

Gain-switched 311-nm Ti:Sapphire laser might be a potential treatment modality for atopic dermatitis

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Abstract Phototherapy with 311-nm narrowband-UVB (NBUVB) is an effective adjuvant treatment modality for atopic dermatitis (AD). In this study, we evaluated the therapeutic effect of the newly developed gain-switched 311-nm Ti:Sapphire laser device using a NC/Nga mouse AD model. A total number of 50 mice were used in this study. Atopic dermatitis (AD) was induced in mice by exposure to *Dermatophagoides farinae*. These, NC/Nga mice were then treated with conventional 311-nm NBUVB or the newly developed gain-switched 311-nm Ti:Sapphire laser. The clinical features, dermatitis severity scores, and scratching behavior were assessed. In addition, serologic analyses including inflammatory cytokines and histological analyses were performed. Gain-switched 311-nm Ti:Sapphire laser improved the AD-like skin lesions, severity, and symptoms of AD in the NC/Nga mouse model. This new laser also modulated the immune response found in the AD model, including

hyper-IgE, upregulated Th2 cytokines, and the Th2-mediated allergic inflammatory reaction. Gain-switched 311-nm Ti:Sapphire laser shows therapeutic promise via an immune-modulation mechanism in an AD mouse model. These data suggest that gain-switched 311-nm Ti:Sapphire laser may be useful as a targeted phototherapy modality for AD.

Keywords Gain-switched 311-nm Ti:Sapphire laser · Atopic dermatitis

Introduction

Atopic dermatitis (AD) is a common, chronic relapsing inflammatory skin disease that is associated with environmental allergies. It is characterized by highly pruritic eczematous skin lesions [1, 2].

The individual therapeutic approach to AD involves the identification and avoidance of aggravating factors. The first-line therapy for the disturbed skin barrier function found in AD includes emollient therapy and appropriate skin cleansing [3]. Topical anti-inflammatory therapies, including glucocorticosteroids and calcineurin inhibitors, have also been mainstays of treatment for mild to moderate AD [4]. Systemic immunosuppressive agents are therapeutic options for severe, refractory AD [5].

Phototherapy with UV radiation can also be used as an adjuvant therapy to treat chronic, pruritic, lichenified forms of AD [5]. Clinical trials have demonstrated that UV irradiation relieves pruritus in AD [6, 7]. Narrowband-UVB (NBUVB) has been found to be more effective than is UVA [1, 8, 9].

NBUVB is the most commonly used phototherapy for AD; it is safe and effective [10]. The main disadvantage of

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conventional NBUVB phototherapy is that AD patients need to expose their entire body surface for UV radiation [11]. Therefore, a UV laser device designed to deliver high fluency UV energy to localized AD skin lesions would be advantageous over conventional NBUVB phototherapy. In this study, we evaluated the therapeutic effectiveness of a new device that employs a gain-switched 311-nm Ti:Sapphire laser in a murine model of atopic dermatitis.

Materials and methods

NC/Nga mouse AD model

A total number of 50, 6-week-old male NC/Nga mice (SaeronBio Inc., Gyeonggi-do, Korea) were used in this study. The mice were housed and bred under conventional conditions (23 ± 3 °C, relative humidity of 55 ± 15 %) at the R&D Center of the College of Medicine in Chung-Ang University, Korea. All animal procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Chung-Ang University in Korea (IACUC: 2015–00030).

The NC/Nga mice were allowed to acclimate to their new environment for 7 days. An electric clipper was used to shave the skin over both ears and the dorsal skin. AD-like skin lesions were induced with 10 % sodium dodecyl sulfate (SDS) spray and 100 mg *Dermatophagoides farinae* (Df) extract ointment (Biostir, Kobe, Japan). Two hundred microliters of 10 % SDS solution was spread on both ears and dorsal skin of the mice using sterilized tips. After 6 h, we applied 100-mg Df extract ointment on both ears and dorsal skin of the mice. We applied Df extract ointment three times per week for 3 weeks. The interval between the application of Df extract ointment was 2 or 3 days.

Treatment device

The newly designed gain-switched Ti:Sapphire laser [12], which emits 311-nm single wavelength, PALLAS® (Laseroptek, Gyeonggi-do, Korea), was used. This device is operated with a pulse duration of 17 ns and fluence of 30–5000 mJ/cm². The Dermalight® 80 Handheld Narrowband UVB Phototherapy Device (National Biological Corporation, Twinsburg, OH, USA) was used as the conventional NBUVB treatment for comparison.

Safety evaluation

In order to determine the appropriate exposure fluence in NC/Nga mice, 50 mice were separated into five groups (with 10 mice/group). Group 1 was not irradiated (negative group). Group 2 was irradiated with gain-switched 311-nm

Ti:Sapphire laser 500 mJ/cm², group 3 with gain-switched 311-nm Ti:Sapphire laser 1000 mJ/cm², group 4 with gain-switched 311-nm Ti:Sapphire laser 1500 mJ/cm², and group 5 with gain-switched 311-nm Ti:Sapphire laser 5000 mJ/cm². At 1, 3, and 7 days after irradiation, the skin changes in the NC/Nga mice were examined macroscopically and histopathologically (data not shown). Irradiation of 1000 mJ/cm² fluence using the gain-switched 311-nm Ti:Sapphire laser was ideal for maintaining effectiveness and safety in the NC/Nga mice.

Experimental protocol

A total of 50 mice were separated into five groups (10 mice/group). Group 1 did not have AD-like skin lesions (normal group). Groups 2, 3, 4, and 5 had AD-like skin lesions. Group 2 was not irradiated (control group). The irradiated groups (3–5) were treated three times per week. Group 3 was irradiated with NBUVB 420 mJ/cm² (NBUVB group), group 4 with gain-switched 311-nm Ti:Sapphire laser 500 mJ/cm² (laser A group), and Group 5 with gain-switched 311-nm Ti:Sapphire laser 1000 mJ/cm² (laser B group) (Fig. 1).

Clinical features and dermatitis severity scores

Clinical changes were measured using digital photographs (Canon 5200D, Canon Inc., Tokyo, Japan). For images with enhanced magnification, we used dermoscope (DermLite Pro, CA, USA) at the same time (days 0, 21, and 28). Ear thickness was measured using a dial caliper (Mitsutoyo, Tokyo, Japan).

The severity of the AD-like skin lesions was evaluated by scoring five clinical signs and symptoms as described previously [13] on days 21 and 28. These included scaling/dryness, itching, erythema/hemorrhage, edema, and excoriation/erosion. Each clinical sign and symptom was scored on the following scale: 0 indicating no signs/symptoms, 1 mild, 2 moderate, and 3 severe. The total severity score of AD-like skin lesions was defined as the sum of all scores (from 0 to 15). Assessments were performed in a blinded manner.

Scratching behavior assay

A scratching behavior assay was performed on day 28 using an automatic video camera (Sony, TRV, Tokyo Japan), as previously described [14], with slight modifications. The number of scratching episodes within 30 min was counted. Each scratching episode was scored from 0 to 4 where 0 indicated no scratching, 2 scratching for <1.5 s, and 4 for >1.5 s. The total scratching behavior score for each mouse was calculated as the sum of the individual scores.

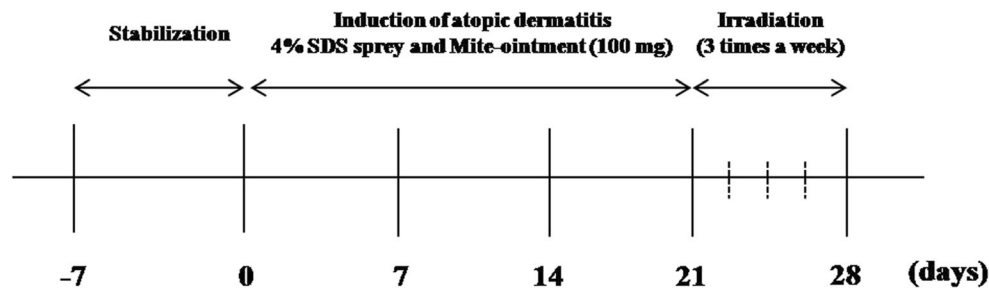


Fig. 1 Experimental protocol. To induce atopic dermatitis lesions, 10 % SDS spray and 100-mg *Dermatophagoides farinae* extract ointment were applied to the skin of the ear and dorsum of NC/Nga mice three times a

week for 3 weeks. The mice were then irradiated three times per week using either NBUVB or the gain-switched 311-nm Ti:Sapphire laser

Transepidermal water loss measurement

Transepidermal water loss (TEWL) was measured using a Tewameter TM300 (Courage and Khazaka Electronic Co., Germany) on days 0, 7, 21, and 28. The mice were acclimated to predetermined conditions (temperature 22–24 °C, relative humidity 50–60 %) for 30 min before the measurements were performed. The TEWL was recorded as an average of minimum deviations among averages measured for 60 s, except the initial results.

Measurement of serum immunoglobulin E and inflammatory cytokines

Mice were anesthetized using local anesthetic agent, which composed of zoletil (30 mg/kg) and rompun (10 mg/kg). Blood was drawn from the retro-orbital plexus on day 28. Serum samples were obtained by centrifuging the blood at 3000 g for 10 min at 4 °C and stored at –80 °C.

Immunoglobulin E (IgE) was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Shibayagi Co., Ltd., Japan) according to the manufacturer's instructions. Interferon (INF)- γ , interleukin (IL)-1 α , IL-1 β , IL-4, IL-6, IL-31, IL-33, and monocyte chemoattractant protein (MCP)-1 were analyzed using the ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. All experiments were performed in duplicate and repeated at least three times.

Histopathological analysis

The skin biopsy was conducted at the last day of experiment (day 28) using 8-mm punch. The dorsal and ear skin tissues were fixed with 4 % paraformaldehyde, embedded in paraffin, and sliced into 5- μ m sections. The sections were transferred to probe-on-plus slides (Fisher Scientific, Pittsburgh, PA, USA). Deparaffinized skin sections were stained with hematoxylin and eosin (H&E) and toluidine blue.

Five sections were stained for immunohistochemical markers using monoclonal antibodies against filaggrin (1:500, ab24584, Abcam, Cambridge, MA, USA), involucrin

(1:500, ab53112, Abcam), loricrin (1:500, ab24722, Abcam), proliferating cell nuclear antigen (PCNA) (1:500, ab15497, Abcam), protease-activated receptor 2 (PAR-2) (1:500, sc8205, Santa Cruz Biotechnology, Santa Cruz, CA, USA), thymic stromal lymphopoietin (TSLP) (1:200, ab188766, Abcam), E-selectin (1:200, NBP1-45545, Novus), Intracellular adhesion molecule-1 (ICAM-1) (1:200, ab2213, Abcam), and IL-31 (1:100, ab102750, Abcam), as described previously [15].

Statistical analysis

Data are presented as means $\pm$ standard deviations. Statistical comparisons between the treated and untreated groups were performed using one-way ANOVA followed by the Tukey post hoc test for direct comparison between specific groups. *P* values <0.05 (*), <0.01 (**), and <0.001 (***) were considered (increasingly) statistically significant.

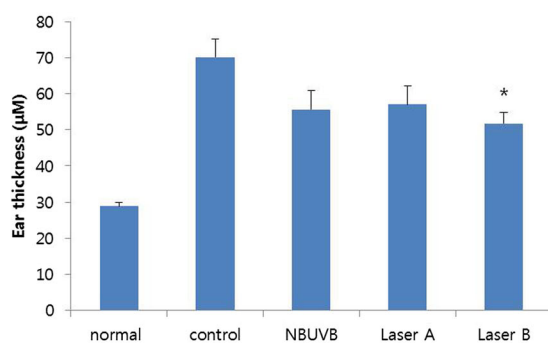
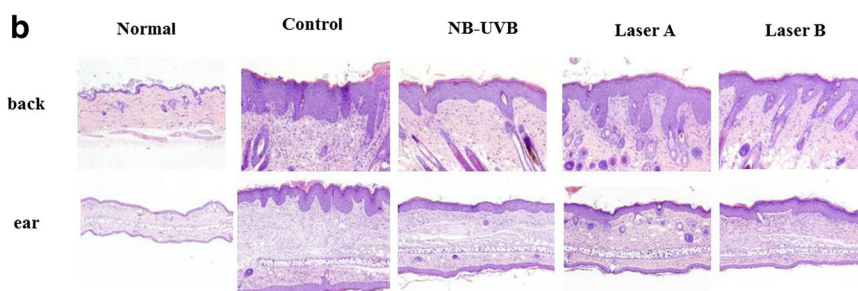
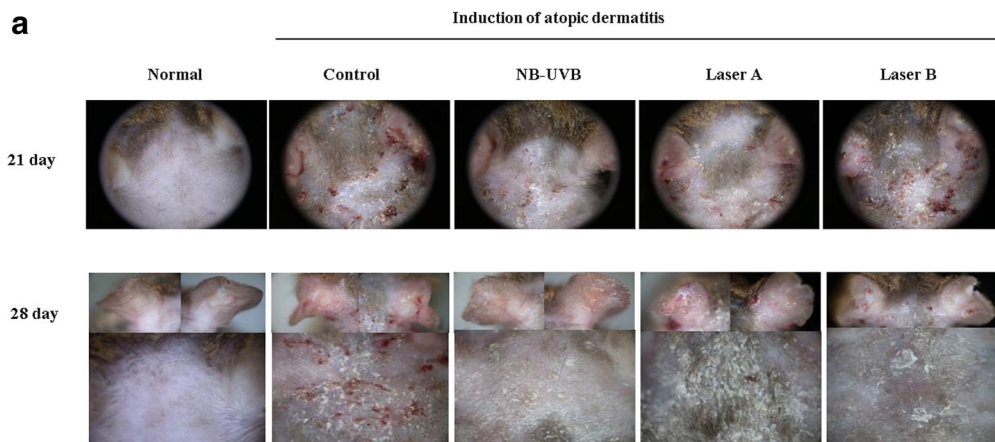
Results

Improvements in AD-like skin lesions with the gain-switched 311-nm Ti:Sapphire laser

Dermoscopy revealed that the clinical features of AD, including erythema, edema, erosions, hemorrhage, and excoriations, developed in the AD-induced group on day 21. These clinical features improved in the NBUVB and laser groups on day 28 (Fig. 2a).

After AD was induced using the Df extract, ear thickness increased by more than two times its baseline value. The NBUVB and laser groups showed decreased ear thickness. In the laser B group, ear thickness decreased significantly from baseline after irradiation (Fig. 2b).

After AD induction, the number of the mast cells was increased in three times in the toluidine blue-stained dorsal skin tissue. The NBUVB and laser groups demonstrated decreases in the number of mast cells (Fig. 2c).



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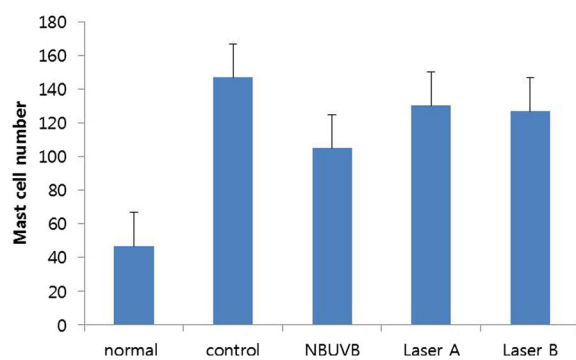
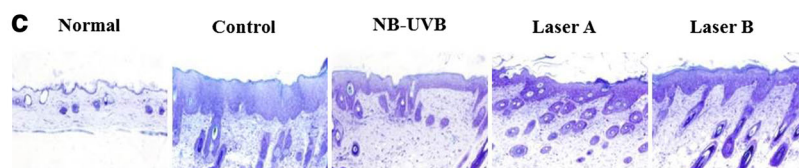


Fig. 2 Effects of the gain-switched 311-nm Ti:Sapphire laser on atopic dermatitis (AD)-like skin lesions. **a** The clinical features of AD-like skin lesions on dermoscopy improved after irradiation (day 28) compared to that before irradiation (day 21). **b** Ear thickness and **c** mast cell number also decreased from pre-radiation levels after irradiation. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. the control group

Improvement in dermatitis and associated symptoms after using the gain-switched 311-nm Ti:Sapphire laser

The dermatitis severity scores in both the ear and dorsal skin increased after NC/Nga mice were exposed to Df extract. On day 21, the severity scores increased from 10 to 12 in the AD-induced group. On day 28, the severity scores were significantly lower in the NBUVB and Laser groups compared to that of the control group (Fig. 3a).

In order to analyze the scratching behavior related to itching, the number of scratching episodes within 30 min was counted. Both NBUVB and Laser B groups had significantly fewer scratching episodes than did the control group (Fig. 3b).

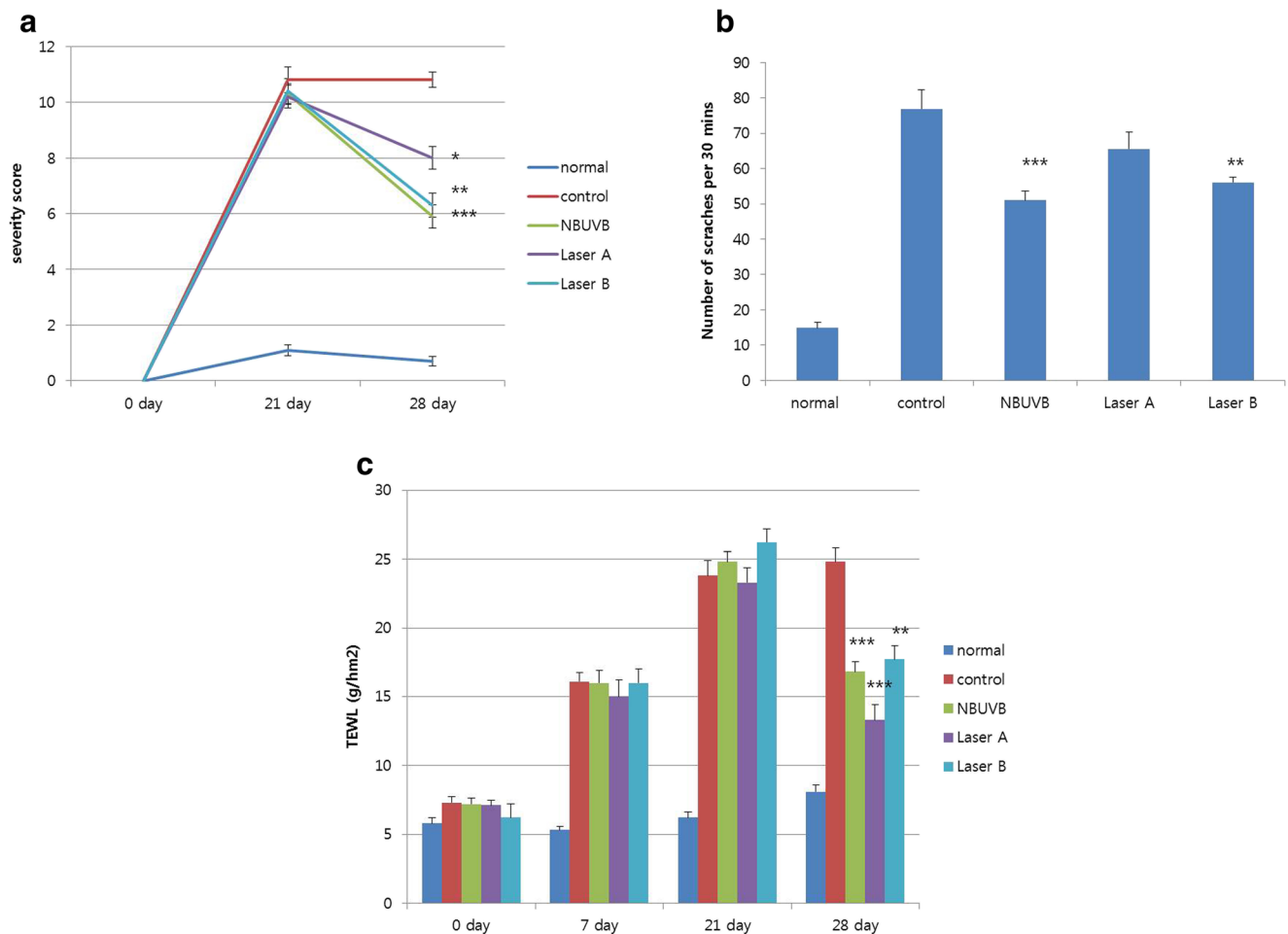


Fig. 3 Effects of the gain-switched 311-nm Ti:Sapphire laser on dermatitis severity and associated symptoms. **a** Dermatitis severity scores before and after irradiation. **b** The number of scratching episodes within

Similar to the severity score, the TEWL increased after Df extract was applied to both the ear and dorsal skin of NC/Nga mice for 3 weeks. The TEWL decreased significantly after irradiation with NBUVB and laser compared to that of the control group (Fig. 3c).

These results suggest that the gain-switched 311-nm Ti:Sapphire laser improves dermatitis severity and alleviates associated symptoms, including itching and dryness.

Effect of the gain-switched 311-nm Ti:Sapphire laser on the production of IgE and inflammatory cytokines

The serum IgE levels were four times higher in the control group than they were in the normal group. After irradiation with NBUVB and the laser, the serum IgE levels decreased significantly compared to that of the control group (Fig. 4a).

The levels of eight cytokines (INF- γ , IL-1 α , IL-1 β , IL-4, IL-6, IL-31, IL-33, and MCP-1) were measured in the serum of NC/

30 min over the course of 28 days. **c** The TEWL values obtained over the 28-day observation period. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. the control group

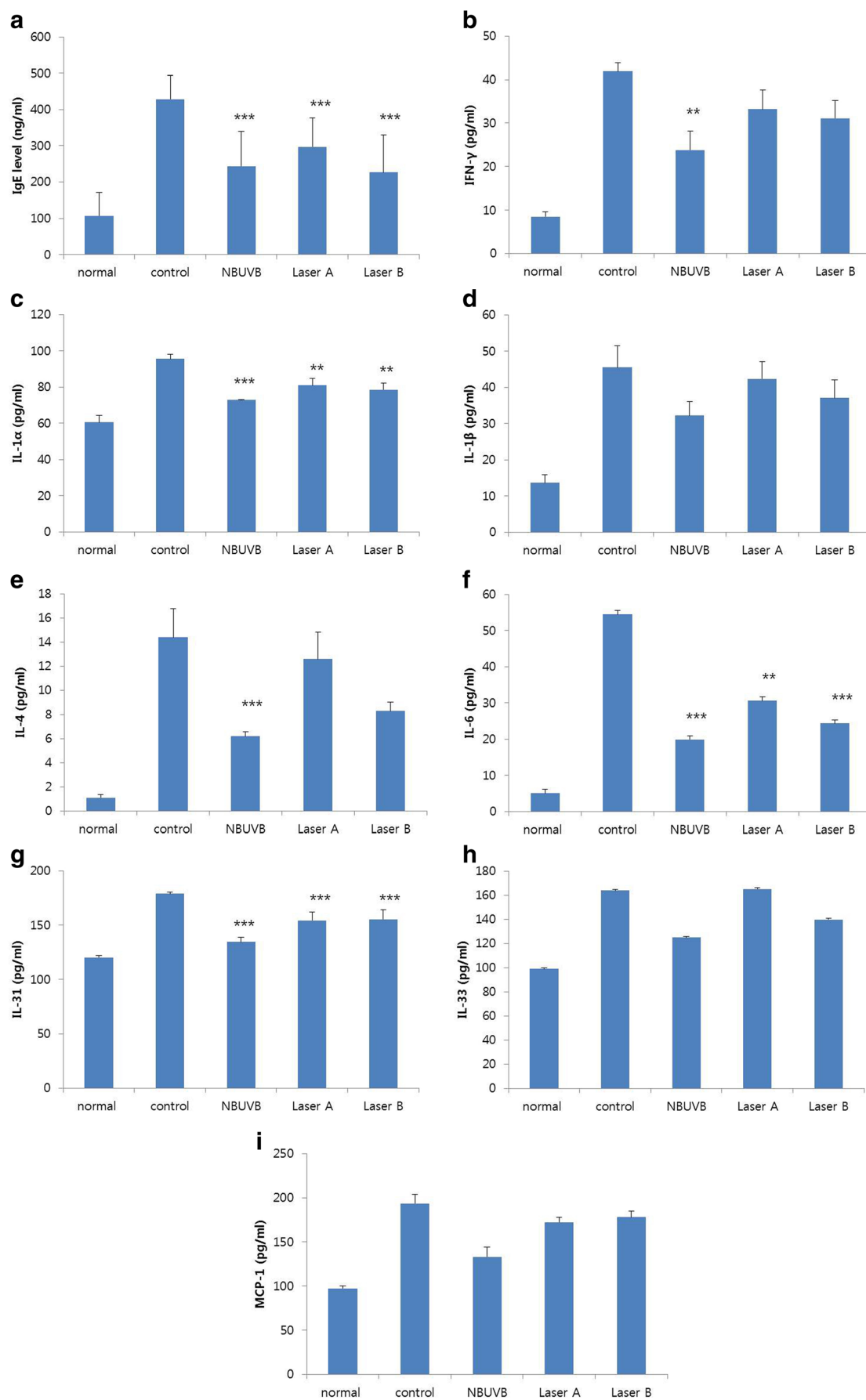
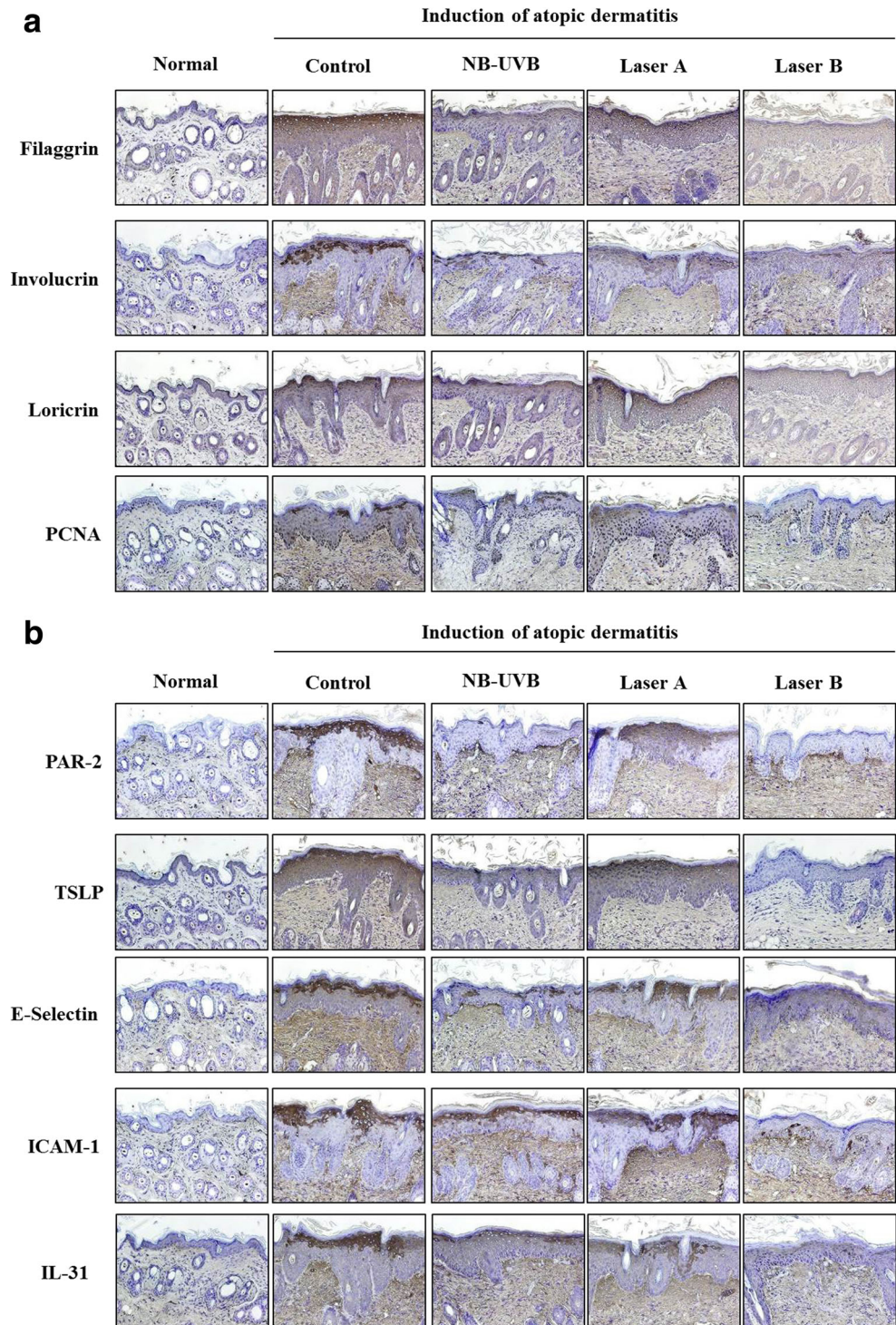


Fig. 4 The effect of the gain-switched 311-nm Ti:Sapphire laser on the production of IgE and inflammatory cytokines. Serum levels of IgE (a), INF- γ (b), IL-1 α (c), IL-1 β (d), IL-4 (e), IL-6 (f), IL-31 (g), IL-33 (h), and MCP-1 (i). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. the control group

Nga mice. Among these, IL-1 α , IL-6, and IL-31 were significantly reduced in the NB-UVB and laser group (Fig. 4b–i).

These results suggest that, in AD mouse models, the gain-switched 311-nm Ti:Sapphire laser suppresses the hyper-production of IgE and of T-helper cell type 2 (Th2) cytokines. However, there is the limitation in the analysis of IgE and cytokines. Because we could not perform blood samples in the baseline (day 0), the results of the experimental group were compared with those of the control group.

Fig. 5 Effect of the gain-switched 311-nm Ti:Sapphire laser on skin barrier function and Th2-mediated allergic inflammation. **a** The skin was immunostained with anti-PCNA, anti-filaggrin, anti-involucrin, and anti-loricrin. **b** Skin tissues were immunostained with anti-PAR-2, anti-TSLP, anti-ICAM-1, anti-E-selectin, and anti-IL-31



Effect of the gain-switched 311-nm Ti:Sapphire laser on skin barrier impairment and Th2-mediated allergic inflammation

The skin tissues were immunostained with anti-PCNA, anti-filaggrin, anti-involucrin, and anti-loricrin to confirm keratinocyte proliferation and differentiation. In both the NBUVB and laser groups, there was decreased expression of PCNA, filaggrin, involucrin, and loricrin compared to those of the control group. These expression levels were more also lower in laser group B than they were in laser group A (Fig. 5a).

In order to investigate the expression patterns of molecules associated with skin barrier function and inflammation-related cytokines, the skin tissues were immunostained with anti-PAR-2, anti-TSLP, anti-ICAM-1, anti-E-selectin, and anti-IL-31. Both NBUVB and laser groups had decreased expression of PAR-2, TSLP, ICAM-1, E-selectin, and IL-31. These expressions were also lower in laser group B than they were in laser group A (Fig. 5b).

Discussion

Phototherapy usually involves UV radiation in the treatment of various skin diseases such as psoriasis, AD, scleroderma, and mycosis fungoides [16]. Among various treatment options, including UVA and UVB, 311-nm NBUVB phototherapy is an effective adjuvant treatment modality in the treatment of AD. Recently, a 308-nm excimer laser was introduced as new adjuvant phototherapy [11] and was approved for use in AD by the US Food and Drug Administration.

We evaluated the therapeutic effectiveness of this gain-switched 311-nm Ti:Sapphire laser device using NC/Nga AD-mouse models. This mouse strain is commonly used as an experimental AD model. Our data demonstrated that the gain-switched 311-nm Ti:Sapphire laser improves the severity and symptoms of AD in this murine model. In addition to the clinical features of AD, the gain-switched 311-nm Ti:Sapphire laser also reduces the skin thickening and mast cell infiltration in inflamed AD skin lesions. Moreover, the gain-switched 311-nm Ti:Sapphire laser was also able to modulate the immune response, including hyper-IgE and upregulated Th2 cytokines. As a result, the gain-switched 311-nm Ti:Sapphire laser improves skin barrier function in AD lesions and inhibits Th-2 mediated inflammatory reaction.

Similar to the 308-nm excimer laser, this newly developed gain-switched 311-nm Ti:Sapphire laser is preferable over conventional 311-nm NBUVB phototherapy. Laser devices enable specific and focal AD lesion treatment. Using the gain-switched 311-nm Ti:Sapphire laser, physicians would be able to directly and precisely deliver an effective UV dose, without irradiating any adjacent, normal skin. In addition, as

compared to conventional phototherapy, targeted phototherapy using a laser device allows for reduced overall treatment time and shortened therapeutic courses.

The newly developed gain-switched 311-nm Ti:Sapphire laser has a circle-shaped spot, which is freely controlled from 1 pi to 12 pi size. The 308-nm excimer laser has a fixed spot, which is 2 × 2 cm in size. Therefore, as compared to the 308-nm excimer laser, the gain-switched 311-nm Ti:Sapphire laser may be more appropriate to treat tiny focal lesions [17].

In conclusion, our study demonstrates that the gain-switched 311-nm Ti:Sapphire laser shows therapeutic effectiveness via immune-modulation in an AD mouse model. These data suggest that the gain-switched 311-nm Ti:Sapphire laser may be useful as a targeted phototherapy modality for AD, as well as other inflammatory skin diseases.

Compliance with ethical standards

Funding sources None

Conflict of interest None declared

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