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Full paper

Effects of antihistamine on up-regulation of histamine H_1 receptor mRNA in the nasal mucosa of patients with pollinosis induced by controlled cedar pollen challenge in an environmental exposure unit

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ABSTRACT

In the present study, we examined the effects of antihistamine on the up-regulation of H1R mRNA in the nasal mucosa of patients with pollinosis induced by controlled exposure to pollen using an environmental exposure unit. Out of 20 patients, we designated 14 responders, whose levels of H1R mRNA in the nasal mucosa were increased after the first pollen exposure and excluded 6 non-responders. Accordingly, the first exposure to pollen without treatment significantly induced both nasal symptoms and the up-regulation of H1R mRNA in the nasal mucosa of the responders. Subsequently, prophylactic administration of antihistamine prior to the second pollen exposure significantly inhibited both of the above effects in the responders. Moreover, the nasal expression of H1R mRNA before the second pollen exposure in the responders pretreated with antihistamine was significantly decreased, as compared with that before the first pollen exposure without treatment. These findings suggest that antihistamines suppressed histamine-induced transcriptional activation of H1R gene in the nasal mucosa, in addition to their blocking effect against histamine on H1R, resulting in a decrease of nasal symptoms. These findings further suggest that by their inverse agonistic activity, antihistamines suppress the basal transcription of nasal H1R in the absence of histamine in responders.

1. Introduction

Allergic rhinitis is one of the most common IgE-mediated diseases and recent reports have shown an increase in its prevalence (1). On the other hand, histamine is a major chemical mediator that induces nasal allergy symptoms through its action on histamine H_1 receptor (H1R). Moreover, the strength of H1R signaling that is involved in nasal symptoms depends on the H1R expression level (2). In fact, it was reported that the expression of H1R mRNA was up-regulated in the nasal mucosa of patients with pollinosis (3,4).

Antihistamines are classified as neutral antagonists or inverse agonists of H1R. Neutral antagonists antagonize histamine by blocking its binding with H1R, whereas inverse agonists not only antagonize histamine on H1R, but also suppress constitutive H1R activity in the absence of histamine (5,6). We also demonstrated that inverse agonist of H1R also suppressed the basal transcription of H1R in the absence of histamine (7,8).

In our previous study using HeLa cells, we reported that histamine increased the expression of H1R mRNA, while antihistamine suppressed its up-regulation (9). We also reported an animal model of nasal allergy using toluene-2,4-diisocynate (TDI)-sensitized rat, and showed that intranasal application of TDI to the nasal mucosa induced nasal symptoms together with an increase of H1R mRNA

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level in the nasal mucosa, and that antihistamine suppressed these effects (10). Compounds that suppressed the up-regulation of H1R gene expression also alleviated nasal symptoms in TDI-sensitized rats (11–16). In another study, we demonstrated that the gene expression of H1R is up-regulated in patients with pollinosis during the pollen season, and its expression level correlated with the severity of nasal symptoms (17). We also showed that prophylactic administration of antihistamines decreased H1R gene up-regulation with suppression of nasal symptoms (17).

However, it is difficult to evaluate the efficacy of the anti-allergic drugs in patients with pollinosis under natural exposure to pollen, because of the unpredictability of various parameters such as pollen concentration, pollen antigenicity and weather conditions. To overcome these limitations, environmental exposure units (EEU) were developed to meet the criteria of stable environmental conditions including temperature, humidity and pollen concentration, and reproducibility of pollen-induced nasal symptoms (18–20).

In the present study, an attempt was made to examine whether controlled exposure to cedar pollen within the EEU induces the up-regulation of H1R mRNA in the nasal mucosa of patients with pollinosis. We then examined the effect of ebastine, a representative antihistamine with inverse agonistic activity (7) administration prior to pollen exposure on the up-regulation of H1R mRNA of the nasal mucosa.

2. Methods

2.1. Patients

The present study included 20 patients with Japanese cedar pollinosis (7 men and 13 women; 30–57 years old; mean age: 45.3 ± 1.5). All showed positive CAP-radioallergosorbent test (>class 2) with serum allergen-specific IgE against cedar pollen (mean class: 3.45 ± 0.23). They did not take any anti-allergic drugs during the period of this study, which was conducted out of the pollen season.

2.2. Study design

After the first exposure to cedar pollen, we divided the patients into two groups: the responder group and non-responder group, based on their changes in the expression levels of H1R gene in the nasal mucosa. Accordingly, the responder group included 14 patients (5 men and 9 women; 36–57 years old; mean age: 46.0 ± 1.7; mean class of cedar pollen: 3.50 ± 0.29; mean number of eosinophils: 196 ± 98/μl), who showed increased levels of H1R mRNA after the first pollen exposure, while the non-responder group included 6 patients (2 men and 4 women; 30–54 years old; mean age: 43.7 ± 3.3 years; mean class of cedar pollen: 3.33 ± 0.42; mean number of eosinophils: 324 ± 169/μl), whose levels of H1R mRNA were not increased after the first pollen exposure. The mean class of cedar pollen and mean number of eosinophils in the responder patients was not different from that in the non-responder patients. After a washout period for 3 months, the 14 responder patients were exclusively exposed to cedar pollen again. Before the second pollen exposure, these patients were treated with oral administration of ebastine, an antihistamine, at a daily dose of 10 mg, p.o. for 3 days prior to the exposure (Fig. 1a).

This study was approved by the Ethical Committees of Tokushima University Hospital, and a written informed consent was obtained from each patient prior to the study.

2.3. Pollen exposure in the EEU

The study was performed out of the Japanese cedar pollen season within the EEU in Wakayama City. The EEU was maintained under comfortable conditions of temperature (20–26 °C) and relative humidity (50–65%). Patients were exposed to cedar pollen at the concentration of 8000 grains/m^3 for 3 h in the EEU, which was enough to induce manifest nasal symptoms in our previous study (18).

2.4. Evaluation of nasal symptoms

Nasal symptoms of sneezing and nasal discharge were evaluated 30 min before the pollen exposure and every 30 min during the exposure for 3 h (Fig. 1b). They were scored according to the frequency of episodes of sneezing and nose-blowing.

2.5. Real-time quantitative RT-PCR

The scrapings of the nasal mucosa were obtained immediately before and after the pollen exposure by scratching the surface of each patients' inferior nasal concha with a small spatula under local anesthesia with 4% lidocaine (Fig. 1b). They were frozen in RNA-later® (Applied Biosystems, Foster City, CA, USA) and stored in a tube at −80 °C until use. Total RNA was isolated using the RNAqueous-Micro Kit (Applied Biosystems) in accordance with the manufacturer's instructions. RNA samples were reverse-transcribed to cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). TaqMan primers and probe were designed using Primer Express primer design software (Applied Biosystems). The sequences of the H1R primers were as follows: sense primer, 5′-CAGAGGATCAGATGTTAGGTGATAGC- 3′; antisense primer, 5′-AGCGGAGCCTCTTCCAAGTAA- 3′. The sequence of the probe was as follows: FAM-CTTCTCTCGAACGGACTCAGATACCACC-TAMRA. The PUM1 primer and probe kit (Hs 00206469-m1, Applied Biosystems) was used as an internal standard (17). The transcripts were used for a 40-cycle, 3-step polymerase chain reaction (PCR) with the GeneAmp 7300 Sequence Detection System (Applied Biosystems). The size and reaction specificity of amplicon were confirmed by agarose gel electrophoresis. Identification of the PCR products was carried out using a genetic analysis system (SEQ8000; Beckman Coulter, Inc., Fullerton, CA, USA).

2.6. Statistical analysis

The results are shown as mean ± standard deviation (SD). Statistical analysis was performed by the paired *t*-test or the Wilcoxon signed-ranks test, and *P* values of <0.05 were considered to be statistically significant.

3. Results

Controlled exposure to Japanese cedar pollen for 3 h in the EEU induced the up-regulation of H1R mRNA in the nasal mucosa of 14 patients (pre-exposure: 1.32 ± 0.74, post-exposure: 2.80 ± 2.18 with $p < 0.01$ vs. pre-exposure), but not in 6 others (pre-exposure: 2.49 ± 1.55, post-exposure: 1.06 ± 0.28 with $p < 0.05$ vs. post-exposure of 14 patients) (Fig. 2). Thus, we designated the former patients as responders and investigated the effect of ebastine on their pollen exposure-induced nasal symptoms and up-regulation of H1R mRNA in the nasal mucosa. The first pollen exposure significantly increased the sum of sneezing and watery rhinorrhea scores at 30, 120, 150 and 180 min in the responder group (pre-exposure: 0.64 ± 1.39, 120 min after provocation: 4.86 ± 4.38, $p < 0.01$ vs. pre-exposure, 180 min after exposure: 3.86 ± 2.30,

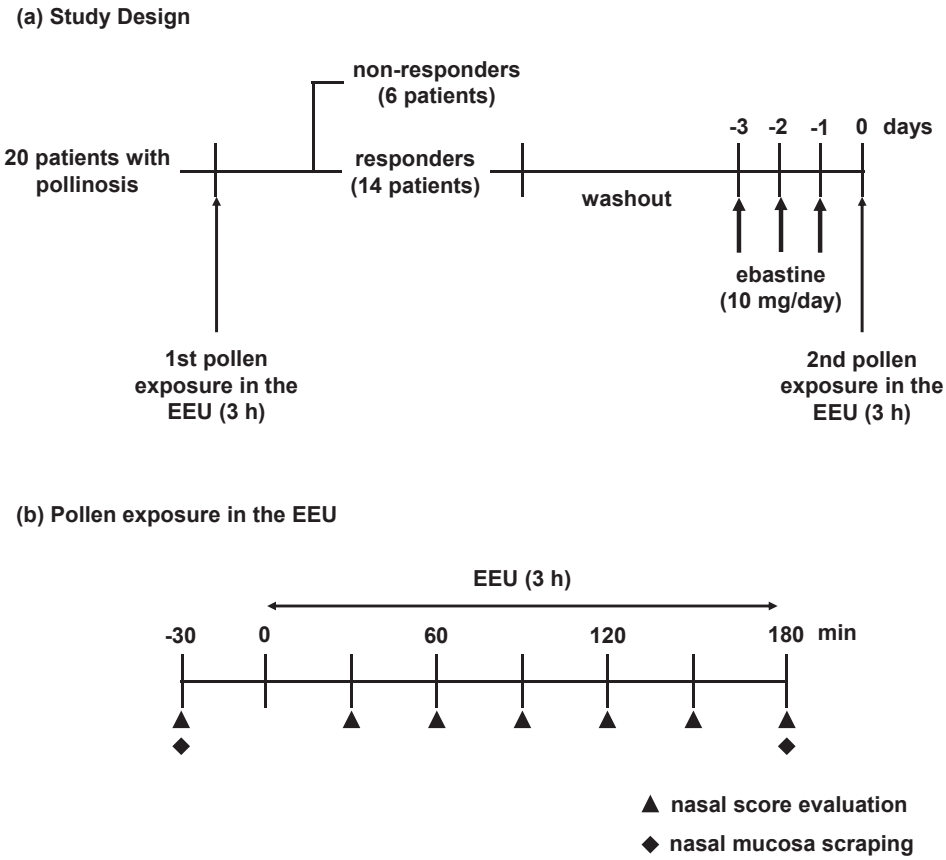

Fig. 1. (a) Study design. Twenty patients were exposed to cedar pollen in the EEU for 3 h. After a wash-out period, 14 responder patients whose levels of H1R mRNA in the nasal mucosa were increased after the 1st pollen exposure were treated with oral administration of ebastine once a daily for 3 day, and then exposed to pollen for 3 h. (b) Pollen exposure in EEU. Nasal symptoms were evaluated 30 min before pollen exposure and every 30 min during pollen exposure in the EEU. Before and after the exposure, the scrapings of the nasal mucosa were obtained, and H1R mRNA was measured.

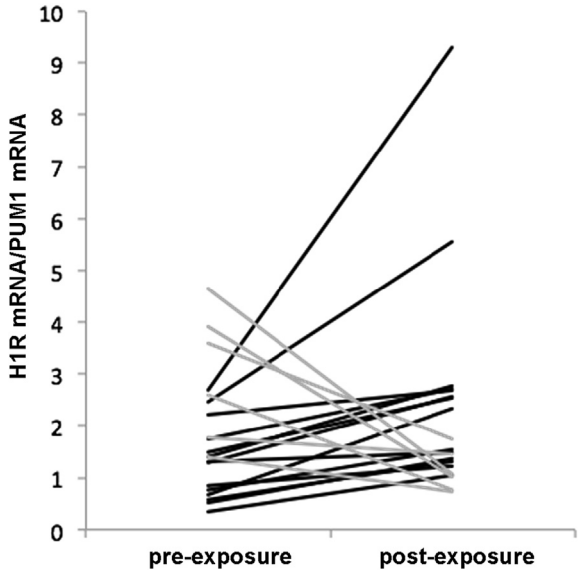

Fig. 2. Effect of the 1st exposure of cedar pollen for 3 h on levels of H1R mRNA in nasal mucosa of 20 patients with pollinosis. Black lines: 14 responder patients whose levels of H1R mRNA were increased after the exposure. Gray lines: 6 non-responder patients whose levels of H1R mRNA were not increased after the exposure. $n = 20$.

$p < 0.01$ vs. pre-exposure; Fig. 3). On the other hand, the pollen exposure did not change the nasal symptom scores (pre-exposure: 0.33 ± 0.52, 120 min after exposure: 5.00 ± 5.40, 180 min after exposure: 3.67 ± 2.34) in 6 non-responder group that was excluded. Three months later to wash out the aftereffect of 1st

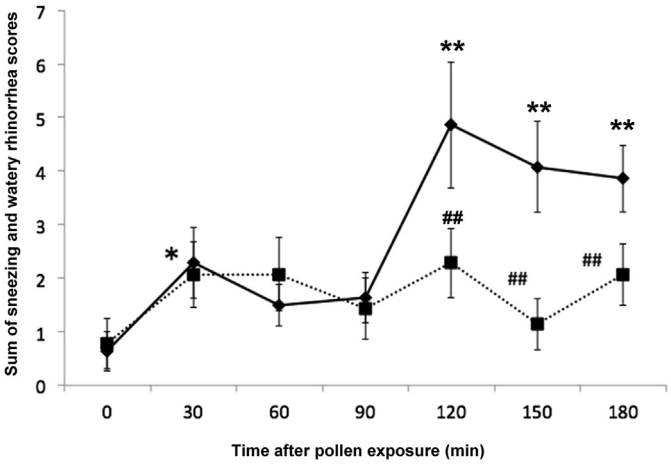

Fig. 3. Effects of the first pollen exposure without treatment and the second exposure after prophylactic administration of antihistamine for 3 days on the sum of sneezing and rhinorrhea scores in the 14 responder patients with pollinosis. ◆: 1st exposure without treatment; ■: 2nd exposure after prophylactic administration of ebastine for 3 days. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. pre-1st exposure, ##$p < 0.01$ vs. 1st exposure. $n = 14$.

pollen exposure, a prophylactic treatment with ebastine at a daily dose of 10 mg was given to these 14 responder patients for 3 days prior to the second pollen exposure. This significantly decreased the nasal symptom scores at 120, 150 and 180 min after the pollen exposure, in comparison with those induced by the first exposure (Fig. 3).

The first pollen exposure significantly increased the levels of H1R mRNA in the nasal mucosa of the responder patients, whereas the ebastine treatment suppressed the pollen exposure-induced up-regulation of H1R mRNA (Fig. 4). Accordingly, the levels of H1R mRNA after the second pollen exposure was significantly decreased, in comparison with those after the first pollen exposure in the responders.

Moreover, the levels of H1R mRNA in the nasal mucosa of the responder patients pretreated with ebastine were significantly decreased before the second pollen exposure, in comparison with those without ebastine treatment before the first pollen exposure (Fig. 4).

4. Discussion

In the present study, we showed that the controlled exposure to Japanese cedar pollen in the EEU induced both nasal symptoms and up-regulation of H1R mRNA in nasal mucosa of 14 responders among the 20 patients with pollinosis. Previously, we reported that in HeLa cells, histamine-induced up-regulation of H1R mRNA through the protein kinase C-δ (PKCδ)/extracellular signal-regulated kinase/poly (ADP-ribose) polymerase-1 signaling pathway (9,21,22). We also reported that H1R in the nasal mucosa was up-regulated at both mRNA and protein levels after provocation in the animal model of allergic rhinitis (11). We further showed that the elevation of the receptor expression increases histamine signaling involved in nasal symptoms (2). Taken together, the present finding suggests that in IgE-mediated response to pollen inhalation, histamine released from mast cells stimulates H1R in the nasal mucosa, resulting in both induction of nasal symptoms and up-regulation of H1R in the responder patients.

In the responder patients who had been prophylactically administrated ebastine for 3 days, the second pollen exposure induced neither nasal symptoms nor the up-regulation of H1R mRNA expression in nasal mucosa. In our previous study with HeLa cells, we reported that antihistamine inhibited histamine-induced up-regulation of H1R mRNA (9). We also showed that preseasonal prophylactic treatment with antihistamine suppressed the gene expression of H1R in the nasal mucosa of patients with pollinosis during the peak pollen period (17). Taken together, the present findings suggest that prophylactic administration of ebastine suppressed the released histamine-induced up-regulation of H1R gene expression in the nasal mucosa of the responder patients after the second pollen exposure. The present findings also suggest that antihistamine reduced histamine signaling by their suppressive effect on histamine-induced transcriptional activation of H1R, in addition to their blocking effect against histamine on H1R, resulting in a decrease of nasal allergy symptoms.

In the responder patients, administration of ebastine for 3 days prior to the second pollen exposure also decreased the gene expression of H1R in the nasal mucosa, as compared with that without ebastine treatment before the first pollen exposure. According to the two-state model of H1R, active and inactive forms of H1R coexist in a dynamic equilibrium, and the inverse agonist of H1R stabilizes the inactive form of H1R, shifting the equilibrium toward the inactive state in the absence of histamine (5,6). We demonstrated that inverse agonist of H1R also suppressed the basal transcription of H1R in the absence of histamine (7,8). As reported previously, ebastine is an inverse agonist of H1R (7), the present finding suggests that antihistamine with inverse agonistic activity suppresses the basal transcription of H1R in the nasal mucosa in the absence of histamine, resulting in the down-regulation of nasal H1R gene expression in absence of pollen exposure.

However, the exposure to pollen did not induce the up-regulation of H1R gene expression in the nasal mucosa of the non-responder patients. Their nasal symptom scores increased after the pollen exposure, but the increase did not reach statistical significance. Thus, it is suggested that in the non-responder patients, the pollen exposure did not release sufficient amount of histamine to induce up-regulation of H1R gene expression, despite a positive allergen-specific IgE against cedar pollen in their serum. However, there was no significant correlation between nasal symptom scores and the levels of H1R mRNA in nasal mucosa, probably because nasal symptoms are partially mediated by chemical mediators other than histamine.

In conclusion, the controlled exposure to cedar pollen in the EEU induced both nasal symptoms and up-regulation of H1R mRNA in the nasal mucosa of the responder patients with pollinosis, and the prophylactic administration with an inverse agonist of H1R suppressed both pollen exposure-induced nasal symptoms and up-regulation of nasal H1R mRNA. Moreover, prophylactic administration with inverse agonist of H1R down-regulated the basal gene expression of H1R mRNA in the nasal mucosa without pollen exposure. These findings suggest that antihistamines with inverse agonistic activity inhibit nasal symptoms in patients with pollinosis through three different mechanisms: 1) blocking histamine effect on H1R, 2) suppressive effect on histamine-induced transcriptional activation of H1R, and 3) suppressive effect on the basal transcription of H1R in the absence of histamine.

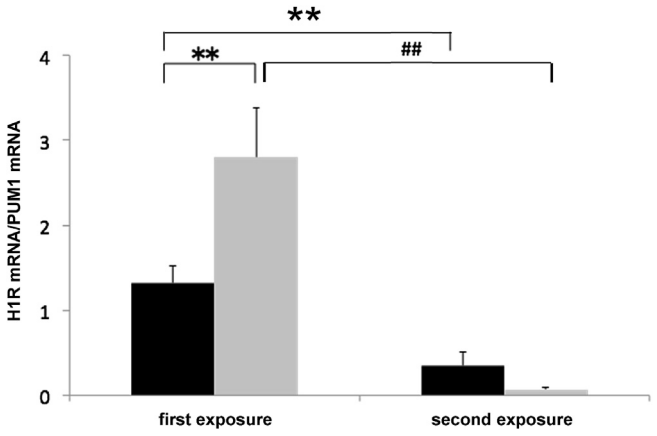

Fig. 4. Effects of the first pollen exposure without treatment and the second exposure after prophylactic administration of antihistamine for 3 days on the levels of H1R mRNA in nasal mucosa of the 14 responder patients with pollinosis. Black columns: pre-1st or 2nd pollen exposure. Gray columns: post-1st or 2nd pollen exposure. $^{**}P < 0.01$, $\#\#P < 0.01$ vs. pre-1st pollen exposure. $\#\#P < 0.01$ vs. post-1st pollen exposure. $n = 14$.

Conflict of interest

The authors declare no financial conflicts of interest.

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