mast cells release a variety of biologically active substances that play important roles in allergic reactions, such as AD (Kawakami et al., 2009). Mast cells are key effecter cells of IgE-mediated hypersensitivity and allergic disorders (Galli et al., 1999). Many studies have reported that serum levels of IgE and TARC are elevated in patients with AD (Kakinuma et al., 2001; Fujisawa et al., 2002). CK treatment significantly reduced serum IgE and TARC levels induced by DNCB, while increasing the level of IL-10 decreased by DNCB. The application of IL-10 is known to block the effector phase of allergic contact hypersensitivity reactions (Kondo et al., 1994). Our data indicated that IL-10 was an important factor in preventing allergic inflammation and suggested that AD lesions might be associated with suppression of regulatory cytokines and chemokine expression. Therefore, we speculate that therapeutic CK application for allergic contact dermatitis would be beneficial.

Th2-like immune responses are key pathogenic mediators of atopic disorders due to the upregulation of IgE. However, Th1 cytokines also contribute to the pathogenesis of chronic atopic dermatitis. In this study, repeated DNCB treatment resulted in the elevation of serum IgE and increased expression of IL-4 and TNF-α mRNA within ear lesions. Elevated serum IgE is a hallmark of AD, and the expression of IL-4

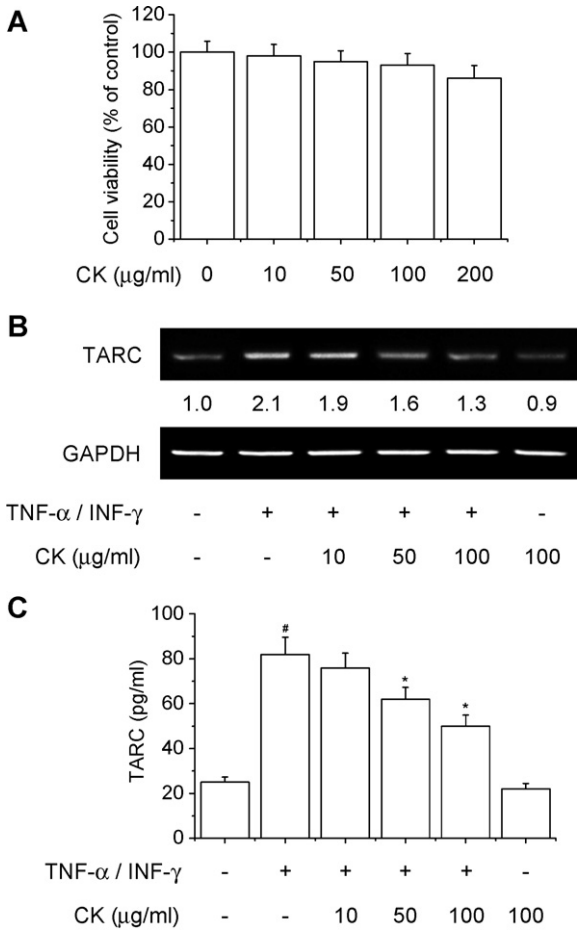

Fig. 4 – Effect of CK on TNF-α- and IFN-γ-induced mRNA expression and production of TARC in HaCaT cells. (A) Cells were cultured in 48-well plates and treated with CK (0, 10, 50, 100, or 200 μg/mL). After 24 h, cell viability was measured using the MTT reduction assay. (B and C) Cells were treated with TNF-α and IFN-γ (each 20 ng/mL) in the presence of CK (0, 10, 50, or 100 μg/mL). After 24 h, total RNA was extracted from cells and mRNA levels of TARC and GAPDH were determined by semi-quantitative RT-PCR (B). Supernatants were also collected and measured for production by ELISA (C). Results are presented as mean ± S.E.M. of three independent experiments. $^{\#}p < 0.05$, compared to the control group; $^{*}p < 0.05$, compared to the TNF-α- and IFN-γ-treated group.

contributes to its elevation. CK treatment ameliorated these DNCB-induced responses.

AD has been described as a Th2-type disease, at least during its initial onset, as lymphocytes that infiltrate the skin primarily produce IL-4 and IL-5 (Vestergaard et al., 1999). The chemokine receptor predominantly expressed on Th2 cells is CC chemokine receptor 4, which binds to TARC (Oshio et al., 2009). It was reported that modulation of TARC expression in keratinocytes may contribute to the pathogenesis of inflammatory skin diseases such as AD (Jeong et al., 2010). TARC production is activated in HaCaT cells after stimulation with TNF-α and IFN-γ (Xiao et al., 2003). Treatment with CK suppressed the mRNA expression and production of TARC induced by TNF-α and INF-γ, suggesting that CK may be beneficial in the treatment of human inflammatory skin diseases. In summary, our data demonstrate that CK inhibited DNCB-induced AD-like skin symptoms by blocking leukocyte accumulation and the expression of pro-inflammatory cytokines. These results indicate that CK may be an effective therapeutic drug for patients with AD.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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REFERENCES

Beltrani, V.S., 2005. Suggestions regarding a more appropriate understanding of atopic dermatitis. Curr. Opin. Allergy Clin. Immunol. 5, 413–418.

Boukamp, P., Petrussevska, R.T., Breitkreutz, D., Hornung, J., Markham, A., Fusenig, N.E., 1998. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J. Cell Biol. 106, 761–771.

Choi, C.Y., Kim, J.Y., Kim, Y.S., Chung, Y.C., Seo, J.K., Jeong, H.G., 2001. Aqueous extract isolated from *Platycodon grandiflorum* elicits the release of nitric oxide and tumor necrosis factor-alpha from murine macrophages. Int. Immunopharmacol. 1, 1141–1151.

Choi, J.H., Hwang, Y.P., Lee, H.S., Jeong, H.G., 2009. Inhibitory effect of Platycodi Radix on ovalbumin-induced airway inflammation in a murine model of asthma. Food Chem. Toxicol. 47, 1272–1279.

Di Cesare, A., Di Meglio, P., Nestle, F.O., 2008. A role for Th17 cells in the immunopathogenesis of atopic dermatitis? J. Invest. Dermatol. 128, 2569–2571.

Fujisawa, T., Fujisawa, R., Kato, Y., Nakayama, T., Morita, A., Katsumata, H., Nishimori, H., Iguchi, K., Kamiya, H., Gray, P.W., Chantry, D., Suzuki, R., Yoshie, O., 2002. Presence of high contents of thymus and activation-regulated chemokine in platelets and elevated plasma levels of thymus and activation-regulated chemokine and macrophage-derived chemokine in patients with atopic dermatitis. J. Allergy Clin. Immunol. 110, 139–146.

Galli, S.J., Maurer, M., Lantz, C.S., 1999. Mast cells as sentinels of innate immunity. Curr. Opin. Immunol. 11, 53–59.

Gao, X.K., Nakamura, N., Fuseda, K., Tanaka, H., Inagaki, N., Nagai, H., 2004. Establishment of allergic dermatitis in NC/Nga mice as a model for severe atopic dermatitis. Biol. Pharm. Bull. 27, 1376–1381.

Homey, B., Steinhoff, M., Ruzicka, T., Leung, D.Y., 2006. Cytokines and chemokines orchestrate atopic skin inflammation. J. Allergy Clin. Immunol. 118, 178–189.

Ingber, A., 2002. What's new in contact dermatitis: allergy to topical steroids in Israeli patients with contact dermatitis. Isr. Med. Assoc. J. 4, 867.

Jeong, S.I., Choi, B.M., Jang, S.I., 2010. Sulforaphane suppresses TARC/CCL17 and MDC/CCL22 expression through heme

oxygenase-1 and NF-κB in human keratinocytes. Arch. Pharm. Res. 33, 1867–1876.
Jiang, J., Yamaguchi, T., Funakushi, N., Kuhara, T., Fan, P.S., Ueki, R., Suto, H., Kase, Y., Ikeda, S., Ogawa, H., 2009. Oral administration of Yokukansan inhibits the development of atopic dermatitis-like lesions in isolated NC/Nga mice. J. Dermatol. Sci. 56, 37–42.
Kakinuma, T., Nakamura, K., Wakugawa, M., Mitsui, H., Tada, Y., Saeki, H., Torii, H., Asahina, A., Onai, N., Matsushima, K., Tamaki, K., 2001. Thymus and activation-regulated chemokine in atopic dermatitis: serum thymus and activation-regulated chemokine level is closely related with disease activity. J. Allergy Clin. Immunol. 107, 535–541.
Kakinuma, T., Nakamura, K., Wakugawa, M., Mitsui, H., Tada, Y., Saeki, H., Torii, H., Komine, M., Asahina, A., Tamaki, K., 2002. Serum macrophage-derived chemokine (MDC) levels are closely related with the disease activity of atopic dermatitis. Clin. Exp. Immunol. 127, 270–273.
Kawakami, T., Ando, T., Kimura, M., Wilson, B.S., Kawakami, Y., 2009. Mast cells in atopic dermatitis. Curr. Opin. Immunol. 21, 666–678.
Kim, K.S., Ezaki, O., Ikemoto, S., Itakura, H., 1995. Effects of *Platycodon grandiflorum* feeding on serum and liver lipid concentrations in rats with diet-induced hyperlipidemia. J. Nutr. Sci. Vitaminol. 41, 485–491.
Kondo, S., McKenzie, R.C., Sauder, D.N., 1994. Interleukin-10 inhibits the elicitation phase of allergic contact hypersensitivity. J. Invest. Dermatol. 103, 811–814.
Kwon, H.K., Lee, C.G., So, J.S., Chae, C.S., Hwang, J.S., Sahoo, A., Nam, J.H., Rhee, J.H., Hwang, K.C., Im, S.H., 2010. Generation of regulatory dendritic cells and CD4+ Foxp3+ T cells by probiotics administration suppresses immune disorders. Proc. Natl. Acad. Sci. U.S.A. 107, 2159–2164.
Lee, E.B., 1973. Pharmacological studies on *Platycodon grandiflorum* A. DC. IV. A comparison of experimental pharmacological effects of crude platycodin with clinical indications of platycodi radix (author's transl). Yakugaku Zasshi 93, 1188–1194.
Lee, K.J., Jeong, H.G., 2002. Protective effect of Platycodi radix on carbon tetrachloride-induced hepatotoxicity. Food Chem. Toxicol. 40, 517–525.
Lee, K.J., Kim, J.Y., Choi, J.H., Kim, H.G., Chung, Y.C., Roh, S.H., Jeong, H.G., 2006a. Inhibition of tumor invasion and metastasis by aqueous extract of the radix of *Platycodon grandiflorum*. Food Chem. Toxicol. 44, 1890–1896.
Lee, K.J., Kim, J.Y., Jung, K.S., Choi, C.Y., Chung, Y.C., Kim, D.H., Jeong, H.G., 2004. Suppressive effects of *Platycodon grandiflorum* on the progress of carbon tetrachloride-induced hepatic fibrosis. Arch. Pharm. Res. 27, 1238–1244.
Lee, K.J., Shin, D.W., Chung, Y.C., Jeong, H.G., 2006b. Chemopreventive effect of saponins derived from roots of *Platycodon grandiflorum* on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in A/J mice. Arch. Pharm. Res. 29, 651–656.
Lee, K.J., You, H.J., Park, S.J., Kim, Y.S., Chung, Y.C., Jeong, T.C., Jeong, H.G., 2001. Hepatoprotective effects of *Platycodon grandiflorum* on acetaminophen-induced liver damage in mice. Cancer Lett. 174, 73–81.
Leung, D.Y., Boguniewicz, M., Howell, M.D., Nomura, I., Hamid, Q.A., 2004. New insights into atopic dermatitis. J. Clin. Invest. 113, 651–657.
Matsuda, H., Watanabe, N., Geba, G.P., Sperl, J., Tsudzuki, M., Hiroi, J., Matsumoto, M., Ushio, H., Saito, S., Askenase, P.W., Ra, C., 1997. Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. Int. Immunol. 9, 461–466.
Metzger, H., 1992. The receptor with high affinity for IgE. Immunol. Rev. 125, 37–48.
Oshio, T., Sasaki, Y., Funakoshi-Tago, M., Aizu-Yokota, E., Sonoda, Y., Matsuoka, H., Kasahara, T., 2009. Dermatophagoides farinae extract induces severe atopic dermatitis in NC/Nga mice, which is effectively suppressed by the administration of tacrolimus ointment. Int. Immunopharmacol. 9, 403–411.
Pokharel, Y.R., Lim, S.C., Kim, S.C., Heo, T.H., Choi, H.K., Kang, K.W., 2008. Sopungyangjae-Tang inhibits development of dermatitis in Nc/Nga mice. Evid. Based Complement. Alternat. Med. 5, 173–180.
Vestergaard, C., Johansen, C., Christensen, U., Just, H., Hohwy, T., Deleuran, M., 2004. TARC augments TNF-alpha-induced CTACK production in keratinocytes. Exp. Dermatol. 13, 551–557.
Vestergaard, C., Yoneyama, H., Murai, M., Nakamura, K., Tamaki, K., Terashima, Y., Imai, T., Yoshie, O., Irimura, T., Mizutani, H., Matsushima, K., 1999. Overproduction of Th2-specific chemokines in NC/Nga mice exhibiting atopic dermatitis-like lesions. J. Clin. Invest. 104, 1097–1105.
Wilson, M.S., Elnekave, E., Mentink-Kane, M.M., Hodges, M.G., Pesce, J.T., Ramalingam, T.R., Thompson, R.W., Kamanaka, M., Flavell, R.A., Keane-Myers, A., Cheever, A.W., Wynn, T.A., 2007. IL-13Ralpha2 and IL-10 coordinately suppress airway inflammation, airway-hyperreactivity, and fibrosis in mice. J. Clin. Invest. 117, 2941–2951.
Xiao, T., Kagami, S., Saeki, H., Sugaya, M., Kakinuma, T., Fujita, H., Yano, S., Mitsui, H., Torii, H., Komine, M., Asahina, A., Nakamura, K., Tamaki, K., 2003. Both IL-4 and IL-13 inhibit the TNF-alpha and IFN-gamma enhanced MDC production in a human keratinocyte cell line, HaCaT cells. J. Dermatol. Sci. 31, 111–117.