Original article

Effect of intranasal corticosteroid on pre-onset activation of eosinophils and mast cells in experimental Japanese cedar pollinosis

Yasuyuki Noyama, Mitsuhiro Okano, Tazuko Fujiwara, Shin Kariya, Sei-ichiro Makihara, Takenori Haruna, Kengo Kanai, Takaya Higaki, Kazunori Nishizaki

Department Otorhinolaryngology, Kagawa Prefectural Central Hospital, Kagawa, Japan
Department Otorhinolaryngology, Kagawa Rosai Hospital, Kagawa, Japan
Department of Otolaryngology-Head & Neck Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Article Info

Article history:
Received 6 September 2015
Received in revised form 27 November 2015
Accepted 14 December 2015
Available online 19 February 2016

Keywords:
Eosinophil cationic protein
Intranasal corticosteroids
Japanese cedar pollinosis
Minimal persistent inflammation
Tryptase

Abbreviations:
AR, allergic rhinitis; ECP, eosinophil cationic protein; FFNS, fluticasone furoate nasal spray; INS, intranasal corticosteroids; JCP, Japanese cedar pollinosis; MPI, minimal persistent inflammation

Abstract

Background: Minimal persistent inflammation (MPI) contributes to hyperreactivity in allergic rhinitis. However, little is known regarding whether pre-onset activation of eosinophils and mast cells is present or not in Japanese cedar pollinosis (JCP). Furthermore, a prophylactic effect of intranasal corticosteroids on such MPI in JCP has not been investigated.

Methods: We designed a double-blinded, randomized, placebo-controlled, crossover trial. Twenty patients with JCP were examined outside the pollen season (UMIN000008410). Nasal provocation with paper discs containing extracts of Japanese cedar pollen was performed once a day for 3 consecutive days. Onset of nasal symptoms was monitored over 15 min after each provocation. The levels of eosinophil cationic protein (ECP) and tryptase in nasal secretions were examined. Fluticasone furoate nasal spray or placebo treatment was started one day before the first provocation.

Results: In the placebo group, 25% of the patients showed onset of nasal symptoms following provocation on the first day. In addition, 75% and 68% of the patients showed symptom onset on the second and third day of provocation, respectively. After the first provocation, the levels of ECP and tryptase in nasal secretions were significantly increased. These increases were seen not only in symptomatic but also in asymptomatic subjects in response to provocation, and the levels were similar between these subjects. Prophylactic treatment with fluticasone significantly suppressed the increase in nasal ECP and tryptase associated with repeated provocations.

Conclusions: These results suggest that pre-onset activation of eosinophils and mast cells is present in experimental JCP, and that prophylactic treatment with intranasal corticosteroids has the potential to control such activation.

Copyright © 2016, Japanese Society of Allergology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

In allergic rhinitis (AR), minimal persistent inflammation (MPI) is characterized by an influx of inflammatory cells such as eosinophils and neutrophils into the nasal mucosa without the onset of nasal symptoms following exposure to low levels of allergen. MPI was originally described after the end of symptoms in both perennial and seasonal allergic rhinitis. It has been suggested that MPI is also present in the initial dispersion of pollen prior to onset of symptoms, which contributes to hyperreactivity and subsequently to onset of full-scale symptoms; however, the precise characterization and clinical implication of this “pre-onset” MPI remains to be elucidated.

Prophylactic, in other words early interventional or initial, treatment starting immediately after pollen release or the onset of symptoms is recommended in patients who annually experience substantial symptoms of pollen-induced seasonal allergic rhinitis. Placebo-controlled studies confirmed that anti-histamines, anti-leukotrienes and intranasal corticosteroids (INS) are effective for
prophylactic treatment in pollinosis. Of these medications, prophylactic treatment with INS has been shown to delay onset and reduce symptom severity. For example, we reported that prophylactic treatment with mometasone furoate nasal spray did not induce the substantial onset of pollinosis, whereas placebo treatment did, in the relatively low pollen dispersal season in Japanese cedar pollinosis (JCP), the most prevalent seasonal allergic rhinitis in Japan. In addition, we have recently demonstrated that this prophylactic treatment significantly delayed the onset of symptoms and alleviated symptom severity compared not only with placebo but also with post-onset treatment with mometasone in the relatively high pollen dispersal season. One of the bases of prophylactic treatment is the control of pre-onset MPI; however, little is known regarding how INS controls pre-onset MPI.

In the present study, we sought to determine whether pre-onset activation of eosinophils and mast cells exists in experimental JCP. In addition, the efficacy of INS for this pre-onset activation was investigated. These results provide a basis for understanding the clinical implications of INS for prophylactic treatment in seasonal AR.

Methods

Patients

Twenty patients with JCP, between the ages of 22 and 52 years (mean 35.3 ± 10.4 years; 6 males and 14 females) were enrolled. All the patients had at least a 2-year history of JCP and were asymptomatic out of the pollen season. Sensitization to Japanese cedar pollen was assessed by a skin prick test. Patients were excluded if they had: (a) sensitization to house dust mite assessed by a skin prick test; (b) comitant sinonasal disease that could potentially affect the outcome of the trial (e.g., nasal polyps, rhinosinusitis, nasal septum deviation); (c) rhinitis medicamentosa and non-infectious, non-allergic rhinitis; (d) cedar pollen-specific immunotherapy; (e) sinonasal surgery including laser vaporization of inferior turbinates within 1 year; (f) medication with anti-allergic drugs including antihistamines, chromones, glucocorticoids and decongestants within 1 year; (g) hypersensitivity to fluticasone furoate nasal spray; (h) systemic infection including mycosis; or (i) were pregnant and breastfeeding. Prior to the study initiation, we estimated the sample size that would be required based on the mean and standard deviation in the groups reported in our previous studies.

Study design

The study was a single-center, double-blinded, randomized, placebo-controlled, crossover trial that was carried out in August, outside the Japanese cedar pollen season (Fig. 1). Allocation concealment was granted by the central registry and computer-generated block randomization. The control nasal provocation, followed by active allergen provocation with a 15 min interval, was given for 3 consecutive days. The allergen provocation test was performed by placing two paper discs (Torii Pharmaceutical, Tokyo, Japan) containing the Japanese cedar pollen extract that is used for skin scratch tests (14.7 μg in 5 μl per disc, Torii), to the surface of the bilateral inferior turbinates for 5 min. Control discs contained 5 μl of the control solution used for the scratch test (Torii Pharmaceutical). After 5 min, the discs with adsorbed nasal secretions were removed, placed into a 1.5 ml micro test tube (Eppendorf AG, Hamburg, Germany), and stored at –80 °C until assayed. The subjects were monitored for 15 min as to whether they showed an onset of nasal symptoms such as sneezing, itching, rhinorrhea and nasal congestion in response to nasal provocation. Fluticasone furoate nasal spray (FFNS: 55 μg per nostril once a day in the morning) or the placebo spray was administered to the subjects starting from one day prior to the first provocation. The placebo spray provided by GlaxoSmithKline K.K. had a white-colored lid and trigger. The lid and trigger of the FFNS bottle, which were initially cyan-colored, were changed to white to ensure that both FFNP and placebo spray appeared the same. This treatment was continued until the third provocation (4 days in total). Wash-out periods of 2 weeks were instituted between the treatments (Fig. 1). The study was approved by the Institutional Review Board of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences (Rinris-1436), and is registered in UMIN (UMIN000008410). Prior to participation in the study, all patients provided written informed consent.

Measurement of nasal ECP and tryptase

The discs were soaked overnight at 4 °C in 0.8 ml of Dulbecco’s phosphate buffered saline (Invitrogen, Grand Island, NY, USA) with gentle rotation. Levels of ECP and tryptase were assayed. The subjects were monitored for 15 min as to whether they showed an onset of nasal symptoms such as sneezing, itching, rhinorrhea and nasal congestion in response to nasal provocation. Fluticasone furoate nasal spray (FFNS: 55 μg per nostril once a day in the morning) or the placebo spray was administered to the subjects starting from one day prior to the first provocation. The placebo spray provided by GlaxoSmithKline K.K. had a white-colored lid and trigger. The lid and trigger of the FFNS bottle, which were initially cyan-colored, were changed to white to ensure that both FFNP and placebo spray appeared the same. This treatment was continued until the third provocation (4 days in total). Wash-out periods of 2 weeks were instituted between the treatments (Fig. 1). The study was approved by the Institutional Review Board of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences (Rinris-1436), and is registered in UMIN (UMIN000008410). Prior to participation in the study, all patients provided written informed consent.

Statistical analysis

Values are expressed as the median value. A non-parametric Mann-Whitney U test and Fisher’s exact probability test were used to compare the data between groups, while the Wilcoxon signed rank test was used for analysis within the groups. Statistical analyses were performed using SAS (Statistical Analysis System) version 9.2, with P < 0.05 considered to be significant.

![Fig. 1. Study design. Subjects received a nasal provocation test with control discs (C) followed by allergen discs (A) for 3 consecutive days. Fluticasone furoate nasal spray (FFNS) or a placebo spray was given to the subjects starting from one day prior to the first provocation. After wash-out for 2 weeks, a crossover trial was performed.](image-url)
Results

Release of ECP and tryptase in nasal secretions following allergen provocation in the placebo treatment group

In the groups treated with placebo, the levels of ECP in nasal secretions extracted from the paper discs remained unchanged 5 min after each provocation. However, the baseline levels of ECP determined in the control disc of the second day ($P = 0.003$) and third day ($P = 0.004$) provocations were significantly increased compared to the baseline ECP on the first day of provocation, suggesting that exposure to Japanese cedar pollen induced release of ECP (Fig. 2).

A significant increase in the levels of tryptase was seen 5 min after the first day provocation ($P = 0.008$). However, unlike the ECP response, the tryptase levels returned to the baseline prior to the active provocation on the second day. The tryptase response on the second and third day provocations was similar to that on the first day of provocation (Fig. 3).

Onset of nasal symptoms after allergen provocation for 3 consecutive days in the placebo treatment group

Fifteen minutes after the first provocation, 5 out of 20 (25%) patients treated with placebo showed an onset of nasal symptoms. In addition, 15 (75%) and 14 (68%) patients showed symptom onset after the second and third day provocation, respectively. No significant differences in age, sex, a history of smoking or complications including bronchial asthma, aspirin intolerance, perennial rhinitis, atopic dermatitis, food allergy and drug allergies were found between patients showing positive (symptomatic, group A, n = 5) and negative (asymptomatic, group B, n = 15) response to the first allergen provocation (Table 1).

Levels of ECP and tryptase after the first day provocation in symptomatic and asymptomatic patients

We next focused on analysis of ECP and tryptase levels in the patients that showed a negative or a positive response to the first day provocation. Nasal ECP and tryptase levels in the control and active (pollen) disc on the first, second, and third day in each group were shown in Table 2. Levels of ECP in the control disc on the second day were similar between the groups (Fig. 4A, $P = 0.861$). In addition, significant elevation in the levels of ECP was seen in asymptomatic patients on day 2 compared to day 1 (Fig. 4B, $P = 0.013$). Levels of tryptase in the allergen disc on the first day were also similar between symptomatic and asymptomatic patients (Fig. 5A, $P = 0.364$). In addition, significant elevation in the levels of tryptase was seen in the allergen discs of asymptomatic patients compared to the control discs (Fig. 5B, $P = 0.012$).

Effect of prophylactic treatment of FFNS on ECP and tryptase release

Prophylactic treatment with FFNS did not inhibit the release of ECP on the first day provocation as compared with the placebo treatment due to a small release even in the placebo treatment. However, a significant decrease in ECP levels was seen in control discs on the second ($P = 0.004$) and third ($P < 0.001$) day provocation in FFNS treatment as compared with the placebo treatment (Fig. 6). Significant inhibition of tryptase levels by FFNS treatment was also seen in allergen discs on the second ($P = 0.034$) and third ($P = 0.023$) day provocation (Fig. 7). The effect of the treatment

Table 1

Demographic characteristics of patients showing symptomatic (group A) and asymptomatic (group B) response to the first provocation.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>33.8 ± 6.6</td>
<td>35.4 ± 11.3</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>1/4</td>
<td>5/10</td>
</tr>
<tr>
<td>History of smoking</td>
<td>0/5</td>
<td>0/15</td>
</tr>
<tr>
<td>Complications Bronchial asthma</td>
<td>0/5</td>
<td>0/15</td>
</tr>
<tr>
<td>Aspirin intolerance</td>
<td>0/5</td>
<td>0/15</td>
</tr>
<tr>
<td>Perennial rhinitis</td>
<td>0/5</td>
<td>1/15</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>2/5</td>
<td>4/15</td>
</tr>
<tr>
<td>Food allergy</td>
<td>0/5</td>
<td>0/15</td>
</tr>
<tr>
<td>Drug allergies</td>
<td>1/5</td>
<td>0/15</td>
</tr>
</tbody>
</table>

Results were shown as mean ± standard deviation.
Wilcoxon's signed rank test.

Effect of the treatment with FFNS was also shown. In addition, treatment with FFNS showed positive response to the first provocation outside the pollen season. In asymptomatic patients with JCP following a single allergen provocation, the levels of nasal ECP and tryptase were released into nasal secretions not only in symptomatic but also in asymptomatic patients with JCP.

**Discussion**

The present results suggest that the prophylactic effect of INS on pollinosis is mediated by the suppression of pre-onset activation of eosinophils and mast cells.

In contrast to the originally described MPI that is seen after the end of symptoms, little is known about “pre-onset” MPI and its regulation. Ricca *et al.* were the first to demonstrate increases in eosinophils, neutrophils and ICAM-1 expression prior to the initial onset of nasal symptoms, which were clinically evident in patients with birch pollinosis only after pollen counts increased dramatically. Several studies investigated MPI in seasonal allergic rhinitis using a nasal provocation test with allergen. Juliusson *et al.* demonstrated an increase in eosinophils and mast cells in asymptomatic patients with strictly seasonal allergic rhinitis to timothy pollen following a single allergen provocation; however, all patients displayed nasal symptoms following the provocation.

Roquet *et al.* demonstrated that although 7 days of exposure to birch pollen allergen at low doses outside the pollen season caused few symptoms, it produced increased ECP but not histamine in lavage fluid from patients with seasonal allergic rhinitis. Our results were consistent with this report by Roquet *et al.* in terms of ECP release by asymptomatic patients, and further demonstrated that pre-onset activation of eosinophils and mast cells can be induced even after a single allergen provocation.

On the other hand, unlike the observation by Roquet *et al.* in which no or minimal symptoms were observed, we found that 25% of the patients showed an onset of nasal symptoms after a single provocation. These different results may be due to the difference in the allergen dose used for provocation. The allergen dose set by Rouquet *et al.* was approximately 1/100 of the cumulative dose until symptoms of itching and sneezing occurred for each individual. We used two paper discs containing a Japanese cedar pollen extract that is used for skin scratch tests (14.7 μg of protein in 5 μl per disc) for provocation. Our dose may be higher than that set by Roquet *et al.*

In the present study, nasal ECP and tryptase were monitored as specific markers for the activation of eosinophils and mast cells, respectively. Previous studies reported that elevation of nasal ECP levels is mainly seen in the late-phase response and is sometimes seen in the early-phase response following allergen provocation. The present study showed that a significant elevation of nasal ECP was seen at 24 h but not at 5 min after a single allergen provocation.

**Table 2**

<table>
<thead>
<tr>
<th>ECP (ng/ml)</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFNS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFNS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Effect of the treatment with FFNS was also shown. P values were determined using Wilcoxon’s signed rank test.**

**Fig. 4.** Nasal ECP levels following a single allergen provocation. (A) Nasal ECP levels in the control disc on the second day in patients with negative (n = 15) or positive (n = 5) response to the first provocation were compared. The bar represents the median value. P values were determined using the Mann–Whitney U test. (B) Changes in nasal ECP levels in the control disc between the first and second day were determined in patients with a negative response to the first provocation. The bar represents the median value. P values were determined using Wilcoxon’s signed rank test.
provocation, suggesting that ECP release is mainly seen in the late-phase response in this experimental model. This result is similar to the report by Raulf-Meimsoth et al. They measured nasal ECP at 30 min, 2, 6 and 24 h after nasal provocation and found a significant elevation of nasal ECP that started at 2 h after provocation and lasted for 24 h. Together with the finding that the elevation of nasal ECP was seen not only in symptomatic but also in asymptomatic subjects, these results suggest a pre-onset activation of eosinophils following exposure to Japanese cedar pollen.

On the other hand, despite it can be speculated that the accumulation of eosinophils is enhanced by repeated stimulation with allergen, the reason why the baseline levels of ECP between the second day and the third day was not significant (P = 0.554) is not clear. Future evidences should be provided whether not only the activation but also the accumulation of eosinophils in nasal secretions is induced in this experimental model.

Tryptase is a neutral protease that is selectively released from mast cells. Previous studies demonstrated that nasal tryptase in nasal secretions or lavages are increased in the early phase but not in the late phase response following nasal provocation with allergens. The present study showed that a significant elevation of nasal tryptase was seen at 5 min but not at 24 h after a single allergen provocation. This result is consistent with the previous reports. Similar to ECP, the elevation of nasal tryptase was seen not only in symptomatic but also in asymptomatic subjects, suggesting that an induction of early-phase minimal inflammation with mast cells occurs following exposure to Japanese cedar pollen. Mast cells also contribute to late-phase responses through upregulation of cytokines and chemokines including GM-CSF, RANTES, eotaxins and TRAC. Although a specific marker of mast cell activation in late-phase responses has not been available, future investigation should determine whether mast cells contribute to “pre-onset” persistent inflammation in pollinosis.

The present study demonstrated that repeated nasal allergen provocations increased the rate of onset of nasal symptoms. This result is consistent with previous reports that demonstrated that repeated allergen provocations during out of pollen season decrease the threshold for nasal symptoms in pollinosis. On the
In conclusion, activation of eosinophils and mast cells was seen in asymptomatic patients with JCP after a single allergen provocation, and prophylactic use of INS was effective in inhibiting this activation. These results suggest that the prophylactic effect of INS on JCP is mediated by the suppression of pre-onset activation of eosinophils and mast cells. Future investigation should consider whether other inflammatory cells including lymphocytes and dendritic cells, as well as constitutive cells including epithelial cells and fibroblasts, contribute to pre-onset activation of eosinophils and mast cells.

Acknowledgments

The authors would like to thank Yuko Okano for her editorial assistance. This work was supported in part by grants from Ministry of Education, Culture, Sports, Science and Technology of Japan (25861563).

Conflict of interest

MO has received lecture fees from GSK and MSD. The rest of the authors have no conflict of interest.

Authors’ contributions

YN, MO and KN designed the study and wrote the manuscript. TF, SK, SM and THA contributed to data collection. KK and THi performed the statistical analysis and interpretation of the results.

References


