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Full paper

Effects of irradiation with narrowband-ultraviolet B on up-regulation of histamine H_1 receptor mRNA and induction of apoptosis in HeLa cells and nasal mucosa of rats

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ABSTRACT

Narrowband-ultraviolet B (NB-UVB) phototherapy is used for the treatment of atopic dermatitis. Previously, we reported that irradiation with 200 mJ/cm^2 of 310 nm NB-UVB suppressed phorbol-12-myristate-13-acetate (PMA)-induced up-regulation of histamine H_1 receptor (H1R) gene expression without induction of apoptosis in HeLa cells. However, the effect of NB-UVB irradiation on nasal symptoms is still unclear. Here, we show that low dose irradiation with 310 nm NB-UVB alleviates nasal symptoms in toluene 2,4-diisocyanate (TDI)-sensitized allergy model rats. Irradiation with 310 nm NB-UVB suppressed PMA-induced H1R mRNA up-regulation in HeLa cells dose-dependently at doses of 75–200 mJ/cm^2 and reversibly at a dose of 150 mJ/cm^2 without induction of apoptosis. While, at doses of more than 200 mJ/cm^2, irradiation with 310 nm NB-UVB induced apoptosis. Western blot analysis showed that the suppressive effect of NB-UVB irradiation on H1R gene expression was through the inhibition of ERK phosphorylation. In TDI-sensitized rat, intranasal irradiation with 310 nm NB-UVB at an estimated dose of 100 mJ/cm^2 once a day for three days suppressed TDI-induced sneezes and up-regulation of H1R mRNA in nasal mucosa without induction of apoptosis. These findings suggest that repeated intranasal irradiation with low dose of NB-UVB could be clinically used as phototherapy of AR.

1. Introduction

Phototherapy with narrowband UVB (NB-UVB, 308–313 nm) has been used for the treatment of a variety of skin diseases including psoriasis and atopic dermatitis (AD).[1,2] Phototherapy with 311 nm NB-UVB was particularly effective at clearing psoriasis with a reduced capacity to produce erythema.[3] Accordingly, NB-UVB fluorescent lamp (Philips TL01) was developed, emitting a peak at 311 ± 2 nm for use in phototherapy. In the American guidelines of AD, phototherapy with NB-UVB is a second line treatment after failure of first-line treatment.[4] However, a recent systematic review recommended UVA1 (340–400 nm) and NB-UVB phototherapy as a first-choice treatment for AD, as evidenced by their sufficient quality.[5] Similar to AD, allergic rhinitis (AR) is characterized by the helper T cell type 1/2 (Th1/Th2) imbalance toward a pronounced Th2 profile. Therefore, it is suggested that NB-UVB might be effective in the treatment of AR. However, it is difficult to irradiate nasal mucosa using the dermatological NB-UVB lamp. Recently, in collaboration with Nichia Corporation, Tokushima, Japan, we developed light-emitting diodes (LEDs) that emit most of their energy at wavelength of 310 nm of NB-UVB and is small enough for intranasal irradiation with NB-UVB.[6]

Histamine is a major chemical mediator in the pathogenesis of AR and the activation of histamine H_1 receptor (H1R) is responsible

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for the development of nasal symptoms, such as rhinorrhea and sneezes. It was reported that H1R gene was up-regulated in the nasal mucosa of patients with AR.[7,8] Recently, we demonstrated that H1R mRNA levels in the nasal mucosa were significantly correlated with the severity of nasal symptoms in patients with AR, and that the pre-seasonal prophylactic treatment with antihistamines suppressed the up-regulation of H1R mRNA in the nasal mucosa.[9] The suppressive effects of pre-treatment with antihistamines on up-regulation of H1R mRNA and nasal allergy symptoms were also proved in toluene 2,4-diisocyanate (TDI)-sensitized rat model of AR.[10] These findings suggest that suppression of H1R signaling is a promising therapeutic strategy for AR. Our preliminary study showed that irradiation with 310 nm NB-UVB suppressed the up-regulation of H1R gene expression in epithelial HeLa cells expressing H1R endogenously, suggesting that phototherapy with NB-UVB might be used for the treatment of AR.[6] However, dose-dependency and reversibility of its effect have not yet been elucidated. Furthermore, the effect of NB-UVB irradiation on nasal symptoms is still unclear.

In the present study, we first investigated whether irradiation with 310 nm NB-UVB dose-dependently and reversibly suppressed Phorbol-12-myristate-13-acetate (PMA)-induced up-regulation of H1R mRNA, and induced apoptosis in HeLa cells, in which PMA increases the expression levels of both mRNA and protein.[11] We then compared the effects of irradiation with 310 nm NB-UVB with those with 305 and 315 nm UVB. Previously, we have shown that protein kinase Cδ (PKCδ)/ERK/PARP-1 signaling pathway was involved in PMA-induced up-regulation of H1R gene expression in HeLa cells, in which PKCδ and ERK were activated by phosphorylation.[12,13] Thus, we further investigated whether irradiation with 310 nm NB-UVB inhibited phosphorylation of PKCδ or ERK. Finally, we investigated the effect of intranasal irradiation with 310 nm NB-UVB on the induction of apoptosis of nasal mucosa and TDI-induced nasal symptom and up-regulation of H1R mRNA in the nasal mucosa of TDI-sensitized rats.

2. Materials and methods

2.1. NB-UVB irradiation and real-time quantitative RT-PCR

LEDs that emit most of their energy at wavelength of 310 nm of NB-UVB, and 305 and 315 nm UVB were developed by Nichia Corporation.[6] HeLa cells were cultured at 37 °C under a humidified atmosphere of 5% CO_2 and 95% air atmosphere in minimal essential medium MEM-α (Invitrogen, Carlsbad, CA, USA) containing 8% fetal calf serum and 1% antibiotic-antimycotic (Invitrogen). The cells cultured in 6-well plates were serum-starved for 12 h and then irradiated with 310 nm NB-UVB, and 305 and 315 nm UVB at doses of 50–200 mJ/cm^2. Before all experiments, we measured the dosage of UVB using a UVB meter (UVX Radiometer, UVP, Upland, CA) because output of LED is changed every day. Duration of LED irradiation is depended on its output and approximately 1 h for 200 mJ/cm^2 irradiation. Then, they were treated with 100 nM PMA for 3 h. After that, the cells were harvested and total RNA was prepared using RNAiso Plus (Takara Bio Inc., Kyoto) as described previously.[12] RNA sample (5 μg) was used for the reverse transcription reaction to synthesize cDNA using a PrimerScript RT reagent Kit (Takara Bio Inc.). and resulting cDNA was subjected to real-time PCR. Real-time PCR was conducted using a GeneAmp 7300 sequence detection system (Applied Biosystems, Foster City, CA, USA). TaqMan primers and the probe were designed using Primer Express Software (Applied Biosystems). The sequences of the primers and TaqMan probe were as follows: forward primer for human H1R, 5′-CAGAGGATCAGATGTTAGGTGATAGC-3'; reverse primer for human H1R, 5′-AGCGGAGCCTCTTCCAAGTAA-3'; and TaqMan probe, FAM-CTTCTCTCTCGAACGGACTCAGATACCACC-TAMRA. To standardize the starting materials, the human GAPDH gene was used, and data were expressed as the ratio of H1R mRNA to GAPDH mRNA.

2.2. Assessment of apoptosis

Apoptotic morphological changes in the nuclear chromatin of cells were detected by Hoechst 33342 staining. HeLa cells were irradiated with 310 nm NB-UVB, and 305 and 315 nm UVB at doses of 50–200 mJ/cm^2. Four hours after the termination of irradiation, the cells seeded on 35 mm dishes were washed with PBS and fixed with 4% formalin in PBS for 30 min, washed twice with PBS, and stained for 20 min with 5 μg/ml of a fluorescent dye Hoechst 33342 (Sigma, St. Louis, MO, USA). Chromosomal condensation was observed using a fluorescence microscopy (TMD 3000-EF; Nikon, Tokyo). Apoptotic cells were also determined with AnnexinV-FITC Apoptosis Detection Kit (Sigma). Four hours after the termination of irradiation, the cells were harvested by trypsinization and re-suspended in 1 × binding buffer and stained with AnnexinV (5 μl) and propidium iodide (PI, 10 μl) for 10 min in the dark. The cells were analyzed by flow cytometry (Becton Dickinson FACS-Verse flow cytometer).

2.3. Immunoblot analysis

HeLa cells were irradiated with 200 mJ/cm^2 of 310 nm NB-UVB, and 305 and 315 nm UVB. Total cell lysates were then prepared and subjected to immunoblot analysis as previously reported after stimulation with PMA for 10 min.[12] Protein samples (15 μg) were separated on a 10% SDS-PAGE gel and phospho-PKCδ (Tyr^{311}) was detected with anti-phospho-PKCδ (Tyr^{311}) antibody (Cell Signaling Technology Japan, Tokyo). For the detection of phospho-ERK, after stimulation with PMA for 5 min, protein samples were separated on 8% SDS-PAGE gel containing 100 μM $MnCl_2$ and 50 μM Phos-tag (Wako Pure Chemical), followed by immunoblotting using anti-phospho-ERK antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). To detect H1R, HeLa cells were serum-starved for 12 h and treated with 200 mJ/cm^2 NB-UVB for 1.5 h. After that, the cells were stimulated with 100 nM PMA for 12 h, and the membrane fraction was prepared using the subcellular protein fractionation kit (Thermo Fisher Scientific, Waltham, MA, USA). The membrane protein (10 μg) were subjected to immunoblot analysis using anti-H1R antibody (AB5654P; Merk Millipore, Billerica, MA, USA). According to the manufacture's instruction, the obtained membrane fraction contains plasma, mitochondria and ER/Golgi membranes, calreticulin was used as a membrane marker and was detected anti-calreticulin antibody (ab22683; Abcam Japan, Tokyo). Protein concentration was determined by the BCA protein assay reagent (Sigma) using bovine serum albumin as a standard.

2.4. Animal studies

Male Brown Norway rats (6-week, 200–250 g, Japan SLC, Hamamatsu) were used. Rats were allowed free access to water and food and kept in a room maintained at 25 ± 2 °C and 55 ± 10% humidity with a 12-h light/dark cycle. Sensitization and provocation with TDI was performed according to the method described by Dev et al.[14] In brief, 10 μl of a 10% solution of TDI (Wako Pure Chemical, Osaka, Japan) in ethyl acetate (EtOAc; Wako Pure Chemical) was applied bilaterally on the nasal vestibule of each rat once a day for five consecutive days (days 1–5). This sensitization procedure was then repeated on days 8–12 at intervals of 2 days. Nine days (days 13–21) after the second sensitization, 10 μl of 10% TDI solution was again applied to the nasal vestibule to provoke

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nasal symptoms on day 22. The control rats were sensitized and provoked with 10 μl of EtOAc using the same procedure. Intranasal irradiation with 310 nm NB-UVB was given to bilateral nostrils once on day 22, 3 times on from days 20–22, and 7 times on days 16–22 under anesthesia with an intraperitoneal injection of pentobarbital at a dose of 30 μg/kg. According to the guideline established by Harkema et al., the nasal mucosa was divided into four regions T1-T4.[15] In this study, we irradiated NB-UVB in front of nostrils and corrected the nasal septum mucosa from No to T2 shown as dashed box in Fig. 1A right panel. We measured the dosages of UVB using a UVB meter (UVX Radiometer, UVP, Upland, CA). The distance between UVB meter from the LED was 5 mm, which is the same distance from the nostrils to the nasal mucosa. Two LEDs that emit 310 nm NB-UVB were placed in front of each nostril at a distance of 2 mm at an angle of 30° outside body axis and 310 nm NB-UVB was irradiated at a dose of 200 mJ/cm^2 (Fig. 1A left panel). Thus, using a rat nasal cavity model and an UVB meter, intranasal irradiation dose of 100 mJ/cm^2 or less was estimated on the nasal septum mucosa at the inmost distance of 3 mm from the nostril. On day 22, after complete recovery from anesthesia, 10 μl of 10% TDI solution was applied to the nasal vestibule to provoke nasal symptom 3 h after the termination of irradiation with NB-UVB. The number of TDI-induced sneezes was measured over a 10-min period immediately after TDI provocation. Rats were then sacrificed and the nasal mucosa was dissected from nasal septum 4 h after the provocation on day 22, and H1R mRNA levels were measured. It is known that UVB causes DNA damage. And the major DNA damage induced by UVB exposure are cyclobutane pyrimidine dimers (CPDs) and (6–4) photoproducts (6–4 PP), and about 70–80% of DNA damage is CPDs and remaining is 6–4 PP.[16] Thus, if we observed CDP-positive cells after UVB-exposure, we can demonstrate the isolated nasal mucosa was exposed to UVB. So, we have checked if the isolated nasal mucosa was exposed by UVB or not by immunofluorescence study using anti-CPD antibody. (TDM-2, COSMO BIO co., ltd; Japan). Serum total IgE concentration was measured using Rat IgE ELISA kit (ab157736, Abcam). All experimental procedures were performed in accordance with the guidelines of the Animal Research Committee of Tokushima University.

2.5. Induction of apoptosis in the nasal mucosa cells was evaluated by TUNEL assay

The nasal mucosa was fixed in 10% neutral buffered formalin for about 12 h, embedded in paraffin, and then cut into 5 μm-thick sections. The cells were permeabilized with 0.2% Triton X-100 in PBS for 5 min. TUNEL reaction was conducted using DeadEnd Fluorometric TUNEL System (Promega KK, Madison, WI, USA) according to the supplier's instructions. After counter-staining with PI (1 μg/ml), the cells were observed using a confocal laser microscope (LSM510; Carl Zeiss, Oberkochen, Germany).

2.6. Statistical analysis

The results are shown as mean ± SEM. Statistical analyses were performed using ANOVA with Dunnett's test using the GraphPad Prism software (GraphPad software INC., La Jolla, CA, USA). $P < 0.05$ were considered statistically significant.

3. Results

3.1. Dose-dependency and time course of suppressive effect of NB-UVB irradiation on PMA-induced up-regulation of H1R gene expression in HeLa cells

PMA induced a significant increase in the expression of H1R mRNA in HeLa cells. Irradiation with 305 nm UVB and 310 nm NB-UVB, but not 315 nm UVB significantly suppressed PMA-induced up-regulation of H1R mRNA at a dose of 150 mJ/cm^2 (Fig. 2A). Irradiation with 310 nm NB-UVB significantly and dose-dependently suppressed PMA-induced up-regulation of H1R mRNA in HeLa cells at doses of 75, 100, 125, 150 and 200 mJ/cm^2 (Fig. 2B). Irradiation with 305 nm UVB almost completely and significantly suppressed the up-regulation of H1R mRNA in HeLa cells regardless of irradiation doses. On the other hand, irradiation with 315 nm UVB showed no effects on the up-regulation regardless of irradiation doses. In time course studies, irradiation with 310 nm NB-UVB at a dose of 150 mJ/cm^2 suppressed PMA-induced up-regulation of H1R mRNA up to 4 h after the termination of irradiation. Then the suppressive effect disappeared more than 6 h after irradiation (Fig. 2C). On the other hand, 305 nm UVB irradiation almost completely suppressed the up-regulation of H1R mRNA up to 4 h after irradiation and irradiated cells were dead more than 6 h after irradiation, while 315 nm UVB irradiation did not suppress the up-regulation of H1R gene expression. Amount of H1R protein was also significantly decreased after irradiation with 310 nm NB-UVB at a dose of 200 mJ/cm^2 (Fig. 2D, Supplementary Fig. 1).

3.2. Effect of NB-UVB irradiation on induction of apoptosis in HeLa cells

Irradiation with 310 nm NB-UVB at a dose of 200 mJ/cm^2 but not 150 mJ/cm^2 showed typical apoptotic morphology of

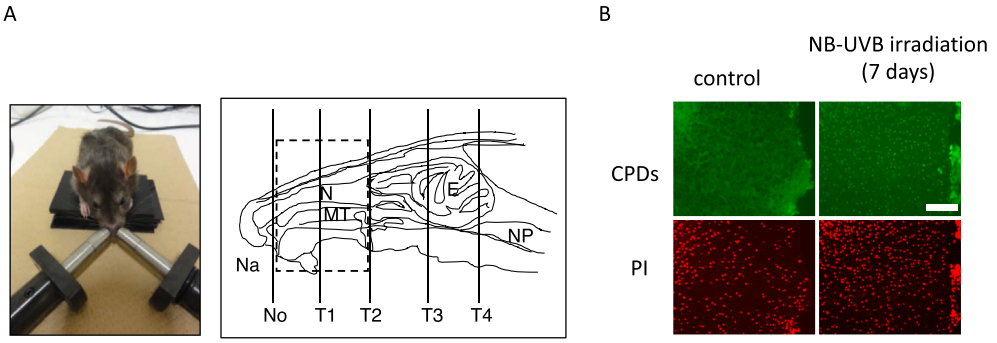

Fig. 1. NB-UVB irradiation equipment used in this study. (A) Left panel; The phototherapy application to the rats. Using a rat nasal cavity model and an UVB meter, intranasal irradiation dose of 100 mJ/cm^2 or less was estimated on the nasal mucosa at the inmost distance of 3 mm from the nostril. Right panel; Schematic presentation of the rat nasal cavity. The nasal septum mucosa from the region nostril (No) to the region T2 was isolated and subjected to the experiments. (B) Detection of CPD formation. The isolated nasal mucosa was fixed in 4% neutral buffered formalin and embedded in paraffin. After the cells were permeabilized with 0.2% Triton X-100, Immunofluorescence study using anti-CPD was conducted. CDP-positive cells were shown as green fluorescence, and nuclei were stained in red fluorescence of PI.

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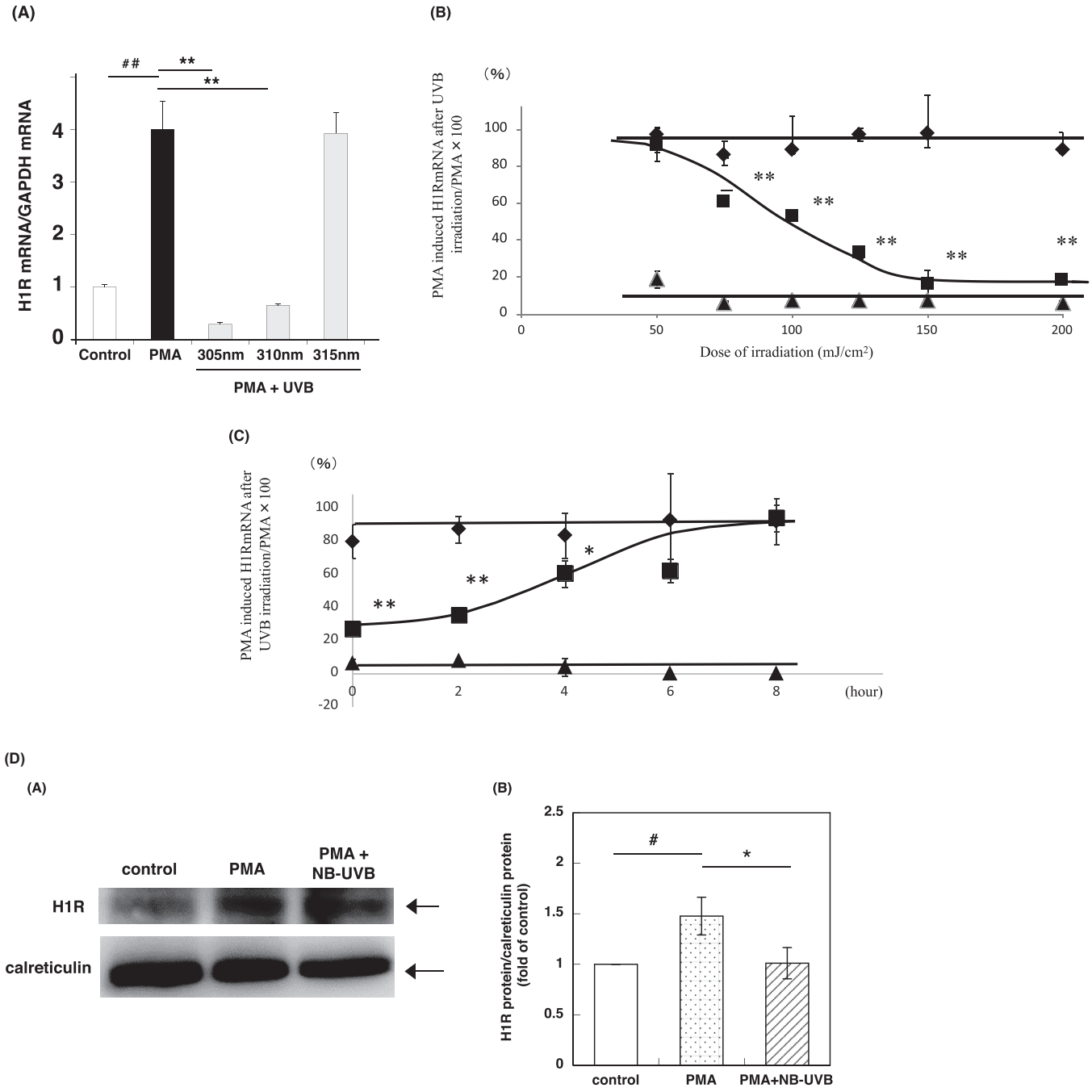

Fig. 2. Effect of irradiation with 310 nm NB-UVB and, 305 and 315 nm UVB on PMA-induced up-regulation of H1R mRNA and protein in HeLa cells. (A) Wavelength dependency; HeLa cells cultured to 70% confluency in 6-well plates were serum-starved for 12 h and irradiated with 310 nm NB-UVB and, 305 and 315 nm UVB at a dose of 150 mJ/cm^2 before PMA stimulation. After 3-h treatment with PMA, the cells were harvested and total RNA was prepared. H1R mRNA level was determined using real-time PCR. (B) Dose-dependency; the cells were irradiated with 50–200 mJ/cm^2. (C) Time course; at the given time intervals after the irradiation with 310 nm NB-UVB and, 305 nm and 315 nm UVB at a dose of 150 mJ/cm^2, cells were harvested and total RNA was isolated. Closed triangles: 305 nm UVB; Closed squares: 310 nm NB-UVB; Closed diamonds: 315 nm UVB. Data are expressed as means ± SEM. $^{\#\#}p < 0.01$ vs. control, $^{*}p < 0.05$, $^{**}p < 0.01$ vs. PMA (n = 4). (D) Immunoblot analysis; HeLa cells were serum-starved for 12 h and irradiated with 310 nm NB-UVB at the dose of 200 mJ/cm^2. Then, the cells were stimulated with 100 nM PMA for 12 h and the membrane fraction was prepared using the subcellular fractionation kit. Immunoblot analysis were performed using 10 μg of membrane protein and H1R protein was detected using anti-H1R antibody. Representative data from five separate experiments are shown (A). In (B), Densitometric analysis was performed using Image J software. Data are expressed as means ± S.E.M. $^{\#}p < 0.05$ vs. control, $^{*}p < 0.05$ vs. PMA (n = 5).

condensed and fragmented nuclei in some HeLa cells. On the other hand, 305 nm UVB induced apoptosis at both doses of 150 and 200 mJ/cm^2 in some HeLa cells, while 315 nm UVB at both doses did not (Fig. 3). The induction of apoptosis was also investigated using AnnexinV/PI staining, in which the percentages of HeLa cells in both early (AnnexinV-positive, PI-negative) and late (AnnexinV-positive, PI-positive) apoptosis were calculated. In a representative investigation, irradiation with 310 nm NB-UVB induced apoptosis at a dose of 200 mJ/cm^2 (40.26 vs. 8.61%: 310 nm vs. control), but not 150 mJ/cm^2 (11.2 vs. 11.1%: 310 nm vs. control) (Fig. 4). Accordingly, the percentages of apoptotic HeLa cells were significantly increased after irradiation with 310 nm NB-UVB at a dose of 200 mJ/cm^2, but not at doses of less than 150 mJ/cm^2, compared with those with control cells

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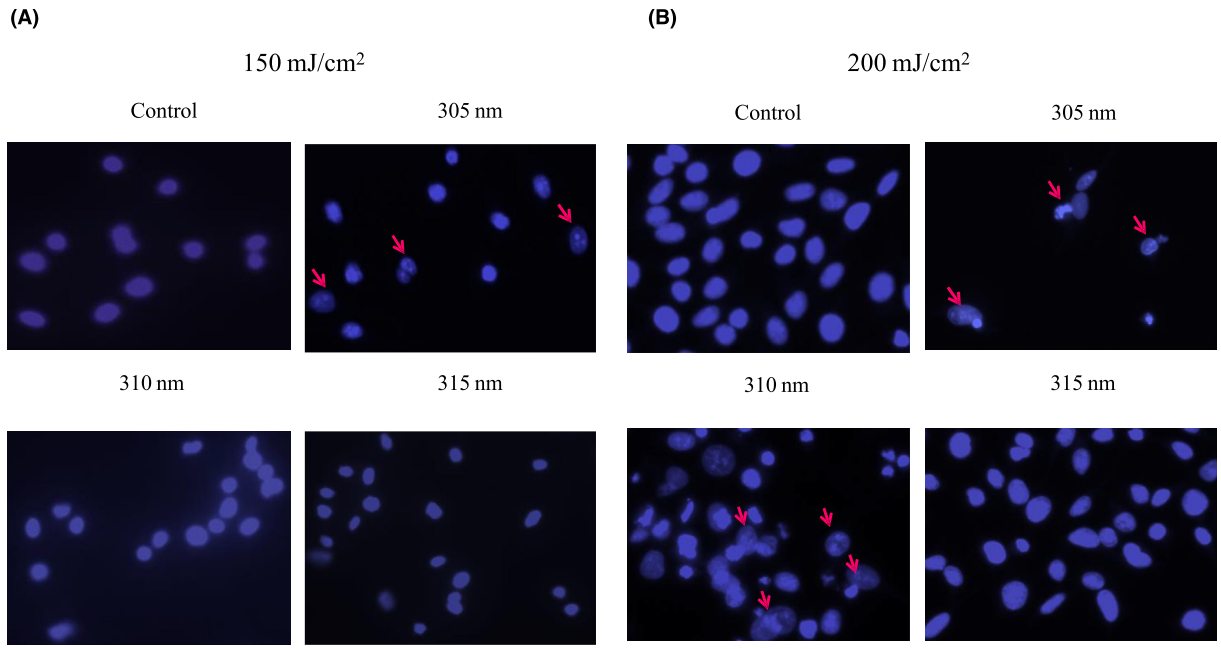

Fig. 3. Effects of irradiation with 310 nm NB-UVB and, 305 and 315 nm UVB on induction of apoptosis in HeLa cells using Hoechst 33342 staining. After irradiation with UVB at doses of 150 (A) and 200 (B) mJ/cm^2, HeLa cells were fixed with 4% formalin in PBS and stained with Hoechst 33342. Chromosomal condensation was observed using a fluorescence microscopy. Apoptotic cells with chromosomal condensation are indicated by red arrows. Control cells were not irradiated.

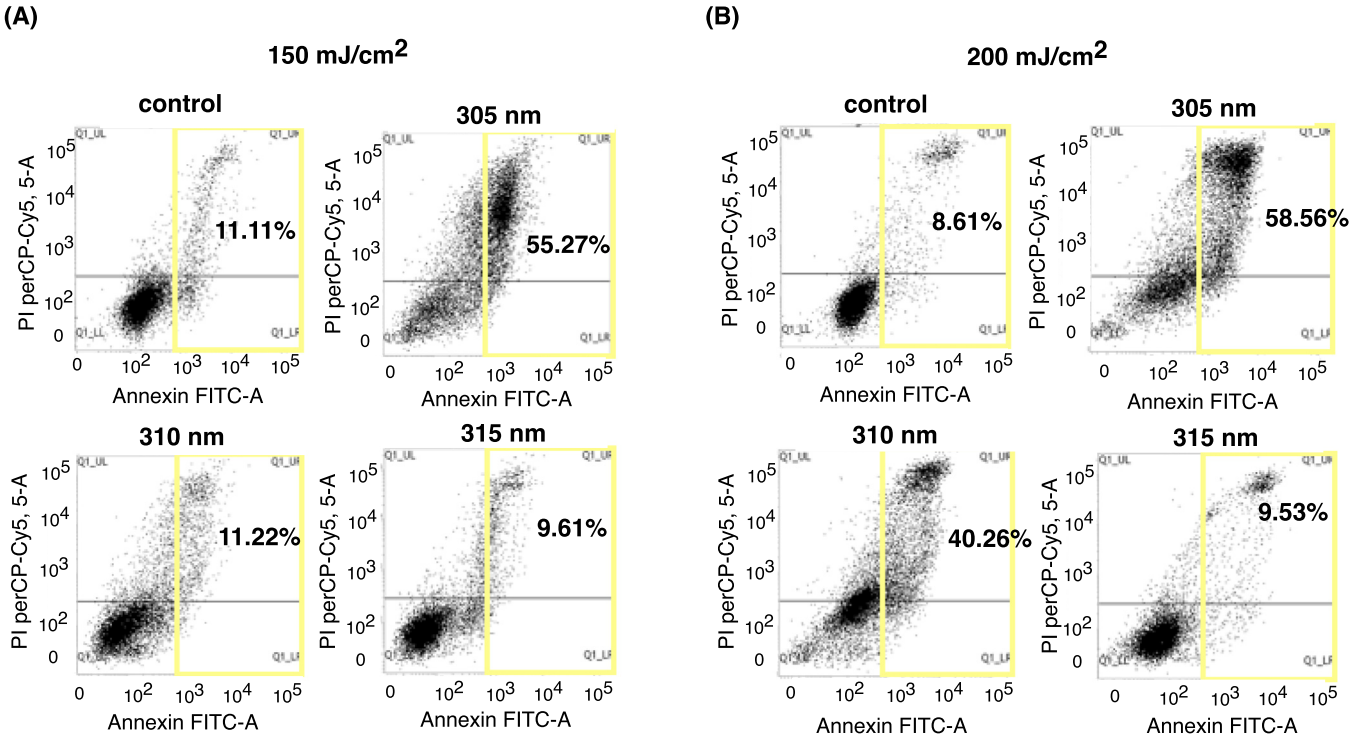

Fig. 4. Effects of irradiation with 310 nm NB-UVB and, 305 and 315 nm UVB on induction of apoptosis in HeLa cells using AnnexinV/PI staining. After irradiation with UVB at doses of 150 (A) and 200 (B) mJ/cm^2, HeLa cells were harvested and stained with AnnexinV and propidium iodide (PI). Flow cytometric analysis was then conducted and the percentage of apoptotic cells were indicated by the summation of both early apoptotic cells (AnnexinV-positive, PI-negative) and late apoptotic cells (AnnexinV-positive, PI-positive). Control cells were not irradiated.

without irradiation (Fig. 5). On the other hand, irradiation with 305 nm UVB induced apoptosis in HeLa cells regardless of the irradiation doses. Moreover, irradiation with 315 nm UVB seemed to induce apoptosis in HeLa cells at doses of more than 600 mJ/cm^2 (inset in Fig. 5).

3.3. Effect of NB-UVB irradiation on PMA-induced PKCδ and ERK phosphorylation in HeLa cells

Irradiation with 310 nm NB-UVB and, 305 and 315 nm UVB at a dose of 200 mJ/cm^2 showed no effect on Tyr^{311} phosphorylation of

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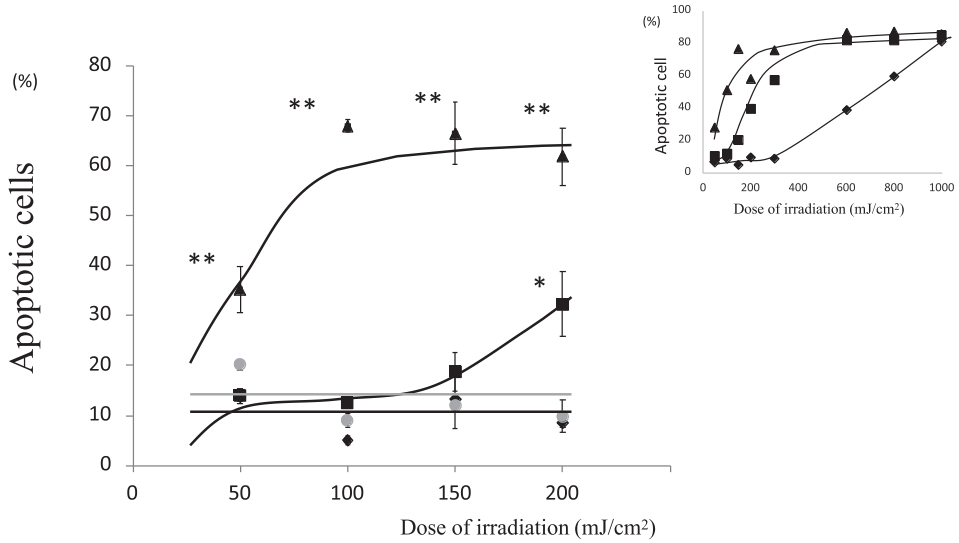

Fig. 5. Dose-dependency of effect of 310 nm NB-UVB and, 305 and 315 nm UVB irradiation on induction of apoptosis in HeLa cells using AnnexinV/PI staining. Flow cytometric analyses were conducted and the percentage of apoptotic HeLa cells was calculated after irradiation with UVB at doses of 50–200 mJ/cm^2. Triangles: 305 nm UVB; Squares: 310 nm NB-UVB; Diamonds: 315 nm UVB; Grey circles: Control. The experiment was performed three times. Data are expressed as means ± SEM. $^*p < 0.05$, $^{**}p < 0.01$ vs. control without irradiation (n = 3). In the inserted graph, the representative experiment was performed at doses of 50–1000 mJ/cm^2.

PKCδ in HeLa cells (Fig. 6A). However, at the same dose, irradiation with 310 nm NB-UVB and 305 nm UVB, but not 315 nm UVB inhibited PMA-induced ERK phosphorylation (Fig. 6B).

3.4. Effect of intranasal irradiation with NB-UVB on TDI-induced nasal symptoms and up-regulation of H1R mRNA in the nasal mucosa of TDI-sensitized rats

At first, we investigated whether NB-UVB exposure actually reach to the nasal septum mucosa of rat by detecting the UVB-induced cyclobutane pyrimidine dimers (CPDs). As shown in Fig. 1B, intranasal irradiation of 200 mJ/cm^2 NB-UVB for 7 days resulted in CDP-positive cells in the nasal mucosa, suggesting that this experimental method is applicable. Intranasal irradiation with 310 nm NB-UVB at an estimated dose of 100 mJ/cm^2 or less, 3 times on days 20–22 significantly suppressed the number of TDI-induced sneezes (Fig. 7A) and TDI-induced up-regulation of H1R gene expression in the nasal mucosa (Fig. 7B) in TDI-sensitized rats. However, intranasal irradiation with NB-UVB at the same dose, once on day 22 and 7 times on days 16–22 suppressed neither TDI-induced sneezes nor H1Rm RNA up-regulation in the nasal mucosa.

3.5. Effect of intranasal irradiation with NB-UVB on apoptosis in the nasal mucosa of TDI-sensitized rats

TUNEL-positive cells were not induced in the nasal mucosa of TDI-sensitized rats after TDI provocation. Intranasal irradiation with 310 nm NB-UVB at an estimated dose of 100 mJ/cm^2 or less, 3 times on days 20–22 induced few TUNEL-positive cells in the nasal mucosa after TDI provocation. However, intranasal irradiation with

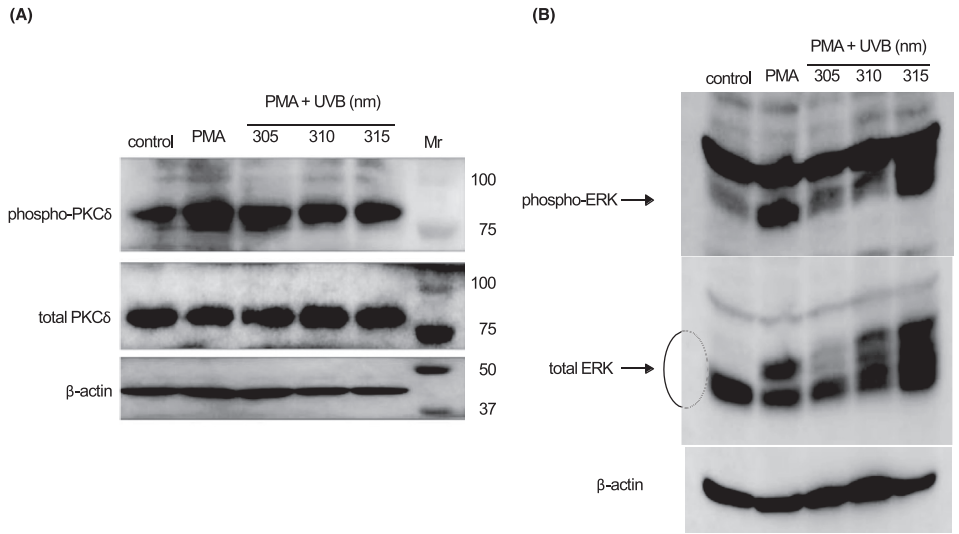

Fig. 6. Effect of irradiation with 310 nm NB-UVB and, 305 and 315 nm UVB on PMA-induced phosphorylation of PKCδ (A) and ERK (B) in HeLa cells. HeLa cells were irradiated with 310 nm NB-UVB, 305 nm and 315 nm UVB at a dose of 200 mJ/cm^2 and, after stimulation with PMA for 10 min (to detect phospho-PKCδ) or 5 min (to detect phospho-ERK). Proteins were separated on a 10% SDS-PAGE gel (to detect phospho-PKCδ) or 8% SDS-PAGE gel containing 100 μM $MnCl_2$ and 50 μM Phos-tag (to detect phospho-ERK), and subjected to immunoblot analysis.

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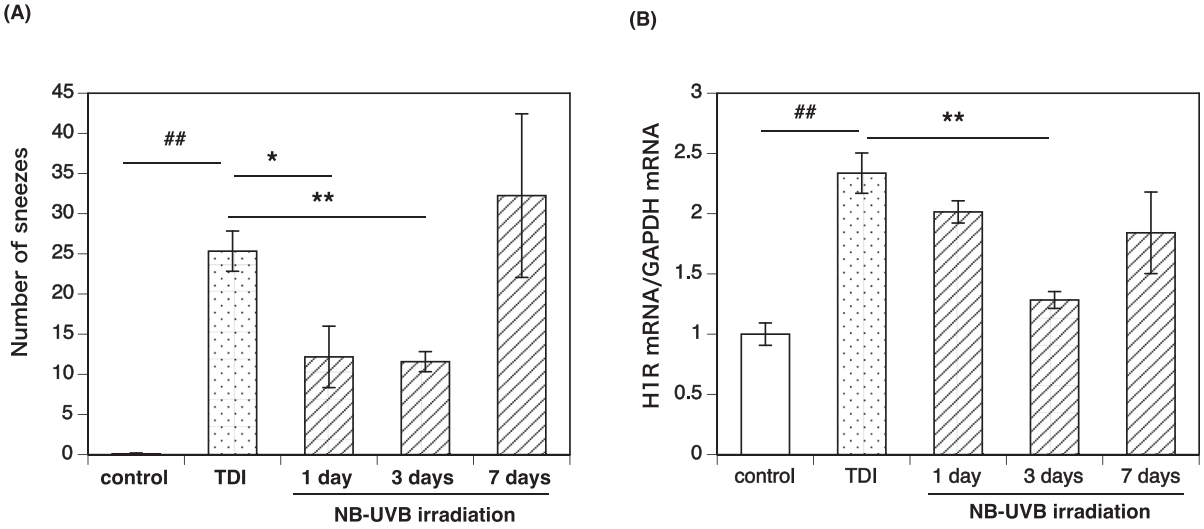

Fig. 7. Effect of irradiation with 310 nm NB-UVB on TDI-induced sneezes (A) and up-regulation of H1R mRNA (B) in the nasal mucosa of TDI-sensitized rats. NB-UVB of 310 nm at an estimated dose of 100 mJ/cm^2 or less was irradiated to the bilateral nostrils of TDI-sensitized rats, once (day 22), three consecutive days (days 20–22) and seven consecutive days (days 16–22) before TDI provocation. Nasal symptoms were measured during 10 min just after TDI provocation on day 22. The rats were sacrificed 4 h after TDI provocation, and total RNA was isolated from the nasal mucosa. H1R mRNA level was determined using real-time PCR. (A) The number of TDI-induced sneezing. (B) The level of TDI-induced up-regulation of H1R mRNA in the nasal mucosa of TDI-sensitized rats. The data are expressed as means ± SEM. n = 6 (irradiation once), 7 (three times), 4 (seven times), 9 (TDI without irradiation), and 10 (control). $^{\#\#}p < 0.01$ vs. control; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. TDI. Control rats were sensitized and provoked with EtOAc. TDI rats were sensitized and provoked with TDI without NB-UVB irradiation.

NB-UVB at the same dose, 7 times on days 16–22 induced a substantial number of TUNEL-positive cells in the nasal mucosa after TDI provocation (Fig. 8).

4. Discussion

In the present study, we showed that irradiation with 310 nm NB-UVB dose-dependently and reversibly suppressed PMA-induced up-regulation of H1R gene expression in HeLa cells without induction of apoptosis at doses of less than 150 mJ/cm^2. Irradiation with 310 nm NB-UVB at a dose of 200 mJ/cm^2 also significantly suppressed PMA-induced increase in H1R protein expression. We also showed that the suppression of PMA-induced up-regulation of H1R mRNA after irradiation with 305 nm UVB or higher doses of 310 nm NB-UVB was due to the induction of apoptosis. Irradiation with 315 nm UVB had no effects on the H1R gene up-regulation. These findings suggested that low-dose irradiation with NB-UVB wavelength-specifically suppressed transcriptional activation of H1R gene without induction of apoptosis.

HeLa cells are not representative for typical target cells involved in allergic reactions. Although, we have not determined which cells in the nasal mucosa are the H1R-expressing cells, epithelial cells are one of the candidates for responsible for histamine-induced up-regulation of H1R gene expression because HeLa cells are the cell line derived from cervical cancer cells arising in epithelial cells. It was reported that H1R mRNA was increased in human nasal epithelial cells after stimulation with diesel exhaust particles that are known to cause chronic airway diseases.[17] Previously, we have demonstrated that PKCδ/ERK/PARP-1 signaling pathway was involved in PMA-induced up-regulation of H1R gene expression in HeLa cells.[12] Involvement of H1R-PKC-ERK signaling pathway in eliciting GM-CSF and IL-8 production from bronchial epithelial cells stimulated by histamine was also reported.[17,18] In addition, involvement of PKCδ was reported by Masaki et al.,[19] Furthermore, it was demonstrated that H1R immunoreactivity was observed in human epithelial cells and vascular endothelial cells.[20] From these findings, we believe that HeLa cells can be a model to analyze molecular mechanism of histamine-induced up-regulation of H1R gene expression in the nasal mucosa although HeLa cells are not representative for typical target cells involved in allergic reactions.

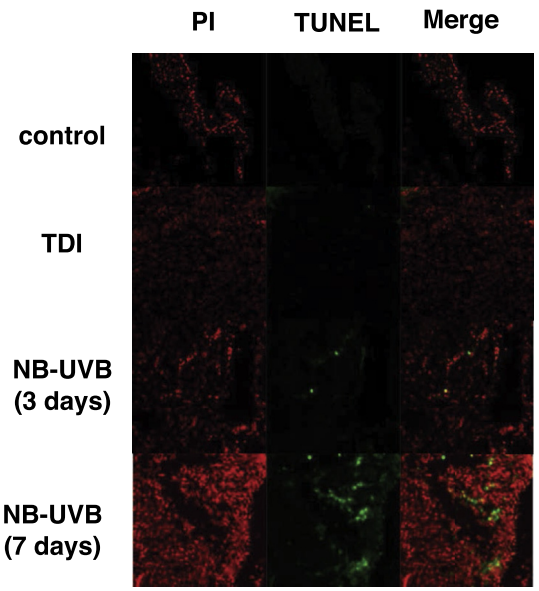

Fig. 8. Effects of irradiation with 310 nm NB-UVB on induction of apoptosis in the nasal mucosa of TDI-sensitized rats. NB-UVB of 310 nm at an estimated dose of 100 mJ/cm^2 or less was irradiated to the bilateral nasal cavity of TDI-sensitized rats, once (day 22), three times (days 20–22) and seven times (days 16–22) before TDI provocation. Four hour after TDI provocation on day 22, nasal mucosa was fixed in 10% neutral buffered formalin and embedded in paraffin. After the cells were permeabilized with 0.2% Triton X-100, TUNEL reaction was conducted. After counter-staining with PI, the cells were observed under a confocal laser microscope. Apoptotic cells were shown as green fluorescence of TUNEL reaction in red background fluorescence of PI. Control rats were sensitized and provoked with EtOAc. TDI rats were sensitized and provoked with TDI without NB-UVB irradiation.

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Immunoblot analysis showed that irradiation with NB-UVB inhibited PMA-induced phosphorylation of ERK, but not that of PKCδ. These findings suggest that ERK is a target molecule of irradiation with NB-UVB, and that NB-UVB-induced inhibition of ERK phosphorylation in PKCδ/ERK/PARP-1 signaling pathway is responsible for NB-UVB-induced suppression of PMA-induced up-regulation of HIR gene expression. Recently, it was reported that low dose laser irradiation at a wavelength of 633 nm induced phosphorylation of ERK, resulting in brain-derived neurotropic factor mRNA transcription *in vitro*.[21] But, to our knowledge, no report of irradiation-induced suppression of ERK phosphorylation has been published.

In the time course studies, PMA-induced up-regulation of H1R mRNA was transiently suppressed up to 4 h after irradiation with low dose of 310 nm NB-UVB in HeLa cells, suggesting that the inhibition of ERK phosphorylation by low dose of NB-UVB irradiation is also reversible. On the contrary, irradiation with 305 nm UVB irreversibly suppressed the up-regulation of H1R mRNA due to its induction of apoptosis of HeLa cells, and 315 nm UVB had no such effects.

In the present study, we used TDI-sensitized rats as a model of AR. Intranasal application of TDI caused neuropeptide-mediated release of histamine from mast cells in the nasal mucosa and led to the development of nasal allergic-like symptoms such as sneezing and watery rhinorrhea in TDI-sensitized guinea pigs and rats.[22–27] In this model, histamine release was triggered by neuropeptide, but not IgE in TDI-sensitized rats. In this study, we measured total IgE level in TDI-sensitized rats using Rat IgE ELISA kit. IgE level of the control rats was 648 ± 44.2 ng/ml (n = 3). On the other hand, it was 1180 ± 65.1 ng/ml (n = 3) in TDI-sensitized rats. So, there was slight increase in total IgE (1.8-fold). Similar result was reported by Ban et al., in which TDI was administrated by inhalation only, increased serum total IgE (~2.5-fold).[28] In this respect, the underlying mechanism of TDI-induced rhinitis is different from the authentic allergen-induced (IgE-dependent) rhinitis and not all the data obtained from this study can be clinically applicable. However, nasal allergic-like symptoms induced by TDI are similar to those observed in AR patients.[29,30] In addition, TDI-sensitized rats also display many of the characteristic features of AR in humans, including infiltration of eosinophils and mast cells,[31] increase in the level of cytokines,[28,32–34] elevation of H1R mRNA and protein level,[25] increase in the HDC mRNA level, HDC activity, and histamine content.[35] Furthermore, the expression of IL-4 and IL-5 mRNAs was also up-regulated in the nasal mucosa of TDI-sensitized rats after provocation with TDI.[36,37] Johnson et al., characterized a murine model of TDI-induced rhinitis.[38] They showed that symptoms of this TDI-induced occupational rhinitis are similar to allergic rhinitis caused by environmental allergens and the underlying mechanisms driving occupational rhinitis also appear to share similarities with allergic rhinitis caused by ubiquitous airborne protein allergens They also showed that TDI-induced inflammation is associated with a Th1/Th2 mixed immune response and that the lower airways showed no evidence of inflammation and the inflammation and gene expression changes were isolated to the upper airways, suggesting that the changes observed in the nasal mucosa were most likely due to local allergen recognition, processing, and presentation to effector cells at the site of exposure. From these reasons, we consider that TDI-sensitized rats can be a model of AR.

Irradiation with 310 nm NB-UVB suppressed TDI-induced nasal symptoms and up-regulation of H1R gene expression in the nasal mucosa of TDI-sensitized rat model of AR. Intranasal pre-irradiation with single low-dose of NB-UVB showed no effects on TDI-induced sneezes and up-regulation of H1R mRNA in the nasal mucosa in TDI-sensitized rats. However, repeated intranasal pre-irradiation with low dose of NB-UVB for three consecutive days suppressed sneezes and up-regulation of H1R mRNA in the nasal mucosa after TDI provocation without induction of apoptosis. When the same exposure dose (200 mJ/cm^2 × 3 = 600 mJ/cm^2) was irradiated at once, the nasal symptom was improved at the same level, but the suppression of H1R gene up-regulation was weak (data not shown). This result was consistent with our previous report showing that repeated pre-treatment with epinastine is more effective than their single treatment in reducing nasal symptoms by causing additional suppression of up-regulations of H1R and IL-4 mRNAs in the nasal mucosa.[10] Treatment with TDI caused the infiltration of mast cells and eosinophils in TDI-sensitized guinea pigs.[31] Irradiation with 200 mJ/cm^2 of NB-UVB did not show any effect on RBL-2H3 cells (data not shown). Thus, it is unlikely that irradiation with NB-UVB affected on mast cells although we have not checked the effect of NB-UVB on mast cells in the nasal mucosa of TDI-sensitized rats.

These findings suggest that prophylactic effect of repeated low-dose irradiation with NB-UVB on nasal symptom is due to its effect in suppressing the up-regulation of both H1R gene and protein expression the nasal mucosa and cumulative effect of NB-UVB irradiation is important to improvement of nasal symptoms without induction of apoptosis in the nasal mucosa of patients with AR. It was also reported that prophylactic effect of dexamethasone is due to its inhibitory effect on the H1R mRNA up-regulation in the nasal mucosa of the rat model.[25] On the other hand, repeated intranasal pre-irradiation with NB-UVB for seven consecutive days induced apoptosis in the nasal epithelium with no effects on TDI-induced sneezes and up-regulation of H1R mRNA in the nasal mucosa of TDI-sensitized rats. Provocation with TDI induced histamine release from mast cells in the nasal mucosa through neurogenic inflammation in TDI-sensitized rats.[39] Because the apoptotic loss of nasal epithelium may expose trigeminal nerve endings in the nasal mucosa to amplify neurogenic inflammation, it is suggested that repeated intranasal pre-irradiation with 310 nm NB-UVB at high cumulative doses that induce apoptosis has no prophylactic effect of TDI-induced nasal symptom and up-regulation of H1R mRNA in the nasal mucosa.

It was reported that intranasal phototherapy with Rhinolight (Rhinolight Ltd., Szeged, Hungary) was effective for the treatment of seasonal AR.[40] The method included a combination of containing UVB (5%), UVA (25%) and visible light (70%) into the nasal cavity. It was reported that Rhinolight suppressed eosinophilic cationic protein and IL-5 in the nasal discharge by inducing apoptosis of T cells and eosinophils.[41] In contrast, our phototherapy with intranasal irradiation with low cumulative dose of NB-UVB suppressed nasal symptom by its inhibitory effect on the up-regulation of H1R mRNA in the nasal mucosa without induction of apoptosis, unlike Rhinolight.

It was reported that UVB dose-dependently caused DNA damage to cell, resulting in the induction of apoptosis in human keratinocytes.[42] And high doses of UVB irradiation induced human keratinocytes to undergo apoptosis.[43] Furthermore, failure to properly repair DNA damage results in mutations and eventually leads to carcinogenesis.[44–47] Indeed, high doses of UVB irradiation has a carcinogenic risk. In contrast, it was reported that low dose of UVB irradiation stimulated repair of UVB-induced DNA damage by changes in expression of p53, a tumor suppressing gene without induction of apoptosis.[43] It was also demonstrated that the UV-induced DNA damage response of respiratory epithelia was similar to that of the human epidermis, and that nasal mucosa was able to efficiently repair UVB-induced DNA damage.[47] Therefore, phototherapy of repeated intranasal irradiation with low cumulative dose of NB-UVB seems to be safe from carcinogenic risk in the nasal mucosa.

In conclusion, our data suggest that repeated intranasal irradiation with low cumulative dose of 310 nm NB-UVB has prophylactic effect on nasal symptoms without induction of apoptosis in the

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nasal mucosa of patients with AR. Phototherapy with low dose of NB-UVB might be clinically used for the treatment of AR.

Conflict of interest

The authors declare no financial conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphs.2018.08.011.

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