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Irradiation with 310 nm and 340 nm ultraviolet light-emitting-diodes can improve atopic dermatitis-like skin lesions in NC/Nga mice†

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Ultraviolet (UV) light produces an immunomodulatory effect on the skin and is widely used for the treatment of chronic inflammatory skin diseases. UV light emitting diodes (UV-LEDs) are a new and promising source of UV radiation. However, their mechanism of action remains largely unknown. In this study, we tested the safety and effectiveness of UV-LED irradiation for the treatment of atopic dermatitis (AD) in an NC/Nga mouse model. Mice were divided into seven groups of eight mice each. Application of *Dermatophagoides farinae* (Df) extract ointment for four weeks induced AD-like skin lesions. Subsequently, the mice were exposed to UV-LEDs, narrow band UVB, or UVA irradiation three times per week. We assessed the immunosuppressive effects of 310 nm (50 mJ cm⁻²) and 340 nm (5 J cm⁻²) UV-LED irradiation. Histological analyses using hematoxylin–eosin, toluidine blue, and immunohistochemical staining were performed. In addition, the serum levels of IgE, inflammatory cytokines and chemokines were measured using enzyme-linked immunosorbent assays (ELISAs). UV-LED irradiation significantly alleviated AD-like skin symptoms, including edema, erythema, dryness, and itching, by modulating Th1 and Th2 responses, transepidermal water loss (TEWL), and scratching behavior in NC/Nga mice. These results suggest that UV-LEDs can improve the treatment of inflammatory skin diseases.

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Introduction

Atopic dermatitis (AD) is a chronic, itchy skin disease that is common in children, but can occur at all ages.¹ Although AD is the most common inflammatory skin disease, only a few therapeutic options exist for these patients (*i.e.*, oral steroids), none of which are completely satisfactory.² Although glucocorticoids are often effective, they cannot be used consistently due to serious side effects.³ New drug forms, such as oral cyclosporine, are equally effective, but are often limited by severe undesirable effects.⁴ Also, dupilumab, a fully human monoclonal antibody aimed against IL-4R α , blocks binding of IL-4 and IL-13 to the receptor.⁵ The result of these studies was

a fast, dose-dependent improvement of skin condition as measured by the Eczema Area and Severity Index (EASI) and the pruritus score.⁶ However, little is known about its potential ocular adverse effects. Therefore, continuous efforts to develop new treatment modalities are necessary.

Ultraviolet (UV) phototherapy is commonly used to treat refractory skin diseases.⁷ UV light wavelengths are classified as UVC (100 to 280 nm), UVB (280 to 315 nm), and UVA (315 to 400 nm).⁸ The most common type of phototherapy used to treat AD is narrowband (NB) ultraviolet B (UVB) light.⁹ Fluorescent lamps emitting NB wavelengths between 311 and 313 nm (NB-UVB) are used in the treatment of refractory skin diseases (psoriasis vulgaris and vitiligo vulgaris), and those emitting a wavelength range of 340–400 nm (UVA1) are used for AD treatment.¹⁰ The safety of NB-UVB light for treating skin diseases has been proven.¹¹ PUVA is a combination treatment that consists of psoralens (P) with a subsequent exposure of the skin to UVA, while NB-UVB treatment uses only ultraviolet light and therefore is not associated with systemic toxicity and allows for treatment at low cost.¹² In patients with psoriasis and AD, UVB irradiation up-regulated regulatory T (Treg)-cell levels and showed immunosuppressive effects.¹³ Therefore, NB-UVB light is effective for the treatment of

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various skin diseases that exhibit a strong immune response. In addition, previous studies have investigated the combined phototherapeutic effects of UVA and UVB.^{14,15}

However, some disadvantages of using fluorescent light as a light source have been reported: (1) a short lifespan and presence of toxic substances, (2) unwanted exposure, and (3) heat dissipation from the light source.¹⁶ To address these issues, newly developed devices based on Light Emitting Diodes (LEDs) should be considered for UVA and UVB irradiation. Some of the most promising applications of UV-LEDs include water disinfection,¹⁷ detection,¹⁸ medication,¹⁹ and plant growth illumination.²⁰ UV-LEDs are a promising source of UV radiation with several advantages over conventional light sources, such as high- and medium-pressure mercury and xenon lamps.²¹ UV sources mainly depend on the strong overlap between the spectral emission of the light source and the wavelengths required by the application, efficient production of the required intensity dose, LED life expectancy, and ease of maintenance. In particular, compared to advanced light sources, such as excimer lasers, UV-LEDs enable the development of devices for the treatment of skin diseases at home.²² This, in turn, cuts down the treatment cost and makes regular treatment more convenient even though the underlying biological mechanism of action induced by UV-LEDs is unclear.

We previously reported important results showing that *in vitro* data combining 310 nm plus 340 nm UV-LED irradiation synergistically reduced adhesion molecules with TNF- α /IFN- γ in HaCaT keratinocytes.²³ Therefore, we propose that combined phototherapy (310 nm and 340 nm) can be a therapeutic strategy for the treatment of AD. The main aims of the current study were to evaluate the mechanisms of action, efficacy, and safety of UV-LED irradiation in NC/Nga mice with AD. As a result, we demonstrated that UV-LEDs were important in reducing the expression of cytokines, chemokines, and adhesion molecules. Therefore, UV-LED irradiation may be useful as a phototherapy for the prevention and treatment of AD.

Materials and methods

Animals and induction of AD-like skin lesions

Six-week-old male NC/Nga mice were obtained from Central Laboratory Animal Inc. (Seoul, Korea) and housed in an air-conditioned room maintained at 24 ± 2 °C and $55 \pm 15\%$ humidity with a 12 h light-dark cycle. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Chung-Ang University and were approved by the Animal Ethics Committee of Chung-Ang University IACUC (approval no. 2018-00019). After the mice were anesthetized with Zoletil 50 (Virbac Laboratories, Carros, France) (50 mg kg⁻¹) and xylazine (10 mg kg⁻¹), their fur was shaved from their dorsal surface using an electric mouse clipper. To disrupt the skin barrier, 200 μ L of 4% sodium dodecyl sulfate (SDS) was applied to the dorsal skin and ears

of mice using sterilized tips 6 h before application of the Df extract (100 mg per mouse) (Biostir, Inc., Hyogo, Japan). These treatments were repeated two times each week for four weeks.²⁴ After applying Df, mice were exposed to UV-LED (310 and 340 nm), NB-UVB, or UVA irradiation three times per week. Mice were irradiated using a UV-LED device with an *in vivo* test module (160 $\times$ 200 mm). Irradiation using the 310 nm UV-LED for *in vivo* experiments had an intensity of 80 μ W cm⁻² at a distance of 20 cm and was applied three times a week for 10 min 40 s with 50 mJ cm⁻² of total energy delivered per exposure. Irradiation using the 340 nm UV-LED for *in vivo* experiments had an intensity of 3.4 mW cm⁻² at 20 cm and was applied three times a week for 25 min with 5 J cm⁻² total energy delivered per exposure. Combined irradiation treatments used UVB (310 nm at 5 J cm⁻²) followed by UVA (340 nm at 50 mJ cm⁻²). Irradiation using the NB-UVB Handisol for *in vivo* experiments had a wavelength of 311–313 nm and a total energy of 630 mJ cm⁻² at 2–3 cm. Application occurred three times a week for 2 min per exposure. Irradiation using the Bio-Spectra system (Bio-Spectra, Vilber Lourmat, Marne-La-Vallée, France) used for *in vivo* experiments had a wavelength of 365 nm (TF-20L), an intensity of 2.8 mW cm⁻² at 20 cm, and was applied 3 times a week for 30 min with 5 J cm⁻² total energy delivered per exposure. Mice were randomly assigned to seven groups ($n = 8$ per group): G1, Normal; G2, Control (Df-sensitized only); G3, 310 nm; G4, 340 nm; G5, 340 plus 310 nm; G6, NB-UVB; and G7, UVA.

Clinical severity score and ear thickness measurement

Dermatitis severity was assessed by scoring the clinical signs and symptoms as previously described.²⁵ The total clinical severity score, 0 (none), 1 (mild), 2 (normal), and 3 (severe), was determined for each of the five dermatitis symptoms, which included itching, erythema/bleeding, edema, excoriation/erosion, and scaling/drying. Ear thickness was measured for 35 days using dial calipers (Mitsutoyo, Tokyo, Japan).

Scratching behavior test

Scratching behavior was monitored and the number of scratching episodes for 28 or 35 days was calculated (with a slight modification) as previously described.²⁵ Briefly, after one minute of therapeutic irradiation, the mice were placed in a transparent cage. The number of scratching episodes over a 30 min time period was scored from 0–4: 0 (no scratches), 2 (scratches less than 1.5 seconds), and 4 (scratches longer than 1.5 seconds). The total scratching behavior count was calculated for each mouse as the sum of each score.

Transepidermal water loss measurement

The amount of TEWL was measured using a Tewameter TM300 (Courage and Khazaka Electronic GmbH, Köln, Germany) at 0, 28, and 35 days. Values were recorded only after signal stabilization, which occurred approximately 10 s after the probe was placed on the skin. Excluding the initial results, only averages with minimum deviations were recorded.

Measurement of inflammatory cytokine levels

Mice were anesthetized and their eyes bled at 35 days. Sera were stored at -80°C . IgE, IL-1 β , IL-4, IL-6, IL-33, and MCP-1/CCL2 levels were measured in mouse sera using an Enzyme-Linked Immunosorbent Assay (ELISA) (eBioscience) in serial, two-fold dilutions for treated or control mice as indicated by the manufacturer. The analysis was performed according to the manufacturer's instructions.

Histopathological analysis

For histological studies, mice were sacrificed 35 days after the start of the experiment. Skin specimens were fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 5 μm -thick sections. These sections were stained with hematoxylin and eosin (H&E) and toluidine blue, in accordance with the established procedures for the histological analysis of skin. Skin specimens were also stained using antibodies for immunohistological analysis according to a previous published method.²⁵ Sections were stained with monoclonal antibodies against ICAM-1 (1 : 200, ab2213, Abcam, Cambridge, MA, USA), E-selectin (1 : 200, NBP1-45545, Novus Biologicals, Abingdon, UK), or IL-31 (1 : 100, ab102750, Abcam, Cambridge, MA, USA) as described previously.²⁴

Statistical analysis

The results are expressed as mean $\pm$ standard error (SE) ($n = 8$). For direct comparison between specific *in vivo* study groups, statistical significance was determined using a one-way ANOVA, followed by a *post-hoc* Tukey test using SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Alleviation of atopic dermatitis-related symptoms using UV-LEDs

To investigate the inhibitory effects of UV-LED irradiation on AD-like skin lesions in the animal model, the dorsal skin and ear symptoms of mice were scored using the criteria established for the major clinical symptoms of AD.²⁶ Clinical severity scores significantly increased with time and reached more than 10 points in the control group (Df-ointment only) compared to those in the normal group (Fig. 1A).

No clinical signs or symptoms were observed in the control group, whereas the control group showed erythema, edema, dryness, and scaling as morphological changes. Clinical severity scores were significantly ameliorated in groups irradiated with UV-LEDs (310 nm and 340 nm), NB-UVB, UVA, and 310 plus 340 nm (Fig. 1B and C). These findings indicated that UV-LED radiation affected the modulation of the immune system in the AD animal model.

Effect of UV-LEDs on serum IgE and inflammatory cytokine production

Serum IgE and type 2 T helper (Th2) cytokines are important in AD pathogenesis.²⁷ Accordingly, we investigated the serum levels of IgE and Th2 cytokines in Df-induced NC/Nga mice

after irradiation with UV-LEDs. Blood samples were collected on the final day of the experiment. Total serum IgE levels were significantly decreased in the NB-UVB, UVA and 310 plus 340 nm groups compared to those in the control group; however, no difference was found among the 310 nm, 340 nm, and control groups (Fig. 2A).

Subsequently, we evaluated the effect of UV-LEDs on the release of cytokines IL-1 β , IL-4, IL-6, IL-33, and MCP-1/CCL2 in serum using an ELISA assay. IL-4 and MCP-1/CCL2 serum levels significantly decreased in 310 nm and 310 plus 340 nm groups compared to those in the control group in the Df-induced NC/Nga mice, but IL-33 serum levels were not significantly different between the other groups. Also, IL-1 β and IL-6 serum levels significantly decreased in all UV-LED groups compared to those in the control group in the Df-induced NC/Nga mice (Fig. 2B–E). IL-1 β is one of the earliest cytokines in proinflammatory response initiation, and IL-6 is a proinflammatory cytokine, with both being produced in acute-phase inflammation. Therefore, IL-1 β may be crucial for AD phototherapy. These results suggested that UV-LED irradiation decreases serum inflammatory cytokine production through a photo-immunosuppression effect.

Effects of UV-LED radiation on scratching behavior and skin barrier function

Controlling scratching behavior is thought to be one of the most effective approaches to AD. Therefore, we measured scratching counts and TEWL in the NC/Nga model. All UV-LED and NB-UVB or UVA groups exhibited a significantly reduced TEWL compared to the control group on day 35 (Fig. 3A). However, scratching counts were significantly inhibited (by 41%) in groups receiving 310 plus 340 nm irradiation compared to those in the control group (Fig. 3B). Scratching counts appeared to be reduced in other groups, but not significantly. These results suggested that UV-LEDs inhibited scratching behavior and improved skin function and epidermis moisture.

Effects of radiation of UV-LEDs through histologic analysis

We investigated the effects of UV-LED radiation on Df-induced infiltration of eosinophils and mast cells into dorsal skin lesions in NC/Nga mice. H&E staining showed that repeated application of Df induced infiltration of inflammatory cells in the control group. In contrast, clear hyperplasia of the epidermis was seen in the normal group. Radiation using UV-LEDs (310 nm and 340 nm), NB-UVB, UVA, or 310 plus 340 nm wavelength, lowered the infiltration of inflammatory cells in the dorsal skin (Fig. 4A).

Staining with toluidine blue showed a significant infiltration of mast cells in the control group (Fig. 4B), but not in the normal group. The number of toluidine blue-stained mast cells in skin lesions of the control group (141.50 ± 17.55 cells) significantly decreased compared to those in the 310 plus 340 nm group (75.50 ± 3.99 cells) (Fig. 4B). Moreover, 50 mJ cm^{-2} 310 nm treatments significantly decreased ear thickness and swelling in Df-induced NC/Nga mice (Fig. 4C) although these differences might depend on the UV penetration depth. These results showed that UV-LEDs might contribute to relieve AD symptoms.

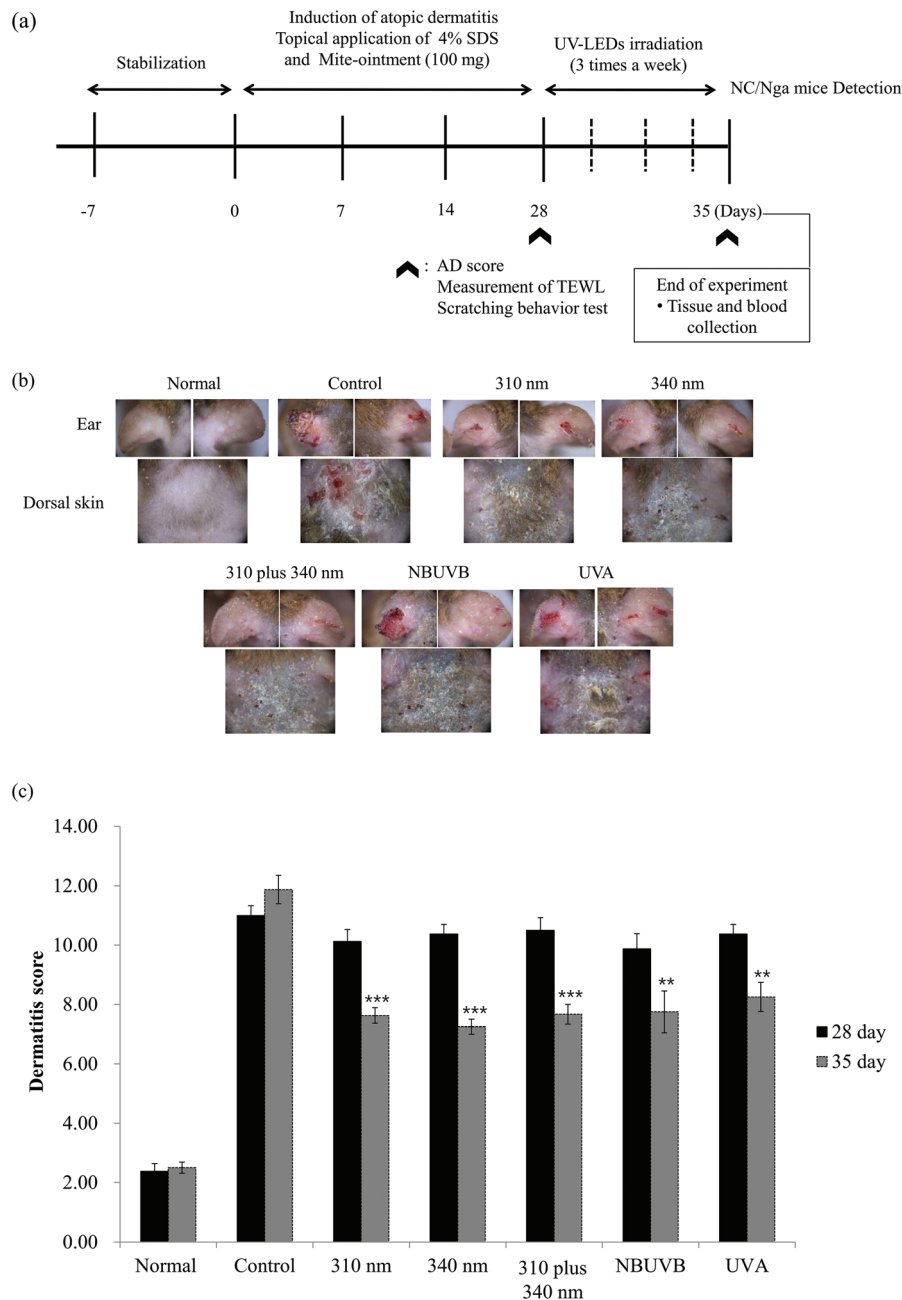


Fig. 1 Effects of 310 nm and 340 nm UV-LED irradiation on dermatitis development in an atopic dermatitis (AD) animal model. (a) Df-ointment was applied to the dorsal skin and ears of NC/Nga mice for four weeks to induce AD-like skin lesions. After application, mice were exposed to UV-LEDs (310 and 340 nm), NB-UVB, or UVA irradiation three times per week. (b) Mice were divided into seven groups: G1, normal; G2, control (Df-sensitized only); G3, 310 nm; G4, 340 nm; G5, 340 nm plus 310 nm; G6, NB-UVB; and G7, UVA. Irradiation using UV-LEDs (310 and 340 nm), NB-UVB, or UVA significantly improved clinical signs on day 35. (c) Changes in total clinical severity scores after UV-LEDs (310 and 340 nm), NB-UVB, or UVA irradiation. The data are presented as means $\pm$ SEM for changes in total clinical severity scores. * $p < 0.05$ and ** $p < 0.01$ compared to controls ($n = 8$).

Effect of UV-LED radiation on inflammation-mediated signaling pathways

We investigated UV-LED phototherapy for treating chronic inflammatory skin disease including AD-mediated cell adhesion molecules. The immunohistochemical data revealed that repeated application of Df induced an AD model with

enhanced formation of a dark brown reaction revealed by the DAB (3,3'-diaminobenzidine) staining patterns of ICAM-1 and E-selectin when compared to the control group. Irradiation with 310 nm or 340 nm, 310 plus 340 nm UV-LEDs diminished the staining patterns of ICAM-1, E-selectin, and IL-31 in the epidermis to the dermis (Fig. 5). These results indicated that the irradiation groups altered functions that blocked an

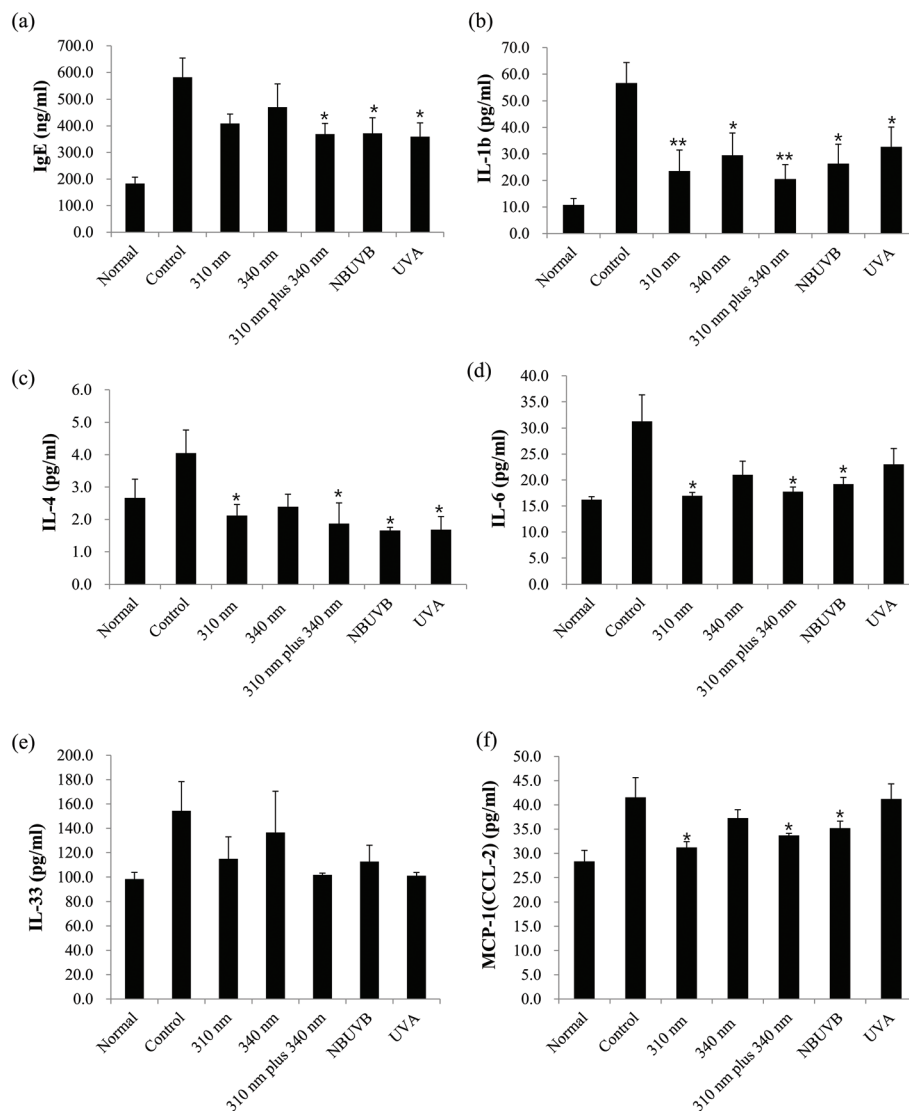


Fig. 2 Effects of 310 nm and 340 nm UV-LED irradiation on serum IgE and cytokine levels in NC/Nga mice with Df-induced AD-like skin lesions. The mice were sacrificed for serum collection after 35 days. The serum levels of (a) IgE, (b) IL-1 β , (c) IL-4, (d) IL-6, (e) IL-33, and (f) MCP-1/CCL2 were measured. IgE serum levels and inflammatory cytokine levels were measured using ELISA kits. The data are presented as means $\pm$ SEM of changes in values. * p < 0.05 and ** p < 0.01 compared to the controls (n = 8).

increase in the inflammation status. This study focused on the key role of UV-LEDs in immunoregulation and provided experimental data that validated the therapeutic potential of UV-LEDs using an NC/Nga mouse model.

Discussion

The main advantages of UV-LEDs include a tunable wavelength, single peak emission, low power consumption, long lifespan, and a small form factor. Kemeny *et al.*²⁸ observed that in clinical tests, a UVB-LED device was an effective treatment for localized psoriasis. However, subsequent studies did not confirm these preclinical tests. Accordingly, we examined the inhibition of AD-like symptoms and the pathogenesis of

310 nm, 340 nm, and 310 plus 340 nm UV-LED irradiation using animal models as a treatment for AD.

NC/Nga mice are commonly used as an experimental model and manifest the clinical characteristics of human AD-like skin symptoms, including increased serum IgE levels.²⁹ We evaluated the therapeutic effect of UV-LEDs in Df-treated NC/Nga mice. Our results suggested that the therapeutic effects of UV-LEDs on the NC/Nga mouse model were associated with down-regulation of inflammatory cytokines and AD-like skin symptoms. Our previous studies showed that combining UVB and UVA wavelength treatments have greater therapeutic effects.²⁴ In this study, combining 310 nm and 340 nm UV-LED irradiation markedly inhibited Df-induced inflammatory cytokines such as IL-1 β .

Recently, IL-31 was linked to the pathogenesis of pruritus in AD skin diseases.³⁰ AD skin has a dense inflammatory cell

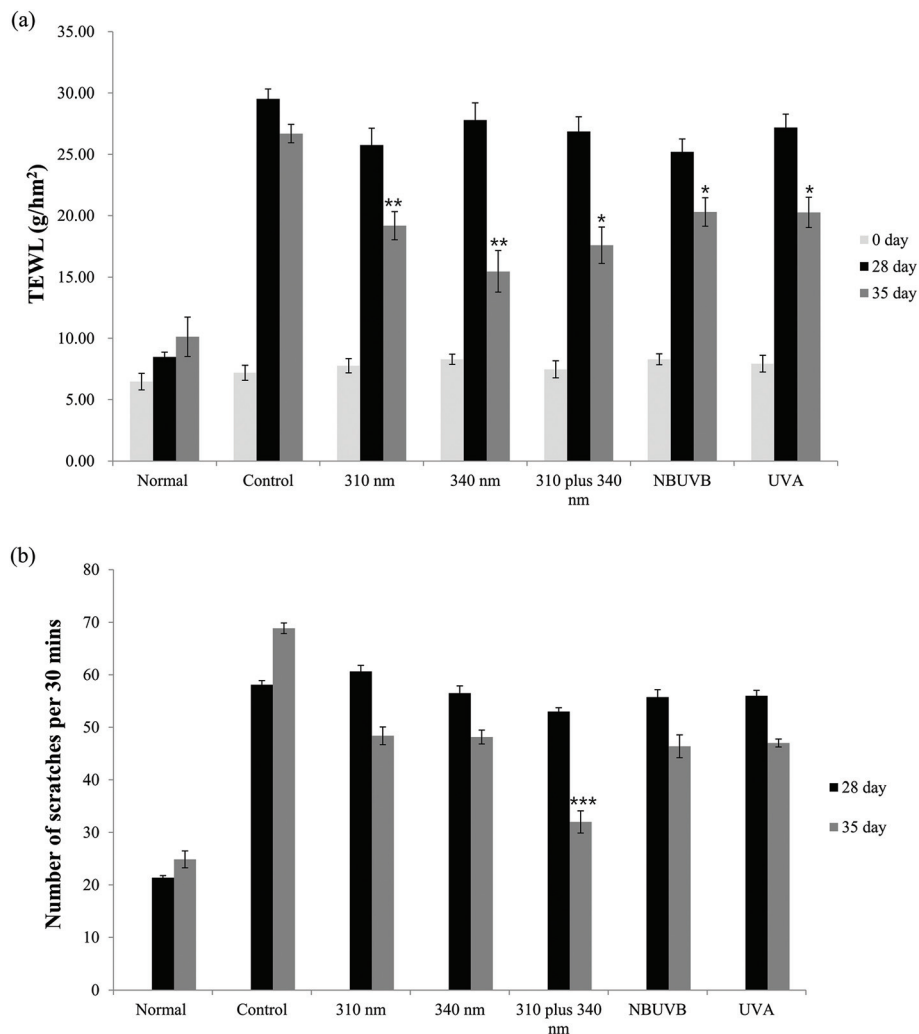


Fig. 3 Effects of 310 nm and 340 nm UV-LED irradiation on transepidermal water loss and scratching behavior in the NC/Nga mice with Df-induced AD-like skin lesions. (a) Measurements of transepidermal water loss (TEWL) were performed under similar conditions on days 0, 28, and 35. (b) Scratching behavior was quantified by counting the number of scratches in 30 min. The data are presented as means $\pm$ SEM of changes in values. * $p < 0.05$ and ** $p < 0.01$ compared to controls ($n = 8$).

infiltrate known to aggravate pruritus. Several studies provided further evidence for a possible therapeutic role for IL-31 in AD.^{31,32} Histological analysis showed that UV-LED phototherapy diminished the infiltration of inflammatory and mast cells in the skin and attenuated the morphological characteristics of AD, such as acanthosis, hyperkeratosis, and spongiosis of the epidermis in the NC/Nga AD model.

Although previous studies have examined combined therapy,^{14,33} it is believed that part of the UVA and UVB treatment effect is due to the depth of skin penetration. The ability of UVA to induce T-lymphocyte apoptosis is probably most relevant to the management of AD. It can also reduce the number of Langerhans cells and mast cells in the dermis.³⁴ UVB has many effects on human skin, including regulation of immune activity and tolerance through depletion of epidermal Langerhans cells.³⁵ In this study, we also found that 310 nm, 340 nm, and 310 plus 340 nm UV-LED radiation markedly

diminished the staining patterns of IL-31, ICAM-1, and E-selectin in the epidermis and dermis. In particular, skin barrier destruction results in the release of IL- α , which represents an early stage of the inflammatory cascade. Activation of IL- α is thought to stimulate additional production of pro-inflammatory cytokines and chemokines, such as IL- β , TNF- α , IL-6, and CXCL8 (IL-8) surrounding epidermal and dermal cells.³⁶ With IL- α , the subsequent action of IL- β involves the activation of dendritic and T-cells, additional cytokine and chemokine production, and upregulation of adhesion molecules, such as E-selectin and ICAM-1 in addition to endothelial fibroblasts,³⁷ resulting in permanent inflammation. Thus, down-regulation of ICAM-1 is an important therapeutic goal in AD regulation.

We suggested that 310 nm and 340 nm UV-LED irradiation could control scratching behavior, inhibit inflammation, and enhance skin barrier function. Based on current data, newer

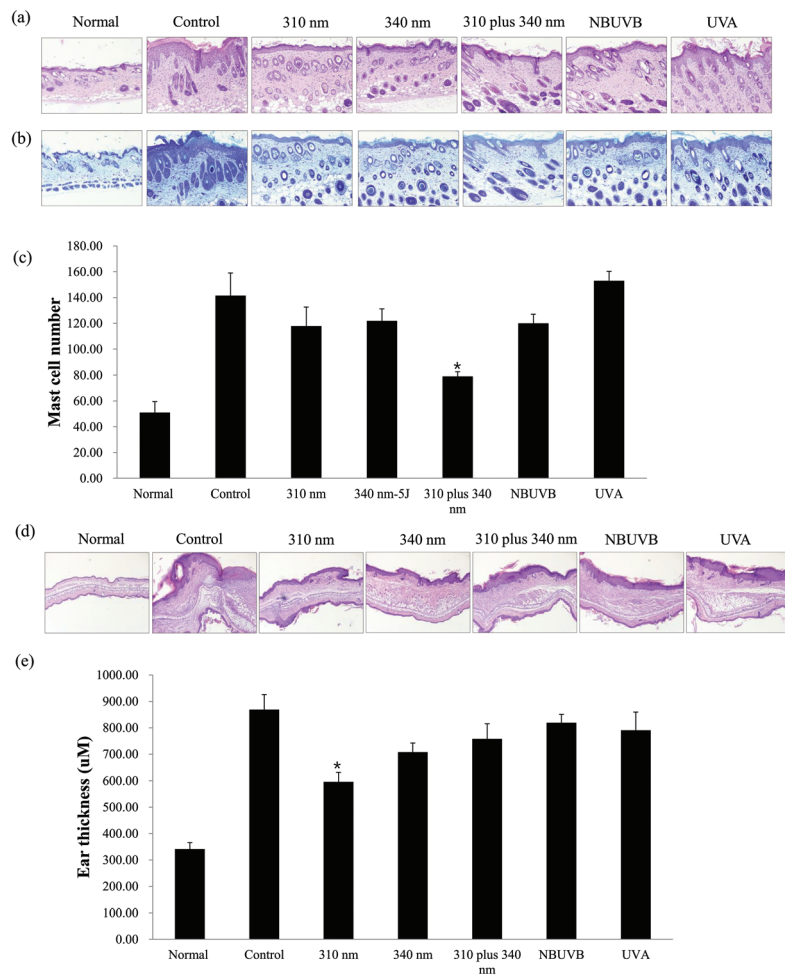


Fig. 4 Histological examination of lesions in NC/Nga mice with Df-induced AD-like skin lesions irradiated with 310 nm and 340 nm UV-LEDs. Dorsal skin specimens from mice irradiated with UV-LEDs (310 and 340 nm), NB-UVB, or UVA three times a week were collected at day 35. Dorsal skin specimens were stained with (a) H&E, or (b and c) toluidine blue for counts or (d and e) ear thickness. Infiltration cell counts and ear thickening measurements were performed using an optical microscope. Values are presented as means $\pm$ SEM ($n = 8$). Significant differences from controls are * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Original magnification $\times 200$.

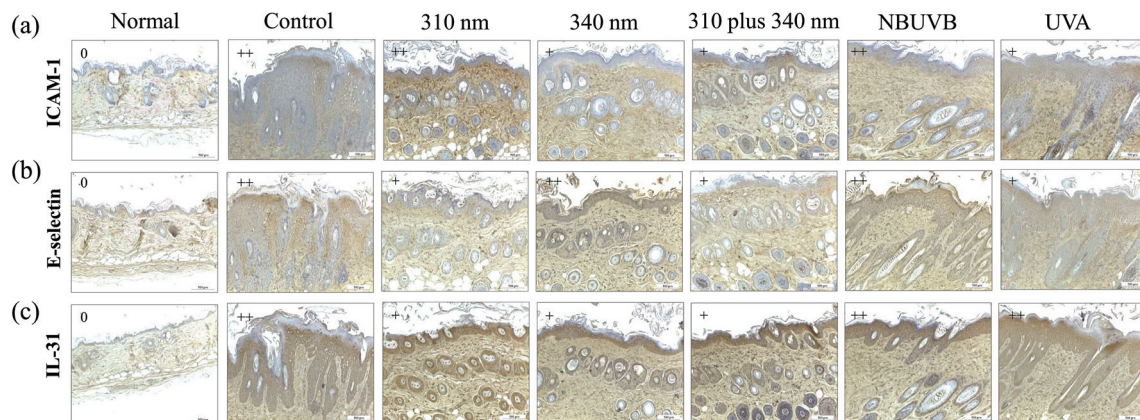


Fig. 5 Anti-inflammatory effects of 310 nm and 340 nm UV-LED irradiation of Df-induced AD-like skin lesions in NC/Nga mice. Paraffin sections of dorsal skin biopsies from G1, normal; G2, control (Df-sensitized only); G3, 310 nm; G4, 340 nm; G5, 340 nm plus 310 nm; G6, NB-UVB; and G7, UVA groups were immunohistochemically (IHC) stained for: (a) ICAM-1, (b) E-selectin, and (c) IL-31. Dark brown dots indicate positively stained cells ($n = 3$ dorsal skin images per group). The IHC intensity score represents the average of the scores in each IHC staining intensity category (++ indicates pronounced findings, + moderate findings and 0 no/scant findings) identified in each core. Original magnification $\times 200$.

UV-LEDs with a narrow emission spectrum, such as 310 and 340 nm, may provide more efficacious wavelengths compared to NB-UVB or UVA.

Collectively, the results demonstrated that UV-LED irradiation significantly alleviated AD-like skin symptoms, scratching behaviors, and TEWL and in NC/Nga mice. These findings suggest that UV-LED phototherapy might be a therapeutic approach for AD.

Conflicts of interest

Authors have no potential conflicts of interest to disclose.

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