Original article

Evaluating the efficacy of epinastine ophthalmic solution using a conjunctivitis allergen challenge model in patients with birch pollen allergic conjunctivitis

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A R T I C L E   I N F O

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Tear histamine level

A B S T R A C T

Background: The efficacy of epinastine 0.05% ophthalmic solution for pollen allergic conjunctivitis has already been shown in a conjunctival allergen challenge (CAC) test using cedar pollen as a challenge. The present study investigated the efficacy of this solution against birch pollen conjunctivitis in a CAC test.

Methods: Ten adult subjects (eight males and two females) with asymptomatic birch pollen conjunctivitis were enrolled in this study. The average age of the subjects was 41.1 years. This study was conducted during a period without birch pollen dispersion. In each subject, the epinastine 0.05% ophthalmic solution was instilled in one eye, and an artificial tear fluid was instilled in the fellow eye in a double-blind manner. Five minutes or 4 h after the drug instillation, both eyes were challenged with an optimal concentration of birch pollen, and ocular itching and conjunctival hyperemia were then graded. Tears were collected before the drug instillation and 20 min after the pollen challenge, and the histamine level was measured.

Results: The ocular itching scores and palpebral conjunctival hyperemia scores of the epinastine-treated eyes were significantly lower than those of the contralateral control eyes when the eyes were pretreated with the drug 4 h before the CAC. There was a significant correlation between the tear histamine level and mean ocular itching score of three time points (3, 5 and 10 min) following the CAC in the control eyes but not the epinastine-treated eyes.

Conclusions: Epinastine is effective in suppressing ocular itching and conjunctival hyperemia in birch pollen conjunctivitis.

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Introduction

Allergic conjunctivitis is a conjunctival inflammatory disease associated with a type 1 allergy. It is classified as seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis. Various species of pollen can be causative antigens of SAC, and variation of pollen depends on the region and season. Cedar pollen is the most common antigen for SAC in Japan, except for Hokkaido prefecture in the north of Japan. In Hokkaido, birch pollen is the most common antigen of SAC, and the birch pollen-related SAC season lasts almost one month, from the end of April to the beginning of June. Birch pollen is also a major allergen trigger in the spring in Europe and North America.

Eye drops containing antiallergic ophthalmic solutions are the main treatment for allergic conjunctivitis. Epinastine has both effects of antihistamine properties, blocking the histamine 1 (H1) receptor, and chemical mediator stabilizer properties, inhibiting the release of mediators, including histamine and leukotrienes. A phase III study demonstrated that epinastine 0.05% ophthalmic solution was effective in suppressing SAC symptoms in patients in a conjunctival allergen challenge (CAC) test using an allergen solution of cedar pollen. To our knowledge, the efficacy of epinastine 0.05% ophthalmic solution in treating SAC caused by other antigens has not been studied. In this study, its efficacy was examined in a CAC test using birch pollen in humans.
Methods

Subjects

Thirty-six healthy adult volunteers with a history of birch pollen allergic conjunctivitis and no ocular symptoms were selected from December 2014 to February 2015 (outside the birch pollen season). The study was approved by the ethics committee of Hokkaido University Hospital (approval number 014-0193) and carried out in accordance with the Declaration of Helsinki and Ethical Guideline for Clinical Studies stipulated by the Ministry of Health, Labor and Welfare, Japan. All the patients who participated in this study provided written consent after they received oral and written information about the study. The study was registered at https://centerumin.ac.jp with the ID code UMIN0000157979.

This was a prospective, double-masked, randomized, placebo-controlled, single-center (Hokkaido University Hospital, Hokkaido, Japan) study.

Allergen solution

An allergen solution was prepared from glycerol 1:20 w/v birch pollen extract solution (Birch Mix PRW HollisterStier, Spokane, WA). Before the challenge tests, the extract was diluted with a diluent of chondroitin sulfate 1:100 w/v, NaCl 2:10,000 w/v and glycerol 2:100 w/v. Diluted allergen solution was prepared at different concentrations (25-fold, 50-fold, 100-fold, and 200-fold).

CAC

The clinical methodology and grading system for allergic conjunctivitis followed those reported earlier. The optimal concentration of the allergen solution was determined individually at the second visit. The allergen solution was instilled into the subject's eye, and the severity of ocular itching and palpebral and bulbar conjunctival hyperemia was evaluated according to the previous study. Table 1 shows the grading of ocular allergic symptoms (ocular itching and conjunctival hyperemia).

Clinical trial design

Figure 1 shows the outline of this study. For the duration of the study, the subjects were instructed not to use corticosteroids, antiallergic drugs, immune suppressants, or immunomodulation therapy, topically nor systemically, and subjects who experienced ocular allergic symptoms including ocular itching and conjunctival hyperemia before the CAC were excluded.

At visit 1, after written informed consent was obtained, subjects were excluded according to demographic data such as age (20 < 65) and pregnancy. Subjects who had a negative response to serum birch pollen-specific Immunoglobulin E (IgE) in a capsulated hydrophilic carrier polymer radioallergosorbent test were excluded from the study.

At visit 2, CAC tests were performed according to the previous study. After the allergen control solution (Allergen Scratch Extract Torii Control Solution, Torii Pharmaceutical, Tokyo, Japan) was instilled into the conjunctiva to exclude subjects who showed a conjunctival inflammatory reaction to the allergen solution without pollen antigen, 30 μL of the lowest allergen solution was instilled into each eye. The allergen concentration was increased until an ocular itching score of at least 2 (continuous itching) was recorded and both palpebral and bulbar conjunctival hyperemia scores of at least 1 (dilation of a few blood vessels) were elicited bilaterally within 10 min. The lowest concentration that produced these symptoms in each subject was the optimal concentration of allergen solution in the CAC. Subjects who failed to show sufficient

Table 1

<table>
<thead>
<tr>
<th>Scale Symptoms</th>
<th>Ocular itching</th>
<th>Palpebral and bulbar conjunctival hyperemia</th>
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</thead>
<tbody>
<tr>
<td>0: None</td>
<td>0: None</td>
<td>0: None</td>
</tr>
<tr>
<td>1: Intermittent itching</td>
<td>1: Dilation of a few blood vessels in part of the palpebral/bulbar conjunctiva</td>
<td></td>
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<tr>
<td>2: Continuous itching</td>
<td>2: Dilation of many blood vessels in the entire palpebral/bulbar conjunctiva</td>
<td></td>
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<tr>
<td>3: Continuous itching with the desire to rub, normal functioning not impaired</td>
<td>3: Redness of entire palpebral/bulbar conjunctiva/individual blood vessels cannot be distinguished</td>
<td></td>
</tr>
<tr>
<td>4: Incapacitating (impairs subject's normal functioning)</td>
<td>4: Incapacitating (impairs subject's normal functioning)</td>
<td></td>
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Fig. 1. Outline of this study. CAC, conjunctivitis allergen challenge; EPI/AT, epinastine hydrochloride ophthalmic solution 0.05% in one eye/artificial tear in the contralateral eye.
symptoms (i.e., itching and blood vessel dilation), even at the highest concentration of the allergen solution, were excluded from this study.

At visit 3, the CAC was performed again, using the optimal concentration of the allergen solution determined at visit 2 to ascertain the reproducibility of the CAC with the allergen solution. After visits 2 and visit 3, 11 subjects left the study. The remaining 10 subjects were included in the randomized arm of the study at visits 4 and 5.

At visit 4, the subjects were randomly assigned to receive epinastine (ALESION ophthalmic solution 0.05%, Santen Pharmaceutical, Osaka, Japan, epinastine hydrochloride ophthalmic solution) in one eye and an artificial tear solution (Soft Santear, Santen Pharmaceutical, Osaka, Japan) in the contralateral eye. An assistant covered the labels on the study drugs to mask their identity. The technician who instilled a drop of the study medication into each eye was not involved in any other aspect of the study. Five minutes after the drug instillation, the CAC was performed using the optimal antigen concentration (50-fold dilution [n = 2], 100-fold dilution [n = 2], and 200-fold dilution [n = 6]), and ocular itching and palpebral and bulbar conjunctival hyperemia were evaluated.

At visit 5, 4 h after the drug instillation, the CAC was performed, and the same ocular allergy symptoms were evaluated.

Tear histamine levels

Tears were collected using a paper Schirmer strip (Color Bar Schirmer, EagleVision, Memphis, TN, USA) before the CAC and 20 min after the challenge at visit 4. The strips were placed in the edge of the lower eyelid for 5 min in the same manner as in a Schirmer I test. The histamine level in the collected tear was measured using commercially available an enzyme-linked immunosorbent assay.

Statistical analysis

The target sample size was determined to be 10 subjects, presuming that the difference in ocular itching following the two treatments (test and artificial tear solutions) was 1.4, with an SD of 0.9 in a paired t-test (two-tailed, significance level 5%, power 80%). The primary endpoint was the mean ocular itching score at three time points (3, 5 and 10 min) following the CAC 4 h after the drug instillation. Statistical analyses were performed with a paired t-test. For all tests, a probability value of less than 0.05 was considered statistically significant.

Results

Characteristics of the subjects

Of the 36 subjects who gave written consent, 10 subjects (two females and eight males, with a mean [SD] age of 41.1 [8.3] years) were included in a randomized trial at visit 4 and visit 5. The majority of the subject exclusions were due to negative birch pollen-specific serum IgE (n = 12) or an insufficient allergic reaction at either visit 2 or visit 3 (n = 9) (Fig. 2).

Efficacy of the drug pretreatment 5 min before the CAC

At visit 4, the CAC was performed 5 min after the drug treatment. The mean (SD) ocular itching scores 3 min, 5 min, and 10 min after the CAC were 1.8 (1.2), 1.8 (1.3), and 1.6 (1.1) in the epinastine-treated eyes and 2.5 (1.0), 2.3 (0.7), 2.2 (0.9) in the control eyes, respectively. The ocular itching score of the epinastine-treated eyes 10 min after the CAC was significantly lower than that of the control eyes (P = 0.024, Fig. 3A).

The palpebral conjunctival hyperemia scores of the epinastine-treated eyes were also significantly lower than those of the control eyes 10 min after the CAC (P = 0.037, Fig. 4A).

Efficacy of drug pretreatment 4 h before the CAC

At visit 5, the CAC was performed 4 h after the drug treatment. The mean (SD) ocular itching scores 3 min, 5 min, and 10 min after the CAC were 0.8 (0.9), 1.0 (0.8), and 0.7 (0.8) in the epinastine-treated eyes and 1.5 (1.2), 1.5 (0.8), 1.6 (1.0) in the control eyes, respectively. The ocular itching scores in the epinastine-treated eyes 3 min and 10 min after the CAC were significantly lower than those of the control eyes (P = 0.025, 0.010, Fig. 3B).
The mean palpebral conjunctival hyperemia scores were also significantly lower in the epinastine-treated eyes than in the control eyes 10 and 20 min after the CAC ($P = 0.045, 0.037$, Fig. 4B).

Furthermore, both the mean ocular itching and the mean palpebral conjunctival hyperemia scores of the three time points (ocular itching at 3, 5 and 10 min, conjunctival hyperemia at 5, 10 and 20 min) of the epinastine-treated eyes were significantly lower than those of the control eyes when they were pretreated 4 h before ($P = 0.017, 0.038$, Table 2). No significant difference was found when they were pretreated 5 min before the CAC.

**Tear histamine levels**

The histamine concentration in the tears was examined before the drug pretreatment and 20 min after the CAC. In 16 eyes (80%), post-CAC histamine levels were elevated compared to the pretreatment levels. However, the pretreatment mean tear histamine level was not significantly different compared to the post-CAC level (Fig. 5). Furthermore, there was a positive correlation between the tear histamine level and the mean of ocular itching score of three time points (3, 5 and 10 min) following the CAC in the control eyes ($r^2 = 0.5002, P = 0.022$, Fig. 6A), whereas no such correlation was found in the epinastine-treated eyes (Fig. 6B). Similarly, there was also a positive correlation between the tear histamine level and the mean of palpebral conjunctival hyperemia score of three time points (5, 10 and 20 min) following the CAC in the control eyes ($r^2 = 0.4579, P = 0.032$, Fig. 7A).

**Discussion**

The CAC was developed by Abelson MB et al. initially, and then, both American Food and Drug Administration and Japanese Pharmaceuticals and Medical Devices Agency recognize the use of

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**Table 2**

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<th>CAC 5 min after the drug instillation</th>
<th>CAC 4 h after the drug instillation</th>
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<tr>
<td></td>
<td>Ocular itching</td>
<td>Palpebral conjunctival hyperemia</td>
</tr>
<tr>
<td>Artificial tear</td>
<td>2.3 ± 0.8</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>Epinastine</td>
<td>1.7 ± 1.2</td>
<td>1.6 ± 0.7</td>
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*P < 0.05.
the CAC test to evaluate the effectiveness of antihistamine ophthalmic solutions.

In Japan, thus far, only cedar pollen has been used in the CAC test. In the present study, birch pollen was used as an antigen. To the best of our knowledge, this is the first worldwide study where birch pollen is used in a CAC test. Birch pollen is a major allergen trigger in the spring in northwest Europe, including Sweden, Finland, the U.K., Germany, Belgium, Switzerland, and Austria, as well as in

Fig. 5. Tear histamine level before drug instillation and 20 min after the allergen challenge at 5 min after the drug instillation. A: Artificial tear-treated eyes, B: Epinastine-treated eyes (n = 10 subjects, mean ± SD).

Fig. 6. The relationship between the tear histamine level and mean ocular itching score of the three time points after the allergen challenge. A: Artificial tear-treated eyes, B: Epinastine-treated eyes (n = 10 subjects).

Fig. 7. The relationship between the tear histamine level and mean palpebral conjunctival hyperemia of the three time points after the allergen challenge. A: Artificial tear-treated eyes, B: Epinastine-treated eyes (n = 10 subjects).
North America and Canada.15 In Sweden and Finland, about 15% of the population is prone to birch pollen allergy, and the allergy is considered a social problem. People with birch pollen allergy present with asthma, rhinitis and conjunctivitis.

Birch pollen can also induce oral allergy syndrome (OAS).16 Some people with birch pollen allergy are also allergic to various fruits, such as apples, peaches, and cherries. OAS is considered to be due to a cross-immune reaction caused by molecular mimicry.17 OAS is a major issue not only in Hokkaido, Japan but also in other countries, such as Sweden and Finland. The prevalence of OAS highlights the need for birch pollen allergy studies.

Epinastine ophthalmic solution is commercially available and its efficacy was demonstrated in a phase III study in which cedar pollen was used in the CAC test. We performed CAC testing using almost the same protocol as the previous phase III study, and differences were 1) the challenging antigen, i.e., ‘cedar’ pollen in the previous study and ‘birch’ pollen in our current study, and 2) the period from the drug instillation to antigen challenge, i.e., ‘15 min’ in the previous study and ‘5 min’ in this study. In the present study, as well, epinastine ophthalmic solution was effective in suppressing allergic conjunctivitis induced by birch pollen. Earlier studies reported the efficacy of epinastine when it was administered 15 min prior to the instillation of an antigen.18 In this study, the epinastine ophthalmic solution was applied 5 min prior to the antigen challenge to assess the rapid-acting efficacy of the solution. The results revealed that it had moderate effectiveness and that its effectiveness reduced compared with the solution which was administered 4 h prior to the antigen challenge. Thus, epinastine ophthalmic solution can be considered to have mild rapid-acting efficacy.

A previous study reported that the histamine concentration in tear fluid was elevated in a CAC test.10 In the present study, the histamine concentration increased in 80% of subjects after the allergen challenge, and the histamine concentration in the tear fluid was positively correlated with the ocular itching score in the control group. On the other hand, there was no correlation between the histamine concentration in the tear fluid and the ocular itching score in the epinastine group. Epinastine has the potential to block the H1 receptor. The absence of a correlation between the histamine concentration and ocular itching score may be due to the H1 receptor blocking action of epinastine, with the solution suppressing ocular itching, even in the presence of a high concentration of histamine in the tears. A previous study also reported that the histamine concentration in tear fluid was correlated with the severity of allergic symptoms in control eyes, whereas it was not correlated with the severity of symptoms in eyes treated with antihistamine ophthalmic solutions.19 Although the study was seasonal observation study but not provocatory study, the results of that study correspond to those of the present work.

In the current study, the pretreatment with epinastine ophthalmic solution had no effect on the histamine concentration in the tear fluid after the allergen challenge. In an earlier study, when patients were premedicated for 5 days with an antihistamine ophthalmic solution, a lower tear histamine level was observed after an allergen challenge.10 In the present study, the eyes were premedicated only once with a drop of epinastine ophthalmic solution 5 min or 4 h before the allergen challenge. To determine the potential of an ophthalmic solution to suppress the release of histamine (i.e., degranulation of mast cells), a longer period of premedication with the ophthalmic solution would be required.

Epinastine is also known to act as an inverse agonist and to down-regulate the gene expression activity of the H1 receptor.20,21 This study involved a single pretreatment with epinastine ophthalmic solution. If the eyes were premedicated with the solution for longer periods, it would likely have shown a greater ability to suppress ocular symptoms.

Acknowledgments
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Conflict of interest
KN received research support from Santen Pharmaceutical. YN is an employee of Santen Pharmaceutical. The rest of the authors have no conflict of interest.

Authors’ contributions
YT and KN designed the study and wrote the manuscript. YT and KN contributed to the implementation of the study, statistical analysis, and interpretation of the results. DI performed the randomization and masking. DJ and SI provided important suggestions about the study design. All the authors read and approved the final manuscript.

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