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Note

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Nodakenin, derived from the roots of *Angelica gigas Nakai*, is an important natural resource and medicinal material with anti-allergic and anti-inflammatory activities. We have previously shown that nodakenin inhibits IgE/Ag-induced degranulation in mast cells. In this study, we investigated the inhibitory effect of nodakenin on 2,4-dinitrochlorobenzene (DNCB)-induced atopic dermatitis (AD)-like skin lesions in ICR mice. Scratching behavior, skin severity score, blood IgE level, and skin thickness were improved in DNCB-induced AD-like ICR mice. Our results showed that nodakenin suppressed the increase of AD-like skin lesions in ICR mice. These results suggest that nodakenin may be a potential therapeutic resource for AD as well as an adjunctive agent to control itching associated with AD.

Key words: atopic dermatitis (AD); nodakenin; skin severity; scratching behavior

Atopic dermatitis (AD) is a allergic-inflammatory skin disease involving complex interactions of the innate and adaptive immune responses based on environmental, pharmacological, and psychological predisposition and triggered by environmental factors.^{1–3} AD affects approximately 10 million people worldwide, and its incidence is continuously increasing in many countries.^{4,5} Despite ongoing development of drugs aimed at the treatment of AD, steroids remain the primary drug for AD therapy. Because long-term administration of steroids is associated with a variety of side effects, there is a significant need for new drugs to treat AD.^{6,7}

Nodakenin, a coumarin isolated from the roots of *Angelica gigas*, has been reported to possess neuroprotective, anti-aggregatory, antibacterial, anti-inflammatory, and memory-enhancing effects.⁸ However, the anti-AD activity of nodakenin has not been evaluated. Our previous studies demonstrated that

nodakenin inhibits IgE-mediated allergic responses by blocking degranulation. In addition, we showed that nodakenin inhibits anaphylactic shock in an animal model and blocks the release of inflammatory cytokines such as IL-4 and TNF- α in mast cells.⁹ With this goal in mind, we examined the inhibitory effects of nodakenin on DNCB-induced AD in ICR mice.

We first investigated the effects of nodakenin on AD-like skin lesions in ICR mice. 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 at Yonsei University (Wonju, Korea) approved the protocol for this study. Nodakenin was kindly donated by Dr Sang Hyeon Sung (Seoul National University, Korea).¹⁰

Mice were divided into three groups (n=6 per group). For the induction of AD-like skin lesions, DNCB was applied to the dorsal skin of mice. DNCB was used on the basis of evidence of its efficacy in creating AD-like skin lesions and IgE hyperproduction in ICR mice.^{11–14} After shaving the dorsal hair of mice, 250 μ L of 1% DNCB solution (dissolved in a 3:1 mixture of acetone and olive oil) was applied to a 7.5 cm² area and sensitization occurred for 4 days. Next, the 7.5 cm² area of sensitized dorsal skin was repeatedly challenged with 200 μ L of a 0.5% DNCB solution for 2 weeks. After inducing AD, 200 μ L of a 2 mg/mL nodakenin solution (dissolved in a 3:1 mixture of acetone and olive oil) was applied to the AD-like dorsal skin of the mice, a total of six times over a two-week period. Control and DNCB-treated mice were treated with the 3:1 mixture of acetone and olive oil without nodakenin on the dorsal skin. Animals were sacrificed 18 days after the first application of DNCB. As shown in Fig. 1(A), repeated topical application of DNCB significantly increased dermatitis symptoms compared with control mice. Specifically, AD induced by repeated application of DNCB resulted in dermatitis symptoms

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Abbreviations: AD, atopic dermatitis; DNCB, 2,4-dinitrochlorobenzene.

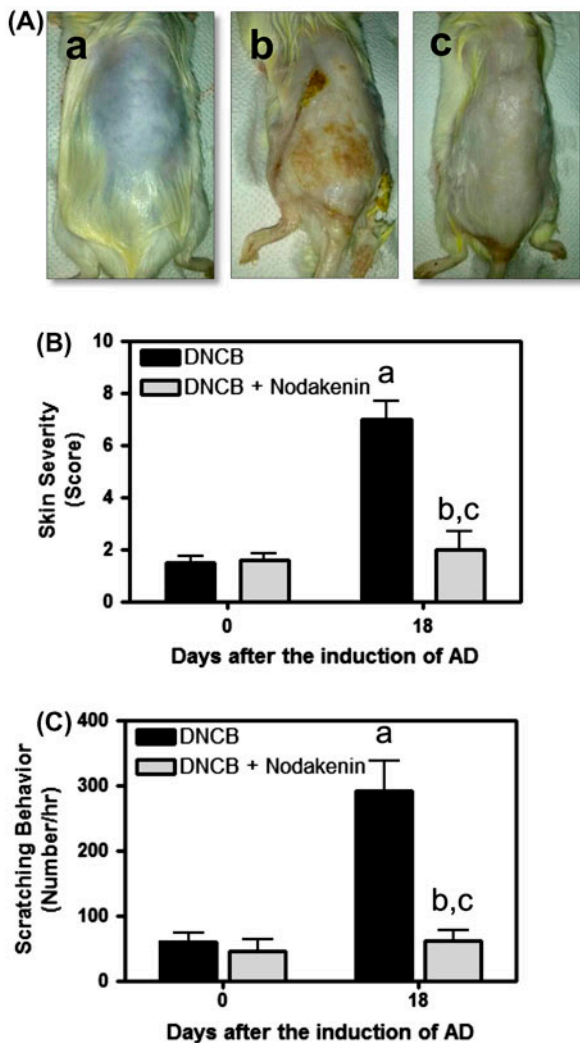


Fig. 1. Effect of nodakenin on skin lesions, skin severity scores, and scratching behavior of DNCB-induced AD-like ICR mice.

Notes: (A) Effect of nodakenin on DNCB-induced AD-like skin lesions in ICR mice. After inducing AD, a 2 mg/mL nodakenin solution (dissolved in a 3:1 mixture of acetone and olive oil) was applied to the dorsal skin of mice, a total of six times over a two-week period. (a) Control group, (b) group treated with DNCB alone, and (c) group treated with nodakenin (2 mg/mL) six times after treatment with DNCB. (B) Effect of nodakenin on skin severity scores of DNCB-induced AD-like ICR mice. The skin severity of mice was assessed macroscopically in a blinded fashion. The extent of AD-like skin dermatitis was scored as follows: 0 (none), 1 (mild), 2 (moderate), or 3 (severe). The sum of individual scores was defined as the dermatitis score. (C) Effect of nodakenin on scratching behavior in DNCB-induced AD-like ICR mice. The scratching behavior of each mouse was videotaped for 60 min after sensitization. Biting of the dorsal skin and scratching with hind paws were observed during a 10 min play back. Licking of the belly and dorsal skin during grooming was disregarded. Data are expressed as mean $\pm$ SD ($n=6$). a: $p < 0.001$ compared to DNCB alone treated group at 0 day, b: $p < 0.001$ compared to DNCB alone treated group at 18 days, and c: no detection compared to DNCB alone treated group at 0 day.

such as severe erythema, hemorrhage, and edema on the dorsal skin. Mice treated with nodakenin decreased dermatitis symptoms after 18 days as compared with DNCB-treated mice. Thus, the symptoms of AD were reduced by administration of nodakenin.

The severity of dermatitis in dorsal skin lesions was evaluated after treatment with nodakenin. To ensure the reliability of skin severity tests, two individuals who

were blinded to the treatment groups independently assessed each mouse. The extent of AD-like skin dermatitis was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). The sum of individual scores was defined as the dermatitis score.¹⁵⁾ Experimental results are expressed as the mean $\pm$ S.D. One-way or two-way analysis of variance (ANOVA) was used for multiple comparisons followed by *post hoc* (Tukey-Kramer) test. SPSS statistical software, Version 18.0 (SPSS, Chicago, IL) was used for statistical analysis. p values of <0.05 were considered statistically significant, as indicated.

As shown in Fig. 1(B), repeated topical application of DNCB increased clinical severity scores of ICR mice. However, application of nodakenin inhibited the symptoms of AD. Stimulation of ICR mice with DNCB potentially increased the skin severity score from 1.5 ± 0.5 to 7.0 ± 1.6 at 18 days. Nodakenin significantly inhibited the skin severity scores of DNCB-induced AD-like ICR mice by 2.0 ± 1.6 at 18 d.

To determine mice activity patterns, we recorded videos each day during the 18-day treatment period. Each mouse was individually housed in a pure plastic cage. After initial sensitization, videos were made to record the behavior patterns of the mice for a total of 60 min for 2 weeks. Scratching behavior was observed for 10 min after videotaping. The number of times scratching behavior was displayed in the video was counted and multiplied by 6. Scratching movements by the hind paws were considered as a scratching behavior episode, and were considered to end when a mouse either licked its hind paw or placed its hind paw back on the floor. Likewise, a series of one or more biting movements were counted as one episode, which was considered to end when the mouse returned to a straight-forward position. Scratching of the rostral back and biting of the caudal back were also observed. Two individuals who were blinded to the treatment groups independently assessed the videos for each mouse.

As shown in Fig. 1(C), repeated topical application of DNCB increased scratching behavior of ICR mice. Treatment with nodakenin suppressed scratching behaviors. Stimulation of ICR mice with DNCB potentially increased scratching behavior from 60.0 ± 32.2 to 291.0 ± 113.0 number/hr at 18 days. Nodakenin significantly inhibited the scratching behavior of DNCB-induced AD-like ICR mice by 61.0 ± 38.4 number/hr at 18 days.

Histological examination of AD typically shows dermal infiltration with eosinophils and mononuclear cells prior to the appearance of clinical symptoms.¹⁶⁾ The dorsal skin was removed from each mouse, fixed in a 4% paraformaldehyde solution, and stabilized with 30% sucrose solution. The prepared skin tissue was subsequently embedded in Tissue-Tek O.C.T Compound (Sakura, USA), sectioned, and stained with hematoxylin and eosin. Histological analysis was performed by light microscopy. Fig. 2 shows the histopathological features of dorsal lesions obtained from DNCB-treated ICR mice.

On day 18, dorsal lesions from DNCB-treated mice exhibited dermal and epidermal thickening, as well as accumulation of inflammatory cells compared with control mice. The effects of DNCB were inhibited by the application of nodakenin, indicating that nodakenin effectively decreased AD symptoms in ICR mice.

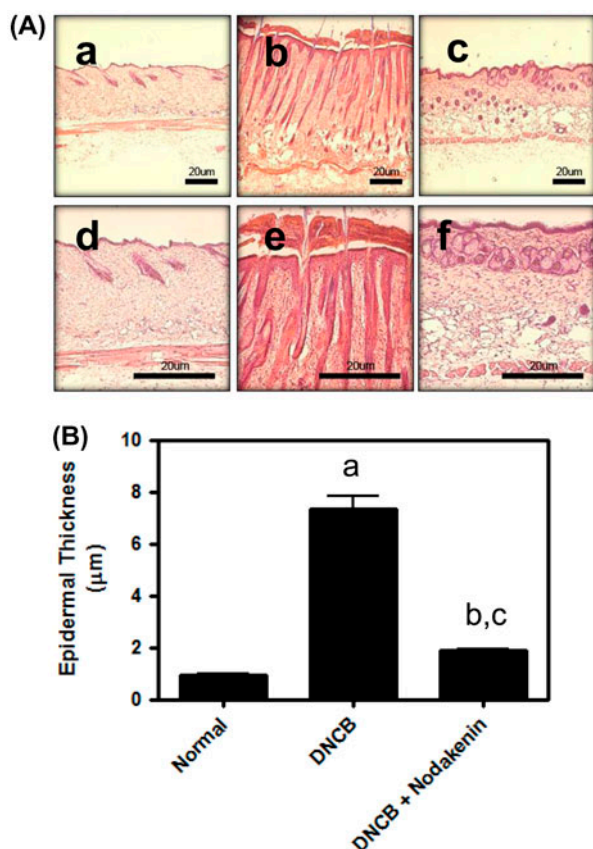


Fig. 2. Effect of nodakenin on thickening of the epidermis and dermis in DNCB-induced AD-like ICR mice.

Notes: (A) Histological analysis was performed by light microscopy. (a,d) Untreated group, (b,e) group treated with DNCB alone, and (c,f) group treated with nodakenin (2 mg/mL) six times after sensitization with DNCB. (a,b,c) X40, (d,e,f) X100. (B) Effect of nodakenin on thickening of the epidermis of DNCB-induced AD-like ICR mice. Dorsal skin thickness was measured with a micrometer (NIKON ECLIPSE TE2000-U, ACT-1 for DXM 1200, JAPAN). Data are expressed as mean $\pm$ SD ($n=6$). a: $p < 0.001$ compared to normal group, b: $p < 0.001$ compared to DNCB alone treated group, and c: no detection compared to normal group.

We previously demonstrated that nodakenin inhibits IgE-mediated allergic responses by blocking degranulation of mast cells.⁹⁾ The IgE level is associated with the severity of AD and contributes to skin barrier abnormality, a key feature of AD. The functions of IgE in allergic inflammation suggest that IgE-mediated activation of mast cells and eosinophils contribute to AD.^{17,18)} Furthermore, an increase in blood serum IgE levels is an important component of AD.^{11,13)} To test the effect of nodakenin on IgE levels in a model of AD-like disease, we collected serum from the blood of mice on the day of sacrifice. Blood serum levels of IgE were measured by sandwich ELISA using a Mouse IgE kit (BioLegend) according to the manufacturer's instructions. IgE concentrations were calculated using a linear regression equation obtained from standard absorbance values. Repeated application of DNCB increased serum IgE levels, while treatment with nodakenin decreased DNCB-increased levels of blood serum IgE effectively. Stimulation of ICR mice with DNCB potently increased IgE levels from 165.1 ± 27.9 to 326.3 ± 47.7 ng/mL at 18 days. Nodakenin significantly inhibited the IgE

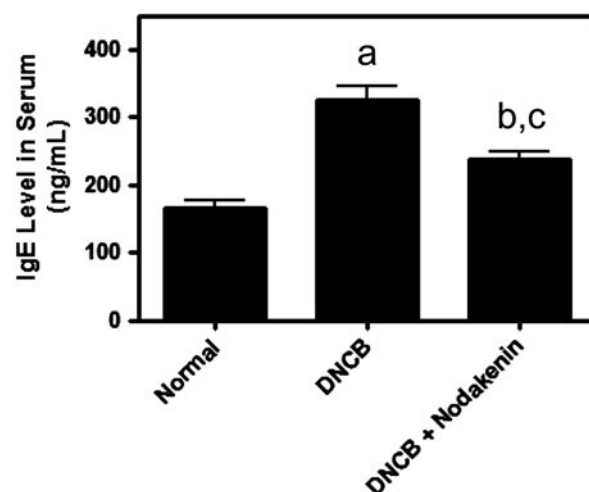


Fig. 3. Effect of nodakenin on blood serum IgE levels of DNCB-induced AD-like ICR mice.

Notes: Serum was collected immediately before sacrifice. Blood serum levels of IgE were measured by sandwich ELISA using a mouse IgE kit according to the manufacturer's instructions. Data are expressed as mean $\pm$ SD ($n=6$). a: $p < 0.001$ compared to normal group, b: $p < 0.01$ compared to DNCB alone treated group, and c: $p < 0.05$ compared to normal group.

level of DNCB-induced AD-like ICR mice by 237.8 ± 27.2 ng/mL at 18 days (Fig. 3).

Together, our results showed that nodakenin suppressed the increase of AD-like skin lesions in ICR mice. Mice with DNCB-induced AD-like symptoms, treated with nodakenin, exhibited improved scratching behavior as well as decreased skin severity scores and blood serum IgE levels. In addition, skin thickness was significantly recovered in the group treated with nodakenin compared with DNCB-induced AD-like ICR mice. Our results suggest that nodakenin may be a useful natural anti-AD agent.

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