

RESEARCH ARTICLE

Anti-allergic effect of ascorbic acid derivative DDH-1 in a mouse model of atopic dermatitis

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Abstract

Atopic dermatitis (AD) is the most common inflammatory skin disease, which is characterized by excessive Th2 immune responses. In AD patients, the expression of the chemokines CCL17 and CCL22 is increased in skin lesions, leading to the infiltration of Th2 cells. In addition, typical pro-inflammatory cytokines, including TNF- α , IL-1 β and IL-6, have also been shown to be associated with the pathogenesis of AD. Recently, DDH-1, an ascorbic acid derivative, has been synthesized and demonstrated to have a more stabilized structure and better skin penetrability. Furthermore, DDH-1 has been shown to suppress pro-inflammatory cytokine expression in vitro and in vivo. Therefore, using an AD mouse model, we evaluated the effect of DDH-1 to reduce allergic skin inflammation. We found that cutaneous administration of DDH-1 significantly reduced the expression levels of TNF- α , IL-1 β and IL-6 in the skin lesions of AD-like mice. Additionally, DDH-1 administration also significantly reduced the expression levels of CCL17 and CCL22, resulting in decreased skin infiltration of Th2 cells. Consequently, DDH-1 reduced ear and epidermal thickness, the serum IgE levels and the number of infiltrating inflammatory cells and mast cells into the AD-like skin lesions. Combination treatment with DDH-1 and corticosteroid more efficiently improved the skin lesions compared with corticosteroid alone. Collectively, our results suggest that DDH-1 has an anti-allergic effect in an AD mouse model by reducing not only the pro-inflammatory cytokine expression but also the Th2-associated chemokine expression. Thus, DDH-1 may be beneficial for AD treatment and prevention as a monotherapy or in combination with corticosteroids.

KEYWORDS

allergy, corticosteroid, inflammation, inflammatory cytokines, vitamin

1 | INTRODUCTION

Atopic dermatitis (AD) is a common inflammatory skin disease associated with skin barrier dysfunction and immune dysregulation including upregulation of Th2 immune responses.¹ CCL17 and CCL22

are Th2-associated chemokines, which can induce Th2 cell migration via their receptor CCR4.² In AD patients, the expression levels of CCL17 and CCL22 are highly upregulated in the skin lesions and considered to recruit CCR4-expressing Th2 cells.^{2,3} Accordingly, Th2 cell-derived cytokines, such as IL-4, IL-5 and IL-13, promote IgE

synthesis in B cells, and subsequent IgE-mediated activation of mast cells triggers various allergic responses.¹ Thus, blocking of the CCR4/CCL17/CCL22 axes is expected to be a novel approach for AD treatment. Furthermore, TNF- α , IL-1 β and IL-6, typical pro-inflammatory cytokines, have been shown to participate in the pathogenesis of AD, and their serum levels are associated with the disease severity.^{4–6} These pro-inflammatory cytokines are also well-established therapeutic targets for AD treatment. Currently, topical corticosteroids are widely used for AD treatment, and they are shown to suppress the expression levels of Th2-associated chemokines and pro-inflammatory cytokines.^{7,8} However, corticosteroids are also known to have several unwanted adverse effects, such as skin barrier damage, erythema, telangiectasias and dyspigmentation.⁷ Thus, the development of new, safe and effective options for AD therapy is needed.

DDH-1, an ascorbic acid derivative, has a more stabilized structure and better skin penetrability than ascorbic acid.^{9,10} Of note, DDH-1 has recently been reported to have an anti-inflammatory effect through the suppression of pro-inflammatory cytokine expression levels *in vitro* and *in vivo*, while ascorbic acid does not have this effect. Accordingly, DDH-1, but not ascorbic acid, has been shown to promote the expression levels of peroxisome proliferator activated receptor (PPAR)- α and PPAR- γ , nuclear factor-kappa B (NF- κ B) negative regulators, in cultured keratinocytes, leading to reduced expression levels of TNF- α , IL-1 β and IL-6.^{9–12} In addition, we have also found that DDH-1 shows an anti-inflammatory effect on a psoriasis mouse model induced by topical imiquimod treatment.¹² Thus, DDH-1 may also be useful to prevent and treat other inflammatory skin diseases including AD.

Dibutyl phthalate (DBP) has been shown to serve as an adjuvant to enhance allergic inflammation by increasing the expression levels of thymic stromal lymphopoietin, an inducer for the production of CCL17 and CCL22.¹³ Furthermore, we previously constructed a transcutaneous delivery system based on a hydrophilic gel patch. The hydrophilic gel patch has been shown to effectively deliver high molecular weight proteins, including antigenic proteins, to dendritic cells in mouse skin.^{3,14–17} In addition, using the hydrophilic gel patch and topical DBP treatment, we could efficiently induce AD-like skin inflammation by delivering the model antigen ovalbumin (OVA) into the mouse skin and enhancing Th2 responses.¹³ In the present study, we evaluated the anti-allergic effect of DDH-1 using this AD mouse model.

2 | MATERIALS AND METHODS

2.1 | Mice

Female BALB/c mice were purchased from Japan SLC (Shimizu, Japan) and housed under specific pathogen-free conditions at Kindai University. For experiments, 6–8 weeks of age mice were used. All mouse experiments in this study were approved by Kindai University's Committee for the Care and Use of Laboratory Animals,

and conducted according to the guidelines and the US National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2 | Hydrophilic gel patch formulation

The hydrophilic gel patch was provided from CosMED Pharmaceutical Co. Ltd. It is composed of cross-linked HiPAS acrylic medical adhesive (10% w/w), octyldodecyl lactate (4.5% w/w), glycerin (0.3% w/w) and sodium hyaluronate (0.02% w/w).^{14–16}

2.3 | Development of AD-like skin inflammation

The mouse model of AD was developed by OVA and DBP using the hydrophilic gel patch as reported previously.^{3,13} Mice were intraperitoneally injected with 300 μ l of PBS containing 25 μ g of OVA and 1000 μ g of alum (Imject Alum; Thermo Fisher Scientific) on Days 0 and 7, followed by application of the hydrophilic gel patch (1 $\times$ 2 cm²) containing 100 μ g of OVA on Days 14, 19 and 24. Each application of the hydrophilic gel patch was continued for 24 h. An adhesive tape was used to support the adherence of the hydrophilic gel patch to the ear skin. From Day 0 to Day 25, DBP solution (1:1 ratio of DBP and Tween 80) was applied to the ear skin. On Day 26, blood and skin tissues were obtained from the AD-like mice for further analysis.

2.4 | Reagents

We synthesized 5-(1,2-dihydroxyethyl)-4-(3-dodecoxy-2-hydroxypropoxy)-3-hydroxyfuran-2(5H)-one, also named DDH-1, as described previously.⁹ DDH-1 assay solutions were prepared by dissolving with distilled water. Betamethasone dipropionate (BD) ointment (0.64 mg/g) was purchased from Shionogi Co., Ltd. The ear skin was treated with DDH-1 daily from Day 14 to Day 25 of the AD induction period. The ear skin was also treated with BD ointment on alternate days from Day 14 to Day 24. To determine a submaximal dose of DDH-1, AD-like mice were treated with 0.02%, 0.1% or 0.5% DDH-1 in preliminary experiments. Because 0.1% and 0.5% DDH-1 showed almost the same therapeutic effect, we used 0.02% and 0.1% DDH-1 to assess the dose-dependent effect. We also previously confirmed that the treatment of 0.02% and 0.1% DDH-1 did not show any changes in the skin of normal mice.¹²

2.5 | Histochemistry

HE staining of the skin was conducted as reported previously.³ Epidermal thickness was measured on HE-stained sections of the ear skin using ImageJ software. Toluidine blue (TB) staining was also conducted as reported previously.³ Inflammatory cells and mast

cells in the dermis were counted in five random fields of each HE or TB section, respectively, under an optical microscope at 400x magnification.

2.6 | ELISA

Serum was collected for the measurement of total IgE levels, and the serum levels were determined using a mouse IgE ELISA kit according to the manufacturer's protocol. Skin tissue samples were lysed with Tissue Extraction Reagent I (Invitrogen) for the measurement of TNF- α , IL-1 β , IL-6 and IL-4 protein levels, and their protein levels were also determined using ELISA kits. All ELISA kits were obtained from BioLegend.

2.7 | Quantitative real-time PCR

The KAPA SYBR Fast qPCR Kit (Kapa Biosystems) was used to perform quantitative real-time PCR on ABI7000 (Applied Biosystems). Thermal cycling conditions were as follows: 95°C for 5 min, followed by 40–45 cycles of 95°C for 30 s and 55°C for 30 s. The sequence of primers for GAPDH, PPAR- α , PPAR- γ , TNF- α , IL-1, IL-4, CCL17 and CCL22 were reported previously.^{12,13} The sequence of primers for IL-6 was as follows; +5'-CTGCAAGAGACTTCCATCCAGTT-3' and -5'-AAGTAGGGAAGGCCGTGTT-3'.

2.8 | Statistical analysis

Statistical analysis was conducted as reported previously.¹⁸ Student's *t*-test was used for pairwise comparisons. One-way ANOVA with Holm post hoc test was used for multiple groups, using the GraphPad PRISM software. *p* values below 0.05 were referred as statistically significant.

3 | RESULTS

3.1 | Inhibitory effect of DDH-1 on pro-inflammatory cytokine expression

To evaluate the effect of DDH-1 on allergic skin inflammation, we employed an AD mouse model induced by a hydrophilic gel patch containing the model antigen OVA and topical DBP treatment.^{13,19} Previously, PPAR- α and PPAR- γ , negative regulators of NF- κ B, are reported to be decreased in the inflamed lesions of psoriasis and AD.^{12,20} On the contrary, DDH-1 has been shown to exert an anti-inflammatory effect by increasing the expression levels of PPAR- α and PPAR- γ .^{9–12} Thus, we first confirmed the mRNA expression levels of PPAR- α and PPAR- γ in the skin lesions of AD-like mice. As shown in Figure 1A, PPAR- α and PPAR- γ mRNA expression levels were found to be decreased in the skin lesions of AD-like mice, while

their mRNA expression levels were significantly increased in the skin lesions of mice applied with DDH-1. Consequently, the mRNA expression levels of TNF- α , IL-1 β and IL-6, which are typical NF- κ B target genes, were strongly increased in the AD-like skin lesions, while their increased mRNA expression was significantly suppressed by DDH-1 treatment (Figure 1A). We also measured the protein expression levels of TNF- α , IL-1 β and IL-6 by using ELISA. As shown in Figure 1B, DDH-1 treatment dose-dependently reduced their protein expression levels in the AD-like skin lesions.

3.2 | Effect of DDH-1 on Th2-associated gene expression

Th2-associated chemokines, including CCL17 and CCL22, are well known to be critically implicated in AD development. Accordingly, we and others have reported that CCL17 and CCL22 serum levels are associated with disease severity of AD.^{21–23} Recently, an ELISA kit for CCL17 (Alaport TARC) has been used for the diagnosis of AD. Thus, we analysed the mRNA expression levels of CCL17 and CCL22 in the skin lesions of AD-like mice. Although these chemokine expression levels were increased in the skin lesions, their increased mRNA expression was significantly suppressed by DDH-1 treatment (Figure 1C). We next analysed the expression levels of IL-4, a Th2 marker, in the AD-like skin lesions. IL-4 mRNA and protein expression levels were also increased in the AD-like skin lesions, while both increased IL-4 mRNA and protein expressions were significantly suppressed by DDH-1 treatment (Figure 1C,D). Thus, DDH-1 seems to inhibit CCR4-mediated Th2 cell infiltration into the AD-like skin lesions by suppressing the expression levels of CCL17 and CCL22.

3.3 | Therapeutic effect of DDH-1 in an AD mouse model

We next evaluated the therapeutic effect of DDH-1 treatment using an AD mouse model. To induce AD-like skin inflammation, mouse ear skin was treated with an OVA-containing hydrophilic gel patch and DBP. Consequently, these mice exhibited increased ear and epidermal thickness, increased serum total IgE levels, and massive infiltration of inflammatory cells and mast cells in the skin lesions (Figure 2). DDH-1 treatment significantly improved the ear and epidermal thickness (Figure 2A–C). Moreover, DDH-1 treatment dose-dependently reduced the serum total IgE levels, although the difference was not statistically significant (Figure 2D). HE and TB staining demonstrated a large number of inflammatory cells and mast cells, respectively, in the dermis of AD-like mice (Figure 2C,E–G). The infiltrated inflammatory cells included mononuclear round cells like lymphocytes and monocytes, and polymorphonuclear cells like eosinophils. DDH-1 treatment also dose-dependently reduced the infiltration of these inflammatory cells and mast cells into the skin lesions (Figure 2C,E–G). There was no significant difference in the therapeutic effect between 0.02% and 0.1% DDH-1 treatment groups.

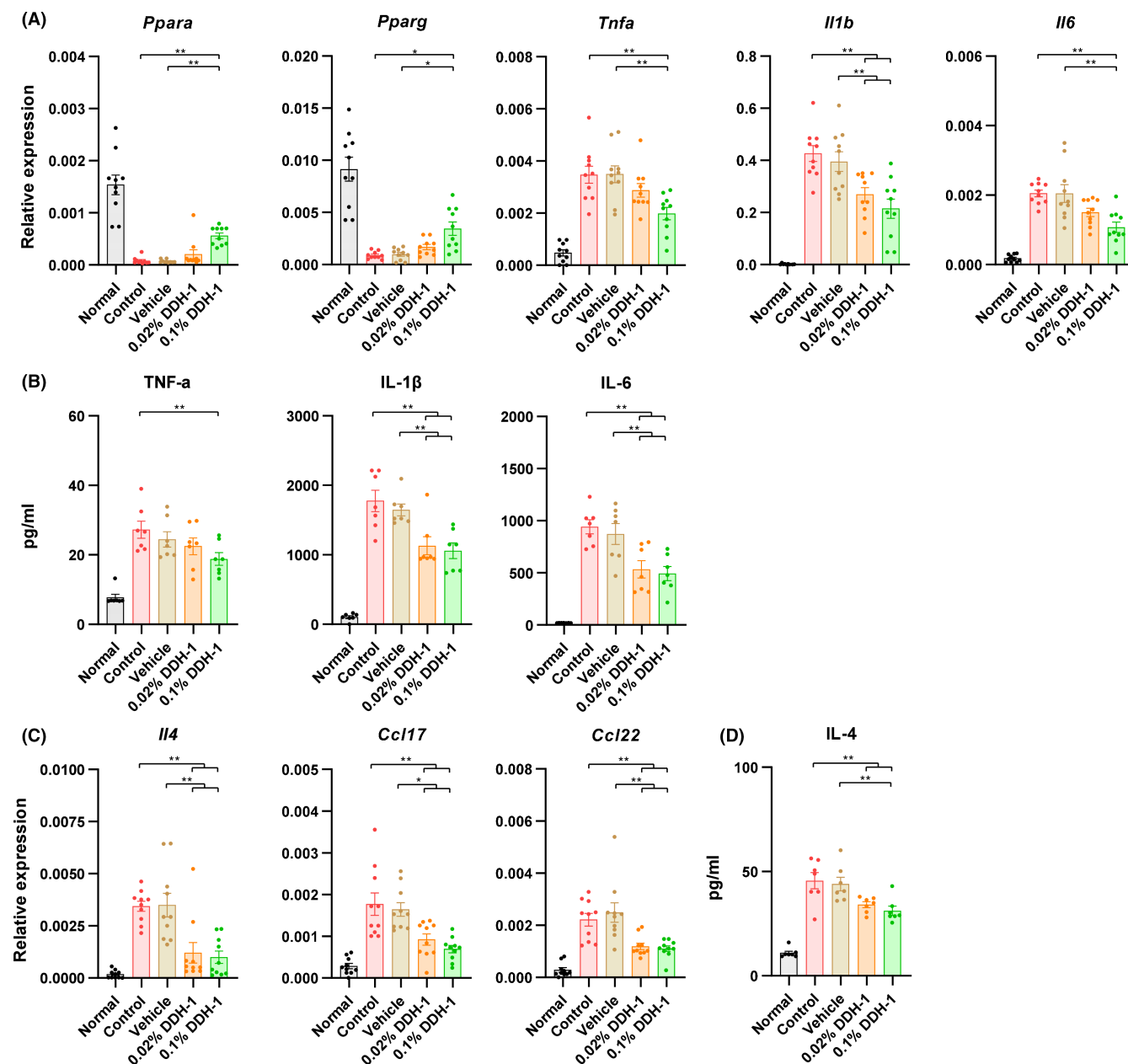


FIGURE 1 Upregulation of PPAR- α and PPAR- γ expression and downregulation of pro-inflammatory cytokine expression by DDH-1 treatment. Mice were intraperitoneally injected OVA-alum on Day 0 and Day 7, followed by the application of OVA-containing hydrophilic gel patch to the ear skin on Day 14, Day 19 and Day 24. Topical DBP was applied daily from Day 0 to Day 25. The skin lesions were daily treated with 20 μ l of DDH-1 solution (0.02% or 0.1%) from Day 14 to Day 25, and skin samples were obtained on Day 26. (A) Quantitative PCR for PPAR- α , PPAR- γ , TNF- α , IL-1 β and IL-6 mRNA expression in the skin lesions of AD-like mice. (B) ELISA of TNF- α , IL-1 β and IL-6 proteins in the skin lesions of AD-like mice. (C) Quantitative PCR for CCL17, CCL22 and IL-4 mRNA expression in the skin lesions of AD-like mice. (D) ELISA of IL-4 proteins in the skin lesions of AD-like mice. Data represent the mean $\pm$ SE of results from ten (A, C) and seven (B, D) skin samples. * p < 0.05, ** p < 0.01

3.4 | Combination treatment of DDH-1 with corticosteroid in AD-like skin lesions

Because corticosteroids are frequently associated with many adverse effects, they have been often used in combination with other medicines to prevent adverse effects in the treatment of inflammatory skin diseases including AD. We thus evaluated the therapeutic effect of a combination treatment of DDH-1 with the corticosteroid

betamethasone dipropionate (BD) ointment in the AD-like mice. We first confirmed the therapeutic effects of low-dose (2.5 mg) and high-dose (12.5 mg) BD ointment monotherapy in the AD-like mice. As shown in Figure 3A,B,D,E, the application of BD ointment dose-dependently improved the ear and epidermal thickness, reduced the serum total IgE levels, and reduced the infiltration of inflammatory cells into the skin lesions. The therapeutic effect of high-dose BD ointment was significantly higher than that of low-dose BD ointment.

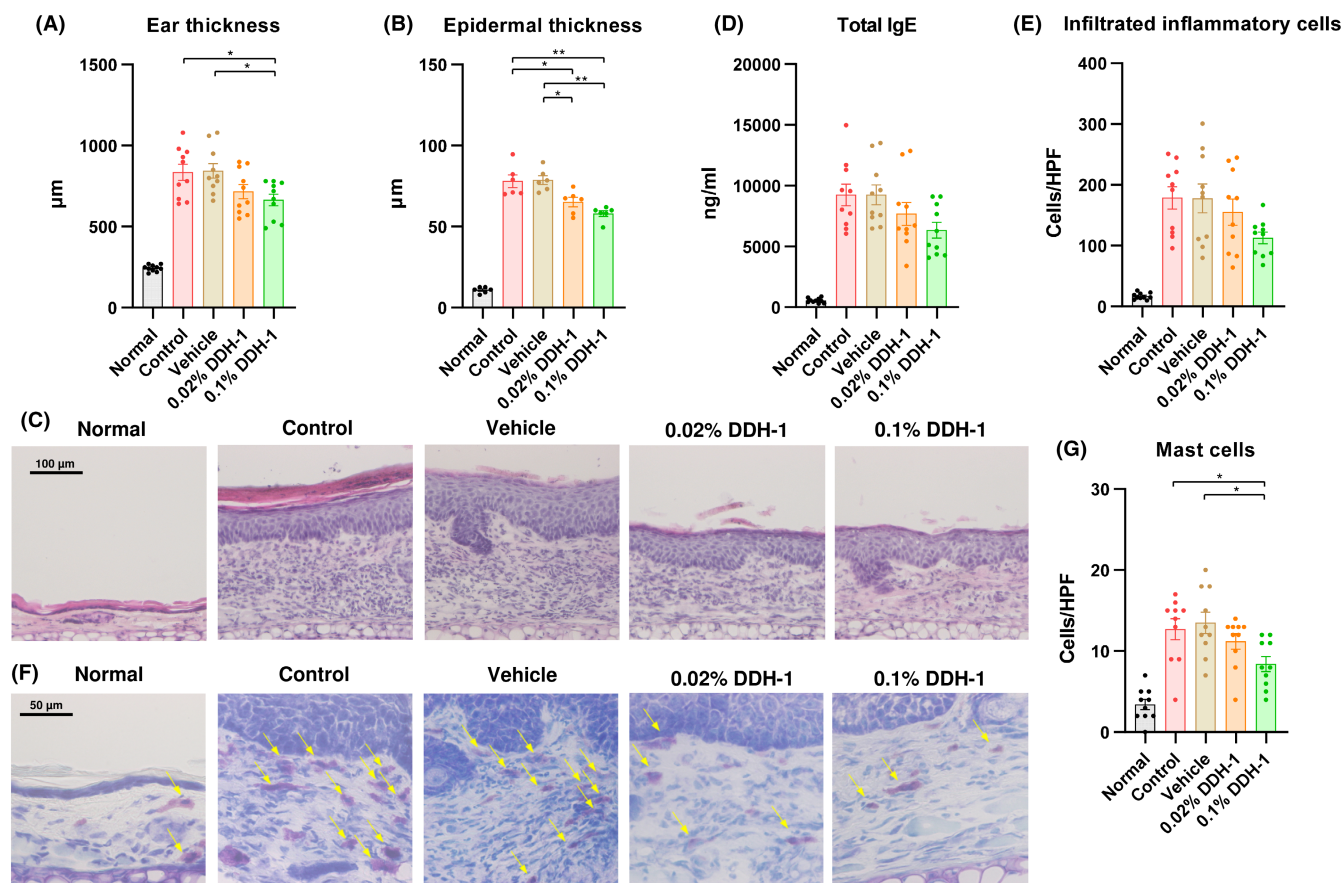


FIGURE 2 Anti-allergic effect of DDH-1 in an AD mouse model. (A) Measurement of ear thickness using a spring-loaded micrometre. (B) Measurement of epidermal thickness from HE-staining images of skin samples. (C) HE staining of skin samples. Scale bar is 100 μm . (D) ELISA of total IgE in the serum of AD-like mice. (E) Count of inflammatory cells in the dermis of AD-like mice. Cells were counted in five random fields of each HE staining image under the optical microscope at 400 $\times$ magnification. (F) TB staining of skin samples. Scale bar is 50 μm . (G) Count of mast cells in the dermis of AD-like mice. Cells were counted in five random fields of each TB staining image under the optical microscope at 400 $\times$ magnification. Data represent the mean $\pm$ SE of results from ten (A, D, E, G) and six (B) skin samples. * $p < 0.05$. ** $p < 0.01$

The application of BD ointment also dose-dependently reduced mast cell infiltration, although there was no significant difference between low-dose and high-dose BD ointment groups. Compared with low-dose BD ointment alone, the combination treatment of DDH-1 with low-dose BD ointment more efficiently improved the ear and epidermal thickness, the serum total IgE levels, and the infiltration of inflammatory cells and mast cells (Figure 3A–F). The therapeutic effect of the combination treatment with BD was also significantly higher than that of DDH-1 treatment alone (Figure 3A–F). Furthermore, we macroscopically confirmed that the ear redness and vasodilation were efficiently improved in AD-like mice with the combination treatment of DDH-1 and low-dose BD ointment more than in AD-like mice with low-dose BD ointment alone (Figure 3G). The therapeutic effect of the combination treatment of 0.1% DDH-1 and low-dose (2.5 mg) BD ointment was almost the same as that of high-dose (12.5 mg) BD ointment alone (Figure 3A–G). There was no significant difference in the therapeutic effect between the combination treatment of 0.02% and 0.1% DDH-1 with low-dose BD ointment groups.

3.5 | Inhibitory effect of the combination treatment of DDH-1 with corticosteroid on AD-related cytokine and chemokine expression

Real-time PCR analysis was conducted to determine the inhibitory effect of the combination treatment of DDH-1 with BD ointment on the expression of pro-inflammatory cytokines and Th2-associated chemokines in AD-like skin lesions. We first confirmed that the combination treatment of DDH-1 with low-dose (2.5 mg) BD ointment significantly increased the mRNA expression levels of PPAR- α and PPAR- γ in the AD-like skin lesions compared with low-dose BD ointment alone (Figure 4A). Consistently, the combination treatment of DDH-1 with low-dose BD ointment significantly reduced the mRNA expression levels of TNF- α , IL-1 β , IL-6 and CCL17 in the AD-like skin lesions compared with low-dose BD ointment alone (Figure 4A). The combination treatment also efficiently reduced IL-4 and CCL22 mRNA expression levels more than low-dose BD ointment alone, although the difference was not statistically significant (Figure 4A). In addition, the inhibitory effect of the combination treatment of DDH-1

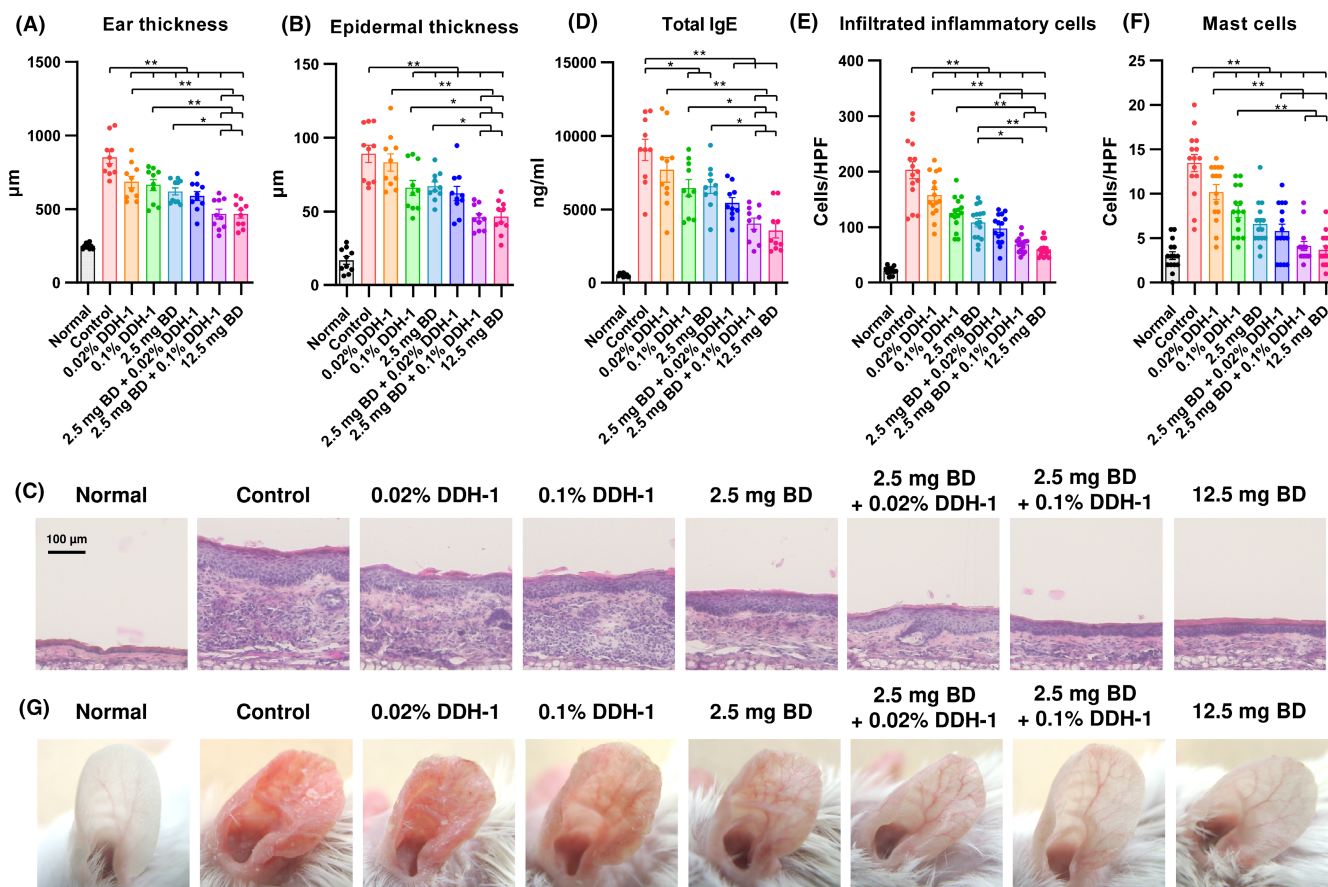


FIGURE 3 Effect of the combination treatment of DDH-1 with corticosteroid in an AD mouse model. Mice were intraperitoneally injected OVA-alum on Day 0 and Day 7, followed by the application of OVA-containing hydrophilic gel patch to the ear skin on Day 14, Day 19 and Day 24. Topical DBP was applied daily from Day 0 to Day 25. The skin lesions were daily treated with 20 μ l of DDH-1 solution (0.02% or 0.1%) from Day 14 to Day 25, and skin samples were obtained on Day 26. For the combination treatment, BD ointment (2.5 mg or 12.5 mg) was topically applied to the skin every other day. (A) Measurement of ear thickness using a spring-loaded micrometre. (B) Measurement of epidermal thickness from HE-staining images of skin samples. (C) HE staining of skin samples. Scale bar is 100 μ m. (D) ELISA of total IgE in the serum of AD-like mice. (E) Count of inflammatory cells in the dermis of AD-like mice. Cells were counted in at five random fields of each HE staining image under the optical microscope at 400 $\times$ magnification. (F) Count of mast cells in the dermis of AD-like mice. Cells were counted in five random fields of each TB staining image under the optical microscope at 400 $\times$ magnification. (G) Macroscopic images of ears from AD-like mice. The representative images are shown. Data represent the mean $\pm$ SE of results from ten (A, B, D) and fifteen (E, F) skin samples. * p < 0.05. ** p < 0.01

with BD ointment was higher than that of DDH-1 alone (Figure 4A). Consistent with their mRNA expression levels, TNF- α , IL-1 β , IL-6 and IL-4 protein expression levels were more efficiently reduced by the combination treatment of DDH-1 with BD ointment (Figure 4B). We next analysed Th2 cells (IL-4⁺CD4⁺ cells) in the skin lesions of AD-like mice by using flow cytometry. As shown in Figure 4C, the number of Th2 cells was strongly increased in the skin lesions of control AD-like mice. DDH-1 or low-dose BD ointment alone significantly reduced the number of Th2 cells in the skin lesions, and the combination treatment of DDH-1 with BD ointment efficiently reduced their number more than the mono-treatments (Figure 4C).

4 | DISCUSSION

In the present study, we have found that DDH-1 has an anti-allergic effect using an AD mouse model. Topical application of DDH-1

improved AD-like symptoms, including skin hypertrophy, increased serum IgE levels, and inflammatory cell and mast cell infiltration. Furthermore, DDH-1 upregulated the expression levels of the NF- κ B negative regulators PPAR- α and PPAR- γ , and reduced the expression levels of TNF- α , IL-1 β and IL-6 in the AD-like mice. Of note, DDH-1 also reduced the expression levels of not only these pro-inflammatory cytokines, but also the Th2-associated chemokines CCL17 and CCL22. CCL17 and CCL22 are well known to be critically associated with AD development by recruiting IL-4-expressing Th2 cells into the skin via their receptor CCR4.^{2,3} Indeed, CCR4-deficiency and a CCR4 antagonist have been shown to ameliorate allergic skin inflammation in mice.^{3,13} Thus, DDH-1 may ameliorate allergic skin inflammation by suppressing CCR4-mediated migration of Th2 cells. Collectively, our findings suggest that the topical application of DDH-1 may be useful for various allergic inflammatory diseases, including AD.

PPARs are members of the nuclear hormone receptor family and function as fatty acid sensors.^{24,25} It has previously been shown

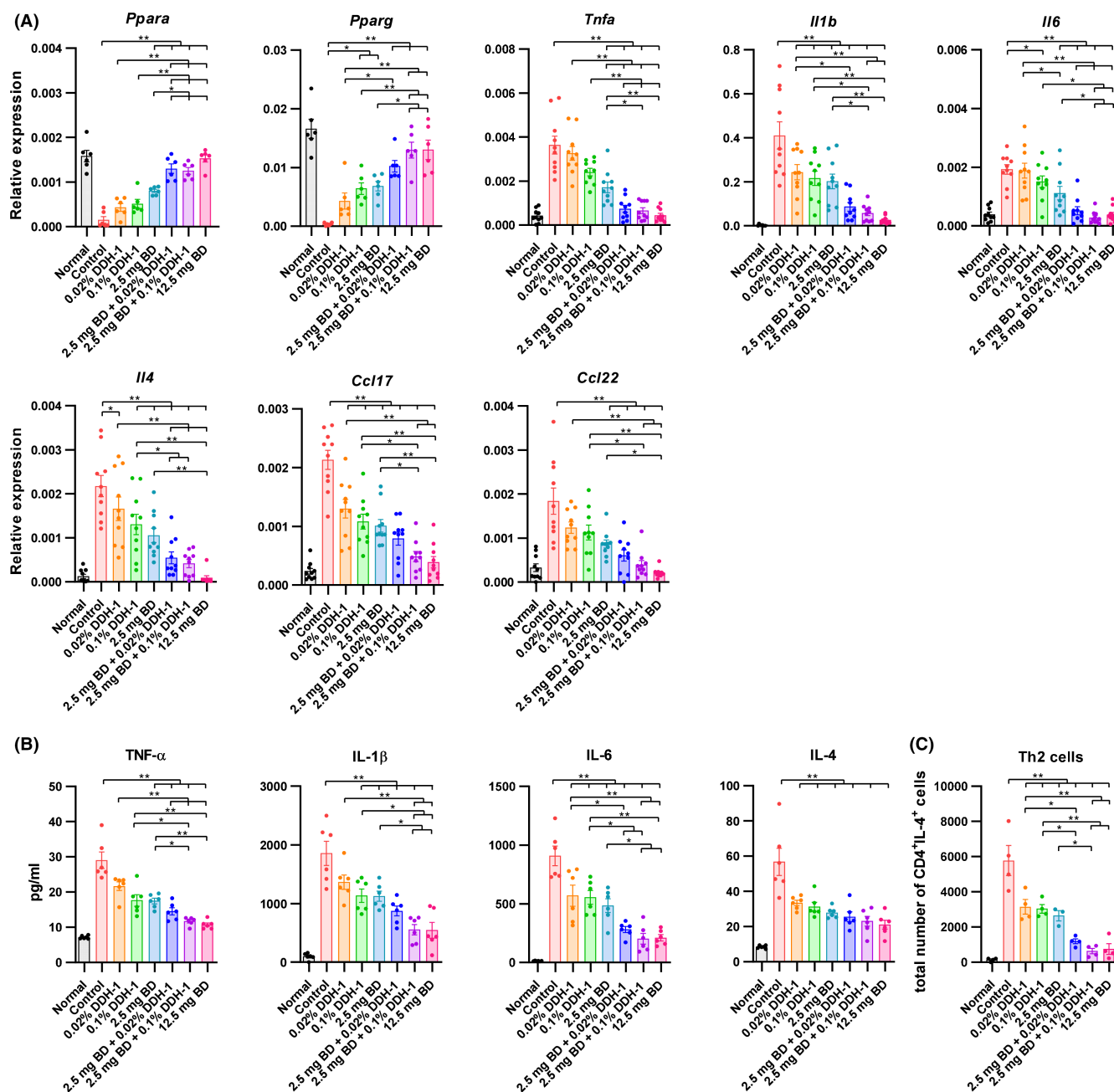


FIGURE 4 Effect of the combination treatment of DDH-1 with corticosteroid on cytokine and chemokine expression. (A) Quantitative PCR for PPAR- α , PPAR- γ , TNF- α , IL-1 β , IL-6, IL-4, CCL17 and CCL22 mRNA expression in the skin lesions of AD-like mice. (B) ELISA of TNF- α , IL-1 β , IL-6 and IL-4 proteins in the skin lesions of AD-like mice. (C) Flowcytometric analysis for Th2 cells. IL-4⁺CD4⁺ Th2 cells were analysed by flow cytometry using the CD45 gate. Data are expressed as the mean $\pm$ SE of results from six or ten (A), six (B) and three or four (C) skin samples. * p < 0.05. ** p < 0.01

that PPAR- α and PPAR- γ have immunomodulatory properties by suppressing transcription factors such as NF- κ B and activator protein-1 (AP-1).²⁴ Furthermore, it has been reported that some agonists of PPAR- α and PPAR- γ show a significant therapeutic effect in mouse models of allergic skin disease.^{26,27} These findings suggest that PPAR- α and PPAR- γ could be new potential candidates for the treatment of allergic skin diseases including AD. Accordingly, we have demonstrated that DDH-1 is capable of increasing the expression levels of PPAR- α and PPAR- γ in the skin lesions of AD-like

mice, although the detailed mechanism is unclear. Thus, DDH-1 may exert its anti-allergic effect by mainly targeting PPAR- α and PPAR- γ . Although Th2 cells are known to express CCR3 and CCR8 in addition to CCR4, the CCR4 ligands CCL17 and CCL22 are the most potent chemotactic factors.² In particular, CCL17 is present on skin endothelial cells under the inflammatory condition and triggers the recruitment of circulating Th2 cells into skin lesions.² At present, CCL17 is considered as a reliable marker of AD, and an ELISA-based assay for serum CCL17 is used to monitor the disease activity of

AD. In the present study, we have also found that DDH-1 reduces the expression levels of CCL17 and CCL22 in AD-like skin lesions. In this regard, it was reported that the expression of CCL17 and CCL22 in B lymphocytes and dermal endothelial cells was transcriptionally controlled by NF- κ B and AP-1.^{28,29} Thus, DDH-1 seems to inhibit the recruitment of Th2 cells through the suppression of these Th2-associated chemokine expressions by PPAR- α and PPAR- γ . Furthermore, medium chain fatty acids, including lauric acid, have been reported to be endogenous agonists for PPAR- γ and partial agonists for PPAR- α .^{30,31} Of note, DDH-1 is synthesized by introducing a laurylglycerol group into ascorbic acid, and lauric acid is included in the laurylglycerol group. Thus, DDH-1 not only induces PPAR- α and PPAR- γ expressions but also may act as an agonist for them in vivo.

Topical corticosteroids are commonly used for the treatment for a variety of inflammatory skin diseases including AD.⁷ The glucocorticoid receptor is the primary receptor for corticosteroids and belongs to the same nuclear hormone receptor family as the PPARs.^{32,33} Numerous studies have demonstrated that glucocorticoid receptors, like PPARs, can directly interact with NF- κ B and AP-1, and suppress their transcriptional activities.^{32,33} Thus, corticosteroids and PPAR agonists are likely to exert an anti-inflammatory effect by targeting similar transcription factors. In addition, some studies reported that PPAR- α agonists reversed many adverse effects associated with the use of topical corticosteroids in mouse skin.^{27,34} Co-treatment of corticosteroids with PPAR- α agonists improved the differentiation and proliferation of keratinocytes and skin barrier function in skin lesions.^{27,34} Taken together, PPAR- α agonists or inducers such as DDH-1 not only may reduce allergic inflammation but also prevent corticosteroid-induced adverse effects in skin lesions.

Topical corticosteroids are often used in combination with other medicines to reduce the risk of their adverse effects in the treatment of various inflammatory diseases.^{35,36} For example, topical corticosteroids combined with vitamin D derivatives are the first-line treatment for psoriasis.³⁷ These vitamin D derivatives have been reported to show favourable safety profiles. They have been also shown to enhance the efficacy of topical corticosteroids by reducing IL-1 β and TNF- α expression levels in the skin lesions of psoriasis.³⁷ Although we found that DDH-1 inhibited allergic skin inflammation using an AD mouse model, the therapeutic effect was relatively mild and less potent than that of corticosteroid. However, the combination treatment of DDH-1 with corticosteroid showed a more potent therapeutic effect compared with corticosteroid alone. Furthermore, the effect of the combination treatment with low-dose (2.5 mg) corticosteroid was almost the same as that of high-dose (12.5 mg) corticosteroid alone. Thus, DDH-1 may be useful for reducing the doses and adverse effects of corticosteroids. In this context, ascorbic acid is well known to have many beneficial effects, including the reduction of radicals and pigmentation, and the enhancement of collagen synthesis,³⁸ which contribute to the prevention of oxidative damage and the regulation of skin barriers. Furthermore, ascorbic acid and its derivatives are generally safe and often used as ingredients in cosmetics.³⁸ Additionally, DDH-1 has anti-inflammatory and corticosteroid-sparing effects possibly due

to the induction of PPAR- α and PPAR- γ production.^{9,10,12} Therefore, the combination of DDH-1 with corticosteroids may be considered a new option to prevent and treat various inflammatory skin diseases including AD.

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CONFLICT OF INTEREST

The authors have declared no conflicting interests.

AUTHOR CONTRIBUTIONS

KK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft. KM: Conceptualization, Data curation, Formal analysis, Project administration, Writing - review & editing. MS: Investigation, Methodology. YS: Investigation, Methodology. YH: Investigation, Methodology. TM: Resources. NO: Conceptualization, Funding acquisition. AK: Conceptualization, Funding acquisition. AO: Conceptualization, Funding acquisition. TN: Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing. All authors have read and approved the final manuscript.

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