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Platycodon grandiflorum root-derived saponins attenuate atopic dermatitis-like skin lesions via suppression of NF-κB and STAT1 and activation of Nrf2/ARE-mediated heme oxygenase-1

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ABSTRACT

Purpose: The consequences of precipitously rising allergic skin inflammation rates worldwide have accelerated the risk of atopic dermatitis (AD). Natural product-based agents with good efficacy and low risk of side effects offer promising prevention and treatment strategies for inflammation-related diseases. We have already reported that *Platycodon grandiflorum* root-derived saponins (Changkil saponins, CKS) have many pharmacological effects, including anti-inflammatory and anti-allergic effects, but its influence on AD remains unclear. Therefore, we evaluated the inhibitory effect of CKS, mainly platycodin D, on AD-like skin symptoms in mice and the possible mechanisms in cells.
Methods: Mice were sensitized and challenged with 2,4-dinitrochlorobenzene (DNCB). Four weeks after challenge, mice were treated with oral administration of CKS for 4 weeks. In addition, cells were used to evaluate the effect of CKS, mainly platycodin D, on the TARC expression regulated mechanism.
Results: CKS attenuated DNCB-induced dermatitis severity, serum levels of IgE and TARC, and mRNA expression of TARC, TNF-α, IFN-γ, IL-4, IL-5, and IL-13 in mice. Histopathological examination showed reduced thickness of the epidermis/dermis and dermal infiltration of inflammatory cells and mast cells in the ears. Moreover, CKS and platycodin D inhibited TNF-α/IFN-γ-induced TARC expression through the suppression of NF-κB and STAT1 and induction of Nrf2/ARE-mediated hemeoxygenase-1 (HO-1) expression in cells.
Conclusion: We suggest that CKS and platycodin D inhibited the development of AD-like skin symptoms by regulating cytokine mediators and may be an effective alternative therapy for AD-like skin symptoms.

Introduction

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease that is triggered by an underlying, complicated interplay between the genetics of the individual and stimulation by allergens (De Benedetto et al., 2012). The basic pathogenic mechanism associated with the development of AD is a T-helper cell (Th)1/Th2 imbalance with allergy sensitization and the acquisition of an allergy to a specific allergen. In the acute stage of AD, Th2-dominant allergic inflammation predominates, leading to increased interleukin (IL)-4, IL-5, and IL-13, but in the chronic stage of AD, Th1-dominant allergic inflammation predominates, leading to increased tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ). Recent studies have reported that Th2 cells overexpression may cause the AD-like skin symptoms

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(Turner et al., 2012). These Th2 cells might increase the secretion of immunoglobulin (Ig)E by B cells. As a result of allergic sensitization, mast cells degranulate and release various biological mediators in allergen-specific IgE-mediated AD (Amin, 2012).

AD affects about 10–20% of the general population. The incidence of AD has increased considerably in recent decades, and AD is one of the most common chronic disorders globally; thus, the accompanying health care cost is a major health and socioeconomic problem (Kim, 2013). The standard therapeutic strategy for treatment of AD has long been application of local or systemic steroids and immunosuppressive agents. These agents remain the first-line treatment for AD because they are effective at reducing symptoms; however, these treatments are often associated with severe side effects and are not sufficiently effective in a substantial number of patients with AD (Aschoff et al., 2011). Therefore, there is a need to develop new and effective therapies for AD.

There is presently widespread interest in developing new and less toxic anti-inflammatory agents from natural sources. The food and traditional oriental medicine, *Platycodon grandiflorum* is used for the treatment of chronic adult diseases and inflammatory diseases (Kim et al., 1995). *Platycodon grandiflorum* root-derived saponins (Changkil saponins, CKS) were shown to exert anti-inflammatory and anti-allergic effects (Kim et al., 2006; Han et al., 2009). A previous study reported that CKS contain triterpenoid saponins, such as deapio-platycoside E, platycoside E, deapio-platycodin D3, platycodin D3, polygalacin D2, platyconic acid A, platycodin D2, platycodin D, and 2′-O-acetylpolygalacin D2 (Noh et al., 2010). Among these triterpenoid saponins, platycodin D is the most abundant and has the greatest pharmacological efficacy including anti-inflammatory and anti-obesity effects (Ahn et al., 2005; Hwang et al., 2013). Although the pharmacological activity of CKS and platycodin D has been investigated, the AD mechanisms remain unknown. Here, we examined the inhibitory effects of CKS and platycodin D on the development of AD-like skin symptoms in NC/Nga mice and the regulatory mechanisms in HaCaT cells.

Materials and methods

Materials

We purchased 2,4-dinitrochlorobenzene (DNCB), MTT, TNF-α, and IFN-γ from Sigma Chemical Co. (St. Louis, MO, USA); LipofectAmine 2000 from Life Technologies, Inc. (Carlsbad, CA, USA); NF-κB luciferase reporter gene from Stratagene (Grand Island, NY, USA); OptEIA™ Set mouse IgE kit from BD Biosciences (San Diego, CA, USA); DuoSet mouse or human CCL17/TARC kit from R&D Systems (Minneapolis, MN, USA); Phospho-NF-κB p65, phospho-IκBα, phospho-STAT1, total-STAT1, and HRP-conjugated anti-IgG secondary antibodies from Cell Signaling Technologies (Beverly, MA, USA); IκBα, NF-κB p65, HO-1, and Nrf2 from Santa Cruz Biotechnology (Santa Cruz, CA, USA). HO-1/ARE luciferase reporter gene was kindly provided by Dr. J. Alam (Tulane University School of Medicine, New Orleans, LA, USA). All chemicals and reagents were of the highest commercially available grade.

Preparation of CKS and platycodin D

The aqueous extract of *Platycodon grandiflorum* root (CK), was supplied by Jangsaeng Doraji Co., Ltd. (Jinju, Korea) and prepared as described previously (Lee and Jeong, 2002). The composition of CK has been reported (Kim et al., 1995). CK was subjected to column chromatography over Amberlite XAD-2, Diaion MCl Gel HP20, or Kogel BG4600. After removing the saccharides and amino acids with water, the column was eluted with methanol to obtain CKS, which is the saponins fraction of CK, as described previously (Kim et al., 2006). Platycodin D was purified and its purity was previously determined (Yoo et al., 2011; Ryu et al., 2012). The purity of the platycodin D was determined to be more than 95%. Chemical structure of platycodin D described as Fig. 6A.

Animals

Animals were purchased and acclimatized as described previously (Choi et al., 2013b,c). All experimental protocols for animal care and standard guideline were performed according to the rules and regulations of the Animal Ethics Committee, Chungnam National University.

Sensitization, challenge, and drug treatment

AD-like immunological and skin lesions were performed as described previously (Choi et al., 2013c). Mice were divided into four groups ($n = 5$ per group). After inducing AD, the CKS-treated group was orally administered a dose of 0.5 or 2 mg/kg of CKS, dissolved in saline, six times per week for 4 weeks. The control and DNCB-treated groups were orally administered saline without CKS. Animals were sacrificed 64 days after the first application of DNCB.

Histopathological studies in ear tissue

Histopathogical right ear lesions were performed as described previously (Choi et al., 2013c). Histopathological changes were examined by light microscopy. An arbitrary scope was given to each microscopic field viewed at a magnification of 100× or 200×.

Measurement of ear thickness

Ear thickness was measured with a micrometer (Mitutoyo, Kawasaki, Japan) on the day of sacrifice. The micrometer was applied near the tip of the ear just distal to the cartilaginous ridges, and the thickness was recorded in micrometers.

Evaluation of skin dermatitis severity

Dermatitis severity in the dorsal skin and ear lesions was evaluated twice per week for 64 days. 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).

Cell cultures

The spontaneously immortalized human keratinocyte cell line HaCaT (a gift from Dr. Fusenig, German Cancer Research Center, Heidelberg, Germany) was cultured in DMEM supplemented with 10% FBS and 1% penicillin and streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2 (Fusenig and Boukamp, 1998).

Measurement of cell viability

MTT reduction assay was performed as previously described (Hwang et al., 2013).

Semi-quantitative reverse transcription (RT)-PCR analysis

RT-PCR analysis was performed as previously described (Choi et al., 2013b).

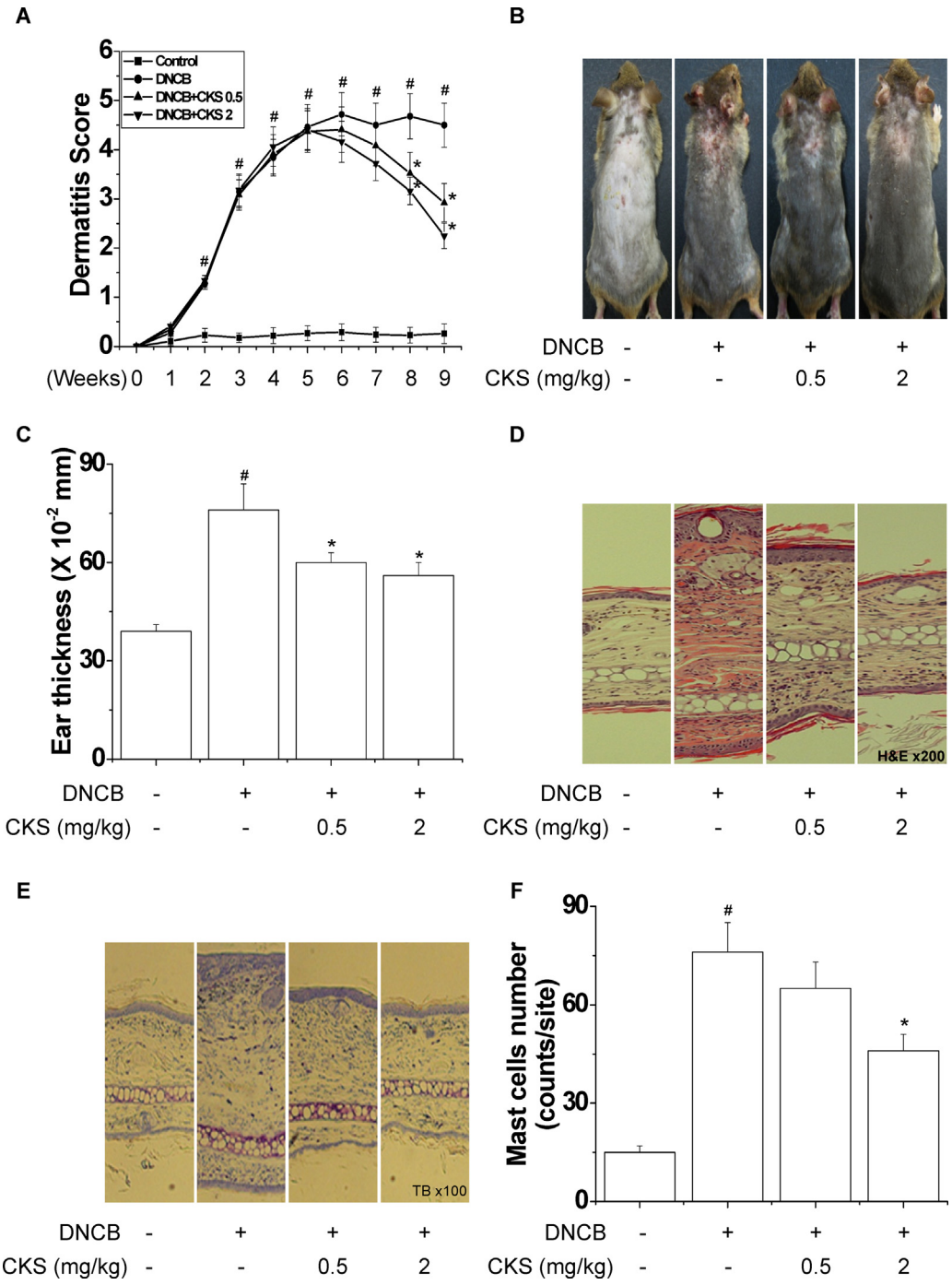

Fig. 1. Effects of CKS on DNCB-induced AD-like skin lesions in NC/Nga mice. (A) The dermatitis score was defined as the sum of scores for seven clinical criteria. (B) Example of the clinical severity of AD-like skin lesions. (C) Ear thickness was measured with a micrometer on the final day. (D) Thin sections were cut and stained with hematoxylin and eosin. (E) Thin sections were cut and stained with toluidine blue. (F) The numbers of mast cells in five sites chosen at random were counted. Results shown are representative of five observations. Data are presented as means ± SD ($n = 5$) and analyzed with ANOVA. $^{\#}p < 0.05$, versus the control group. $^{*}p < 0.05$, versus the DNCB-treated group.

Transient transfection and luciferase assay

Cells were transfected with a NF-κB or HO-1/ARE reporter vector, and *Renilla* luciferase reporter vector using serum- and antibiotic-free LipofectAmine 2000 reagent. At 5 h after transfection, the transfection medium was replaced with growth medium. The cells were then treated with CKS for 1 h and stimulated with TNF-α/IFN-γ for 24 h. Luciferase activity analysis was performed as previously described (Choi et al., 2013b).

Western blotting

Cells were treated with CKS for 1 h and then stimulated with TNF-α/IFN-γ for 15 min, 30 min, 6 h, or 24 h. Western blot analysis was performed as previously described (Choi et al., 2013b).

ELISA

Level of IgE and TARC in serum or level of TARC in culture medium were measured by sandwich ELISA using the OptEIA™

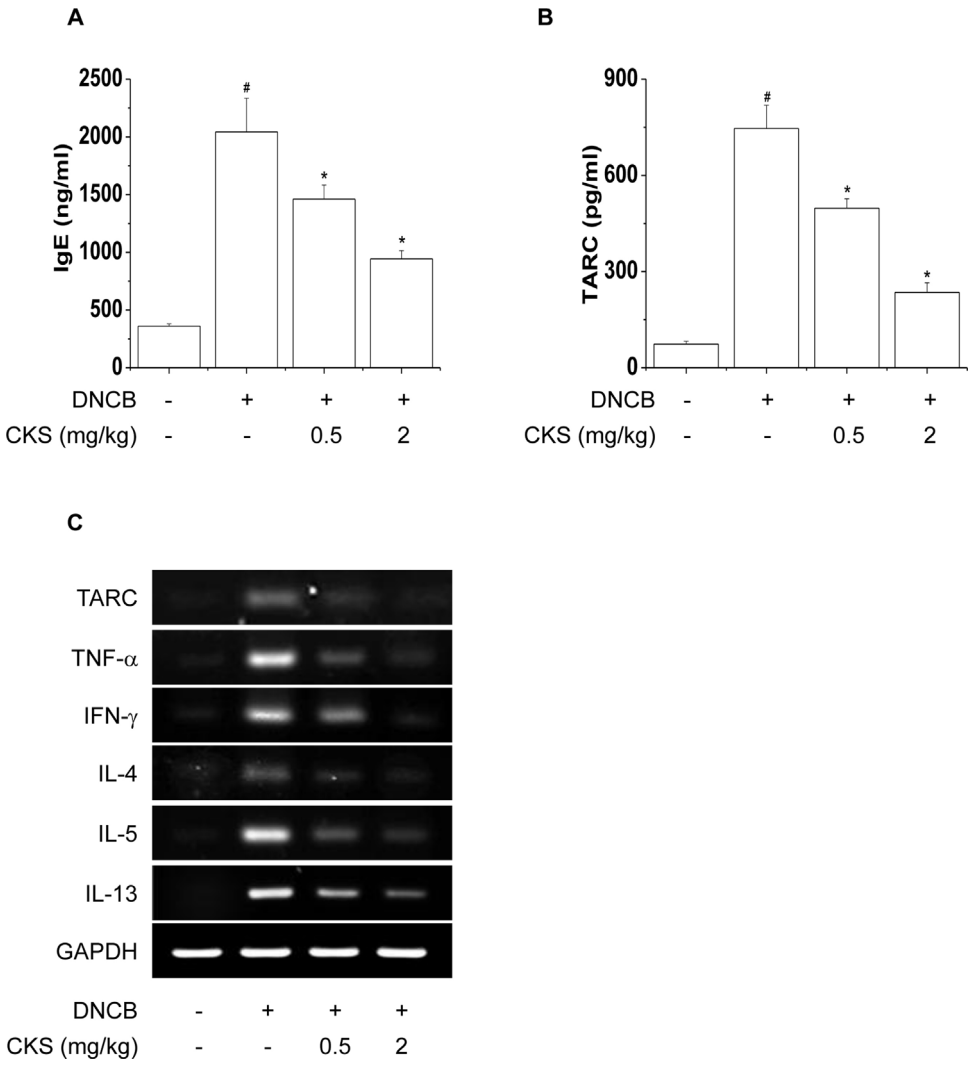

Fig. 2. Effects of CKS on DNCB-induced serum level of IgE and TARC and mRNA expression of TARC chemokine and Th1 and Th2 cytokines in NC/Nga mice. Serum levels of (A) IgE and (B) TARC were determined by ELISA. (C) Total RNA was extracted from ear tissue, and the mRNA expression of TARC, TNF-α, IFN-γ, IL-4, IL-5, and IL-13 was determined by semi-quantitative RT-PCR. Results shown are representative of five observations. Data are presented as means ± SD ($n = 5$) and analyzed with ANOVA. $^{\#}p < 0.05$, versus the control group. $^{*}p < 0.05$, versus the DNCB-treated group.

Set mouse IgE kit and the DuoSet mouse or human CCL17/TARC kit, according to the respective manufacturer's instructions.

Statistical analysis

All experiments were repeated at least three times. The data were expressed as means of ±SD. Statistical significance was determined using an ANOVA followed by the Tukey–Kramer test, setting $p < 0.01$ or $p < 0.05$ as the level of significance.

Results

Inhibitory effects of CKS on DNCB-induced AD-like skin severity in NC/Nga mice

Repeated topical application of DNCB significantly increased dermatitis severity and ear thickness in NC/Nga mice. Furthermore, ear histopathological lesions showed thickening of the dermis and epidermis as well as infiltration of inflammatory cells and mast cells in the DNCB-treated group compared with the control group. CKS significantly reduced DNCB-induced AD severity, ear thickness, and infiltration of inflammatory cells and mast cells (Fig. 1).

Inhibitory effects of CKS on DNCB-induced serum level of IgE and TARC in NC/Nga mice

A previous study reported that IgE and TARC, which are elevated in AD, were induced by the chemical hapten, DNCB (Choi et al., 2013b,c). The anti-AD effect of CKS was evaluated in DNCB-induced AD-like skin symptoms model. CKS significantly reduced DNCB-increased serum level of IgE and TARC (Fig. 2A and B).

Inhibitory effects of CKS on DNCB-increased mRNA expression of TARC, TNF-α, IFN-γ, IL-4, IL-5, and IL-13 in NC/Nga mice

In the acute phase, there are predominantly Th2 cytokines (IL-4, IL-5, and IL-13), whereas the chronic phase is characterized by Th1 cytokines (TNF-α and IFN-γ) (Leung and Bieber, 2003). TARC is also correlated with AD severity and skin keratinocytes have been found to be key players in AD pathogenesis (Saeki and Tamaki, 2006). We examined the mRNA expression of TARC chemokine and Th1 and Th2 cytokines in the ear lesions. CKS inhibited DNCB-induced mRNA expression of TARC chemokine and Th1 and Th2 cytokines (Fig. 2C).

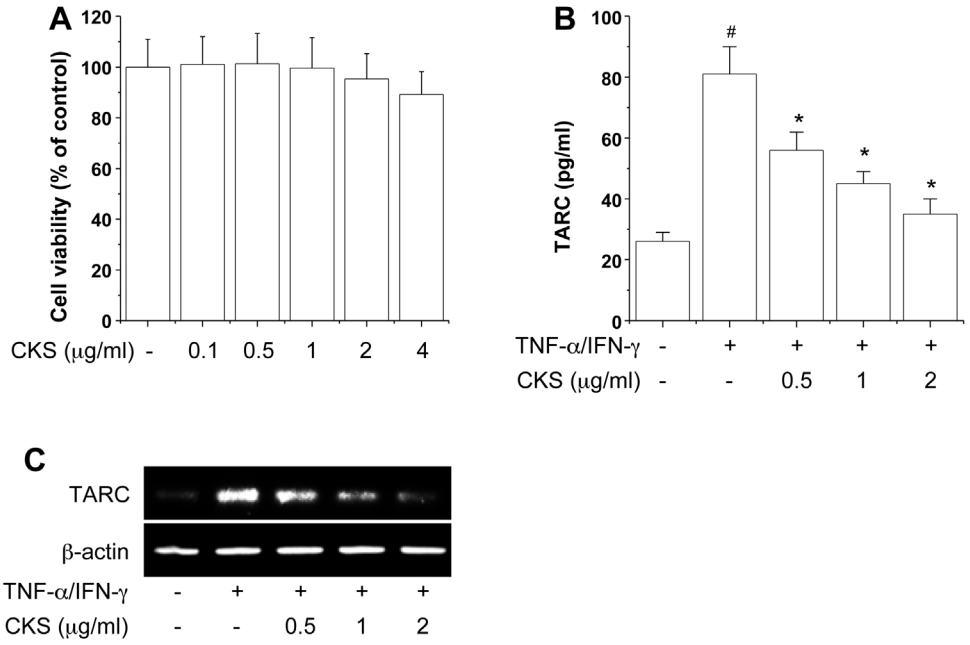

Fig. 3. Effects of CKS on TNF-α/IFN-γ-induced TARC level in HaCaT cells. (A) Cells viability was measured using the MTT reduction assay. (B) TARC production in the supernatants was measured by ELISA. (C) TARC mRNA expression was determined by semi-quantitative RT-PCR. Data are presented as means ± SD ($n = 3$) and analyzed with ANOVA. $^{\#}p < 0.01$, versus the control group. $^{*}p < 0.01$, versus the TNF-α/IFN-γ-treated group.

Inhibitory effects of CKS on TNF-α/IFN-γ-induced expression of TARC in HaCaT cells

To examine the possibility that the cytotoxicity of CKS contributes to its suppressive effect on TARC expression, cell viability was examined by the MTT reduction assay (Fig. 3A). Thus, CKS at 0.5–2 μg/ml were used in subsequent experiments. Also, TNF-α/IFN-γ-induced mRNA expression and protein production of TARC were decreased by CKS treatment (Fig. 3B and C).

Inhibitory effects of CKS on TNF-α/IFN-γ-induced activation of NF-κB and STAT1 in HaCaT cells

Since NF-κB and STAT1 are pivotal regulators of TNF-α- and IFN-γ-induced immune responses, we examined the contribution of NF-κB and STAT1 pathways in TNF-α/IFN-γ-induced TARC activation. CKS significantly inhibited TNF-α/IFN-γ-induced NF-κB luciferase activity, NF-κB p65 phosphorylation, IκBα phosphorylation and degradation, and NF-κB p65 nuclear translocation (Fig. 4A–C). Furthermore, a NF-κB pathway inhibitor, JSH-23, inhibited TNF-α/IFN-γ-induced TARC mRNA expression (Fig. 4D). We further evaluated the effect of CKS on TNF-α/IFN-γ-induced activation of STAT1. CKS inhibited TNF-α/IFN-γ-induced STAT1 phosphorylation (Fig. 4E). A JAK/STAT pathway inhibitor, AG490, also inhibited TNF-α/IFN-γ-induced TARC mRNA expression (Fig. 4F).

Inhibitory effects of CKS on TNF-α/IFN-γ-induced TARC expression via up-regulation of Nrf2/ARE-mediated HO-1 expression in HaCaT cells

Hemeoxygenase-1 (HO-1) has been reported to play a regulatory role in skin inflammation, such as AD (Listopad et al., 2007). We examined the effect of CKS on the expression of anti-oxidant enzymes in cells. CKS increased HO-1 expression, Nrf2 nuclear translocation, and HO-1/ARE luciferase activity (Fig. 5A–C). ZnPP, HO-1 pathway inhibitor, completely neutralized the inhibitory effect of CKS on TNF-α/IFN-γ-induced up-regulation of TARC (Fig. 5D).

Inhibitory effects of platycodin D on TNF-α/IFN-γ-induced TARC expression via up-regulation of Nrf2/ARE-mediated HO-1 expression and down-regulation of NF-κB and STAT1 activation in HaCaT cells

Platycodin D reported the most potent CKS triterpenoid saponins with regard to its effect on radical scavenging activity and its pharmacological effects (Ryu et al., 2012). The anti-AD effect of platycodin D was evaluated in TNF-α/IFN-γ-induced TARC activation. First, to examine the possibility that the cytotoxicity of platycodin D contributes to its suppressive effect on TARC expression, cell viability was examined by the MTT reduction assay. We observed no significant alterations in cell viability following platycodin D treatment at these concentrations (data not shown). Second, we examined the inhibitory effect of platycodin D on TNF-α/IFN-γ-increased TARC mRNA expression in cells. TNF-α/IFN-γ-induced TARC mRNA expression was decreased by platycodin D treatment (Fig. 6B). Third, we examined the inhibitory effect of platycodin D on TNF-α/IFN-γ-induced NF-κB and STAT1 activation in cells. Platycodin D inhibited TNF-α/IFN-γ-induced NF-κB p65 phosphorylation, IκBα phosphorylation and degradation, and STAT1 phosphorylation (Fig. 6C and D). Finally, we examined the effect of platycodin D on the expression of anti-oxidant enzymes in cells. Platycodin D increased HO-1 expression, Nrf2 nuclear translocation, and HO-1/ARE luciferase activity (Fig. 6E–G).

Discussion

Natural product-based agents with good efficacy and low risk of side effects offer promising prevention and treatment strategies for inflammation-related diseases (Dey et al., 2006). Natural immune modulators from herbal extracts or herbal derivatives have been reported an inhibitory effect on AD (Han et al., 2012). Many researchers are searching for alternative treatments to replace steroids for AD therapy (Yang et al., 2013). Thus, we

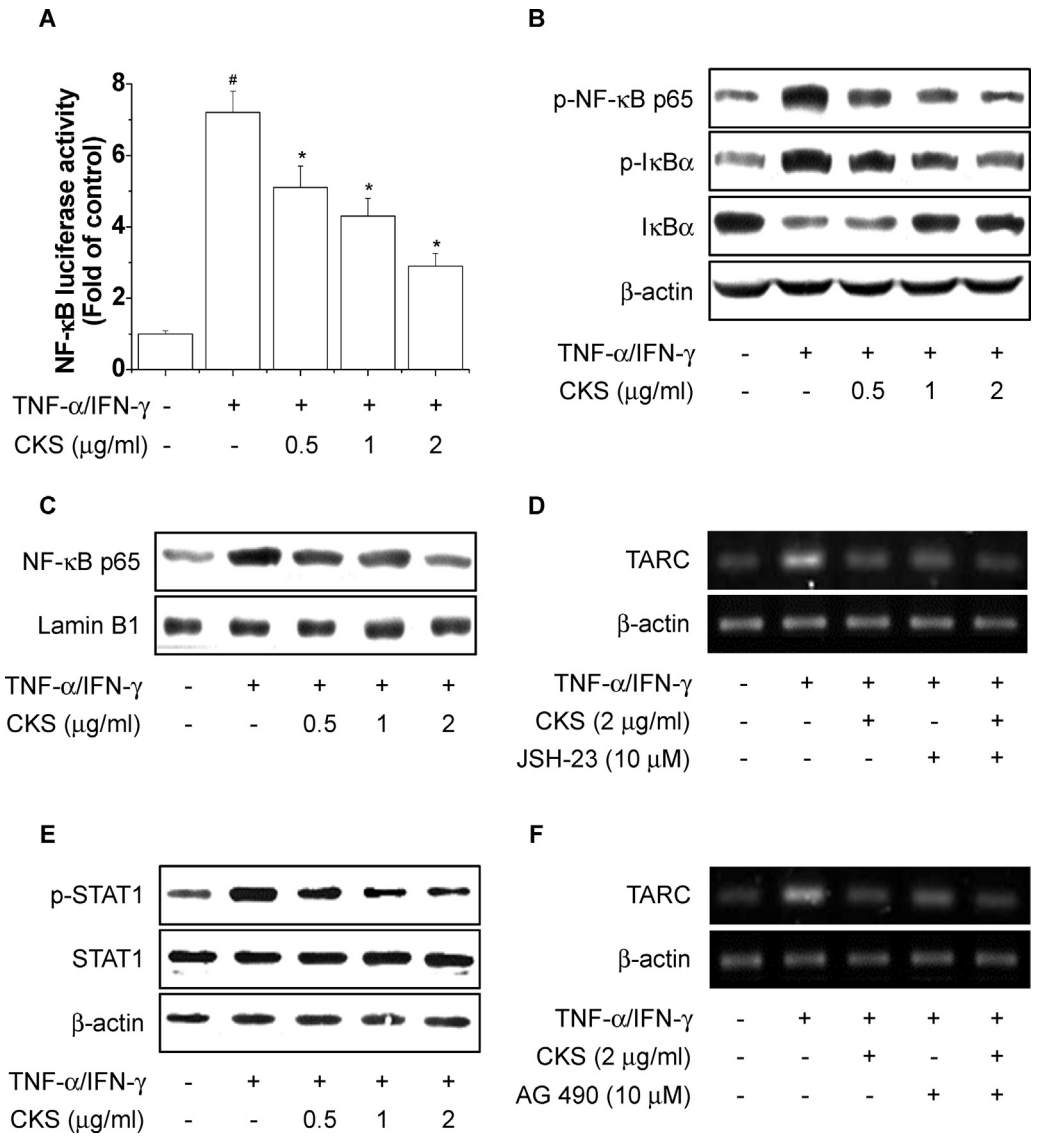

Fig. 4. Effects of CKS on TNF-α/IFN-γ-induced NF-κB and STAT1 activation in HaCaT cells. (A) NF-κB luciferase activity was normalized to *Renilla* activity and expressed as fold increase over the control. (B) Total protein was analyzed by western blotting using antibodies against phospho-NF-κB p65, phospho-IκBα, IκBα, and β-actin. (C) Nuclear protein was analyzed by western blotting using antibodies against NF-κB p65 and Lamin B1. (D) Total RNA was extracted from the cells and the TARC mRNA expression was determined by semi-quantitative RT-PCR. (E) Total protein was subjected to western blotting using antibodies against phospho-STAT1, total-STAT1, and β-actin. (F) Total RNA was extracted from the cells and the TARC mRNA expression was determined by semi-quantitative RT-PCR. Data are presented as means ± SD ($n = 3$) and analyzed with ANOVA. $^{\#}p < 0.01$, versus the control group. $^{*}p < 0.01$, versus the TNF-α/IFN-γ-treated group.

demonstrated the molecular mechanisms of CKS, mainly platycodin D, on AD-like skin symptoms both *in vivo* and *in vitro*. Our finding contributes to the understanding that dietary CKS, mainly platycodin D, treatment reduces the occurrence of AD-like skin symptoms, and this nutritional strategy represents alternatives to pharmaceutical approaches for reducing inflammatory skin disorders.

AD is a biphasic inflammatory skin disease provoked by an imbalance in Th1 and Th2 immune responses. The initial activation of Th2 cells during the acute phase followed by activation of Th1 cells accounts for the persistence of the inflammatory response in AD (Leung and Bieber, 2003). AD is a major allergic disease that results from skin inflammation and is characterized by erythema, edema, erosion, pruritus, and dryness. AD-like skin symptoms in NC/Nga mice are similar to human AD; thus, NC/Nga mice are used to investigate the mechanisms of AD development and for evaluating the effects of various drug candidates against AD (Jin et al., 2009). Consistent with this, we used NC/Nga mice to evaluate the inhibitory effects of CKS on the progression of AD-like skin symptoms by application of the chemical hapten, DNCB. CKS attenuated AD-like skin symptoms, such as AD severity and histopathological examination. One of the main characteristics of AD is an elevated serum IgE level. Elevated serum IgE is known to cause both acute and chronic AD-like skin symptoms, and repeated application with DNCB elevated serum IgE levels in NC/Nga mice. Various mediators, including inflammatory cytokines and leukocytes, are involved in the immunological and inflammatory processes of AD. The DNCB-mediated increases in serum IgE and Th1/Th2 cytokine expression were reduced by CKS treatment. Furthermore, we reported the inhibitory mechanism of CKS on IgE-mediated allergic response in mast cells (Han et al., 2009). These results suggested that CKS inhibited DNCB-induced development of AD-like skin symptoms by suppressing IgE-mediated mast cell activation and Th1/Th2 inflammatory cytokine release.

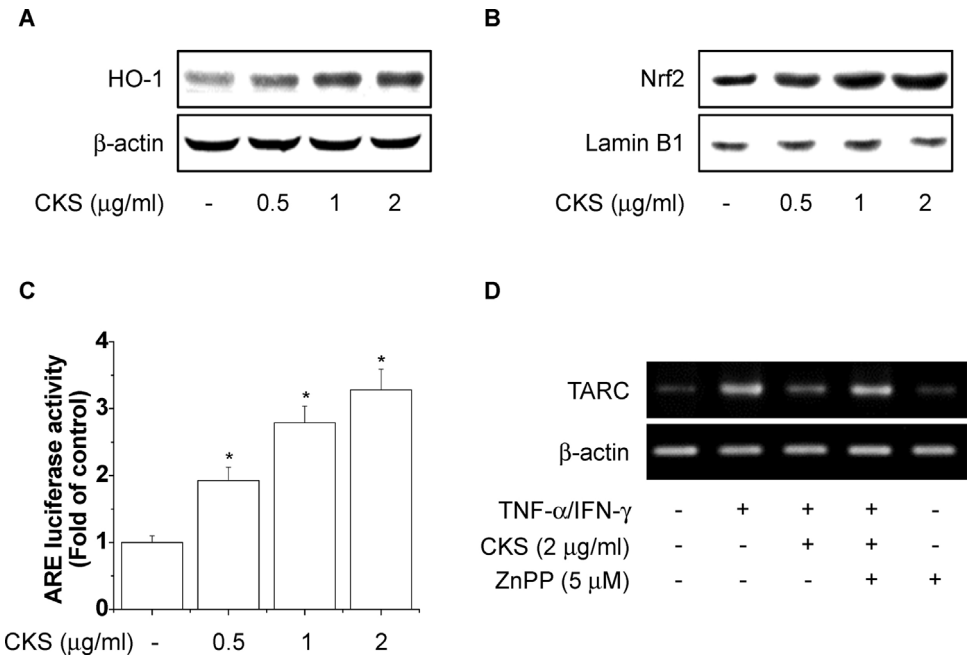

Fig. 5. Effect of CKS on Nrf2/ARE-mediated HO-1 activation in HaCaT cells. (A) Total protein was subjected to western blotting using antibodies against HO-1 and β-actin. (B) Nuclear protein was subjected to western blotting using antibodies against Nrf2 and Lamin B1. (C) HO-1/ARE luciferase activity was normalized to *Renilla* activity and expressed as fold increase over the control. (D) Total RNA was extracted from the cells and the TARC mRNA expression was determined by semi-quantitative RT-PCR. Data are presented as means ± SD ($n = 3$) and analyzed with ANOVA. $^{\#}p < 0.01$, versus the control group.

TARC is a chemokine involved in leukocyte trafficking to the skin. TARC is characteristically observed during the development of Th2-mediated AD. TARC attracts Th2 cells into the skin from circulating blood. TARC is selectively increased in AD patients in proportion to the severity of AD (Tamaki et al., 2006). Determination of serum TARC level is used as an indicator of AD severity. We showed that DNCB-induced serum TARC level was reduced by CKS treatment in mice. Also, treatment with CKS or platycodin D significantly inhibited TNF-α/IFN-γ-induced TARC expression in cells.

NF-κB and STAT1 are pivotal regulators of TNF-α/IFN-γ-induced inflammatory responses, which are involved in the transcriptional activation of responsive genes. TARC promoter region contains NF-κB- and STAT-binding sequences; thus, these transcription factors may mediate the transcription of TARC. NF-κB is a transcriptional regulator of TARC activation. TNF-α/IFN-γ-induced NF-κB activity is responsible for expression of pro-inflammatory cytokines. Upon pro-inflammatory cytokine stimulation, the IκBα protein is phosphorylated and leads to the ubiquitination and proteasomal degradation of IκB; NF-κB can then translocate into the nucleus, where it binds specific promoter regions of target genes and activates the expression of inflammatory cytokines. We show that TNF-α/IFN-γ-induced phosphorylation of NF-κB p65 and IκBα, degradation of IκBα, nuclear translocation of NF-κB p65, and NF-κB luciferase activity were all inhibited in cells by CKS or platycodin D treatment. These results suggest that CKS and platycodin D inhibited TARC expression via blockade of TNF-α/IFN-γ-induced NF-κB activity in cells.

STAT1 activation is regulated by activation of JAK. IFN-γ activates JAK1/2, which then phosphorylates STAT1 protein. Phosphorylated STAT1 protein is then translocated to the nucleus, where it activates TARC expression (Darnell et al., 1994). We examined the inhibitory effect of CKS or platycodin D on TNF-α/IFN-γ-induced TARC expression through the suppression of STAT1 activation in cells. TNF-α/IFN-γ-induced phosphorylation of STAT1 was inhibited in cells by CKS or platycodin D treatment. These results suggest that CKS or platycodin D inhibited TARC expression via blockade of TNF-α/IFN-γ-induced STAT1 activity in cells.

Various natural compounds can induce phase II detoxifying and antioxidant responsive genes. An important role of these antioxidant enzymes is to protect cells against the harmful effects of oxidative stress (Chen and Kong, 2004). Enhancement of the antioxidant defense system is an important strategy in the amelioration of AD symptoms. Upon stimulation, Nrf2 is translocated from the cytosol to the nucleus, where it subsequently binds to the ARE, resulting in a cytoprotective response characterized by up-regulation of antioxidant enzymes and decreased sensitivity to oxidative stress injury. Nrf2/ARE-mediated HO-1 expression was induced by plant-derived polyphenols (Balogun et al., 2003). Our previous study reported the Platycodi Radix root attenuated DMN-induced liver fibrosis through the activation of Nrf2-mediated antioxidant enzymes both *in vivo* and *in vitro* (Choi et al., 2013a). CKS or platycodin D increased Nrf2 nuclear translocation-activated ARE luciferase activity and HO-1 expression in cells. CKS or platycodin D promoted the transcription of key antioxidant genes by triggering the translocation of Nrf2 into the nucleus. These results suggest that CKS or platycodin D inhibited TARC expression via Nrf2/ARE-mediated HO-1 induction in cells.

In conclusion, our results revealed the anti-inflammatory actions of CKS or platycodin D on AD-like skin lesions in *in vivo* and *in vitro* systems. Treatment with CKS inhibited DNCB-induced AD-like skin symptoms, including infiltration of inflammatory cells and mast cells, serum level of IgE and TARC, mRNA expression of TARC, Th1 and Th2, and dermatitis severity in mice. CKS or platycodin D inhibited TNF-α/IFN-γ-induced TARC expression through the suppression of NF-κB and STAT1 and induction of Nrf2/ARE-mediated HO-1 expression in cells. Our findings indicate that CKS or platycodin D inhibited the development of AD-like skin symptoms by regulating Th1/Th2 cytokines and leukocytes and may be viable alternatives or complementary therapeutic drugs for the treatment of patients with AD.

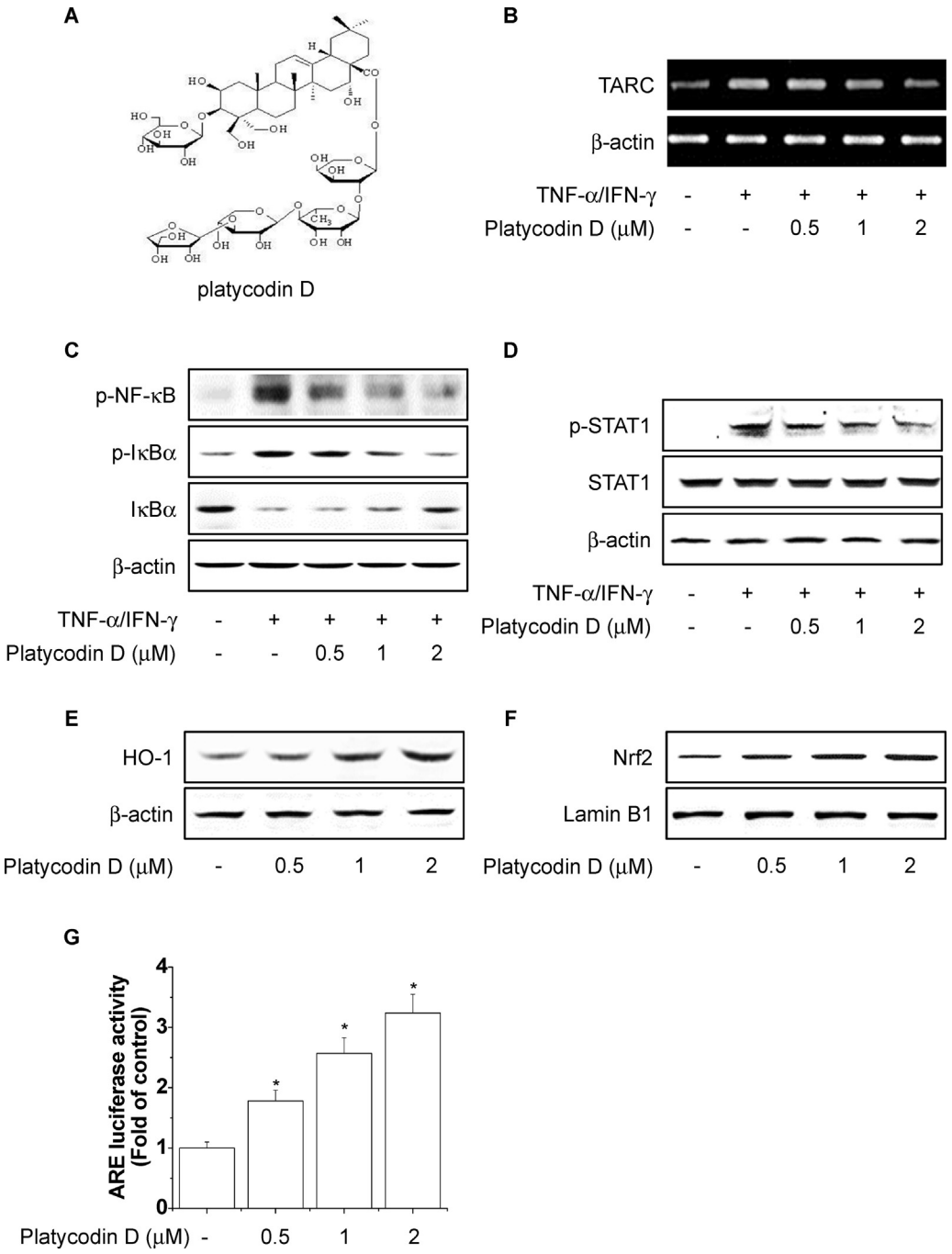

Fig. 6. Effects of platycodin D on TNF-α/IFN-γ-induced TARC expression through activation of Nrf2/ARE-mediated HO-1 expression and suppression of NF-κB and STAT1 activity in HaCaT cells. (A) Chemical structure of platycodin D. (B) Total RNA was extracted from the cells and the TARC mRNA expression was determined by semi-quantitative RT-PCR. (C) Total protein was subjected to western blotting using antibodies against phospho-NF-κB p65, phospho-IκBα, IκBα, and β-actin. (D) Total protein was subjected to western blotting using antibodies against phospho-STAT1, total-STAT1, and β-actin. (E) Total protein was subjected to western blotting using antibodies against HO-1 and β-actin. (F) Nuclear protein was subjected to western blotting using antibodies against Nrf2 and Lamin B1. (G) HO-1/ARE luciferase activity was normalized to *Renilla* activity and expressed as fold increase over the control. Data are presented as means ± SD ($n = 3$) and analyzed with ANOVA. $^{\#}p < 0.01$, versus the control group.

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References

Ahn, K.S., Noh, E.J., Zhao, H.L., Jung, S.H., Kang, S.S., Kim, Y.S., 2005. Inhibition of inducible nitric oxide synthase and cyclooxygenase II by *Platycodon grandiflorum* saponins via suppression of nuclear factor-kappaB activation in RAW 264.7 cells. Life Sci. 76, 2315–2328.

Amin, K., 2012. The role of mast cells in allergic inflammation. Respir. Med. 106, 9–14.

Aschoff, R., Schmitt, J., Knuschke, P., Koch, E., Bräutigam, M., Meurer, M., 2011. Evaluation of the atrophogenic potential of hydrocortisone 1% cream and

pimecrolimus 1% cream in uninvolved forehead skin of patients with atopic dermatitis using optical coherence tomography. Exp. Dermatol. 20, 832–836.
Balogun, E., Hoque, M., Gong, P., Killeen, E., Green, C.J., Foresti, R., Alam, J., Motterlini, R., 2003. Curcumin activates the haem oxygenase-1 gene via regulation of Nrf2 and the antioxidant-responsive element. Biochem. J. 371, 887–895.
Chen, C., Kong, A.N., 2004. Dietary chemopreventive compounds and ARE/EpRE signaling. Free Radic. Biol. Med. 36, 1505–1516.
Choi, J.H., Jin, S.W., Kim, H.G., Khanal, T., Hwang, Y.P., Lee, K.J., Choi, C.Y., Chung, Y.C., Lee, Y.C., Jeong, H.G., 2013a. Platycodi Radix attenuates dimethylnitrosamine-induced liver fibrosis in rats by inducing Nrf2-mediated antioxidant enzymes. Food Chem. Toxicol. 56, 231–239.
Choi, J.H., Jin, S.W., Park, B.H., Kim, H.G., Khanal, T., Han, H.J., Hwang, Y.P., Choi, J.M., Chung, Y.C., Hwang, S.K., Jeong, T.C., Jeong, H.G., 2013b. Cultivated ginseng inhibits 2,4-dinitrochlorobenzene-induced atopic dermatitis-like skin lesions in NC/Nga mice and TNF-α/IFN-γ-induced TARC activation in HaCaT cells. Food Chem. Toxicol. 56, 195–203.
Choi, J.H., Kim, H.G., Jin, S.W., Han, E.H., Khanal, T., Do, M.T., Hwang, Y.P., Choi, J.M., Chun, S.S., Chung, Y.C., Jeong, T.C., Jeong, H.G., 2013c. Topical application of *Pleurotus eryngii* extracts inhibits 2,4-dinitrochlorobenzene-induced atopic dermatitis in NC/Nga mice by the regulation of Th1/Th2 balance. Food Chem. Toxicol. 53, 38–45.
Darnell Jr., J.E., Kerr, I.M., Stark, G.R., 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 264, 1415–1421.
De Benedetto, A., Kubo, A., Beck, L.A., 2012. Skin barrier disruption: a requirement for allergen sensitization? J. Invest. Dermatol. 132, 949–963.
Dey, M., Ribnicky, D., Kurmukov, A.G., Raskin, I., 2006. In vitro and in vivo anti-inflammatory activity of a seed preparation containing phenethylisothiocyanate. J. Pharmacol. Exp. Ther. 317, 326–333.
Fusenig, N.E., Boukamp, P., 1998. Multiple stages and genetic alterations in immortalization, malignant transformation, and tumor progression of human skin keratinocytes. Mol. Carcinog. 23, 144–158.
Han, E.H., Park, J.H., Kim, J.Y., Chung, Y.C., Jeong, H.G., 2009. Inhibitory mechanism of saponins derived from roots of *Platycodon grandiflorum* on anaphylactic reaction and IgE-mediated allergic response in mast cells. Food Chem. Toxicol. 47, 1069–1075.
Han, N.R., Moon, P.D., Kim, H.M., Jeong, H.J., 2012. Effect of Pyeongwee-San (KMP6) on 2,4-dinitrofluorobenzene-induced atopic dermatitis-like skin lesions in NC/Nga mice. Life Sci. 90, 147–153.
Hwang, Y.P., Choi, J.H., Kim, H.G., Khanal, T., Song, G.Y., Nam, M.S., Lee, H.S., Chung, Y.C., Lee, Y.C., Jeong, H.G., 2013. Saponins, especially platycodin D, from *Platycodon grandiflorum* modulate hepatic lipogenesis in high-fat diet-fed rats and high glucose-exposed HepG2 cells. Toxicol. Appl. Pharmacol. 267, 174–183.
Jin, H., He, R., Oyoshi, M., Geha, R.S., 2009. Animal models of atopic dermatitis. J. Invest. Dermatol. 129, 31–40.
Kim, K.H., 2013. Overview of atopic dermatitis. Asia Pacific Allergy 3, 79–87.
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. (Tokyo) 41, 485–491.
Kim, J.Y., Kim, D.H., Kim, H.G., Song, G.Y., Chung, Y.C., Roh, S.H., Jeong, H.G., 2006. Inhibition of tumor necrosis factor-alpha-induced expression of adhesion molecules in human endothelial cells by the saponins derived from roots of *Platycodon grandiflorum*. Toxicol. Appl. Pharmacol. 210, 150–156.
Lee, K.J., Jeong, H.G., 2002. Protective effect of Platycodi radix on carbon tetrachloride-induced hepatotoxicity. Food Chem. Toxicol. 40, 517–525.
Leung, D.Y., Bieber, T., 2003. Atopic dermatitis. Lancet 361, 151–160.
Listopad, J., Asadullah, K., Sievers, C., Ritter, T., Meisel, C., Sabat, R., Döcke, W.D., 2007. Heme oxygenase-1 inhibits T cell-dependent skin inflammation and differentiation and function of antigen-presenting cells. Exp. Dermatol. 16, 661–670.
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.
Noh, J.R., Kim, Y.H., Gang, G.T., Yang, K.J., Kim, S.K., Ryu, S.Y., Kim, Y.S., Lee, C.H., Lee, H.S., 2010. Preventative effects of *Platycodon grandiflorum* treatment on hepatic steatosis in high fat diet-fed C57BL/6 mice. Biol. Pharm. Bull. 33, 450–454.
Ryu, C.S., Kim, C.H., Lee, S.Y., Lee, K.S., Choung, K.J., Song, G.Y., Kim, B.H., Ryu, S.Y., Lee, H.S., Kim, S.K., 2012. Evaluation of the total oxidant scavenging capacity of saponins isolated from *Platycodon grandiflorum*. Food Chem. 132, 333–337.
Saeki, H., Tamaki, K., 2006. Thymus and activation regulated chemokine (TARC)/CCL17 and skin diseases. J. Dermatol. Sci. 43, 75–84.
Tamaki, K., Kakinuma, T., Saeki, H., Horikawa, T., Kataoka, Y., Fujisawa, T., Sato, S., Takehara, K., Nakahara, T., Fukagawa, S., Furue, M., 2006. Serum levels of CCL17/TARC in various skin diseases. J. Dermatol. 33, 300–302.
Turner, M.J., Travers, J.B., Kaplan, M.H., 2012. T helper cell subsets in the development of atopic dermatitis. J. Drugs Dermatol. 11, 1174–1178.
Yang, G., Choi, C.H., Lee, K., Lee, M., Ham, I., Choi, H.Y., 2013. Effects of *Catalpa ovata* stem bark on atopic dermatitis-like skin lesions in NC/Nga mice. J. Ethnopharmacol. 145, 416–423.
Yoo, D.S., Choi, Y.H., Cha, M.R., Lee, B.H., Kim, S.J., Yon, G.H., Hong, K.S., Jang, Y.S., Lee, H.S., Kim, Y.S., Ryu, S.Y., Kang, J.S., 2011. HPLC-ELSD analysis of 18 platycosides from balloon flower roots (Platycodi Radix) sourced from various regions in Korea and geographical clustering of the cultivation areas. Food Chem. 129, 645–651.