A Thesis for the Degree of Master of Science

The essential role of JNK1 for the development of atopic dermatitis and the preventive effect of JNK1 inhibiting phytochemicals luteolin and licochalcone A

아토피 피부염 발병에서의 JNK1의 역할과 JNK1 억제능을 갖는 식물유래화합물의 효능

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August, 2013

Abstract

Atopic dermatitis (AD) is a common allergic disease, imposing large social and economic burdens worldwide. AD is characterized by eczematous skin lesions and immunoglobulin E (IgE) hypersecretion. I investigated the role of c-Jun N-terminal kinase 1 (JNK1) and the effect of JNK1-inhibiting phytochemicals on the development of AD in mice. The vitamin D3 analogue MC903 was used to induce AD in wild-type (WT) and JNK1−/− mice. The symptoms of AD were less severe in JNK1−/− mice compared to WT mice. JNK1−/− mice showed less ear thickening and infiltration of eosinophils and mast cells in AD-like lesions than did WT mice when treated with MC903. MC903-treated JNK1−/− mice also showed lower levels of serum IgE, which was significantly elevated in MC903-treated WT mice. Splenocytes isolated from MC903-treated WT and JNK1−/− mice were stimulated with anti-CD3 and anti-CD28 monoclonal antibodies. Splenocytes from JNK1−/− mice

produced lower levels of T-helper (Th)2 cytokines (interleukin-4 and -13) and transcription factor GATA-binding protein 3, and produced increased levels of the Th1 cytokines interferon-γ and transcription factor T-box expressed in T cells. The JNK1-inhibiting phytochemicals luteolin and licochalcone A prevented MC903-induced ear thickening and the increase in serum IgE, suggesting that these compounds could prevent the development of AD. Our results indicate that JNK1 plays an important role in the pathogenesis of AD and may be a useful target for food-based therapies to prevent AD.

Key Words: Atopic dermatitis; JNK1; luteolin; licochalcone A; ear swelling; IgE; IL-4; IL-13; IFN-γ; GATA-3; T-bet

Student ID: 2011-21323

Contents

I. Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by itchy eczematous skin lesions [1]. Although the underlying causes of AD are complex and incompletely understood, genetic predisposition, nutrition, and a combination of allergic and nonallergic factors appear to be important in determining disease expression [2]. Skin lesions associated with AD involve severe rash, edema, hemorrhage, and desquamation [3]. Pathological changes associated with AD include epidermal thickening and marked infiltration of inflammatory cells, such as eosinophils and mast cells [4].

At least two forms of AD have been delineated to date: an "extrinsic" form associated with immunoglobulin E (IgE)-mediated sensitization that affects 70–80% of patients, and an "intrinsic" form lacking IgE-mediated sensitization that affects 20–30% of patients [1, 5]. In extrinsic AD, memory T cells expressing the skin homing receptor cutaneous lymphocyte-associated antigen (CLA)

produce increased levels of T-helper 2 (Th2) cell cytokines, including interleukin (IL)-4 and IL-13, which are known to induce isotype switching to IgE synthesis, and IL-5, which plays an important role in eosinophil development and survival [1]. These CLA^+ T cells also produce abnormally low levels of interferon (IFN)-γ, a Th1 cytokine known to inhibit Th2 cell function [1]. Production of IL-4 and IL-13 is lower in intrinsic than extrinsic AD [1].

Intracellular signaling via mitogen-activated protein kinases (MAPKs) has been implicated in a variety of cellular events, including the activation and differentiation of T helper cells [6]. Several studies have shown that c-Jun N-terminal kinase (JNK), also known as stress-activated protein kinase, phosphorylates the transcription factor c-Jun and increases activator protein-1 (AP-1) transcription activity [7-9]. Signals from both the T-cell receptor-CD3 complex and the costimulatory factor CD28 are required for JNK and AP-1 activation in T cells. These signals may be integrated to mediate T-cell activation [7, 10]. AP-1 reportedly plays an

important role in regulating cytokine genes in Th1 and Th2 cells [7, 11, 12]. Dong et al. generated JNK1-deficient mice and found that they exhibit deficiencies in Th differentiation characterized by an exaggerated Th2 response [7]. The mechanism by which JNK1 regulates the development of AD has not been elucidated.

Phytochemicals have many biological and medicinal properties, and demonstrate antioxidative [13], anti-inflammatory [14], antimicrobial [15, 16], anticancer [17], and antidiarrheal [18] activities upon interaction with MAPKs associated with several signaling pathways [19]. Our research group previously found that the phytochemicals luteolin and licochalcone A inhibit JNK1 (unpublished data). However, how luteolin- and licochalcone A-mediated inhibition of JNK1 affects the development of AD has yet to be reported.

Ⅱ. Materials and Methods

2.1. Animals

Female wild-type (WT) and JNK1−/− C57BL/6J mice (12-15 weeks old) were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed in individual ventilated cages under specific pathogen-free conditions at 22 ± 2°C with a 12-hour light-dark cycle. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Seoul National University, Seoul, Korea.

2.2. Topical application

To induce AD-like skin lesions in mice, 1 nmol MC903 (Sigma, St. Louis, MO, USA) in ethanol was applied topically to both ears daily for 14 days. To investigate the effect of

phytochemicals on the development of AD, mice were given topical applications of 4 nmol of either luteolin (Indofine Chemical Co., Hillsborough, NJ, USA) or licochalcone A (Santa Cruz Biotechnology, Dallas, TX, USA) in 200 l acetone on the left ear 2 hours before MC903 application.

2.3. Blood and tissue sampling

Blood samples were collected from anesthetized mice by cardiac puncture on the last day of the experiment and were stored at −80°C until use. After blood collection, the mice were sacrificed and the ears were excised for histologic evaluation. The spleen was aseptically removed to evaluate cytokine and transcription factor production by splenocytes.

2.4. Histological examination

The ear tissues from WT and JNK1−/− mice were fixed with 4% paraformaldehyde, embedded in paraffin, and 4-μm-thick

sections were cut and transferred onto slides. Deparaffinized skin sections were stained with hematoxylin-eosin, toluidine blue, and Congo red. Tissue sections were examined using an Olympus AX70 microscope (Olympus, Tokyo, Japan) to assess histological changes and to count the number of eosinophils and mast cells. Stained cells were counted at 400× magnification.

2.5. Measurement of ear thickness

After MC903 treatment, the ears of mice became swollen, so we could confirm the severity of AD was evaluated by measuring the ear thickness. The measurements were performed by an investigator who was blinded to the experimental conditions. During the 14-day MC903 treatment, ear thickness was measured and recorded three times per week using a vernier caliper (Mitutoyo, Kanagawa, Japan).

2.6. Determination of total serum IgE

The total amount of IgE in the serum was measured using

an enzyme-linked immunosorbent assay (ELISA) kit (Shibayagi, Gunma, Japan) according to the manufacturer's instructions.

2.7. Th1 and Th2 cytokine production in splenocytes

The spleen was aseptically removed from the animals. Red blood cells (RBC) were lysed using RBC lysis buffer (Quiagen, Hilden, Germany), and the resulting lysate was centrifugated at 1500 rcf for 10 minutes at a temperature of 4°C. The prepared splenocytes (5×10^6 cells/ml) in RPMI1640 (Welgene, Daegu, Korea) with 10% FBS media were stimulated with plate-bound anti-CD3 (1 μg/ml) and anti-CD28 (3 μg/ml) monoclonal antibodies (mAbs) (BD bioscience, San Jose, CA, USA) at 37°C in 5% CO_2 for 48 hours, at which time the culture supernatant was collected and stored at −80°C until use. The levels of IL-4, IL-13, and IFN-γ produced by the splenocytes were determined using a multiplex

MCYTOMAG-70K assay (Millipore, Billerica, MA, USA), according to the manufacturer's instructions. The levels of GATA-binding protein 3 (GATA-3) and T-box expressed in T cells (T-bet) were determined using Western blot analysis.

2.8. Western blot analysis

Western blotting was performed as described previously [20]. The protein concentration of each sample was determined with a dye-binding protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA), as described in the manufacturer's manual. Cell lysates were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the separated proteins were transferred to a polyvinylidene difluoride membrane (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The membrane was blocked with 5% skim milk and incubated with specific primary antibodies against GATA-3 and T-bet (Abcam, Cambridge, UK) diluted 1000 times in TBST, followed by

incubation with horseradish peroxidase-conjugated secondary antibodies. Protein bands were visualized using a chemiluminescence detection kit (Amersham Pharmacia Biotech).

2.9. Statistical analysis

Data are expressed as the mean and standard error of the mean (SEM), and the significance of differences was determined using the Student's t test. Probability values of $p < 0.05$, 0.01, and 0.001 were used as criteria for statistical significance.

Ⅲ. Results

3.1. The severity of MC903-induced AD-like symptoms was lower in JNK1−/− mice

To investigate the role of JNK1 on AD-like symptoms, JNK1−/− and WT mice were treated daily with the vitamin D3 analogue calcipotriol (MC903) by topical application to the ear for 2 weeks to induce AD-like symptoms. Ethanol was used as vehicle control. After 2 weeks of treatment, the ears of MC903-treated WT mice became dry, scaly, red, swollen, and lichenified (Fig. 1A). In contrast, the ears of MC903-treated JNK1−/− mice showed less severe AD-like symptoms. I found no marked differences in ear histology between the vehicle-treated JNK1−/− and vehicle-treated WT mice.

The ear thickness of MC903- and vehicle-treated JNK1−/− and WT mice was also measured over the course of the 2-week treatment period. The ears of MC903-treated WT mice were

significantly thicker (0.37 ± 0.01 mm) than those of vehicle-treated WT mice (0.24 mm) (Fig. 1B). In contrast, the ears of MC903-treated JNK1−/− mice were thinner (0.32 ± 0.01 mm) than those of MC903-treated WT mice (0.37 mm). I found no significant difference in ear thickness between vehicle-treated JNK1−/− mice (0.23 ± 0.01 mm) and vehicle-treated WT mice (0.24 mm). Overall, MC903-induced AD-like symptoms were suppressed in JNK1−/− mice, suggesting that JNK1 plays an important role in the development of AD symptoms.

3.2. MC903-induced infiltration of eosinophils and mast cells in AD-like skin lesions was reduced in JNK1−/− mice

To investigate whether the infiltration of eosinophils and mast cells into AD-like skin lesions is modulated in JNK1−/− mice, tissue sections were stained with Congo red and toluidine blue to discriminate eosinophils and mast cells, respectively. No difference

was observed with respect to the number of eosinophils in the skin of the ears of vehicle-treated WT (0.43 ± 0.03 cells per mm^2) and vehicle-treated JNK1−/− (0.29 ± 0.28 cells per mm^2) mice. Repeated MC903 administration over the course of 14 days induced significant infiltration of eosinophils into the AD-like skin lesions of WT mice (23.50 ± 1.85 cells per mm^2). However, the number of eosinophils that infiltrated into the AD-like skin lesions was significantly lower in JNK1−/− mice (10.00 ± 1.32 cells per mm^2) than WT mice (23.50 ± 1.85 cells per mm^2) (Fig. 2A and B).

No significant difference was found with respect to the number of mast cells in the skin of the ears of vehicle-treated WT (9.00 ± 1.07 cells per mm^2) and vehicle-treated JNK1−/− mice (7.86 ± 0.67 cells per mm^2). Repeated MC903 administration for 14 days induced infiltration of mast cells into AD-like skin lesions (19.70 ± 2.54 cells per mm^2) in WT mice. The number of mast cells that infiltrated into the lesions was significantly lower in JNK1−/− mice (9.00 ± 1.12 cells per mm^2) than in WT mice (19.70 ± 2.54 cells per mm^2) (Fig. 2A and C). Overall, MC903-induced infiltration of

eosinophils and mast cells into AD-like skin lesions was suppressed in JNK1−/− mice, suggesting that JNK1 plays an important role in recruiting immune cells into the lesion.

3.3. The level of serum IgE was reduced in JNK1-/- mice

Elevation in the serum IgE level is a hallmark of AD [21, 22]; therefore, I examined whether JNK1 is associated with elevation of serum IgE in MC903-treated mice. The serum concentration of IgE in MC903-treated WT mice was 4372 ± 790 ng/ml (Fig. 3), whereas that of MC903-treated JNK1−/− mice was significantly lower (483 ± 68 ng/ml), suggesting that JNK1 is involved in the elevation of serum IgE in AD.

3.4. The level of cytokines IL-4, IL-13, and IFN-γ were altered in JNK1−/− splenocytes

AD is caused by an imbalance in Th1 and Th2 responses [23]. To

examine the role of JNK1 in the production of Th1 and Th2 cytokines, splenocytes derived from MC903-treated WT and JNK1−/− mice were stimulated with anti-CD3 and anti-CD28 mAbs. An ELISA specific for IL-4, IL-13, and IFN-γ was performed using splenocyte supernatants. As shown in Fig. 4A and B, splenocytes derived from JNK1−/− mice produced lower levels of the Th2 cytokines IL-4 (883 ± 163 pg/ml) and IL-13 (54 ± 8 pg/ml) than splenocytes derived from WT mice (IL-4, 1625 ± 253 pg/ml; IL-13, 86 ± 27 pg/ml). In contrast, splenocytes derived from JNK1−/− mice produced a higher level of the Th1 cytokine IFN-γ (8953 ± 138 pg/ml) than those from WT mice (7768 ± 405 pg/ml) (Fig. 4C).

3.5. Levels of the immunomodulatory transcription factors GATA-3 and T-bet were altered in JNK1−/− splenocytes

The level of the immunomodulatory transcription factor GATA-3, which is selectively expressed in Th2 cells [5], was 0.3-

fold lower in splenocytes from JNK1−/− mice compared to those from WT mice (Fig. 5A and B). The level of T-bet, which is selectively expressed in Th1 cells [24], was 6.7-fold higher in splenocytes from JNK1−/− mice compared to those from WT mice (Fig. 5A and C). These data indicate that JNK1 plays an important role in regulating the levels of transcription factors that balance Th1 and Th2 cytokine production.

3.6. The JNK1-inhibiting phytochemicals luteolin and licochalcone A prevented development of AD-like symptoms

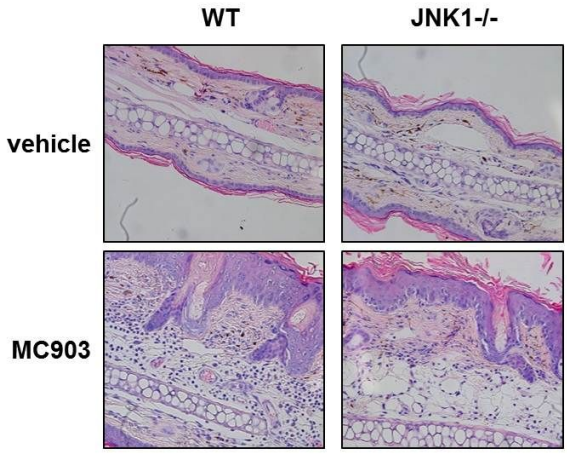
I previously found that the phytochemicals luteolin and licochalcone A inhibit JNK1 (unpublished results). To determine whether JNK1-inhibiting phytochemicals prevent development of AD, luteolin (4 nmol) or licochalcone A (4 nmol) were applied topically to the ears of mice 2 hours before each MC903 treatment (1 nmol) for 2 weeks. The baseline ear thickness was 0.21-0.22 mm.

Ear thickness determined on Day 14 was significantly lower in mice treated with luteolin (0.44 ± 0.01 mm) or licochalcone A (0.42 ± 0.02 mm) compared to untreated controls (0.49 ± 0.02 mm) (Fig. 6A).

The effect of luteolin and licochalcone A on serum IgE in MC903-treated mice was also examined. The serum IgE level in luteolin-treated (4288 ± 940 ng/ml) and licochalcone A-treated (4052 ± 1248 ng/ml) mice was significantly lower than in control mice (9442 ± 1570 ng/ml) (Fig. 6B). These results suggest that inhibition of JNK1 may prevent development of AD.

Figure 1

A


WT
JNK1-/-
vehicle
MC903

B

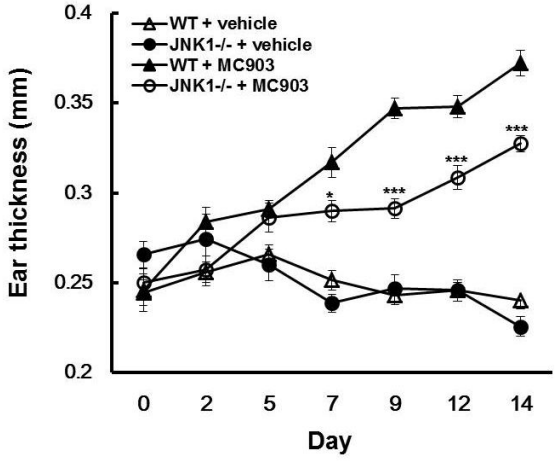

WT + vehicle
JNK1-/- + vehicle
WT + MC903
JNK1-/- + MC903
Ear thickness (mm)
0.4
0.35
0.3
0.25
0.2
0
2
5
7
9
12
14
Day
*
***
***
***

Figure 1. MC903-induced AD-like symptoms in WT and JNK−/− mice.

A. Hematoxylin and eosin staining of ear skin of WT and JNK1−/− mice treated with vehicle (ethanol) or MC903 (1 nmol) for 2 weeks (×400). **B.** Ear thickness of WT and JNK1−/− mice treated with vehicle (ethanol) or MC903 (1 nmol) measured every other day for 2 weeks. Data are the means ± SEM (n = 7 or 8). *, $p < 0.05$ and ***, $p < 0.001$, MC903-treated WT vs. JNK1−/− mice.

Figure 2

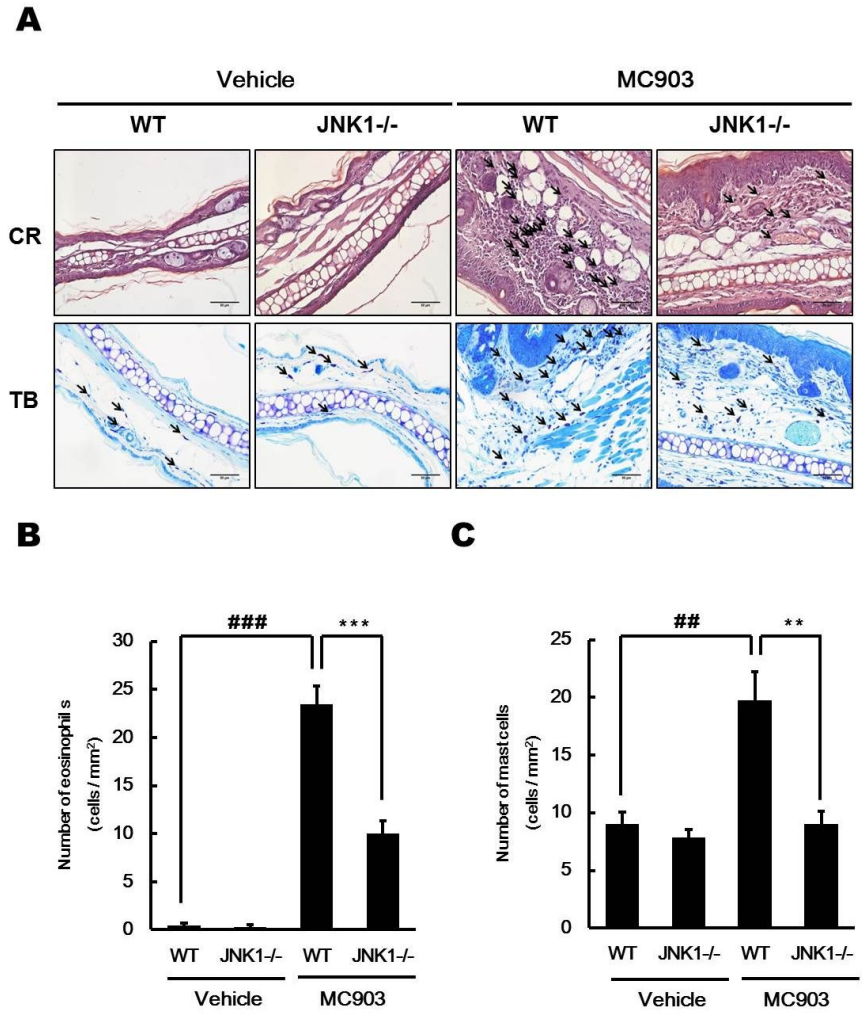

A
Vehicle
MC903
WT
JNK1-/-
WT
JNK1-/-
CR
TB
B
###
***
Number of eosinophils (cells / mm²)
30
25
20
15
10
5
0
WT
JNK1-/-
WT
JNK1-/-
Vehicle
MC903
C
##
**
Number of mast cells (cells / mm²)
25
20
15
10
5
0
WT
JNK1-/-
WT
JNK1-/-
Vehicle
MC903

Figure 2. The number of eosinophils and mast cells in ear skin of WT and JNK1−/− mice.

A. Representative histological analysis of sections of ear skin from WT and JNK1−/− mice treated with vehicle or MC903 (1 nmol) (×400). Arrows indicate eosinophils (upper, Congo red (CR) staining) and mast cells (lower, toluidine blue (TB) staining). **B and C.** Number of eosinophils (B) and mast cells (C) per 0.025 mm^2 of ear skin from WT and JNK1−/− mice treated with vehicle or MC903 (1 nmol). Data are the means ± SEM (n = 7 or 8). ##, $p < 0.01$, ###, $p < 0.001$, vehicle- vs. MC903-treated WT mice. **, $p < 0.01$, ***, $p < 0.001$, MC903-treated WT vs. JNK1−/− mice.

Figure 3

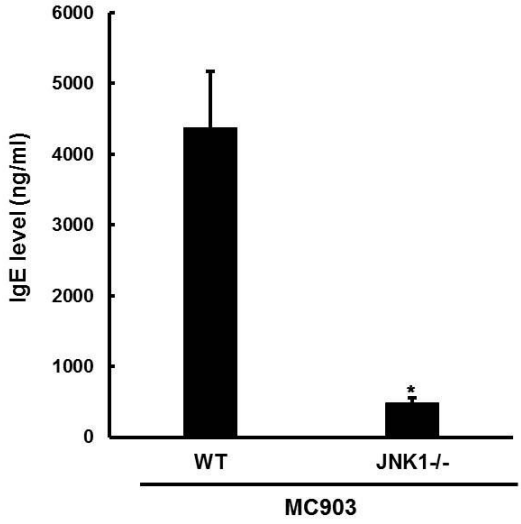
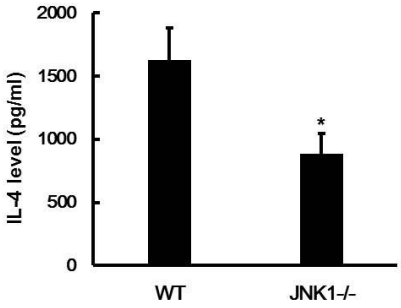


Figure 3. Serum IgE level in MC903-treated WT and JNK1−/− mice.

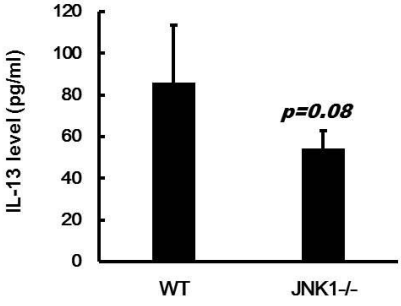
The level of serum IgE was measured using an ELISA. Blood was collected from WT and JNK1−/− mice after 14 days of treatment with MC903 (1 nmol). Data are the means ± SEM (n = 4). *, $p < 0.05$, MC903-treated WT vs. JNK1−/− mice.

Figure 4

A


2000
1500
1000
500
0
IL-4 level (pg/ml)
*
WT
JNK1-/-

B


120
100
80
60
40
20
0
IL-13 level (pg/ml)
p=0.08
WT
JNK1-/-

C

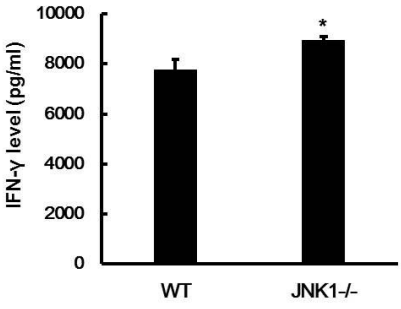

10000
8000
6000
4000
2000
0
IFN-γ level (pg/ml)
*
WT
JNK1-/-

Figure 4. IL-4, IL-13, and IFN-γ levels in splenocytes derived from MC903-treated WT and JNK1−/− mice.

Splenocytes were collected from WT and JNK1−/− mice after 14 days of treatment with MC903 (1 nmol) and stimulated with anti-CD3 and anti-CD28 mAbs for 48 hours. The levels of **(A)** IL-4, **(B)** IL-13, and **(C)** IFN-γ were measured using a multiplex assay. Data are the means ± SEM (n = 5). *, $p < 0.05$, MC903-treated WT vs. JNK1−/− mice.

Figure 5

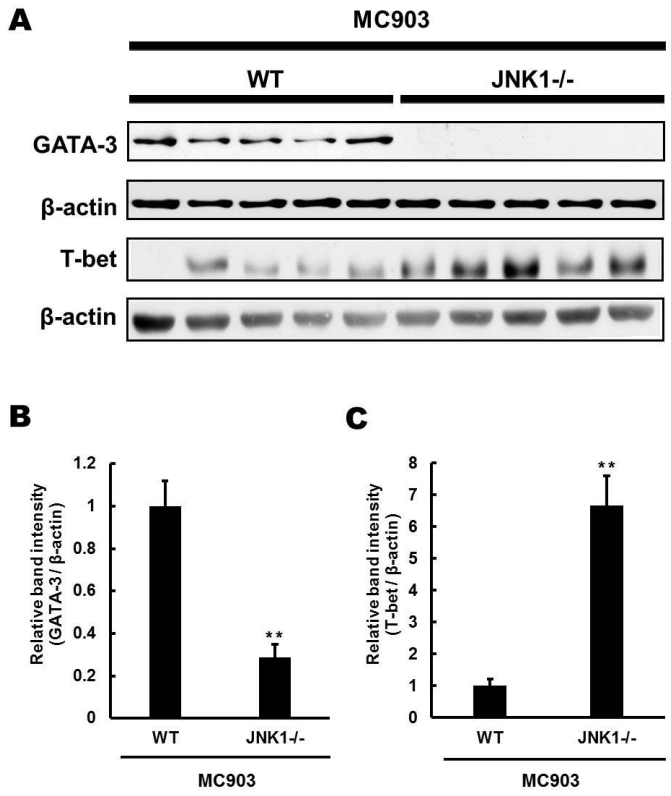
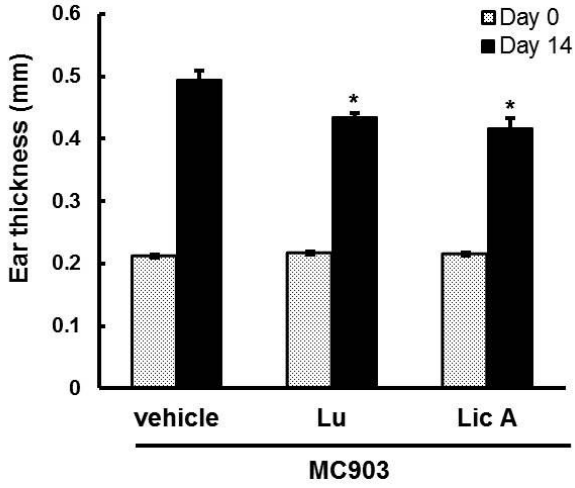

A
MC903
WT
JNK1-/-
GATA-3
β-actin
T-bet
β-actin
B
Relative band intensity (GATA-3 / β-actin)
1.2
1
0.8
0.6
0.4
0.2
0
**
WT
JNK1-/-
MC903
C
Relative band intensity (T-bet / β-actin)
8
7
6
5
4
3
2
1
0
**
WT
JNK1-/-
MC903

Figure 5. Levels of GATA-3 and T-bet in splenocytes derived from MC903-treated WT and JNK1−/− mice.

Splenocytes were collected from WT and JNK1−/− mice after 14 days of treatment with MC903 (1 nmol) and stimulated with anti-CD3 and anti-CD28 mAbs for 48 hours. **A.** The levels of GATA-3, T-bet, and β-actin were determined with Western blotting. **B and C.** The ratio of GATA-3 (B) and T-bet (C) to β-actin was determined with densitometry and normalized to the levels in WT mice. Data are the means ± SEM (n = 5). **, $p < 0.01$, MC903-treated WT vs. JNK1−/− mice.

Figure 6

A


Day 0
Day 14
Ear thickness (mm)
0.6
0.5
0.4
0.3
0.2
0.1
0
*
*
vehicle
Lu
Lic A
MC903

B

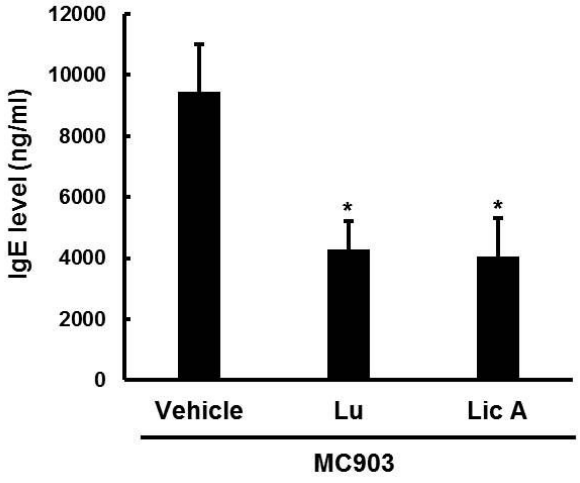

IgE level (ng/ml)
12000
10000
8000
6000
4000
2000
0
*
*
Vehicle
Lu
Lic A
MC903

Figure 6. Effects of the JNK1-inhibiting phytochemicals luteolin and licochalcone A on AD-like symptoms in MC903-treated mice.

A. Ear thickness of MC903-treated mice measured before and after treatment with luteolin and licochalcone A. Luteolin (4 nmol) or licochalcone A (4 nmol) was applied topically to the ear 2 hours before MC903 (1 nmol). **B.** The level of serum IgE was measured using an ELISA. Blood was collected from mice after 14 days of treatment with MC903 alone, luteolin and MC903, or licochalcone A and MC903. Vehicle: acetone, Lu: luteolin, Lic A: licochalcone A. Data are the means ± SEM (n = 5). *, $p < 0.05$, vehicle vs. luteolin or vehicle vs. licochalcone A.

Ⅳ. Discussion

Dong et al. implicated JNK signaling pathways in immune responses mediated by the activation and differentiation of CD4 Th cells into Th1 and Th2 effector cells, and reported that JNK1−/− T cells hyperproliferate, exhibit decreased activation-induced cell death, and preferentially differentiate into Th2 cells [7]. However, the role of JNK1 in the pathogenesis of AD has not been determined. Li et al. used a mouse model of AD induced by the vitamin D3 derivative MC903 to characterize its pathogenesis from onset to fully established disease phenotype. Their model is highly useful for preclinical studies of potential AD therapies [25]. I used this model to investigate the role of JNK1 in the pathogenesis of AD and to evaluate the effect of JNK1-inhibiting phytochemicals on AD development.

The systemic balance between Th1 and Th2 responses is critical for proper cellular and humoral immune function, and is normally maintained by immunoregulatory cytokines [26].

Imbalances in Th1 and Th2 responses often cause pathological conditions [27]. For example, an excess number of Th1 cells is associated with autoimmunity, but an excess number of Th2 cells is associated with hypersensitivity or allergy [28]. Compared to normal skin or uninvolved skin, acute skin lesions associated with AD have a significantly greater number of cells expressing IL-4, IL-5, and IL-13 mRNAs, but fewer cells expressing IFN-γ or IL-12 mRNAs [1]. I found that splenocytic production of the Th2 cytokines IL-4 and IL-13 was lower in MC903-treated JNK1−/− mice than WT mice, but production of the Th1 cytokine IFN-γ was higher (Fig. 4), suggesting that JNK1 is involved in IL-4 and IL-13 production and in maintaining a low IFN-γ level in AD.

The regulatory mechanisms of Th1 and Th2 cell polarization have been studied extensively. One of the most important factors that influence differentiation of Th cells is the transcription factor [29]. Some transcription factors are expressed selectively in Th1 or Th2 cells, and thus are identified as Th1 or

Th2 specific [30]. These transcription factors regulate the expression of Th1- or Th2-specific cytokine genes [5, 24]. For example, GATA-3 is selectively expressed in Th2 cells and activates transcription of the genes encoding IL-4 and IL-13 [28, 31, 32]. In contrast, T-bet is selectively expressed in Th1 cells and activates transcription of the IFN-γ gene [33]. Our study showed that GATA-3 expression was downregulated and T-bet expression was upregulated in MC903-treated JNK1−/− mice compared to WT mice (Fig. 5), suggesting that JNK1 plays an important role in regulating GATA-3 and T-bet levels in AD.

One of the main characteristics of AD in humans is elevated serum IgE [34, 35]. Topical application of MC903 reportedly increases serum IgE levels [25]. I found that the serum IgE level was significantly lower in MC903-treated JNK1−/− mice than WT mice (Fig. 3). The Th2 cytokines, particularly IL-4 and IL-13, regulate IgE synthesis by B cells [1], suggesting that JNK1 is

involved in increasing serum IgE by stimulating IL-4 and IL-13 production.

In the skin, Th2 cytokines induce proliferation and activation of eosinophils [36]. Eosinophils defend against parasites and mediate allergic reactions, and are thus found in large numbers in many AD patients [10, 37]. I observed extensive eosinophil infiltration in affected tissues of MC903-treated WT mice but not in MC903-treated JNK1−/− mice (Fig. 2A and B). Mast cells can be activated by allergen-sensitized IgE [38]. Mast cells are key effectors in IgE-mediated allergic disorders and are activated by cross-linking with a high affinity IgE receptor [39]. An appropriate animal model of AD should thus involve infiltration of mast cells into the epidermis and dermis [40]. I found that the number of mast cells in AD-like skin lesions was significantly reduced in MC903-treated JNK1−/− mice compared to WT mice (Fig. 2A and C). Our results suggest that JNK1 plays important roles in recruiting

eosinophils to AD lesions and in inducing mast cell activation and histamine release in AD.

Luteolin (2-[3,4-dihydroxyphenyl]-5,7-dihydroxy-4-chromenone) is commonly found in fruits and vegetables, such as chili, perilla, onion, celery, and broccoli [41], and reportedly has antioxidant [42, 43], anticancer [44, 45], and antiallergic [46] activities. Licochalcone A (4′,4-dihydroxy-3-α,α-dimethylallyl-6-methoxychalcone) is a major phenolic constituent of the *Glycyrrhiza* plant, the root of which is commonly called licorice [47]. Licochalcone A has anti-inflammatory [48, 49], antiparasitic [50], anticancer [51, 52], and antihyperglycemic [53] activities. Previously, I found that the phytochemicals luteolin and licochalcone A inhibit JNK1 (unpublished results). Here, I found that luteolin and licochalcone A inhibited both MC903-induced ear thickening and increased serum IgE levels associated with AD (Fig. 6), suggesting that JNK1-inhibiting phytochemicals would be effective anti-AD therapeutic agents.

In conclusion, I found that MC903-induced AD-like symptoms are less severe in JNK1−/− mice compared to WT mice, and that treatment with JNK1-inhibiting phytochemicals prevents development of MC903-induced AD-like symptoms. In contrast to Dong et al., who reported that Th2 cytokine production is enhanced in JNK1−/− mice [7], our results clearly suggest that JNK1 plays an important role in the pathogenesis of AD including inhibition of Th2 cytokines production in JNK1-/- mice and that inhibition of the JNK1 signaling pathway may be helpful in treating AD. I therefore propose that new therapeutic strategies to treat AD be developed that target JNK1.

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VI. 초록

아토피피부염은 습진성 피부 병변과 혈액 내 높은 이뮤노글로블린 수치를 갖게하는 만성 염증성 피부 질환이다. 최근 알레르기 질환의 급증으로 전세계적으로 매년 아토피피부염 환자가 증가하고 있어, 경제적, 사회적으로도 큰 문제가 되고 있다. 본 연구는 인산화 효소의 하나인 JNK1의 아토피 피부염 발병에 있어서의 기능과 JNK1 억제 효능을 가지는 천연식물 유래 물질의 항아토피피부염 효능에 대해 규명하였다. 정상생쥐와 JNK1 유전자가 소실된 생쥐의 귀에 비타민 디 유도체인 MC903을 발라 아토피를 유도하였다. 아토피의 증상으로서 귀 두께의 증가 정도를 측정하였고, 면역세포의 침윤, 혈중 IgE, Th1과 Th2 세포 관련 싸이토카인의 양을 측정하였다. 또한, T세포 전사인자인 GATA-3와 T-bet의 단백질 양을 확인하였다. 그 결과 정상생쥐에 비해

JNK1이 소실된 생쥐에서 여러 아토피피부염 관련 증상들이 억제됨을 확인하였고 JNK1이 아토피피부염 발병에 있어 중요한 역할을 한다는 결론을 얻었다. 더 나아가 천연식물 유래 물질인 루테올린과 리코찰콘에이를 처리 후 아토피피부염을 유도한 결과 물질 처리군에서 귀 두께가 덜 증가되고, 혈중 IgE양이 억제됨을 관찰하여, 이 두 물질이 모두 아토피피부염 예방 효능이 있음을 확인하였다. 본 연구 결과, JNK1은 아토피피부염 발병에 있어 중요한 역할을 하고 있으며, 이를 예방하기 위한 식의약품을 개발하는 것에 있어 유용한 타겟이 될 가능성을 보여 주었다.

주요어 : 아토피피부염; JNK1; 루테올린; 리코찰콘에이; IgE; 싸이토카인; GATA-3; T-bet;