



## ORIGINAL RESEARCH

# Effects of narrow-band UVB on nasal symptom and upregulation of histamine H<sub>1</sub> receptor mRNA in allergic rhinitis model rats

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## Abstract

**Background:** Phototherapy with narrow-band ultraviolet B (narrow-band UVB) is clinically effective treatment for atopic dermatitis. In the present study, we examined the effects of intranasal irradiation with narrow-band UVB on nasal symptom, upregulation of histamine H<sub>1</sub> receptor (H1R) gene expression and induction of DNA damage in the nasal mucosa of allergic rhinitis (AR) model rat.

**Methods:** AR model rats were intranasally irradiated with 310 nm of narrow-band UVB. Nasal mucosal levels of H1R mRNA were measured using real-time quantitative reverse transcriptase (RT)-PCR. DNA damage was evaluated using cyclobutane pyrimidine dimer (CPD) immunostaining.

**Results:** In toluene 2,4-diisocyanate (TDI)-sensitized rats, TDI provoked sneezes and H1R gene expression in the nasal mucosa. Intranasal pre-irradiation with 310 nm narrow-band UVB at doses of 600 and 1400, but not 200 mJ/cm<sup>2</sup> significantly inhibited the number of sneezes and upregulation of H1R gene expression provoked by TDI. CPD-positive cells appeared in the nasal mucosa after intranasal narrow-band UVB irradiation at a dose of 1400, but not 200 and 600 mJ/cm<sup>2</sup>. The suppression of TDI-provoked sneezes and upregulation of H1R gene expression lasted 24 hours, but not 48 hours, after narrow-band UVB irradiation with a dose of 600 mJ/cm<sup>2</sup>.

**ABBREVIATIONS:** AD, atopic dermatitis; AR, allergic rhinitis; CPD, cyclobutane pyrimidine dimer; H1R, histamine H<sub>1</sub> receptor; LED, light-emitting diode; narrow-band UVB, narrowband-ultraviolet B; TDI, toluene 2,4-diisocyanate; TUNEL, TdT-mediated dUTP nick end labeling.

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**Conclusions:** Intranasal pre-irradiation with narrow-band UVB dose-dependently inhibited sneezes and upregulation of H1R gene expression of the nasal mucosa in AR model rats, suggesting that the inhibition of nasal upregulation of H1R gene expression suppressed nasal symptom. The suppression after narrow-band UVB irradiation at a dose of 600 mJ/cm<sup>2</sup> was reversible without induction of DNA damage. These findings indicated that low-dose narrow-band UVB phototherapy could be effectively and safely used for AR treatment in a clinical setting.

**Level of Evidence:** NA.

**KEYWORDS**

allergic rhinitis, apoptosis, histamine H<sub>1</sub> receptor, narrow-band ultraviolet B, phototherapy

## 1 | INTRODUCTION

Phototherapy with narrow-band ultraviolet B (narrow-band UVB, wavelength from 308-313 nm) is clinically effective treatment for skin diseases, such as atopic dermatitis (AD) and psoriasis.<sup>1-3</sup> Because allergic rhinitis (AR) shares a common pathophysiology with AD, narrow-band UVB phototherapy could be effective treatment for AR. However, the dermatological Philips TLO1 narrow-band UVB lamp is too big for intranasal irradiation. To overcome this problem, light-emitting diodes (LEDs) that emit their energy mostly at 310 nm wavelength narrow-band UVB, have been developed; these LEDs are small enough for intranasal irradiation.<sup>4</sup>

Histamine is a main chemical mediator in the AR development and nasal symptoms are mediated by histamine signaling via the histamine H<sub>1</sub> receptor (H1R).<sup>5</sup> H1R gene expression is upregulated in the nasal mucosa of patients with AR,<sup>6,7</sup> and levels of H1R mRNA are correlated with the intensity of nasal symptoms in AR patients.<sup>8</sup> Therefore, suppression of the upregulation of H1R mRNA in the nasal mucosa is a promising therapeutic strategy for the treatment of AR.<sup>9-14</sup>

In a previous study, we reported that irradiation with the 310 nm narrow-band UVB wavelength-specifically, reversibly, and dose-dependently suppresses phorbol-12-myristate-13-acetate (PMA)-induced upregulation of H1R gene expression in human epithelial HeLa cells.<sup>4</sup> We also reported that irradiation with narrow-band UVB inhibits phosphorylation of extracellular signal-regulated kinase (ERK) in the H1R signaling pathway, suggesting that the suppression of ERK phosphorylation is underlying mechanism of the inhibition of PMA-induced upregulation of H1R gene expression in HeLa cells.<sup>15,16</sup> In this study, we examined whether irradiation with narrow-band UVB suppressed upregulation of H1R mRNA in vivo. For this purpose, we used rats sensitized with toluene 2,4-diisocyanate (TDI) as an AR model. We examined whether intranasal narrow-band UVB irradiation suppressed TDI-provoked upregulation of H1R mRNA in the nasal mucosa and nasal symptom of TDI model rats. We also examined whether intranasal irradiation with narrow-band UVB induced apoptosis and DNA damage in nasal mucosal cells.

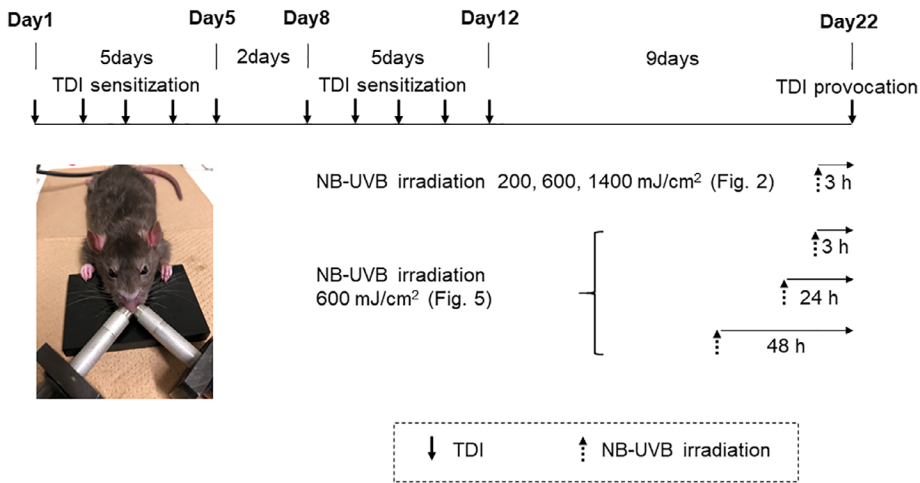
## 2 | METHODS

### 2.1 | Animals

Brown Norway rats (male, six-week-old, 200-250 g, Japan SLC, Hamamatsu, Japan) were used for this study. Rats were allowed free access to water and food. Room temperature and humidity were maintained at 25 ± 2°C and 55 ± 10%, respectively, with a 12-hours light/dark cycle. Rats sensitization and provocation with TDI were performed by technique described by Nurul et al.<sup>9</sup> Briefly, 10 µL of a 10% solution of TDI in ethyl acetate (Wako Pure Chemical, Osaka, Japan) was applied bilaterally to the rats nasal vestibule once a day for 5 days (days 1-5). After a 2-days interval, the sensitization procedure was repeated (days 8-12). Nine days after the second sensitization, 10 µL of TDI solution was applied to the nasal vestibule again to provoke nasal symptom in TDI-sensitized rats (Figure 1). Control rats were treated with ethyl acetate using the same procedure. Immediately after TDI provocation, the number of sneezes was counted for 10 minutes. The nasal septum mucosa was dissected 4 hours after TDI provocation. Samples were stocked in RNAlater (Thermo Fisher Scientific, Waltham, Massachusetts) at -80°C until assayed. We performed all experimental procedures in accordance with the guidelines and approval of the Animal Research Committee of Tokushima University.

### 2.2 | Narrow-band UVB irradiation

An LED that emits its energy mostly at 310 nm wavelength, within the narrow-band UVB (Nichia Corporation, Tokushima, Japan), was used for intranasal irradiation through the bilateral nostrils of TDI-sensitized rats on day 22. An UVX Radiometer (UVP, Upland, California) was used to measure the dosages of narrow-band UVB. The distance between the sensor of the UVB meter and the tip of the probe, where the LED was embedded, was 5 mm, that was the same as the distance from the rats nostrils to nasal mucosa. Two probes were placed in front of each rat nostril and adjusted 30° angle outside to the body axis. Narrow-band UVB at 310 nm was used to irradiate rat



**FIGURE 1** Experimental protocol. Rats were sensitized with 10  $\mu\text{L}$  of a 10% solution of TDI in ethyl acetate, which was applied to the bilateral nasal vestibule on day 1-5 and day 8-12. Narrow-band UVB of 310 nm was irradiated intranasally at doses of 200, 600 and 1400  $\text{mJ}/\text{cm}^2$  on day 22. Three hours after narrow-band UVB irradiation, we applied TDI to the bilateral nasal vestibule again to provoke nasal symptom. Immediately after TDI provocation, the number of sneezes was counted during 10 minutes

nostrils at doses of 200, 600, and 1400  $\text{mJ}/\text{cm}^2$  under anesthesia with pentobarbital with a dose of 30 mg/kg, intraperitoneally. Three hours after irradiation, we applied TDI to nasal vestibule bilaterally to provoke nasal symptom (Figure 1).

### 2.3 | Real-time quantitative reverse transcriptase (RT)-PCR

We homogenized nasal mucosa samples using a polytron homogenizer (PT-K; Kinematica, AG, Luzern, Switzerland). We then isolated total RNA using the RNAiso Plus reagent (Takara Bio Inc., Kyoto, Japan). RNA samples were reverse transcribed to cDNA using a PrimeScript RT Reagent Kit (Takara Bio Inc., Shiga, Japan). TaqMan primers and the probe were designed using Primer Express Software (Applied Biosystems, Foster City, CA, USA) as follows: forward primer for rat H1R, 5'-TATGTGTCGGGGCTGCACT-3'; reverse primer for rat H1R, 5'-CGCCATGATAAAACCCAACTG-3'; and TaqMan probe, FAM-CCGAGAGCGGAAGGCAGCCA-TAMURA. To standardize the starting material, endogenous rodent GAPDH control reagents (Applied Biosystems) were used, and we expressed data as a ratio of GAPDH mRNA.

### 2.4 | Induction of apoptosis evaluated by TdT-mediated dUTP nick end labeling (TUNEL) staining

Narrow-band UVB-induced apoptosis in the nasal mucosa cells was detected by TUNEL staining in TDI-sensitized rats. Three hours after narrow-band UVB irradiation with doses of 200, 600 and 1400  $\text{mJ}/\text{cm}^2$ , we applied TDI to the nasal vestibule to provoke nasal symptom. Four hours after provocation, we dissected and fixed the nasal mucosa in 4% paraformaldehyde for approximately 24 hours, washed in phosphate buffered saline (PBS), and transferred to 30% sucrose for cryoprotection. The nasal mucosa was embedded in optimal cutting temperature (OCT) compound (Tissue-Tek; Sakura Fine Technical, Tokyo, Japan), rapidly frozen on dry ice, and then cut into

10  $\mu\text{m}$ -thick sections using a cryostat (CM3050s; Leica Instrument, Nussloch, Germany). The TUNEL staining was performed by using the DeadEnd Fluorometric TUNEL System (Promega, Madison, Wisconsin). The tissue sections on a glass slide were dried, washed in PBS to remove the OCT compound, incubated with 20  $\mu\text{g}/\text{mL}$  Proteinase K for 10 minutes at 25°C, washed in PBS for 5 minutes, and immersed in 4% paraformaldehyde for 5 minutes. They were then washed in PBS for 5 minutes, immersed in 0.2% Triton-X100 for 5 minutes, and washed in PBS for 5 minutes. Equilibration Buffer (100  $\mu\text{L}$ ) was added to the tissue sections and incubated for 10 minutes at 25°C. The TdT reaction mixture (50  $\mu\text{L}$ ) was added to the tissue sections and incubated for 60 minutes at 37°C in a humidified chamber. The tissue sections were then immersed in 2 $\times$  saline sodium citrate (SSC) for 15 minutes 25°C, washed in PBS three times for 5 minutes each, counterstained with 1  $\mu\text{g}/\text{mL}$  propidium iodide for 15 minutes, and washed in PBS three times for 5 minutes each. Thereafter, the tissue sections were observed using a fluorescent microscope (BZ-X710; Keyence, Osaka, Japan). As a positive control, the tissue sections dissected from control rats were treated with RQ1 RNase-Free DNase (Promega). Before the equilibration buffer was added, 500  $\mu\text{L}$  RQ1 DNase reaction buffer was added to the tissue sections and incubated for 5 minutes at 25°C. After the tissue sections were dried, 10 u/mL RQ1 RNase-Free DNase was added to the tissue sections and incubated for 10 minutes at 25°C. The tissue sections were washed in PBS, and then the TUNEL reaction was performed the same as described above.

### 2.5 | DNA damage evaluated by cyclobutane pyrimidine dimer (CPD) immunostaining

Narrow-band UVB-induced DNA damage in nasal mucosa cells was detected by using CPD immunostaining in TDI-sensitized rats. Three hours after narrow-band UVB irradiation at doses of 200, 600, and 1400  $\text{mJ}/\text{cm}^2$ , we applied TDI to the nasal vestibule to provoke nasal symptom. Four hours after provocation, we dissected the nasal mucosa, then fixed in 4% paraformaldehyde for approximately

24 hours, washed in PBS, and transferred to 30% sucrose for cryoprotection. The nasal mucosa was embedded in OCT compound (Tissue-Tek; Sakura Fine Technical, Tokyo, Japan), rapidly frozen on dry ice, and then cut into 10  $\mu\text{m}$ -thick sections using a cryostat (CM3050s; Leica Instrument, Nussloch, Germany). The tissue sections on a glass slide were dried, washed in PBS-Tween (PBST) to remove the OCT compound, and subsequently boiled at 85°C in 10 mM citric acid solution for 10 minutes. The tissue sections were incubated on ice for 1 hour, washed in PBST for 5 minutes, blocked for 1 hour at 25°C in a solution of 3% goat serum in PBST, and then incubated with anti-CPD antibody (Cosmo Bio Co., Ltd., Tokyo, Japan) diluted 1:400 in PBST at 4°C overnight. Finally, the tissue sections were incubated with secondary antibody, fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG antibody (Jackson ImmunoResearch, West Grove, Pennsylvania) diluted 1:400 in PBST at 25°C for 2 hours, counterstained with 1  $\mu\text{g}/\text{mL}$  propidium iodide for 15 minutes, and washed in PBST three times for 5 minutes each. Thereafter, the tissue sections were observed using a fluorescent microscope (BZ-X710; Keyence, Osaka, Japan).

## 2.6 | Time course after narrow-band UVB irradiation

We used 310 nm of narrow-band UVB at a dose of 600  $\text{mJ}/\text{cm}^2$  to intranasally irradiate TDI-sensitized rats, and TDI provocation was performed 3, 24, and 48 hours later on day 22 (Figure 1). We counted the number of sneezes for 10 minutes, then dissected the nasal mucosa 4 hours after provocation, and samples were stored as described above. Nasal mucosa levels of H1R mRNA were measured using real-time quantitative RT-PCR, as described above.

We detected narrow-band UVB-induced apoptosis and DNA damage in nasal mucosa cells using TUNEL staining and CPD immunostaining, respectively, as described above.

## 2.7 | Statistical analysis

The results are presented as the mean  $\pm$  SEM. Statistical analyses were performed using analysis of variance with Dunnett's test using the GraphPad Prism software (GraphPad Software Inc., La Jolla, California). *P* values less than .05 were considered statistically significant.

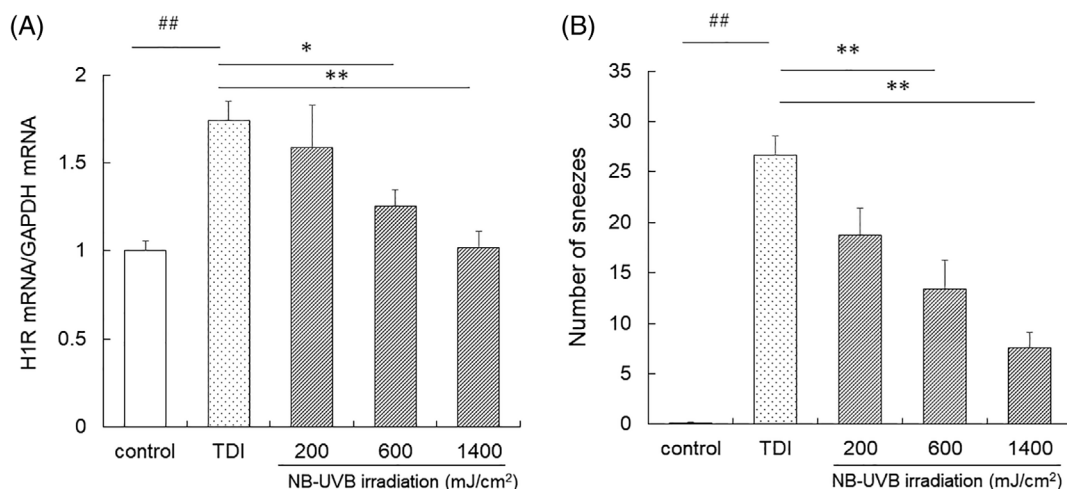
## 3 | RESULTS

### 3.1 | Effect of narrow-band UVB irradiation on TDI-induced upregulation of H1R mRNA of the nasal mucosa and sneezes of TDI-sensitized rats

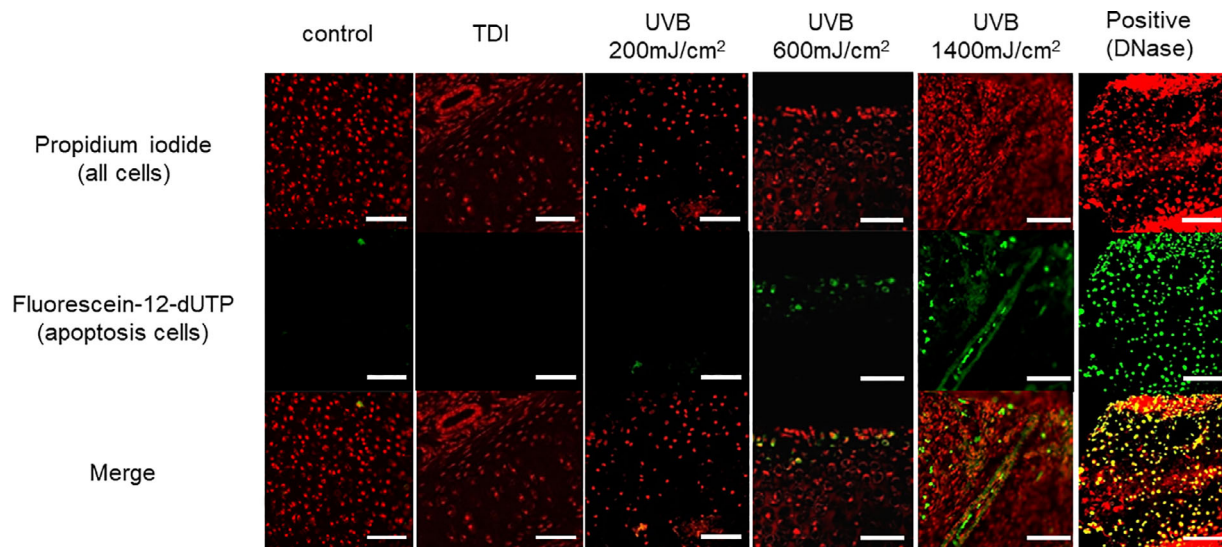
In TDI-sensitized rats, TDI provocation significantly increased H1R mRNA levels in the nasal mucosa. Intranasal pre-irradiation with narrow-band UVB with doses of 600 and 1400, but not 200  $\text{mJ}/\text{cm}^2$  significantly inhibited TDI-provoked H1R mRNA upregulation (Figure 2A). TDI provocation significantly increased the number of sneezes in TDI-sensitized rats. Intranasal pre-irradiation with narrow-band UVB with doses of 600 and 1400, but not 200  $\text{mJ}/\text{cm}^2$  significantly inhibited the number of TDI-induced sneezes of TDI-sensitized rats (Figure 2B).

### 3.2 | Induction of apoptosis after narrow-band UVB irradiation in nasal mucosa cells of TDI-sensitized rats

After intranasal narrow-band UVB irradiation with a dose of 200  $\text{mJ}/\text{cm}^2$ , no TUNEL-positive cells appeared in the nasal mucosa of TDI-sensitized rats. However, after intranasal narrow-band UVB irradiation with doses of 600 and 1400  $\text{mJ}/\text{cm}^2$ , a few and some TUNEL-positive cells appeared in the nasal mucosa, respectively (Figure 3).



**FIGURE 2** Effects of intranasal irradiation with 310 nm narrow-band UVB on TDI-induced up-regulation of H1R mRNA of the nasal mucosa, A, and sneezes, B, of TDI-sensitized rats. Narrow-band UVB at doses of 200, 600, and 1400  $\text{mJ}/\text{cm}^2$  was irradiated intranasally through bilateral nostrils of rats. Three hours after narrow-band UVB irradiation, TDI was applied again to provoke nasal symptom. Immediately after TDI provocation, the number of sneezes was counted during 10 minutes. The nasal mucosa of rats was dissected 4 hours after TDI provocation. We determined H1R mRNA level using real-time PCR. Control rats were treated with vehicle. The data are expressed as means  $\pm$  SEM. *n* = 9 (200  $\text{mJ}/\text{cm}^2$ ), 8 (600  $\text{mJ}/\text{cm}^2$ ), 7 (1400  $\text{mJ}/\text{cm}^2$ ), 20 (TDI without irradiation), 20 (control). ##*P* < .01 vs control; \**P* < .05, \*\**P* < .01 vs TDI



**FIGURE 3** Effects of intranasal irradiation with 310 nm narrow-band UVB on induction of apoptosis in nasal mucosa cells of TDI-sensitized rats. Narrow-band UVB at doses of 200, 600, and 1400 mJ/cm<sup>2</sup> was irradiated intranasally through bilateral nostrils of rats. Three hours after narrow-band UVB irradiation, TDI was applied to the nasal vestibule to induce nasal symptom. Four hours after provocation, the nasal mucosa of rats was dissected, fixed in 4% paraformaldehyde, embedded in OCT compound, and rapidly frozen. The tissue was cut into 10  $\mu$ m-thick sections. The tissue sections were stained using DeadEnd Fluorometric TUNEL System, counter-stained with propidium iodide and observed using a fluorescent microscope. Control rats were treated with vehicle. As a positive control, the nasal mucosa of control rats was treated with DNase. Apoptotic cells were indicated as green fluorescence and nuclei were indicated as red fluorescence. Scale bar indicates 50  $\mu$ m

### 3.3 | Induction of DNA damage after narrow-band UVB irradiation in nasal mucosa cells of TDI-sensitized rats

After intranasal narrow-band UVB irradiation at doses of 200 and 600 mJ/cm<sup>2</sup>, no CPD-positive cells appeared in the nasal mucosa of TDI-sensitized rats. Meanwhile, after intranasal narrow-band UVB irradiation at a dose of 1400 mJ/cm<sup>2</sup>, many CPD-positive cells appeared in the nasal mucosa (Figure 4).

### 3.4 | Time course after narrow-band UVB irradiation

Intranasal narrow-band UVB pre-irradiation at a dose of 600 mJ/cm<sup>2</sup> significantly inhibited TDI-provoked sneezes and upregulation of H1R gene expression 3 and 24 hours, but not 48 hours, after irradiation (Figure 5). A few TUNEL-positive cells appeared in the nasal mucosa 3 hours, but not 24 and 48 hours, after narrow-band UVB irradiation at a dose of 600 mJ/cm<sup>2</sup> (Figure 6A). No CPD-positive cells appeared in the nasal mucosa 3, 24, and 48 hours after narrow-band UVB irradiation at a dose of 600 mJ/cm<sup>2</sup> (Figure 6B).

## 4 | DISCUSSION

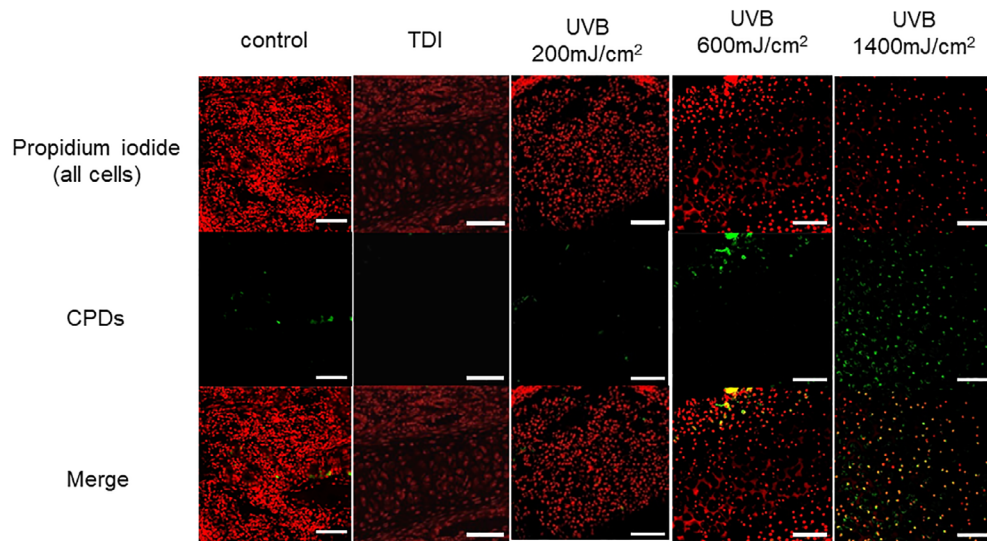
In the present study, we showed that intranasal pre-irradiation with 310 nm narrow-band UVB dose-dependently suppressed H1R mRNA

upregulation in the nasal mucosa and TDI-provoked sneezes of TDI-sensitized rats as an AR model. Our previous study showed that nasal hypersensitivity to histamine was increased in TDI-sensitized rats, because of an increase of levels of both H1R mRNA and protein in their nasal mucosa.<sup>14</sup> Because sneeze is mediated by nasal trigeminal reflex through H1R,<sup>17</sup> and H1R immunohistochemically localized in the epithelium, vessels and nerves of human nasal mucosa,<sup>18</sup> the present findings suggest that suppression of TDI-induced increase in amount of H1R in the nasal mucosal nerves by narrow-band UVB is responsible for the suppression of TDI-induced sneezes in TDI-sensitized rats after intranasal irradiation with narrow-band UVB. Moreover, in our previous *in vitro* study, narrow-band UVB suppressed upregulation of both H1R mRNA and protein in human epithelial HeLa cells that endogenously express H1R.<sup>16</sup> Our previous clinical study also showed that levels of H1R mRNA of the nasal mucosa was correlated with the intensity of allergic symptom in patients with AR.<sup>8</sup> Therefore, it is suggested that intranasal pre-irradiation with narrow-band UVB suppressed upregulation of neural H1R in the nasal mucosa, resulting in the suppression of nasal symptom in a rat model of AR.

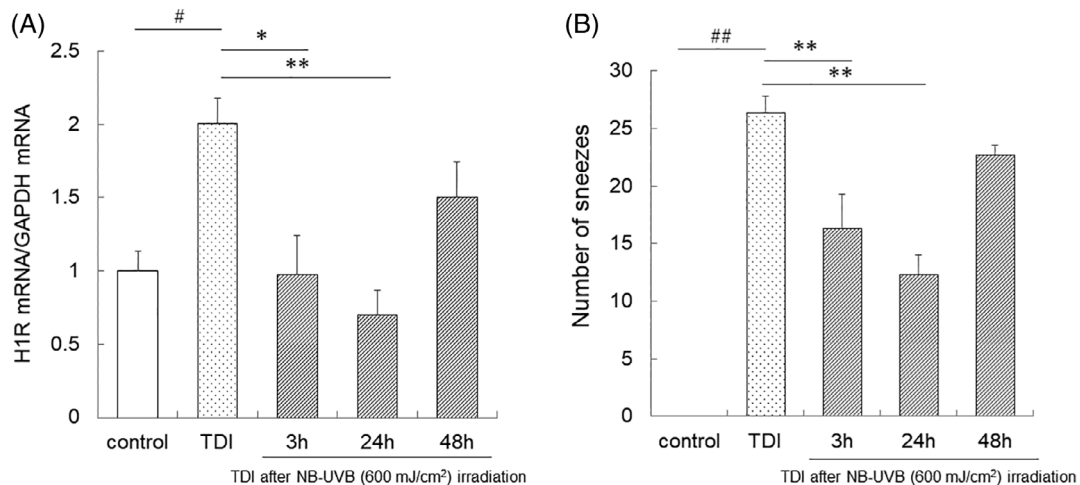
We reported that the suppression of ERK phosphorylation induced by narrow-band UVB in the protein kinase C delta (PKC $\delta$ )/ERK/poly (ADP-ribose) polymerase 1 (PARP-1) signaling pathway is responsible for narrow-band UVB-induced inhibition of PMA-induced upregulation of H1R mRNA in HeLa cells.<sup>15,16</sup> Therefore, it was suggested that the inhibition of TDI-provoked upregulation of H1R mRNA in the nasal mucosa of TDI-sensitized rats after intranasal narrow-band UVB irradiation was also due to its inhibition of ERK phosphorylation.

It has been reported that intranasal irradiation using Rhinolight (Rhinolight Ltd., Szeged, Hungary), which contains visible light (70%),





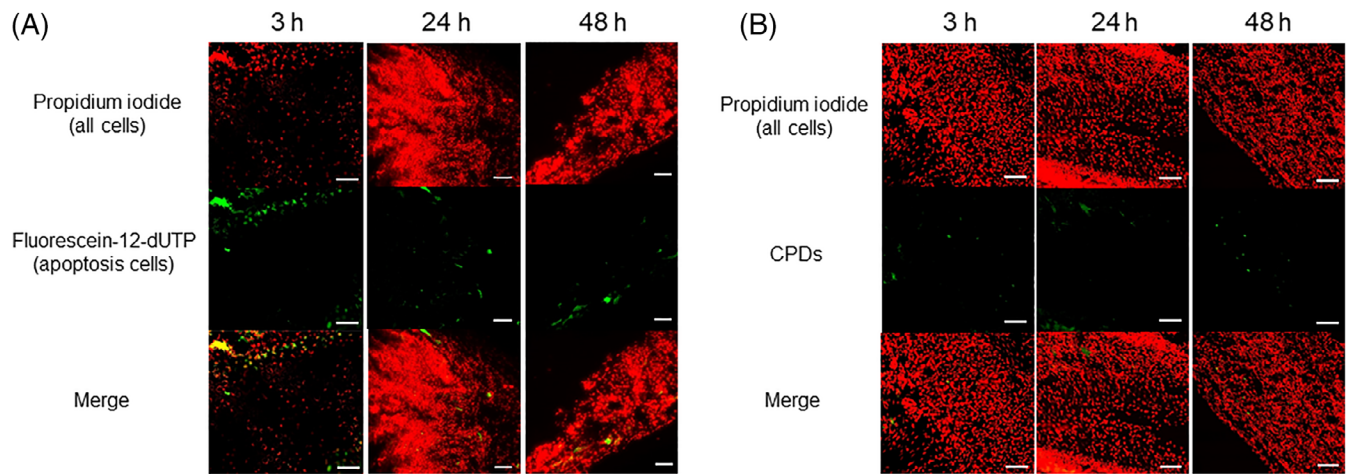
**FIGURE 4** Effects of intranasal irradiation with 310 nm narrow-band UVB on UV-induced DNA damage in nasal mucosa cells of TDI-sensitized rats. Narrow-band UVB at doses of 200, 600, and 1400 mJ/cm<sup>2</sup> was irradiated intranasally through bilateral nostrils of rats. Three hours after narrow-band UVB irradiation, TDI was applied to the nasal vestibule to induce nasal symptom. Four hours after provocation, the nasal mucosa of rats was dissected, fixed in 4% paraformaldehyde, embedded in OCT compound, and rapidly frozen. The tissue was cut into 10 μm-thick sections. The tissue sections were immunostained using anti-CPD antibody, counter stained with propidium iodide, and observed using a fluorescent microscope. Control rats were treated with vehicle. CPD-positive cells were indicated as green fluorescence, and nuclei were indicated as red fluorescence. Scale bar indicates 50 μm



**FIGURE 5** Time course of TDI-induced H1R mRNA upregulation in the nasal mucosa, A, and sneezes, B, after intranasal irradiation with 310 nm narrow-band UVB of TDI sensitized rats. Narrow-band UVB at a dose of 600 mJ/cm<sup>2</sup> was irradiated intranasally through bilateral nostrils of rats. TDI provocation was performed 3 hours, 24 hours, and 48 hours later. Immediately after TDI provocation, we counted the number of sneezes during 10 minutes. Four hours after TDI provocation, the nasal mucosa was dissected. H1R gene expression level was determined using real-time PCR. Control rats were treated with vehicle. The data are presented as means ± SEM. *n* = 3 (3 hours), 4 (24 hours), 3 (48 hours), 4 (TDI without irradiation), 4 (control). #*P* < .05, ##*P* < .01 vs control; \**P* < .05, \*\**P* < .01 vs TDI

UVA (25%) and UVB (5%) was effective for AR treatment.<sup>19,20</sup> It has also been reported that Rhinolight induced apoptosis of T cells and eosinophils in the nasal mucosa, and decreased levels of IL-5 and eosinophilic cationic protein in the nasal discharge.<sup>19</sup> Accordingly, it was possible that narrow-band UVB-induced suppression of H1R mRNA upregulation was due to induction of apoptosis in immunocompetent cells in the nasal mucosa. However, the hypothesis was unlikely, because only a few

TUNEL-positive nasal mucosal cells were induced after intranasal irradiation with 310 nm narrow-band UVB at a dose of 600 mJ/cm<sup>2</sup> that inhibited upregulation of H1R gene expression. Moreover, the suppressive effects of upregulation of H1R gene expression lasted 24 hours, but disappeared 48 hours after irradiation with narrow-band UVB. Thus, apoptosis could not account for the reversibility of narrow-band UVB-induced inhibition of the upregulation of H1R mRNA.



**FIGURE 6** Time course of induction of apoptosis, A, and DNA damage, B, in the nasal mucosa after intranasal irradiation with 310 nm narrow-band UVB of TDI-sensitized rats. Narrow-band UVB at a dose of  $600 \text{ mJ/cm}^2$  was irradiated intranasally through bilateral nostrils of rats. TDI provocation was performed 3 hours, 24 hours, and 48 hours after narrow-band UVB irradiation. Four hours after provocation, the nasal mucosa of rats was dissected fixed in 4% paraformaldehyde, embedded in OCT compound, and rapidly frozen. The tissue was cut into  $10 \text{ }\mu\text{m}$ -thick sections. The tissue sections were stained using DeadEnd Fluorometric TUNEL System and anti-CPD antibody, counter-stained with propidium iodide, and observed using a fluorescent microscope. A, Apoptotic cells and B, CPD-positive cells were indicated as green fluorescence, and nuclei were indicated as red fluorescence. Scale bar indicates  $50 \text{ }\mu\text{m}$

In the present study, intranasal narrow-band UVB irradiation with 310 nm at  $600 \text{ mJ/cm}^2$  induced no CPD-positive cells with DNA damage in the nasal mucosa of TDI-sensitized rats. Usually, UVB light causes specific DNA damage to produce CPD and pyrimidine pyrimidone (6-4) photoproducts.<sup>21,22</sup> DNA damage is considered to be the most potent cause of UVB-induced apoptosis.<sup>23</sup> However, intranasal narrow-band UVB irradiation at a dose of  $600 \text{ mJ/cm}^2$  induced apoptosis in a few nasal mucosal cells without inducing DNA damage. Therefore, it is suggested that apoptosis after low-dose narrow-band UVB ( $600 \text{ mJ/cm}^2$ ) irradiation is caused by mechanisms other than DNA damage, such as UVB-generated reactive oxygen species in cells.<sup>24-26</sup> It has been reported that DNA damage of human nasal mucosa induced by a single UV irradiation is efficiently removed within a few days,<sup>27</sup> and that failure to properly repair DNA damage may cause gene mutations, finally leading to carcinogenesis.<sup>27-30</sup> Because low-dose narrow-band UVB ( $600 \text{ mJ/cm}^2$ ) did not induce DNA damage in nasal mucosal cells in rats, intranasal phototherapy with 310 nm narrow-band UVB at a dose of  $600 \text{ mJ/cm}^2$  is safely applicable for the treatment of AR.<sup>4,15,16</sup>

In both Japanese and American practice guidelines for AD, narrow-band UVB phototherapy is the second line treatment for refractory AD that is intractable to first line treatments, such as a topical agent.<sup>1,3</sup> A recent review article concluded that phototherapy with UVA1 (340-400 nm) and narrow-band UVB are valid therapeutic options for patients with AD.<sup>31</sup> narrow-band UVB phototherapy is usually performed 3 times a week for 6 weeks,<sup>32</sup> after the irradiation dose is calculated based on the Minimal Erythema Dose or the patient's skin phototype.<sup>32,33</sup> Whereas, phototherapy with narrow-band UVB is the first line treatment for psoriasis.<sup>2</sup> Japanese practice guidelines for psoriasis recommend that psoriasis is treated with narrow-band UVB phototherapy once to three times a week.<sup>34</sup> In the present study, the suppression of nasal symptom after pre-irradiation

with low-dose narrow-band UVB lasted 24 hours, but disappeared after 48 hours in a rat model of AR, suggesting that a protocol of intranasal phototherapy with narrow-band UVB 3 times a week for the treatment of AR might work the same way as phototherapy for AD and psoriasis. However, further research is necessary to determine the appropriate frequency and intervals of intranasal narrow-band UVB phototherapy in patients with AR.

In conclusion, intranasal 310 nm narrow-band UVB irradiation dose-dependently inhibited nasal symptom and H1R mRNA upregulation in the nasal mucosa of AR model rats. These findings suggested that intranasal narrow-band UVB irradiation inhibited the upregulation of H1R gene expression in the nasal mucosa, resulting in the suppression of nasal symptom. The suppression after intranasal narrow-band UVB irradiation at a dose of  $600 \text{ mJ/cm}^2$  was reversible without induction of DNA damage. It was suggested that low-dose narrow-band UVB phototherapy could be effectively and safely used for the AR treatment in a clinical setting.

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#### CONFLICT OF INTEREST

The authors have no conflicts of interest directly relevant to the content of this article.

## AUTHOR CONTRIBUTIONS

Seiichiro Kamimura and Yoshiaki Kitamura designed the study and wrote the manuscript. Seiichiro Kamimura, Tatsuya Fujii, and Kentaro Okamoto carried out experimental work. Nanae Sanada, Natsuki Okajima, and Tomoharu Wakugawa contributed to data collection and interpretation of results. Hiroyuki Fukui, Hiroyuki Mizuguchi, and Noriaki Takeda supervised the research and wrote the manuscript. All authors read and approved the final manuscript.

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