

Effect of Eriodictyol on the Development of Atopic Dermatitis-Like Lesions in ICR Mice

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Atopic dermatitis (AD) is a chronic, allergic, and inflammatory skin disease associated with eczema and dermatitis symptoms. Our previous studies have reported that eriodictyol extract inhibits immunoglobulin E (IgE)/Ag-induced type I hypersensitivity by suppressing the activation of proinflammatory cytokines, such as interleukin-4 (IL-4), and the expression of ceramide kinase. In this study, we investigated the inhibitory effect of eriodictyol on 2,4-dinitrochlorobenzene (DNCB)-induced AD-like skin lesions in ICR mice. Treatment with 2 mg/mL eriodictyol for DNCB-induced AD-like skin lesions in ICR mice improved scratching behavior and skin severity score. Histological analysis demonstrated that thickening of the skin lesions were significantly reduced in the eriodictyol-treated group. Also, eriodictyol suppressed the DNCB-mediated elevation of IgE serum levels. These results suggest that eriodictyol may be a potential therapeutic resource for AD and an adjunctive agent to control itchiness in AD.

Key words atopic dermatitis; eriodictyol; skin severity; scratching behavior

The rate of atopic dermatitis (AD) has been increasing recently in many industrialized countries and its occurrence is usually during early infancy and childhood, but can also be in adulthood.^{1,2)} AD is a chronic, relapsing, and inflammatory skin disease in humans and is associated with eczematous symptoms and immunoglobulin E (IgE) hyperproduction. AD is caused by a complex interrelationship among genetic, immunologic, and skin barrier dysfunction factors.^{3–5)}

The earliest event involved in the pathogenesis of AD is considered to be a disruption of skin barrier function after scratching. Itching is a serious problem in AD patients because scratching worsens the dermatitis.⁶⁾ Reduction of itching-associated scratching is the most effective therapeutic strategy for improving the quality of life for AD patients.

AD is associated with the paradigm of an allergic T helper (Th) 2-mediated disease, characterized by abnormal IgE production, peripheral eosinophilia, and mast cell activation as well as upregulation of Th2 and Th1 cytokines in chronic skin lesions.^{7,8)}

Topical steroids and immunosuppressive agents have been standard treatments for severe cases of AD. However, many patients are still worried about the long-term use of these agents and their potential adverse effects.^{9–11)} Therefore, there is a great need for the development of new and effective therapies for AD.

Eriodictyol is a unique constituent of the painted maple (*Acer mono*) and yerba santa (*Eriodictyon californicum*). Pharmacological activities attributed to eriodictyol include the control of blood vessel permeability in arthralgia and fracture, as well as antioxidant and antimicrobial effects.^{12,13)}

Recently, our previous studies demonstrated that eriodictyol inhibits IgE-mediated allergic responses by blocking degranulation associated with ceramide kinase. Also, we showed that eriodictyol inhibits anaphylactic shock in an animal model and blocks the release of inflammatory cytokines such as interleukin-4 (IL-4) in mast cells.¹⁴⁾

Although eriodictyol has been reported by bioactivity assays, the effect of eriodictyol as an anti-atopic agent for AD remains unclear. In this study, we investigated the inhibitory effect of eriodictyol on the development of AD in male ICR mice. The efficacy of eriodictyol was tested by scratching behavior, skin severity score, blood IgE level, and histopathological examination. Histopathologic analysis revealed thickening of the epidermis in AD animals. We found that eriodictyol suppressed scratching behavior, skin severity score, and blood IgE level along with reducing the thickness of the epidermis. These results suggest that eriodictyol may be a useful natural resource for use as an anti-atopic dermatitis agent.

MATERIALS AND METHODS

Animals and Treatment Eriodictyol were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Male ICR mice (aged 6 weeks) were purchased from Orient Bio (Gangneung, Korea) and housed in wire cages at 20–22°C and at a relative humidity of 40–50%. All animals were given access to standard laboratory chow and water *ad libitum*. The Institutional Animal Care and Use Committee (IACUC) at Yonsei University (Wonju, Korea) approved the protocol for this study. The mice were divided into 3 groups ($n=6$ per group). To induce AD-like skin lesions, 1% 2,4-dinitrochlorobenzene (DNCB) was applied to the dorsal skin of mice in 7.5 cm² area. After complete removal of dorsal hairs, the 250 μ L of 1% DNCB solution (dissolved in a 3:1 mixture of acetone and olive oil) was applied on the first day and sensitization occurred for 4 d. Four days after sensitization, 7.5 cm² area of the dorsal skin was challenged with 200 μ L of 0.5% DNCB solution, repeatedly, for 2 weeks. After inducing AD, the 200 μ L of 2 mg/mL eriodictyol solution (dissolved in a 3:1 mixture of acetone and olive oil) was applied to the DNCB-induced dorsal skin of the mice a total of 6 times during 2 weeks. Control and DNCB-treated mice were treated with the 3:1 mixture of acetone and olive oil without eriodictyol on the dorsal skin. Animals were sacrificed 18 d after the first application of DNCB.

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Evaluation of Skin Dermatitis Severity The severity of dermatitis in the dorsal skin lesions was evaluated after treatment with eriodictyol. To ensure the reliability of skin severity test, two people who do not know about the exact group of mice did blind test and the test was double-checked. The evaluated symptoms consisting of (1) erythema, (2) pruritus and dry skin, (3) edema and excoriation, (4) erosion, and (5) lichenification were scored as follows: none=0; mild=1; moderate=2; severe=3. The sum of the scores for each evaluated symptom (maximum score: 15) was considered as the skin severity score.¹⁵⁾

Evaluation of Scratching Behavior Each mouse was housed individually in a pure plastic cage. After initial sensitization, we made a video to record the behavior patterns of the mice during 2 weeks and their behavior was watched for 10 min after video recorded. We count the numbers of scratching behavior in video and multiply that by 6. Two people who do not know about the exact group of mice did blind test and it was double-checked. Scratching of the rostral back and biting of the caudal back were observed. Also the scratching movements by the hind paw was defined as a scratching bout which ended when the mice either licked its hind paw or placed its hind paw back on the floor and a series of one or more biting movements were counted as one episode which ended when the mouse returned to the straight-forward position.¹⁶⁾

Histopathological Studies The dorsal skin was isolated from each mouse, fixed in 4% paraformaldehyde solution, and stabilized in 30% sucrose solution. It was subsequently embedded in Tissue-Tek O.C.T Compound (Sakura, U.S.A.), sectioned, and stained with hematoxylin and eosin. Histological analysis was used by light microscopy. Dorsal skin thickness was measured with a micrometer (NIKON ECLIPSE TE2000-U, ACT-1 for DXM 1200, Japan).

Measurement of Blood IgE Content IgE level in the blood serum was measured by sandwich enzyme-linked immunosorbent assay (ELISA) using the mouse IgE kit from BioLegend (U.S.A.), according to the manufacturer's instructions. IgE concentrations were calculated using a linear regression equation obtained from standard absorbance values.

Statistical Analysis Experimental results were expressed as mean±S.D. One-way analysis of variance (ANOVA) was followed by Dunnett's test for multiple comparisons. *p* values of <0.05 and <0.01 were considered statistically significant, as indicated.

RESULTS

Effect of Eriodictyol on Skin Severity Score of DNCB-Induced ICR Mice The repeated application of DNCB for 18 d in mice induced dermatitis symptoms of erythema, pruritus, dryness, edema, excoriation, erosion, and lichenification (Fig. 1A). The skin conditions were evaluated every other day for 18 d using the dermatitis severity score. As shown in Fig. 1B, repeated topical application of DNCB increased the clinical severity scores in ICR mice. However, the application of eriodictyol inhibited these symptoms of AD.

Effect of Eriodictyol on Scratching Behavior of DNCB-Induced ICR Mice To measure the patterns of mice activities, we made a video recording every day during 2 weeks. Scratching behavior was evaluated by the number of relevant

behaviors observed, such as scratching dorsal parts with hind paws or biting the dorsal skin. As shown in Fig. 2, repeated topical application of DNCB increased the scratching behavior on ICR mice. However, the application of eriodictyol inhibited scratching behaviors.

Effect of Eriodictyol on Thickening of the Epidermis and Dermis of DNCB-Induced ICR Mice The histopathological features of the dorsal lesions in DNCB-induced ICR mice are shown in Fig. 3A. On day 17 after the initial sensitization, it was found that dorsal lesions showed thickening of the dermis and epidermis in DNCB-treated mice compared with the control mice. On the other hand, the thickening of dorsal skin was reduced by application of eriodictyol so that it was more similar to the skin of the control mice (Fig. 3B). These results indicate that eriodictyol effectively decreases AD symptoms in ICR mice.

Effect of Eriodictyol on IgE Level of DNCB-Induced ICR Mice We previously demonstrated that eriodictyol inhibits IgE-mediated allergic responses by blocking degranulation of mast cells.¹⁴⁾ Mast cells are known as key effector cells in IgE-mediated allergic disorders.^{1,2)} An increase in serum IgE levels is an important component of AD.¹⁵⁾ Repeated application of DNCB increased IgE levels in serum. However, treatment of eriodictyol effectively restored the levels of serum IgE (Fig. 4).

DISCUSSION

Mast cells, which have high-affinity receptors for IgE (FcεRI), are significantly increased in AD skin lesions as compared with healthy skin.¹⁷⁾ Activated mast cells release a variety of biologically active substances that play important roles in allergic reactions, such as those of AD.¹⁸⁾ However, the effect of eriodictyol on AD-like skin dermatitis had remained unclear. Therefore, in this study, we investigated the effects of eriodictyol on AD-like skin lesions induced by DNCB in ICR mice.

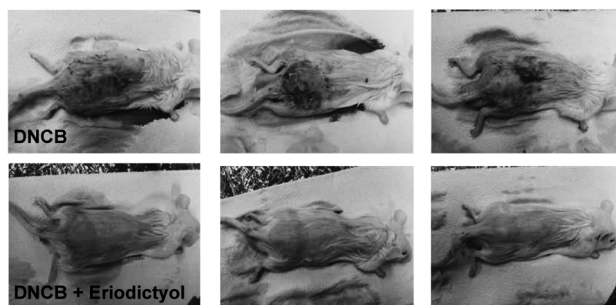
AD is an increasingly common inflammatory skin disorder.^{19,20)} There is currently much research being done to investigate the cause of AD and new treatments are being developed. However, the agents being used for treatment, such as steroids and anti-histamine group drugs, can have severe side effects which limit their clinical applications. Because of these problems, there is a need for natural resources that are relatively more stable than chemical agents like steroids.

Experimental animal models of AD use DNCB treatment to simulate the clinical features of human AD-like skin symptoms, such as erythema, pruritus, dryness, edema, excoriation, erosion, and lichenification.²¹⁾ Eriodictyol treatment suppresses the AD-like skin symptoms induced by DNCB, indicating its therapeutic potential for human AD-like skin symptoms. DNCB is an extensively studied animal model of AD and is preferred due to its reproducibility and repeatability.^{22,23)}

In this study, we investigated the inhibitory effect of eriodictyol on DNCB-induced AD-like skin lesions in ICR mice. We separated the groups into control, AD-like model, and eriodictyol treatment groups.

Histopathological analysis indicated that dorsal lesions showed thickening of the dermis and epidermis in DNCB-treated mice compared with the control mice. However, the thickening of the dorsal skin was reduced by the application of

A



B

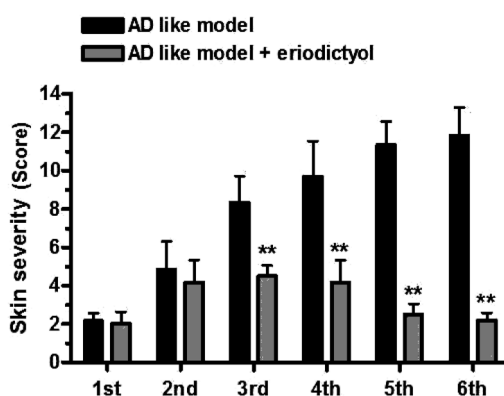


Fig. 1. Effect of Eriodictyol on Skin Severity Score of DNCB-Induced ICR Mice

(A) Effect of eriodictyol on DNCB-induced atopic dermatitis-like model in ICR mice. After inducing AD, 2mg/mL eriodictyol solution (dissolved in a 3:1 mixture of acetone and olive oil) was applied to the dorsal skin of the mice a total of 6 times during 2 weeks. (B) Effect of eriodictyol on skin severity score. The skin severity of mice was assessed macroscopically in a blinded fashion as mentioned in Materials and Methods. The development of AD-like skin dermatitis was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). The sum of the individual scores was defined as the dermatitis score. Data are expressed as mean $\pm$ S.D. ($n=6$). ** $p<0.01$.

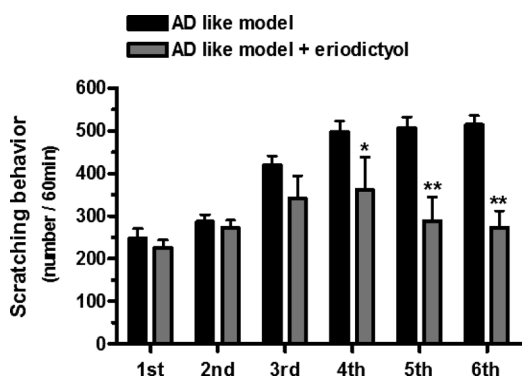


Fig. 2. Effect of Eriodictyol on Scratching Behavior of DNCB-Induced ICR Mice

The scratching behavior of each mouse was videotaped for 60min after sensitization. Biting of the dorsal skin and scratching with hind paws were observed during play back. Licking of the belly and dorsal skin during grooming was disregarded. Data are expressed as mean $\pm$ S.D. ($n=6$). * $p<0.05$, ** $p<0.01$.

eriodictyol (Fig. 3).

In the scratching behavior test and measurement of skin severity score test, we found that repeated application of DNCB increased scratching behavior in ICR mice, but the application of eriodictyol suppressed scratching behavior (Fig. 2). Furthermore, repeated application of DNCB increased clinical severity scores in ICR mice, but the application of eriodictyol

inhibited these symptoms and clinical severity scores of AD (Fig. 1).

Several studies have demonstrated that serum IgE levels are elevated in patients with AD.¹⁵⁾ In the progression of AD, T helper type 2 (Th2) responses with expression of the blood IgE, eosinophilia, and low-level Th2 cytokines are increased.²⁴⁾ Because of this observation, elevated IgE level is considered to be a hallmark of AD. In this study, eriodictyol suppressed the DNCB-mediated elevation of IgE serum levels (Fig. 4). In our previous report, we demonstrated that eriodictyol has anti-allergic effects. Eriodictyol significantly blocked degranulation in mast cells associated with ceramide kinase. It also inhibited anaphylactic shock in animal models and blocked the release of inflammatory cytokines such as IL-4 in mast cells.¹⁴⁾ Therefore, we believe that eriodictyol suppress the blood IgE and eosinophilia by blocking the allergic activity through inhibition of ceramide kinase and IL-4 expression.

In conclusion, this study demonstrated that topical application of eriodictyol suppressed the development of AD-like skin lesions in ICR mice. Treatment with eriodictyol for DNCB-induced AD-like skin lesions improved scratching behavior, skin severity score, and IgE level. Histological analysis demonstrated that thickening of the skin lesions was significantly reduced in the eriodictyol group. Our results indicate that eriodictyol treatment could provide an effective alternative therapy for the management of AD.

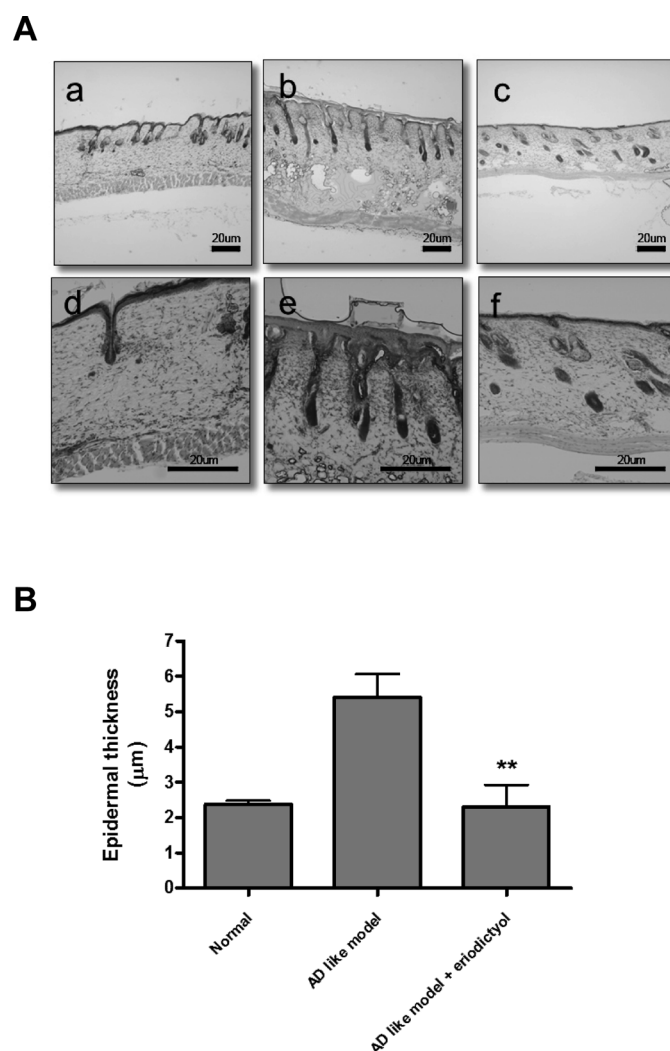


Fig. 3. Effect of Eriodictyol on Thickening of the Epidermis and Dermis of DNCB-Induced ICR Mice

(A) Effect of eriodictyol on histopathological features of the dorsal lesions in DNCB-induced ICR mice. Dorsal skins were removed and fixed with 4% paraformaldehyde solution. To stabilize skins, they were soaked in 30% sucrose solution overnight. (a,d) untreated group, (b,e) group treated with DNCB alone, (c,f) group treated with eriodictyol (2mg/mL) six times after treatment with DNCB. a, b, c: $\times 40$, d, e, f: $\times 100$. (B) Effect of eriodictyol on thickening of the epidermis of DNCB-induced ICR mice. Dorsal skin thickness was measured with a micrometer. Data are expressed as mean $\pm$ S.D. ($n=6$). ** $p<0.01$.

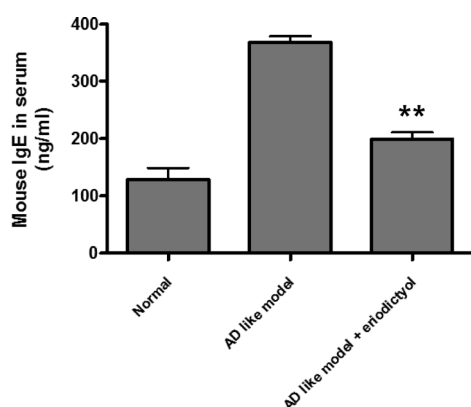


Fig. 4. Effect of Eriodictyol on IgE Level of DNCB-Induced ICR Mice

Serum was collected immediately before sacrifice of the mice. IgE level in the blood serum was measured as mentioned in Materials and Methods. Data are expressed as mean $\pm$ S.D. ($n=3$). ** $p<0.01$.

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