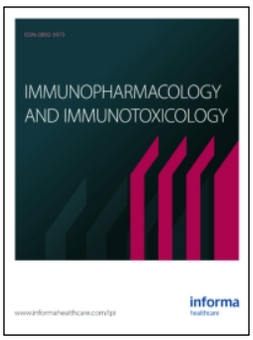

Immunopharmacology and Immunotoxicology

ISSN: 0892-3973 (Print) 1532-2513 (Online) Journal homepage: https://www.tandfonline.com/loi/iipi20

dl-Malic acid as a component of α-hydroxy acids: effect on 2,4-dinitrochlorobenzene-induced inflammation in atopic dermatitis-like skin lesions *in vitro* and *in vivo*

Bina Lee, Jun Heo, SooYeon Hong, Eun-Young Kim, Young Joo Sohn & Hyuk-Sang Jung

To cite this article: Bina Lee, Jun Heo, SooYeon Hong, Eun-Young Kim, Young Joo Sohn & Hyuk-Sang Jung (2019): dl-Malic acid as a component of α-hydroxy acids: effect on 2,4-dinitrochlorobenzene-induced inflammation in atopic dermatitis-like skin lesions *in vitro* and *in vivo*, Immunopharmacology and Immunotoxicology, DOI: 10.1080/08923973.2019.1680688

To link to this article: https://doi.org/10.1080/08923973.2019.1680688

Published online: 24 Oct 2019.

Submit your article to this journal

Article views: 3

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=iipi20

IMMUNOPHARMACOLOGY AND IMMUNOTOXICOLOGY
https://doi.org/10.1080/08923973.2019.1680688

Taylor & Francis
Taylor & Francis Group

Check for updates

ORIGINAL ARTICLE

DL-Malic acid as a component of α-hydroxy acids: effect on 2,4-dinitrochlorobenzene-induced inflammation in atopic dermatitis-like skin lesions *in vitro* and *in vivo*

Bina Lee*, Jun Heo*, SooYeon Hong, Eun-Young Kim, Young Joo Sohn and Hyuk-Sang Jung

Department of Anatomy, College of Korean Medicine, Kyung Hee University, Seoul, Republic of Korea

ABSTRACT

Background: dl-Malic acid (dl-M) is used widely in cosmetic formulations as a pH-adjuster or as a preservative. dl-M is used as an exfoliator in the form of α-hydroxy acids. However, the role of dl-M in skin diseases (including atopic dermatitis (AD)) has not been studied deeply. We wished to reveal the effect of dl-M on AD induced by 2,4-dinitrochlorobenzene (DNCB) in Balb/c mice.

Methods: The thickness and immune-cell infiltration into the dermis and epidermis were evaluated. Moreover, serum levels of cytokines, as well as expression of mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) in tissue were measured in AD mice. We also studied the effect of dl-M on inflammatory mediators in a human keratinocyte (HaCaT) cell line.

Results: The dl-M (high) group improved skin condition compared with the DNCB-treated group. The dl-M (high) group inhibited phosphorylation of MAPK and NF-κB in skin tissue. dl-M reduced serum levels of interleukin-4 and IgE. Finally, dl-M decreased the expression of thymus and activation-regulated chemokine, monocyte chemoattractant protein-1 and intercellular cell adhesion molecule induced by interferon-gamma/tumor necrosis factor-α in HaCaT cells.

Discussion: These results suggest that dl-M can improve the skin conditions of AD mice.

ARTICLE HISTORY
Received 23 May 2019
Accepted 12 October 2019

KEYWORDS
DL-Malic acid; atopic dermatitis; inflammation; cytokines; eosinophils

Introduction

Atopic dermatitis (AD) is an inflammatory skin condition characterized by intense itching and skin pain [1–3]. According to one report, AD patients had to pay US $442 as direct costs for treatment in 2013 [4]. Besides financial problems, AD can lead to reduced sleep duration, increased sleep disturbances, and fatigue, all of which affect daily activities [5]. Furthermore, suicidal ideation or anxiety among AD patients has been reported [6].

The mechanism of AD involves thickening of the dermis and epidermis, increased serum levels of immunoglobulin (Ig)E, and infiltration of immune cells (e.g. eosinophils, mast cells (MCs)) to skin [1,7–10]. Therapeutic strategies for AD have been based on glucocorticosteroids [11]. The latter have been shown to reduce serum levels of IgE in an AD model in Balb/c mice using 2,4 dinitrochlorobenzene (DNCB) [12]. Glucocorticosteroids have also been shown to reduce epidermis thickness in a DNCB-induced AD model using Nc/Nga mice [13]. However, long-term use or overuse of corticosteroids can cause severe adverse effects in AD patients, such as skin atrophy, flushing, hypertrichosis, and capillary dilatation [14]. Thus, the discovery of alternative therapeutic agents/compounds that reduce the inflammation associated with AD with fewer side effects is crucial. DL-Malic acid (DL-M) is used widely in cosmetic formulations as a pH-adjuster or preservative [15]. DL-M or L-malic acid are synthesized chemically at strong pressure and heat through hydration of maleic acid or fumaric acid [16,17]. L-Malic acid is found in fruits such as apples [18], peach cultivars [19], and *Crataegus pinnatifida* [20]. L-Malic acid has an acidic taste [21]. DL-M is also used as an exfoliator in the form of α-hydroxy acids (AHAs) [22]. The latter are a class of chemical compounds comprising a carboxylic acid substituted with a hydroxyl group on the adjacent carbon. AHAs are topical agents used for cosmetic purposes [23]. AHAs are composed of glycolic acid, lactic acid, malic acid and tartaric acid, and are known to be nontoxic [22,23]. DL-M and L-malic acid are considered to have the same function as food acidulants [24], but thorough understanding of these two materials is lacking. One study showed that a complex of malic acid, citric acid and succinic acid had antithrombotic and antiplatelet activities [25]. Citric acid and succinic acid have been reported to be used against skin diseases [26,27]. Moreover, the effect of lactic acid (another component of AHAs) on skin injury due to AD has been demonstrated [28]. However, studies of DL-M against skin diseases (including AD) are lacking.

Materials and methods

Reagents

Female BALB/c mice were supplied from the Animal Center of NARA Biotech (Seoul, Korea). DL-M ($HO_2CCH_2CH(OH)CO_2H$;

CONTACT Hyuk-Sang Jung jhs@khu.ac.kr Department of Anatomy, College of Korean Medicine, Kyung Hee University, 26, Kyungheedae-ro, Dongdaemun-gu, Seoul 02447, Republic of Korea
*These authors contributed equally to this work.

© 2019 Informa UK Limited, trading as Taylor & Francis Group

molecular weight = 429.645 g mol^{-1}; purity = 99.5%; catalog number = PHR1273) (Figure 1) was provided by Sigma–Aldrich (Saint Louis, MO, USA), as was DNCB. Enzyme-linked immunosorbent assay (ELISA) kits for mouse IgE and interleukin (IL)-6 were acquired from BD Biosciences (San Jose, CA, USA). Phosphorylated extracellular signal-regulated kinase (*p*-ERK), ERK, phosphorylated-P38, P38, phosphorylated-*c*-Jun *N*-terminal kinase (*p*-JNK), JNK and nuclear factor-kappa B (NF-κB) were purchased from Cell Signaling Technologies (Danvers, MA, USA). Peroxidase IgG was obtained from Jackson ImmunoResearch (West Grove, PA, USA). Fetal bovine serum (FBS; catalog number 16000-044), penicillin/streptomycin and phosphate-buffered saline (PBS) were provided by Invitrogen (Carlsbad, CA, USA). CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay was obtained from Promega (Madison, WI, USA).

Animals

Animal-care and experimental procedures were approved by the Animal Care and Use Committee of Kyung Hee University (KHUASP(SE)-15-116; Seoul, Republic of Korea). Female BALB/c mice (7 weeks) were allowed to adjust to a laboratory environment for 1 week before experimentation. Mice were housed under controlled light (12-h light–dark cycle), temperature (23 ± 3 °C) and relative humidity (50%–60%) conditions and had *ad libitum* access to food and water.

The procedures for sensitization and challenge are described in Figure 2. The backs of mice were shaved. The mice were divided into two groups; Control group ($n = 8$) and DNCB group ($n = 24$). Then, DNCB group was administrated 200 μL of 0.5% DNCB in acetone/olive oil for 3 days. Control group was applied only acetone/olive oil without DNCB. After an incubation period of 10 days, the mice were then randomly divided into four groups; Control group ($n = 8$), DNCB group ($n = 8$), DL-M low ($n = 8$, 1 mM) and DL-M high group ($n = 8$, 10 mM). DNCB group, DL-M low group and DL-M high group were applied onto the backs of mice 1% DNCB every 3 days. DL-M low and high group in 200 μL of physiologic (0.9% saline containing 10% olive oil was applied to dorsal skin once-daily for 16 days). Control group was applied vehicle mixture. Mice were killed and blood collected via cardiac puncture from each experimental group. Blood was centrifuged at 900 (×g) and 4 °C for 10 min to obtain serum.

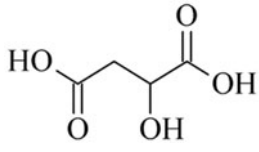

Figure 1. Chemical structure of malic acid.

Measurement of IgE and IL-6

Levels of IgE and IL-6 were measured with an ELISA kit according to manufacturer instructions.

Histology

Skin tissues was separated and embedded in 10% neutral-buffered formalin overnight. Skin tissues were embedded in paraffin and sectioned at a thickness of 4 μm. Sections were stained with hematoxylin and (H & E; eosin (Sigma–Aldrich) for eosinophil counting). Sections were stained with toluidine blue for MC counting. All sections were viewed under a light microscope (DP73; Olympus, Tokyo, Japan) at ×100 and ×400 magnification.

Western blotting

Skin tissues was separated and frozen at −70 °C. They were homogenized in RIPA buffer (Thermo Scientific, Waltham, MA, USA) for extraction of mitogen-activated protein kinase (MAPK) and a NE-PER Nuclear and Cytoplasmic Extraction kit (Pierce, Rockford, IL, USA) for NF-kB. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (using 10% gels) and transferred to polyvinylidene fluoride (PVDF) membranes. The latter were maintained for 1 h at room temperature with 5% skimmed milk, incubated overnight at 4 °C with primary antibody, and incubated with a peroxidase-conjugated secondary antibody for 1 h. Enhanced chemiluminescence was employed for detection. Protein expression was measured using ImageJ (National Institutes of Health, Bethesda, MD, USA).

Cell viability and expression of proinflammatory cytokines

HaCaT cells (American Type Culture Collection, Manassas, VA, USA) were incubated in Dulbecco's modified Eagle's medium (DMEM) with 100 U mL^{-1} of penicillin/streptomycin and 10% heat-inactivated FBS at 37 °C in an atmosphere of 5% CO_2 and humidity of 95%. Cell viability was tested with the MTS colorimetric assay. HaCaT cells were incubated with DL-M (0.1, 0.5, 1 mM) for 1 h followed by 10 ng mL^{-1} of interferon-gamma/tumor necrosis factor-alpha (IFNγ/TNFα) for 24 h.

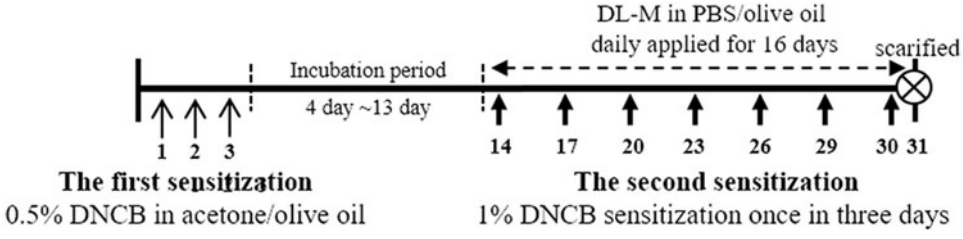

Figure 2. Experimental schedule (schematic). Application was to dorsal skin. The normal group was treated with vehicle (PBS).

Cell supernatants were used to measure expression of proinflammatory cytokines. Expression of monocyte chemoattractant protein (MCP)-1 (BD Biosciences), intercellular adhesion molecule (ICAM) (Boster Biological Technology, Pleasanton CA, USA), monocyte-derived chemokine (MDC; Boster Biological Technology), and thymus and activation-regulated chemokine (TARC; R&D Systems, Minneapolis, MN, USA) was measured using a sandwich ELISA kit according to manufacturer instructions.

Expression of TARC mRNA in HaCaT cells

HaCaT cells were incubated with DL-M for 1 h and then IFNγ/TNFα for 24 h. Expression of TARC in HaCaT cells was measured via polymerase chain reaction (PCR). Total RNA was extracted using TRIzol®, and complimentary cDNA molecules were prepared using SuperScript™ II reverse transcriptase (Invitrogen). Primers were designed to match with human TARC (forward: 5′-ACT GCT CCA GGG ATG CCA TCG TTT TT-3′; reverse: 5′-ACA AGG GGA TGG GAT CTC CCT CAC TG-3′). PCR was analyzed on a 1.5% agarose gel and visualized by NαBI (Neoscience, Suwon, Republic of Korea).

Statistical analyses

Data are the mean ± SEM. Results were evaluated using PRISM (GraphPad, San Diego, CA, USA). One-way ANOVA was used to evaluate significance, followed by Dunnett's multiple comparison test. $p < .05$ was considered significant.

Results

DL-M reduced the thickness of the epidermis and dermis of AD mice in vivo

To understand the effect of DL-M application in the skin of AD mice, we measured the thickness of the epidermis and dermis. The DNCB group had increased thickness of the dermis and epidermis compared with that of the normal group. The thickness of the epidermis in the DL-M (low and high) groups was reduced significantly. The thickness of the dermis in the DL-M (high) group was reduced significantly compared with that of the DNCB group (Figure 3).

DL-M inhibited immune cell infiltration on DNCB-induced AD mice

DL-M (low and high) groups showed a reduced number of eosinophils compared with that of the control group (Figure 4(A)). DL-M (low and high) groups revealed a reduced number of eosinophils and MCs in the dermis. The number of eosinophils and MCs in the DL-M (high) group was reduced more than twofold compared with that in the control group (Figure 4(B,C)).

DL-M reduced serum levels of IgE and IL-6 in AD mice

DL-M suppressed serum levels IgE and of the cytokine IL-6 in AD mice (Figure 5). DL-M (low and high) groups had a significantly decreased serum level of IgE. The DL-M (high) group had a significantly reduced serum level of IL-6.

DL-M reduced expression of NF-κB protein and MAPK protein in AD mice

DL-M suppressed NF-κB activation in AD mice. Expression of NF-κB protein was reduced significantly in the DL-M (high) group (Figure 6). Western blotting was done to evaluate the inhibitory effect of DL-M upon MAPK in AD mice. Expression of P-ERK and P-JNK was repressed in DL-M (low and high) groups, but that of P-P38 did not change significantly.

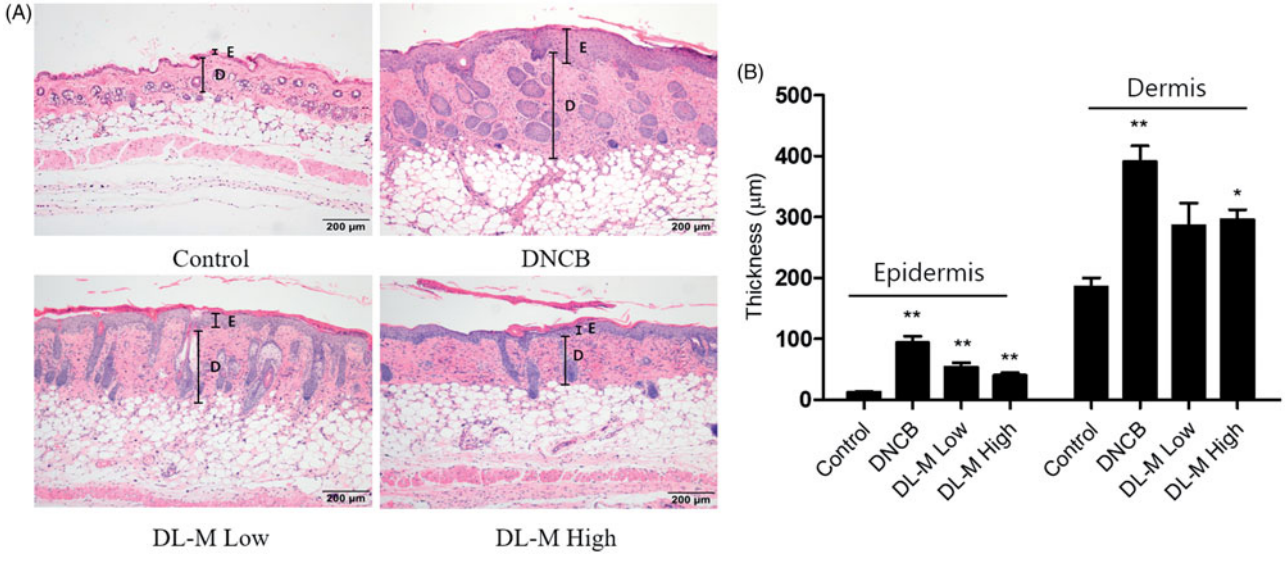

Figure 3. DL-M inhibited thickening of the epidermis and dermis in mice after DNCB-induced AD. (A) The thickness of the epidermis and dermis was examined by H&E staining of skin sections (×100 magnification; scale bar: 200 μm). (B) Measurement of the thickness of the epidermis and dermis. Data are the mean ± SEM, $^{*}p < .05$ and $^{**}p < .01$.

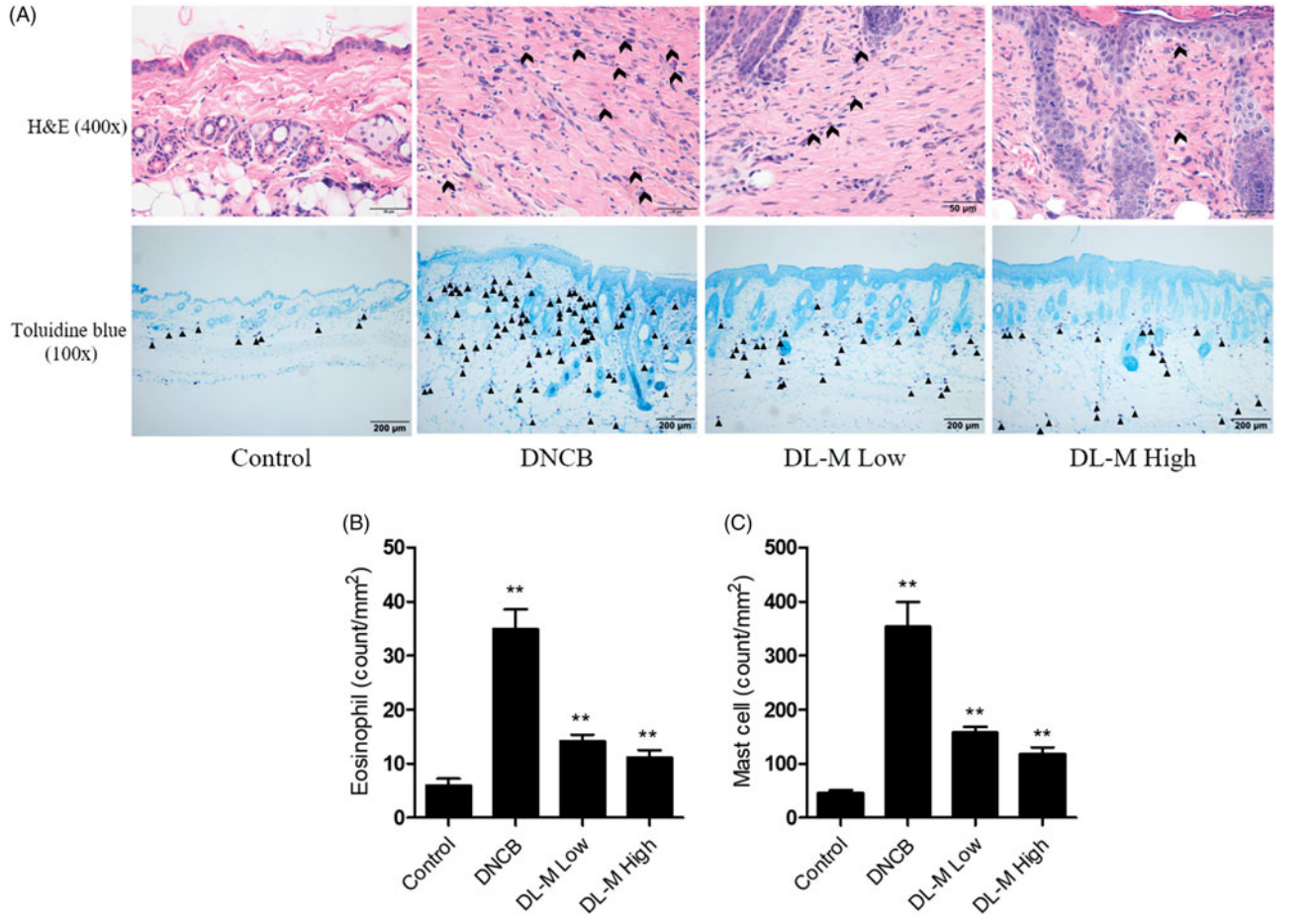

Figure 4. DL-M prevented infiltration of mast cells and eosinophils into the dermis. (A) Infiltration of eosinophils in dermal lesions was examined by H&E staining of skin sections in the upper section (×400 magnification; scale bar = 50 μm). The lower section shows infiltration of mast cells in dermal lesions after staining by toluidine blue of skin sections (×100 magnification; scale bar = 200 μm). (B) The number of eosinophils in the dermis was counted in six fields per section. (C) The number of mast cells in skin tissues was counted in three fields per section. Data are the mean ± SEM, $^{**}p < .01$.

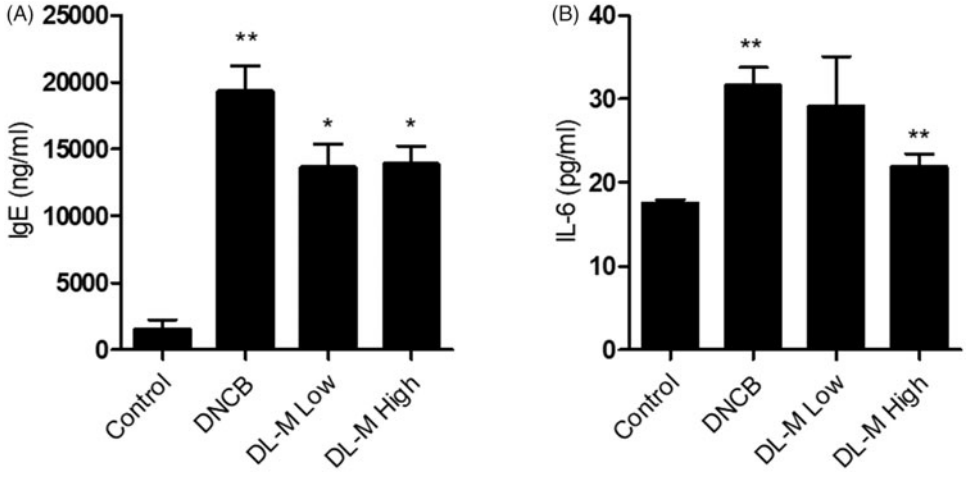

Figure 5. DL-M had an inhibitory effect on serum levels of (A) IgE and (B) IL-6. Data are the mean ± SEM, $^{*}p < .05$ and $^{**}p < .01$.

DL-M had an inhibitory effect on expression of proinflammatory cytokines/chemokines in HaCaT cells

HaCaT cells were treated with DL-M at various concentrations to test their viability (Figure 7(A)). A representative characteristic of AD patients is high expression of TARC [29]. Therefore, we identified the effect of DL-M on expression of TARC mRNA in HaCaT cells (Figure 7(B)). DL-M (1 mM) reduced expression of MCP-1, ICAM and TARC induced by IFNγ/TNFα in HaCaT cells (Figure 7(C–F)) significantly. DL-M reduced MDC expression in HaCaT cells, but not significantly so.

Discussion

We wished to determine the anti-AD effect of DL-M as a component of AHAs, and to search for the mechanism of DL-M in

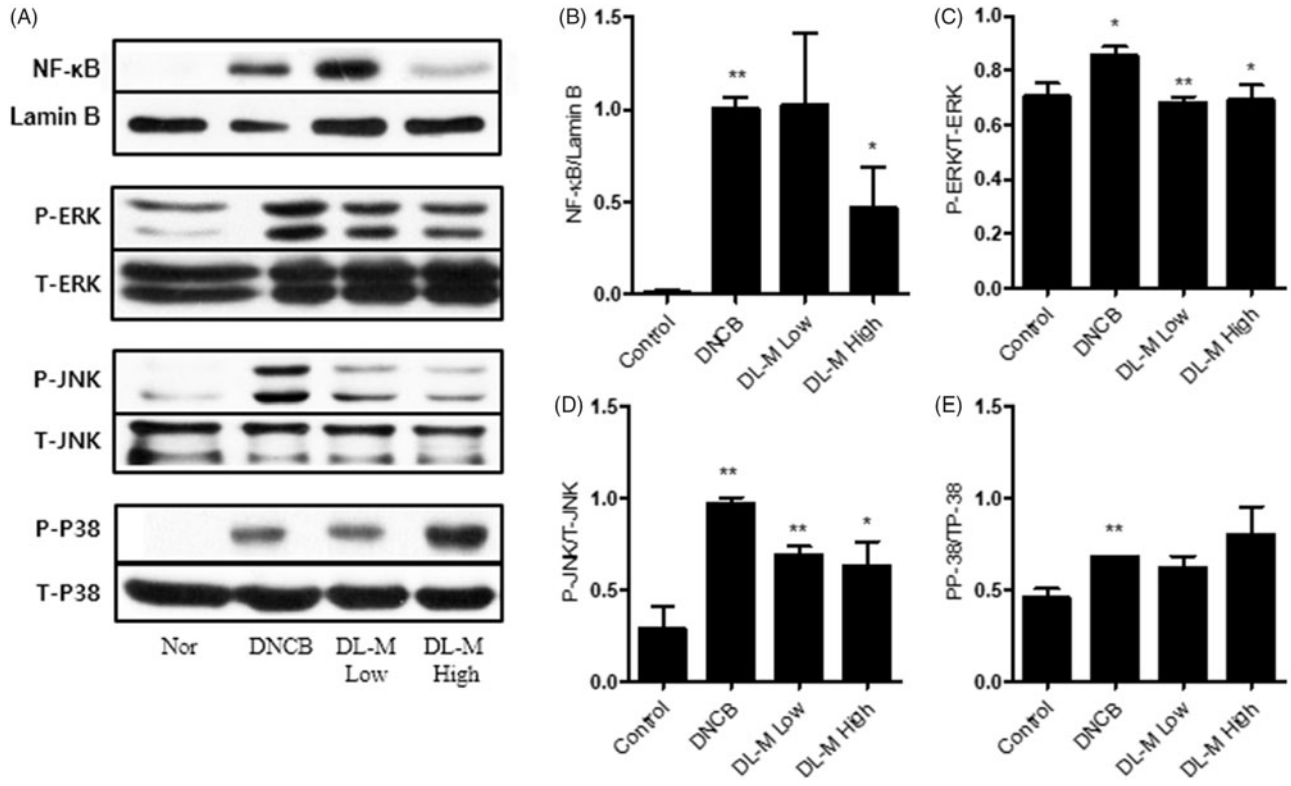

Figure 6. DL-M decreased expression of NF-kB protein and MAPK protein in AD mice. (A) Western-blotting bands of NF-κB, P-ERK1/2, P-JNK and P-P38. (B–D) Quantified with imageJ software. Data are the mean ± SEM, $^{*}p < .05$ and $^{**}p < .01$.

atopic dermatitis *in vivo* and *in vitro*. DL-M decreased: (i) infiltration of eosinophils and MCs in skin lesions; (ii) expression of proinflammatory cytokines and protein expression of MAPK and NF-κB in skin tissue; (iii) reduced expression of TARC, ICAM, and MCP-1 in HaCaT cells.

Measurement of serum levels of IgE has been considered as a diagnostic tool for AD because 80% of AD patients have IgE reactivity to allergens [30,31]. When IgE–antigen complexes stimulate FcεRI-receptors, bone marrow- and skin-derived mast cells produce IL-6 [32]. The latter has a crucial role in inflammatory and immune reactions [33]. IL-6 can induce the acute-phase allergic response [34] and T-cell proliferation [35,36], and is produced by fibroblasts and keratinocytes [33,37]. DL-M can inhibit AD by decreasing serum levels of IgE and IL-6 in AD mice. In the present study, DL-M decreased the epidermis thickness, dermis thickness as well as the number of MCs and eosinophils. These results suggest that DL-M has an inhibitory effect on DNCB-induced AD through suppression of expression of IgE and IL-6.

In chronic AD, the epidermis thickens, and infiltration of eosinophils and MCs is observed [10,38–41]. MCs release cytokines such as MCP-1 that can regulate the effect of adhesion molecules on endothelial cells, and activated endothelial cells induce expression of ICAM-1 [42,43]. The latter has a pivotal role in the transmigration of monocytes, neutrophils and eosinophils during chronic allergic diseases [42]. ICAM can also stimulate activation of naïve cluster of differentiation (CD)4 T cells. TARC and MDC are expressed in inflammatory keratinocytes in skin diseases such as AD and psoriasis [44]. In AD patients, increases in expression of TARC and MDC have been observed in skin lesions [29]. TARC and MDC are produced by CD-4 T cells [45], and recruit T-helper (Th)2 cells [46]. Th2 cells are involved in the early stages of tissue inflammation through induction of cytokine release that results in IgE production and eosinophil activation [47]. In the present study, expression of ICAM, MCP-1 and TARC in keratinocytes was suppressed by DL-M.

MAPK is involved in the growth, proliferation, differentiation, and survival of various cells, including keratinocytes [48,49]. ERK and JNK have been reported to regulate expression of cytokine genes in response to inflammation [50,51]. According to one report, phosphorylation of MAPK is promoted by MCP-1 in endothelial cells [52]. Moreover, MAPK affects activation of the NF-κB pathway [51]. NF-κB has an important role in the inflammatory response because it regulates expression of proinflammatory cytokine genes [53]. We found that DL-M inhibited the NF-κB pathway and inhibited phosphorylation of ERK and JNK.

In conclusion, our studies demonstrated DL-M inhibited DNCB-induced atopic dermatitis through inhibition of inflammatory cytokines, MAPK and NF-κB phosphorylation. DL-M attenuated cytokines and chemokines in human keratinocyte. Especially TARC, which is known to produce CD-4 T cells, were reduced significantly. Although our findings have only shown that DL-M reduces several inflammatory mediators in animal tissues and human keratinocytes, these results suggest that it will be possible to do research for mechanism of DL-M as antiatopic dermatitis agents.

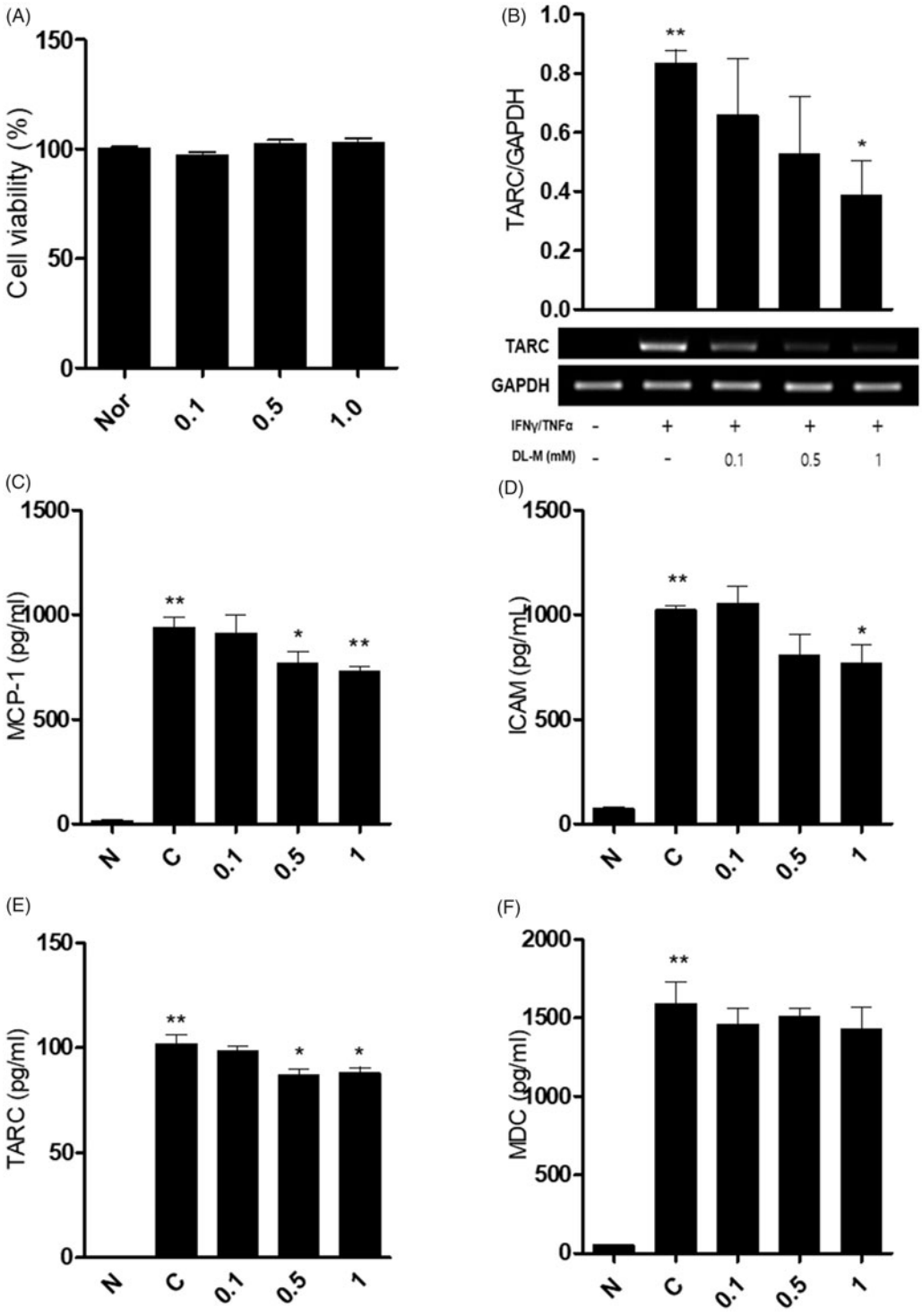

Figure 7. (A) DL-M is not toxic to HaCaT cells. (B) Effect of DL-M on expression of cytokine mRNA in HaCaT cells. Bands were quantified using ImageJ software. (C–F) Graphs showing the effect of DL-M upon expression of MCP-1, ICAM, TARC, and MDC induced by IFNγ/TNFα in HaCaT cells. Data are mean values of three independent experiments. Data are the mean ± SEM, $*p < .05$ and $**p < .01$.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017R1D1A1B03028505).

References

[1] Leung DY, Bieber T. Atopic dermatitis. Lancet. 2003;361(9352):151–160.
[2] Leung DY. Atopic dermatitis: new insights and opportunities for therapeutic intervention. J Allergy Clin Immunol. 2000;105(5):860–876.
[3] Vakharia PP, Chopra R, Sacotte R, et al. Burden of skin pain in atopic dermatitis. Ann Allergy Asthma Immunol. 2017;119(6):548–552 e3.

[4] Lim HW, Collins SAB, Resneck JS, Jr, et al. The burden of skin disease in the United States. J Am Acad Dermatol. 2017;76(5): 958–972 e2.
[5] Yu SH, Attarian H, Zee P, et al. Burden of sleep and fatigue in US adults with atopic dermatitis. Dermatitis. 2016;27(2):50–58.
[6] Picardi A, Lega I, Tarolla E. Suicide risk in skin disorders. Clin Dermatol. 2013;31(1):47–56.
[7] Leung DY. Atopic dermatitis: immunobiology and treatment with immune modulators. Clin Exp Immunol. 1997; 107(Suppl 1):25–30.
[8] Bieber T. Atopic dermatitis. N Engl J Med. 2008;358(14): 1483–1494.
[9] Marsella R, Olivry T, Carlotti DN, et al. Current evidence of skin barrier dysfunction in human and canine atopic dermatitis. Vet Dermatol. 2011;22(3):239–248.
[10] Soter NA. Morphology of atopic eczema. Allergy. 1989;44(s9): 16–19.
[11] Moghadam-Kia S, Werth VP. Prevention and treatment of systemic glucocorticoid side effects. Int J Dermatol. 2010;49(3): 239–248.
[12] Sohn EH, Jang SA, Lee CH, et al. Effects of Korean red ginseng extract for the treatment of atopic dermatitis-like skin lesions in mice. J Ginseng Res. 2011;35(4):479–486.
[13] Park HS, Hwang YH, Kim MK, et al. Functional polysaccharides from *Grifola frondosa* aqueous extract inhibit atopic dermatitis-like skin lesions in NC/Nga mice. Biosci Biotechnol Biochem. 2015;79(1):147–154.
[14] Saeki H, Nakahara T, Tanaka A, et al. Clinical practice guidelines for the management of atopic dermatitis 2016. J Dermatol. 2016; 43(10):1117–1145.
[15] Knuf C, Nookaew I, Remmers I, et al. Physiological characterization of the high malic acid-producing *Aspergillus oryzae* strain 2103a-68. Appl Microbiol Biotechnol. 2014;98(8):3517–3527.
[16] Qi Zhang YY, Cheng L, Cao C, et al. Thermodynamic models for determination of the solubility of DL-malic acid in methanol plus (acetonitrile, *N*,*N*-dimethylformamide, isopropyl alcohol) binary solvent mixtures. J Chem Thermodyn. 2015;85:148–154.
[17] Moon SY, Hong SH, Kim TY, et al. Metabolic engineering of *Escherichia coli* for the production of malic acid. Biochem Eng J. 2008;40(2):312–320.
[18] Beruter J. Carbohydrate metabolism in two apple genotypes that differ in malate accumulation. J Plant Physiol. 2004;161(9): 1011–1029.
[19] Di Vaio C, Marallo N, Graziani G, et al. Evaluation of fruit quality, bioactive compounds and total antioxidant activity of flat peach cultivars. J Sci Food Agric. 2015;95(10):2124–2131.
[20] Wu J, Peng W, Qin R, et al. Crataegus pinnatifida: chemical constituents, pharmacology, and potential applications. Molecules. 2014;19(2):1685–1712.
[21] Seymour Jet GB, Tucker GA, editors. Biochemistry of fruit ripening. Vol. 4. London: Chapman & Hall; 1993.
[22] Van Scott EJ, Ditre CM, Yu RJ. Alpha-hydroxyacids in the treatment of signs of photoaging. Clin Dermatol. 1996;14(2): 217–226.
[23] Kim TH, Choi EH, Kang YC, et al. The effects of topical alpha-hydroxyacids on the normal skin barrier of hairless mice. Br J Dermatol. 2001;144(2):267–273.
[24] Israel Goldberg RAW, Williams R. Biotechnology and food ingredients. Vol. 1. New York: van Nostrand Reinhold; 1991.
[25] Zhang QC, Zhao Y, Bian HM. Antiplatelet activity of a novel formula composed of malic acid, succinic acid and citric acid from *Cornus officinalis* fruit. Phytother Res. 2013;27(12):1894–1896.
[26] Findlay GH. Succinic dehydrogenase in human epidermis. Br J Dermatol. 1965;77(2):88–91.
[27] Inoue H, Someno T, Kawada M, et al. Citric acid inhibits a bacterial ceramidase and alleviates atopic dermatitis in an animal model. J Antibiot. 2010;63(10):611–613.
[28] Tokudome Y. Influence of oral administration of lactic acid bacteria metabolites on skin barrier function and water content in a murine model of atopic dermatitis. Nutrients. 2018;10(12): 1858.
[29] Horikawa T, Nakayama T, Hikita I, et al. IFN-gamma-inducible expression of thymus and activation-regulated chemokine/CCL17 and macrophage-derived chemokine/CCL22 in epidermal keratinocytes and their roles in atopic dermatitis. Int Immunol. 2002; 14(7):767–773.
[30] Brenninkmeijer EE, Schram ME, Leeflang MM, et al. Diagnostic criteria for atopic dermatitis: a systematic review. Br J Dermatol. 2008;158(4):754–765.
[31] Mittermann I, Wikberg G, Johansson C, et al. IgE sensitization profiles differ between adult patients with severe and moderate atopic dermatitis. PLoS One. 2016;11(5):e0156077.
[32] Church MK, Levi-Schaffer F. The human mast cell. J Allergy Clin Immunol. 1997;99(2):155–160.
[33] May LT, Helfgott DC, Sehgal PB. Anti-beta-interferon antibodies inhibit the increased expression of HLA-B7 mRNA in tumor necrosis factor-treated human fibroblasts: structural studies of the beta 2 interferon involved. Proc Natl Acad Sci USA. 1986;83(23): 8957–8961.
[34] Gauldie J, Richards C, Harnish D, et al. Interferon beta 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. Proc Natl Acad Sci USA. 1987;84(20):7251–7255.
[35] Lotz M, Jirik F, Kabouridis P, et al. B cell stimulating factor 2/interleukin 6 is a costimulant for human thymocytes and T lymphocytes. J Exp Med. 1988;167(3):1253–1258.
[36] Garman RD, Jacobs KA, Clark SC, et al. B-cell-stimulatory factor 2 (beta 2 interferon) functions as a second signal for interleukin 2 production by mature murine T cells. Proc Natl Acad Sci USA. 1987;84(21):7629–7633.
[37] Kirnbauer R, Kock A, Schwarz T, et al. IFN-beta 2, B cell differentiation factor 2, or hybridoma growth factor (IL-6) is expressed and released by human epidermal cells and epidermoid carcinoma cell lines. J Immunol. 1989;142(6):1922–1928.
[38] Liu FT, Goodarzi H, Chen HY. IgE, mast cells, and eosinophils in atopic dermatitis. Clinic Rev Allerg Immunol. 2011;41(3): 298–310.
[39] Wessler I, Reinheimer T, Kilbinger H, et al. Increased acetylcholine levels in skin biopsies of patients with atopic dermatitis. Life Sci. 2003;72(18/19):2169–2172.
[40] Irani AM, Sampson HA, Schwartz LB. Mast cells in atopic dermatitis. Allergy. 1989;44(s9):31–34.
[41] Leiferman KM, Ackerman SJ, Sampson HA, et al. Dermal deposition of eosinophil-granule major basic protein in atopic dermatitis. Comparison with onchocerciasis. N Engl J Med. 1985;313(5): 282–285.
[42] Bochner BS, Luscinskas FW, Gimbrone MA, Jr, et al. Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1-activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. J Exp Med. 1991;173(6): 1553–1557.
[43] Gudbjornsson B, Hallgren R, Nettelbladt O, et al. Phenotypic and functional activation of alveolar macrophages, T lymphocytes and NK cells in patients with systemic sclerosis and primary Sjogren's syndrome. Ann Rheum Dis. 1994;53(9):574–579.
[44] Staumont-Salle D, Fleury S, Lazzari A, et al. CX(3)CL1 (fractalkine) and its receptor CX(3)CR1 regulate atopic dermatitis by controlling effector T cell retention in inflamed skin. J Exp Med. 2014; 211(6):1185–1196.
[45] Hirata H, Arima M, Cheng G, et al. Production of TARC and MDC by naive T cells in asthmatic patients. J Clin Immunol. 2003;23(1): 34–45.
[46] Vestergaard C, Deleuran M, Gesser B, et al. Thymus- and activation-regulated chemokine (TARC/CCL17) induces a Th2-dominated inflammatory reaction on intradermal injection in mice. Exp Dermatol. 2004;13(4):265–271.
[47] Campbell JJ, Haraldsen G, Pan J, et al. The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. Nature. 1999;400(6746):776–780.

[48] Cargnello M, Roux PP. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. Microbiol Mol Biol Rev. 2011;75(1):50–83.
[49] Thiel G, Ekici M, Rossler OG. Regulation of cellular proliferation, differentiation and cell death by activated Raf. Cell Commun Signal. 2009;7(1):8.
[50] Tournier C, Whitmarsh AJ, Cavanagh J, et al. The MKK7 gene encodes a group of c-Jun NH2-terminal kinase kinases. Mol Cell Biol. 1999;19(2):1569–1581.
[51] Azzolina A, Guarneri P, Lampiasi N. Involvement of p38 and JNK MAPKs pathways in substance P-induced production of TNF-alpha by peritoneal mast cells. Cytokine. 2002;18(2):72–80.
[52] Werle M, Schmal U, Hanna K, et al. MCP-1 induces activation of MAP-kinases ERK, JNK and p38 MAPK in human endothelial cells. Cardiovasc Res. 2002;56(2):284–292.
[53] Werle M, Schmal U, Hanna K, et al. MCP-1 induces activation of MAP-kinases ERK, JNK and p38 MAPK in human endothelial cells. Cardiovasc Res. 2002;56(2):284–292.