Journal of Dermatological Science 62 (2011) 91–97

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.elsevier.com/jds

Inhibitory effects of UV-based therapy on dry skin-inducible nerve growth in acetone-treated mice

Atsuko Kamo [a], Mitsutoshi Tominaga [a], Suhandy Tengara [a], Hideoki Ogawa [b], Kenji Takamori [a,b,*]

[a] Institute for Environmental and Gender Specific Medicine, Juntendo University Graduate School of Medicine, 2-1-1 Tomioka, Urayasu, Chiba 279-0021, Japan
[b] Department of Dermatology, Juntendo University Urayasu Hospital, 2-1-1 Tomioka, Urayasu, Chiba 279-0021, Japan

ARTICLE INFO

Article history:
Received 12 October 2010
Received in revised form 4 January 2011
Accepted 6 January 2011

Keywords:
Axonal guidance molecules
Dry skin
Nerve fibers
UV-based therapy
Pruritus

ABSTRACT

Background: UV-based therapy has anti-pruritic effects in inflammatory skin diseases, such as atopic dermatitis and psoriasis. These anti-pruritic effects may be partly due to inhibition of intraepidermal nerve growth, but they have not been fully characterized.
Objective: This study was performed to characterize the anti-nerve growth effects of UV-based therapies in acetone-treated mice as an acute dry skin model.
Methods: Nerve fibers penetrate into the epidermis 24 h after acetone treatment in mice, and nerve growth peaks 48 h after acetone treatment. To investigate the effects of UV-based therapies on the epidermal nerve fibers, including combination treatment with corticosteroid ointment, the mice were treated with psoralen ultraviolet A (PUVA), PUVA and betamethasone valerate ointment (PUVA + BV), narrowband ultraviolet B (NB-UVB) or an excimer lamp. Each therapy was provided 24 h after acetone treatment, and skin samples were taken 48 h later. Nerve fiber densities and expression levels of nerve growth factor (NGF) and semaphorin 3A (Sema3A) in the epidermis were examined by immunohistochemistry.
Results: Penetration of nerve fibers into the epidermis was observed in the acetone-treated mice, concomitant with increased NGF and decreased Sema3A levels in the epidermis. The acetone-induced intraepidermal nerve growth was significantly decreased by PUVA, PUVA + BV, NB-UVB, and excimer lamp treatments compared with controls. In addition, PUVA + BV and NB-UVB normalized the abnormal expression of NGF and Sema3A in the epidermis, but no such normalization was observed with excimer lamp treatment.
Conclusion: UV-based therapies, especially NB-UVB and excimer lamp treatments, may be effective therapeutic methods for pruritus involving epidermal hyperinnervation.

1. Introduction

Itching, or pruritus, an unpleasant sensation associated with the desire to scratch, frequently accompanies a variety of inflammatory skin conditions and systemic diseases. Histamine is the best-known pruritogen in humans and is also used as an experimental itch-causing substance. Clinically, antihistamines, *i.e.*, histamine H_1 antagonists, are commonly used to treat all types of itch resulting from renal and liver diseases, as well as from serious skin diseases, such as atopic dermatitis (AD). However, antihistamines often lack efficacy in patients with chronic itch [1–3].

Increased epidermal nerve density is considered as one cause of antihistamine-resistant itch, suggesting that the lesioned skin is susceptible to stimulation and sensitive to itching [4]. The sprouting of epidermal nerve fibers associated with pruritus is found in AD [5,6], xerosis [7], allergic contact eczema [8], and prurigo nodularis [9], and in experimental animal models [10,11]. Nerve growth factor (NGF) produced by keratinocytes is an important growth factor that determines skin innervation [12], and increased plasma concentrations of NGF in AD patients are correlated with disease severity [13]. We recently demonstrated that levels of semaphorin 3A (Sema3A), which induces retraction of NGF-responsive sensory afferents, are decreased in the epidermis of AD patients [14], suggesting that epidermal innervation is regulated by the fine balance between nerve elongation factors

Abbreviations: AD, atopic dermatitis; AP-1, activator protein-1; BSA, bovine serum albumin; BV, betamethasone valerate; IL, interleukin; 8-MOP, 8-methoxypsoralen; NB-UVB, narrowband ultraviolet B; NDS, normal donkey serum; NGF, nerve growth factor; PBS, phosphate-buffered saline; PGP9.5, protein gene products 9.5; PUVA, psoralen ultraviolet A; Sema3A, semaphorin 3A; TEWL, transepidermal water loss; TNF, tumor necrosis factor.

* Corresponding author at: Department of Dermatology, Juntendo University Urayasu Hospital, 2-1-1 Tomioka, Urayasu, Chiba 279-0021, Japan.
Tel.: +81 47 353 3171; fax: +81 47 353 3178.
E-mail address: ktakamor@juntendo.ac.jp (K. Takamori).

0923-1811/$36.00
doi:10.1016/j.jdermsci.2011.01.004

(e.g., NGF, amphiregulin, gelatinase) [6,10] and nerve repulsion factors (e.g., Sema3A, anosmin-1) [14,15].

UV-based therapies, such as psoralen ultraviolet A (PUVA) and narrowband-ultraviolet B (NB-UVB), are efficacious in the treatment of chronic pruritus in patients with AD [16], psoriasis [17], and lichen amyloidosus [18]. Recently, excimer laser has been shown to ameliorate dermatitis in psoriasis patients and also pruritus in AD patients [19]. Our previous study showed that PUVA therapy reduces epidermal hyperinnervation in AD patients by normalizing abnormal Sema3A and NGF expression in their epidermis [6]. However, the anti-nerve growth effects of other UV-based therapies, such as NB-UVB and excimer lamps, have not been fully characterized.

In this study, we examined the anti-nerve growth effects of UV-based therapies, including combination therapy with corticosteroid ointment, in acetone-treated mice, which show epidermal hyperinnervation. Here, we describe an effective UV-based therapeutic strategy for epidermal hyperinnervation that partly involves the regulation of itch.

2. Materials and methods

2.1. Animals

Male ICR mice (10 weeks old; SLC Japan, Shizuoka, Japan) were maintained in the experimental animal facility of Juntendo University Graduate School of Medicine under a 12 h light:12 h dark cycle at a regulated temperature of 22–24 °C, with food and tap water provided *ad libitum*. Care and handling of all animals conformed to the NIH guidelines for animal research, and all animal procedures were approved by the Institutional Animal Care and Use Committee of the Juntendo University Graduate School of Medicine.

2.2. Antibodies and reagents

The primary antibodies used in this study were anti-protein gene product 9.5 (PGP9.5, 1:4000 dilution; BIOMOL International Corporation, Plymouth Meeting, PA, USA), rabbit anti-NGF (1:500 dilution; Millipore Corporation, Billerica, MA, USA) and rabbit anti-Sema3A (1:200 dilution; Abcam Inc., Cambridge, MA, USA). Secondary antibodies conjugated with Alexa Fluor dye (1:300 dilution) were purchased from Molecular Probes (Eugene, OR, USA).

Bovine serum albumin (BSA) and 8-methoxypsoralen (8-MOP) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Normal donkey serum (NDS) was purchased from Chemicon (Temecula, CA, USA). Vectashield mounting medium was purchased from Vector Laboratories (Burlingame, CA, USA).

2.3. Treatment of cutaneous barrier disruption

The protocol for cutaneous barrier disruption has been described previously [11]. Briefly, the hair over the rostral part of the back was shaved at least 3 days before acetone treatment, and each shaved area was treated with acetone-soaked cotton balls for 5 min. In the control group, the shaved area was treated with sterile water.

Following anesthesia with sevoflurane (Abbott Japan, Osaka, Japan), transepidermal water loss (TEWL) was measured using a Tewameter® TM210 (Courage & Khazawa, Cologne, Germany) for 30 s.

2.4. UV-based therapy

The skin of acetone-treated mice was treated with UV-based therapies 24 h after acetone treatment ($n = 6$, each group). An untreated group was also used as a control.

2.4.1. Psoralen and ultraviolet A (PUVA) therapy or combination of PUVA and betamethasone valerate (BV) ointment

Following intraperitoneal injection of 4 mg/kg body weight of 8-MOP, mice were irradiated with 4 J/cm^2 of UVA (320–375 nm) using a 6 W UVL-56 Handheld UV Lamp (UVP Inc., Upland, CA, USA) [20]. 8-MOP was dissolved in absolute ethanol to 5 g/L and diluted 1:10 with sterile water immediately prior to injection as described [21]. In the PUVA + BV group, mice were treated with 100 mg per site of BV ointment (Rinderon®V; Shionogi & Co., Ltd., Osaka, Japan) after PUVA therapy. Control groups were treated with 8-MOP or BV alone.

2.4.2. Narrow-band ultraviolet B (NB-UVB) therapy

Mice were irradiated with 1 J/cm^2 of NB-UVB (311 nm) [22], using an 18 W 120UVB-NB model (Solark Systems, Inc., Ontario, Canada).

2.4.3. Excimer lamp therapy

A xenon-chloride gas 308-nm excimer lamp (E500-308P model; Excimer, Inc., Kanagawa, Japan) was used to irradiate mice at a dose of 250 mJ/cm^2 [23].

Each UV irradiance was measured using a radiometer (Sato Shouji, Inc., Kanagawa, Japan).

2.5. Immunohistochemistry

Skin samples were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 4 h. After washing with phosphate buffered saline (PBS, pH 7.4), the samples were immersed successively in PBS solutions containing 10%, 15%, and 20% sucrose. The skin samples were embedded in Optimal Cutting Temperature (OCT) compound (Sakura Finetek Japan Co., Ltd., Tokyo, Japan), frozen in liquid nitrogen, and cut into cryosections (20 μm thick for PGP9.5 staining or 8 μm thick for NGF staining) using a CM1850 cryostat (Leica Microsystems, Wetzlar, Germany). The sections were mounted on silane-coated glass slides. After blocking in PBS with 5% NDS and 2% BSA, the sections were incubated with antibodies against PGP9.5 and NGF overnight at 4 °C.

For immunofluorescence staining of Sema3A, skin samples were embedded in OCT compound without fixation, and cryosections 8 μm thick were fixed with ice-cold acetone for 10 min at −20 °C. The sections were rehydrated in PBS, blocked in PBS with 5% NDS and 2% BSA, and incubated with antibodies against Sema3A overnight at 4 °C. After washing with PBS, the sections were incubated with secondary antibodies for 1 h at room temperature and mounted in Vectashield mounting medium. Immunoreactivity was viewed with a confocal laser-scanning microscope (DMIRE2; Leica Microsystems).

2.6. Semi-quantification of epidermal nerve fibers

Three skin specimens from each mouse were stained with anti-PGP9.5 antibody. Using a confocal microscope, optical sections 0.9 μm thick were scanned through the z-plane of the stained specimens (thickness 20 μm), and the images were reconstructed in three dimensions using Leica Confocal Software (Leica Microsystems). The numbers of epidermal nerves were determined by analyzing at least 27 confocal images from each group. The numbers of epidermal nerve fibers in areas of 1.6×10^3 μm^2 were hand-counted in the images by two researchers (M.T. and S.T.) blinded to treatment. All values are reported as the means ± standard deviation (SD) of three experiments.

2.7. Semi-quantitative measurements of immunohistochemical fluorescence intensity

Three skin specimens from each mouse were stained with anti-NGF or anti-Sema3A antibody, and the fluorescence intensity of

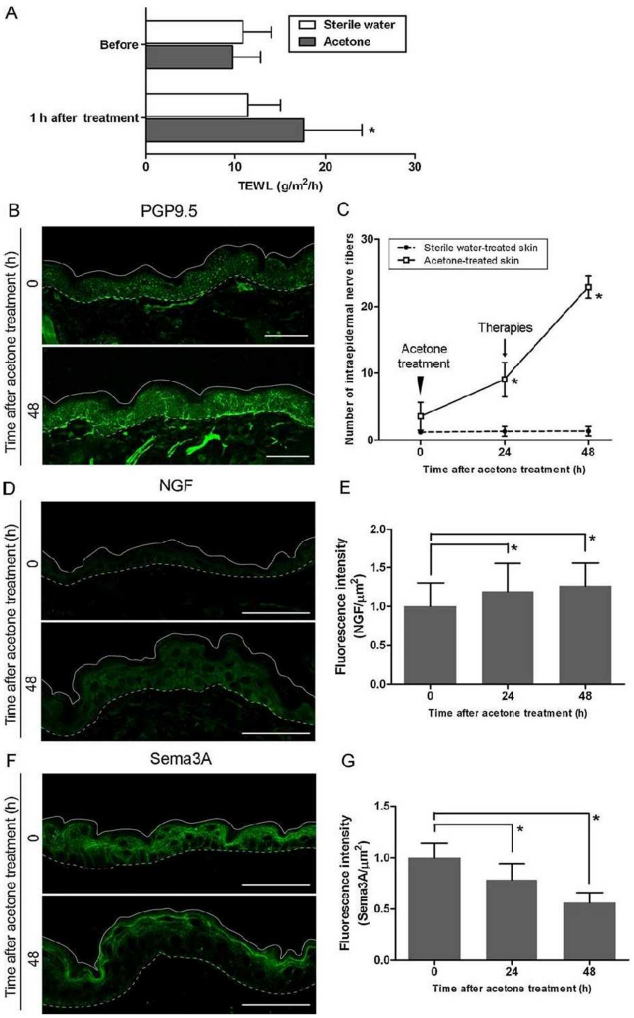

Fig. 1. Distribution of nerve fibers and NGF and Sema3A levels in the epidermis of acetone-treated mice. (A) TEWL was significantly increased 1 h after acetone treatment. $^{*}P < 0.01$ *vs.* sterile water-treated control mice. (B) Acetone treatment significantly increased the penetration of nerve fibers into the epidermis. (C) Increased numbers of nerve fibers were observed after acetone treatment, but not in skin treated with sterile water. $^{*}P < 0.001$ *vs.* sterile water-treated control mice at each time point. Arrowheads and arrows show the time points of acetone treatment and therapies, respectively. (D) Expression pattern of epidermal NGF in sterile water- and acetone-treated mice. (E) The fluorescence intensity of epidermal NGF was significantly increased after acetone treatment ($^{*}P < 0.05$). (F) Expression pattern of epidermal Sema3A in sterile water- and acetone-treated mice. (G) Significant decreases in the levels of epidermal Sema3A were observed after acetone treatment. ($^{*}P < 0.05$). Scale bars, 50 μm. White and broken lines indicate the skin surface and the border between the epidermis and dermis, respectively.

each mouse was evaluated using 15 confocal images. Exposure and acquisition settings were fixed such that no signal saturation occurred. The total fluorescence intensity in the epidermis of each mouse was measured using Leica Confocal Software (Leica Microsystems), and fluorescence intensity per unit area was calculated. All values are reported as the means ± SD of three experiments.

2.8. Statistical analysis

Data were analyzed using Prism 5 (GraphPad Software Inc., La Jolla, CA, USA). The differences between means were analyzed by Tukey's multiple comparison tests.

3. Results

3.1. Distribution of nerve fibers and NGF and Sema3A levels in the epidermis of acetone-treated mice

We confirmed the skin dryness and cutaneous barrier disruption by TEWL measurement. TEWL was increased and peaked at 1 h after acetone treatment (Fig. 1A). The distribution of intraepidermal nerve fibers was examined immunohistochemically in the skin of acetone-treated mice. Intraepidermal nerve fibers were occasionally observed in control mice treated with sterile water (data not shown). Penetration of nerve fibers into the epidermis was observed in the acetone-treated skin, and nerve growth was increased 48 h after acetone treatment (Fig. 1B and C).

Immunohistochemical measurements of the levels of expression of NGF and Sema3A in the epidermis of acetone-treated mice showed that epidermal NGF levels were significantly increased (Fig. 1D and E), while epidermal Sema3A levels were significantly decreased (Fig. 1F and G), 24 and 48 h after acetone treatment.

3.2. Anti-nerve growth effects of UV-based therapies on acetone-treated mice

Acetone-induced epidermal hyperinnervation was significantly decreased in PUVA, PUVA + BV, NB-UVB, and excimer lamp treated groups compared with that in the nontreated group (Fig. 2A and B). Both NB-UVB and excimer lamp treatments had a particularly strong effect on intraepidermal nerve growth (Fig. 2B). No signs of redness or burning of the skin of the mice within the treated site were observed in all groups (data not shown).

3.3. Effects of UV-based therapies on epidermal NGF and Sema3A levels in acetone-treated mice

Epidermal NGF levels were significantly decreased in the skin treated with BV, PUVA, PUVA + BV and NB-UVB, while no alteration was observed in excimer lamp treated group (Fig. 3A). The significant increase of the levels of epidermal Sema3A was observed in the PUVA + BV and NB-UVB treated groups (Fig. 3B).

4. Discussion

Epidermal hyperinnervation is found in the skin of patients with AD, psoriasis, and xerosis, and is associated with intractable itch [4]. In clinical settings, PUVA therapy is often used together with corticosteroid ointment as adjunctive treatment in patients with inflammatory skin diseases [24], although the effects of each therapy on nerve growth in the skin remain unclear. In the present study, the rank order of anti-nerve growth effect was: BV < PUVA < PUVA + BV. Our data also showed that the epidermal NGF and Sema3A levels were significantly normalized in the PUVA + BV-treated group. Previous studies have shown that UV-based therapies reduce the number of epidermal nerve fibers [6,25] and concomitantly normalize the levels of Sema3A and NGF in the epidermis of AD patients [6]. These observations suggest that epidermal hyperinnervation is regulated by the fine balance between nerve elongation and repulsion factors. These findings may explain the differences in the degree of anti-nerve growth effects among the treatments used in this study.

The expression of axonal guidance molecules controlled by PUVA treatment was examined in this study. Psoralen acts by interfering with activator protein-1 (AP-1) in murine keratinocytes, thereby inhibiting the ability of AP-1 to bind to DNA [26]. Although the Sema3A promoter has not yet been investigated, the NGF promoter contains an AP-1 element important for transcriptional activity [27,28]. PUVA has been reported to affect chromatin structure in human epithelial cells [29,30], and these changes in chromatin structure may influence the DNA binding activity of transcription factors [31]. These findings may explain the mechanism of PUVA-regulated gene expression in epidermal keratinocytes.

Another possibility is the gene regulation of axonal guidance molecules by inflammatory cytokines produced by cutaneous cells, such as keratinocytes and immune cells. A recent study indicated that tumor necrosis factor (TNF)-α enhances NGF production *via* the Raf-1/MEK/ERK pathway in cultured normal human epidermal keratinocytes [32]. Although UV irradiation induces cytokine secretion from cultured keratinocytes, successful UV-based therapy of atopic dermatitis is known to be associated with downregulation of cytokine production in the inflamed skin [33]. Our preliminary experiments also showed that Sema3A expression in cultured normal human epidermal keratinocytes is controlled by some inflammatory cytokines (Tengara et al., unpublished observations). Therefore, these findings raise the possibility that PUVA regulates the expression of axonal guidance molecules by reducing the cytokine levels in the skin.

Glucocorticoids have also been reported to reduce the expression of proinflammatory genes by repression of key inflammatory transcription factors, including AP-1 and NF-κB [34]. Thus, BV may also inhibit NGF expression in epidermal keratinocytes through repression of AP-1 transcription factor expression. A more recent study indicated that TNF-α and interleukin (IL)-1β were upregulated in UVA-irradiated keratinocytes and its effect was inhibited by Jun-N-terminal kinase (JNK) inhibitor [35]. JNKs can induce the expression of AP-1 proteins and also increase the transduction activity of AP-1 complexes by phosphorylation [36]. These data suggest that BV application after PUVA therapy suppresses the UVA-induced inflammation response. Therefore, the anti-nerve growth activity of combination treatment may be greater than either treatment alone.

Interestingly, we also found that the anti-nerve growth effects of NB-UVB and excimer lamp treatments were more effective than PUVA + BV treatment. It is generally accepted that UVA penetrates into the dermis, while UVB is limited almost exclusively to the epidermis [37]. This suggests that the high efficacy of UVB irradiation is restricted to the epidermal region alone, and may explain the different anti-nerve growth effects among the UV-based therapies used in this study. This is supported by clinical studies using PUVA, NB-UVB, or excimer lamp therapies [38,39].

Photobiologically, the wavelengths of NB-UVB and excimer lamp are very close to each other, and therefore their therapeutic effects seem to be similar [40]. Although our study showed a strong inhibitory effect of NB-UVB and excimer lamp on epidermal nerve growth, these UV-based therapies had different effects on the expression of axonal guidance molecules in the epidermis of acetone-treated mice.

Short-wave radiation, such as UVB, excites DNA directly and generates photoproducts, such as cyclobutane pyrimidine dimers

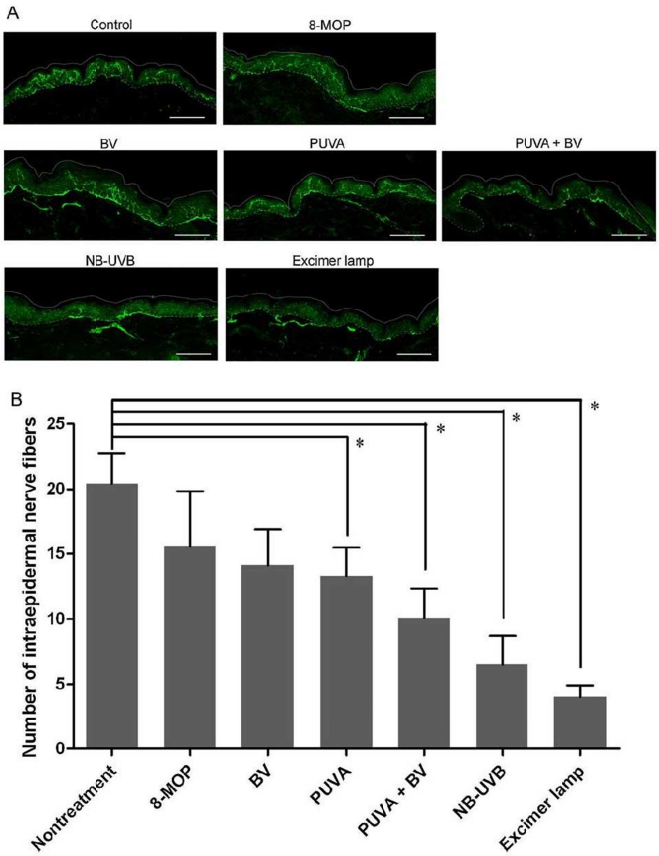

Fig. 2. Effects of UV-based therapy on intraepidermal nerve fibers in acetone-treated mice. (A) Distributions of intraepidermal nerve fibers after a single topical application of UV-based therapy including combination treatment with BV ointment in acetone-treated mice were examined immunohistochemically with anti-PGP9.5 antibody. White and broken lines indicate the skin surface and the border between the epidermis and dermis, respectively. Scale bars, 50 μm. (B) A marked decrease in the number of intraepidermal nerve fibers was observed in the group of mice treated with PUVA, PUVA + BV, NB-UVB and excimer lamp (*$P < 0.001$). All values represent the means ± SD of 6 animals.

and (6–4) photoproducts, which induce considerable bending of the DNA [41]. A recent study demonstrated that 311–313 nm UVB radiation (dose: 750 mJ/cm^2) induced AP-1 binding to DNA [42]. These findings suggest that NB-UVB has the potential to modulate the expression of NGF in keratinocytes. Moreover, several groups have proposed that UV irradiation induces ligand-independent activation of cell-surface receptors, such as epidermal growth factor receptor [43,44]. These observations also suggest that NB-UVB may modulate the expression of Sema3A in keratinocytes. This was supported by recent findings that epidermal growth factor increases the expression of Sema3A at both mRNA and protein levels in human corneal epithelial cells [45]. However, as photoproducts are among the factors involved in skin carcinogenesis, further studies are required to determine adequate irradiation doses with low DNA damage potential.

Excimer lamp treatment, the most effective form of therapy on intraepidermal nerve fibers, did not alter the epidermal expression of axonal guidance molecules. Experimentally, keratinocytes were more resistant than lymphocytes to UVB-induced apoptosis [46]. Therefore, although the mechanisms remain obscure, the anti-nerve growth effects may depend on the sensitivity of cutaneous cells to different UV wavelengths. This may also partly explain the greater inhibitory effect of NB-UVB on intraepidermal nerve fibers compared with PUVA + BV, although NB-UVB restored the expression balance of axonal guidance molecules to the same level as PUVA + BV.

In conclusion, the results presented here indicated that NB-UVB and excimer lamp treatment may be an effective therapeutic method for patients with pruritus involving epidermal hyperinnervation. Combination therapy consisting of PUVA + BV may be

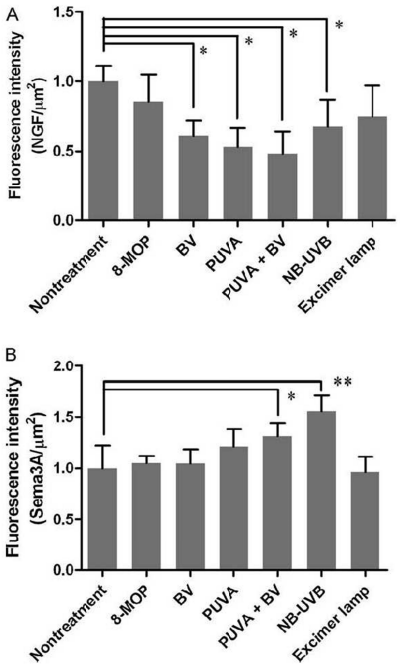

Fig. 3. Effects of UV-based therapy on epidermal NGF and Sema3A levels in acetone-treated mice. (A) The fluorescence intensity of epidermal NGF was significantly decreased in mice treated with BV, PUVA, PUVA + BV and NB-UVB compared with the control mice ($^{*}P < 0.05$). (B) The fluorescence intensity of epidermal Sema3A was significantly increased in PUVA + BV and NB-UVB-treated mice ($^{*}P < 0.05$, $^{**}P < 0.001$). All values represent the means ± SD of 6 animals.

recommended for anti-pruritic therapy rather than either treatment alone. These findings also expand our knowledge regarding effective treatments for pruritic skin diseases.

Acknowledgments

This work was supported by a Health Labor Sciences Research Grant for Research on Allergic Disease and Immunology from the Japanese Ministry of Health, Labor, and Welfare, by the 'High-Tech Research Center' Project for Private Universities: with a matching fund subsidy from MEXT, and by a grant from the Kashiwado Memorial Foundation for Medical Research.

References

[1] Stander S, Weisshaar E, Luger TA. Neurophysiological and neurochemical basis of modern pruritus treatment. Exp Dermatol 2008;17:9–161.
[2] Paus R, Schmelz M, Biro T, Steinhoff M. Frontiers in pruritus research: scratching the brain for more effective itch therapy. J Clin Invest 2006;116:86–1174.
[3] Ikoma A, Steinhoff M, Stander S, Yosipovitch G, Schmelz M. The neurobiology of itch. Nat Rev Neurosci 2006;7:47–535.
[4] Tominaga M, Takamori K. Recent advances in pathophysiological mechanisms of itch. Expert Rev Dermatol 2010;5:197–212.
[5] Urashima R, Mihara M. Cutaneous nerves in atopic dermatitis. A histological, immunohistochemical and electron microscopic study. Virchows Arch 1998;432:70–363.
[6] Tominaga M, Tengara S, Kamo A, Ogawa H, Takamori K. Psoralen-ultraviolet A therapy alters epidermal Sema3A and NGF levels and modulates epidermal innervation in atopic dermatitis. J Dermatol Sci 2009;55:6–40.
[7] Takamori K, Takimoto R, Hase T. Mechanisms of itch in dry skin-NGF induces the elongation/penetration of nerve fibres into the epidermis. In: Proceedings of the International Workshop for the Study of Itch; 2001.p. pp36.
[8] Kinkelin I, Motzing S, Koltenzenburg M, Brocker EB. Increase in NGF content and nerve fiber sprouting in human allergic contact eczema. Cell Tissue Res 2000;302:7–31.
[9] Abadia Molina F, Burrows NP, Jones RR, Terenghi G, Polak JM. Increased sensory neuropeptides in nodular prurigo: a quantitative immunohistochemical analysis. Br J Dermatol 1992;127:51–344.
[10] Tominaga M, Ozawa S, Ogawa H, Takamori K. A hypothetical mechanism of intraepidermal neurite formation in NC/Nga mice with atopic dermatitis. J Dermatol Sci 2007;46:199–210.
[11] Tominaga M, Ozawa S, Tengara S, Ogawa H, Takamori K. Intraepidermal nerve fibers increase in dry skin of acetone-treated mice. J Dermatol Sci 2007;48:11–103.
[12] Albers KM, Wright DE, Davis BM. Overexpression of nerve growth factor in epidermis of transgenic mice causes hypertrophy of the peripheral nervous system. J Neurosci 1994;14:32–1422.
[13] Toyoda M, Nakamura M, Makino T, Hino T, Kagoura M, Morohashi M. Nerve growth factor and substance P are useful plasma markers of disease activity in atopic dermatitis. Br J Dermatol 2002;147:9–71.
[14] Tominaga M, Ogawa H, Takamori K. Decreased production of semaphorin 3A in the lesional skin of atopic dermatitis. Br J Dermatol 2008;158:4–842.
[15] Tengara S, Tominaga M, Kamo A, Taneda K, Negi O, Ogawa H, et al. Keratinocyte-derived anosmin-1, an extracellular glycoprotein encoded by X-linked Kallmann syndrome gene, is involved in modulation of epidermal nerve density in atopic dermatitis. J Dermatol Sci 2010;58:64–71.
[16] Meduri NB, Vandergriff T, Rasmussen H, Jacobe H. Phototherapy in the management of atopic dermatitis: a systematic review. Photodermatol Photoimmunol Photomed 2007;23:12–106.
[17] Evers AW, Kleinpenning MM, Smits T, Boezeman J, van de Kerkhof PC, Kraaimaat FW, et al. Itch and scratching as predictors of time to clearance of psoriasis with narrow-band ultraviolet B therapy. Br J Dermatol 2009;161:6–542.
[18] Oiso N, Yudate T, Kawara S, Kawada A. Successful treatment of lichen amyloidosus associated with atopic dermatitis using a combination of narrowband ultraviolet B phototherapy, topical corticosteroids and an antihistamine. Clin Exp Dermatol 2009;34:6–833.
[19] Baltas E, Csoma Z, Bodai L, Ignacz F, Dobozy A, Kemeny L. Treatment of atopic dermatitis with the xenon chloride excimer laser. J Eur Acad Dermatol Venereol 2006;20:60–657.
[20] Miyauchi-Hashimoto H, Okamoto H, Sugihara A, Horio T. Therapeutic and prophylactic effects of PUVA photochemotherapy on atopic dermatitis-like lesions in NC/Nga mice. Photodermatol Photoimmunol Photomed 2005;21:30–125.
[21] Shuler CF, Latt SA. Sister chromatid exchange induction resulting from systemic, topical, and systemic-topical presentations of carcinogens. Cancer Res 1979;39:4–2510.
[22] Shintani Y, Yasuda Y, Kobayashi K, Maeda A, Morita A. Narrowband ultraviolet B radiation suppresses contact hypersensitivity. Photodermatol Photoimmunol Photomed 2008;24:7–32.
[23] Kollner K, Wimmershoff MB, Hintz C, Landthaler M, Hohenleutner U. Comparison of the 308-nm excimer laser and a 308-nm excimer lamp with 311-nm narrowband ultraviolet B in the treatment of psoriasis. Br J Dermatol 2005;152:4–750.
[24] Morison WL. Phototherapy and photochemotherapy of skin disease. 3rd ed., NY: Taylor & Francis Group; 2005.
[25] Wallengren J, Sundler F. Phototherapy reduces the number of epidermal and CGRP-positive dermal nerve fibres. Acta Derm Venereo. 2004;84:5–111.
[26] Marley CA, Vetrano AM, Whittemore MS, Mariano TM, Heck DE, Laskin DL, et al. Inhibition of interferon-gamma signaling by a mercurio-substituted dihydropsoralen in murine keratinocytes. Biochem Pharmacol 2005;70:34–1726.
[27] Hengerer B, Lindholm D, Heumann R, Ruther U, Wagner EF, Thoenen H. Lesion-induced increase in nerve growth factor mRNA is mediated by c-fos. Proc Natl Acad Sci USA 1990;87:903–3899.
[28] D'Mello SR, Heinrich G. Structural and functional identification of regulatory regions and cis elements surrounding the nerve growth factor gene promoter. Brain Res Mol Brain Res 1991;11:64–255.
[29] Ree K, Johnsen AS, Hovig T. Ultrastructural studies on the effect of photoactivated 8-methoxy psoralen. Nuclear changes in a human epithelial cell line. Acta Pathol Microbiol Scand A 1981;89:81–90.
[30] Gasparro FP, Felli A, Schmitt IM. Psoralen photobiology: the relationship between DNA damage, chromatin structure, transcription, and immunogenic effects. Recent Results Cancer Res 1997;143:27–101.
[31] Park SW, Huq MD, Loh HH, Wei LN. Retinoic acid-induced chromatin remodeling of mouse kappa opioid receptor gene. J Neurosci 2005;25:7–3350.
[32] Takaoka K, Shirai Y, Saito N. Inflammatory cytokine tumor necrosis factor-alpha enhances nerve growth factor production in human keratinocytes, HaCaT cells. J Pharmacol Sci 2009;111:91–381.
[33] Morita A, Werfel T, Stege H, Ahrens C, Karmann K, Grewe M, et al. Evidence that singlet oxygen-induced human T helper cell apoptosis is the basic mechanism of ultraviolet-A radiation phototherapy. J Exp Med 1997;186:8–1763.

[34] Newton R, Holden NS. Separating transrepression and transactivation: a distressing divorce for the glucocorticoid receptor? Mol Pharmacol 2007;72:799–809.
[35] An L, Dong GQ, Gao Q, Zhang Y, Hu LW, Li JH, et al. Effects of UVA on TNF-alpha IL-1beta, and IL-10 expression levels in human keratinocytes and intervention studies with an antioxidant and a JNK inhibitor. Photodermatol Photoimmunol Photomed 2010;26:28–35.
[36] Silvers AL, Bowden GT. UVA irradiation-induced activation of activator protein-1 is correlated with induced expression of AP-1 family members in the human keratinocyte cell line HaCaT. Photochem Photobiol 2002;75:10–302.
[37] Meinhardt M, Krebs R, Anders A, Heinrich U, Tronnier H. Wavelength-dependent penetration depths of ultraviolet radiation in human skin. J Biomed Opt 2008;13:044030.
[38] Van Weelden H, Baart de la Faille H, Young E, van der Leun JC. Comparison of narrow-band UV-B phototherapy and PUVA photochemotherapy in the treatment of psoriasis. Acta Derm Venereol 1990;70:5–212.
[39] Ortel B, Perl S, Kinaciyan T, Calzavara-Pinton PG, Honigsmann H. Comparison of narrow-band (311 nm) UVB and broad-band UVA after oral or bath-water 8-methoxypsoralen in the treatment of psoriasis. J Am Acad Dermatol 1993;29:40–736.
[40] Asawanonda P, Kijluakiat J, Korkij W, Sindhupak W. Targeted broadband ultraviolet b phototherapy produces similar responses to targeted narrow-band ultraviolet B phototherapy for vitiligo: a randomized, double-blind study. Acta Derm Venereol 2008;88:81–376.
[41] Kielbassa C, Roza L, Epe B. Wavelength dependence of oxidative DNA damage induced by UV and visible light. Carcinogenesis 1997;18:6–811.
[42] Hopper BD, Przybyszewski J, Chen HW, Hammer KD, Birt DF. Effect of ultraviolet B radiation on activator protein 1 constituent proteins and modulation by dietary energy restriction in SKH-1 mouse skin. Mol Carcinog 2009;48:52–843.
[43] Wang HQ, Quan T, He T, Franke TF, Voorhees JJ, Fisher GJ. Epidermal growth factor receptor-dependent, NF-kappaB-independent activation of the phosphatidylinositol 3-kinase/Akt pathway inhibits ultraviolet irradiation-induced caspases-3, -8, and -9 in human keratinocytes. J Biol Chem 2003;278:45–45737.
[44] Fisher GJ, Talwar HS, Lin J, Lin P, McPhillips F, Wang Z, et al. Retinoic acid inhibits induction of c-Jun protein by ultraviolet radiation that occurs subsequent to activation of mitogen-activated protein kinase pathways in human skin in vivo. J Clin Invest 1998;101:40–1432.
[45] Ko JA, Morishige N, Yanai R, Nishida T. Up-regulation of semaphorin 3A in human corneal fibroblasts by epidermal growth factor released from cocultured human corneal epithelial cells. Biochem Biophys Res Commun 2008;377:8–104.
[46] Krueger JG, Wolfe JT, Nabeya RT, Vallat VP, Gilleaudeau P, Heftler NS, et al. Successful ultraviolet B treatment of psoriasis is accompanied by a reversal of keratinocyte pathology and by selective depletion of intraepidermal T cells. J Exp Med 1995;182:68–2057.