

Effect of Antihistamine Eye Drops on the Conjunctival Provocation Test with Japanese Cedar Pollen Allergen

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ABSTRACT

Background: Approximately 16.2% of the Japanese population suffer from cedar pollinosis, with various manifestations such as ophthalmic, laryngo-pharyngeal and skin symptoms in addition to nasal symptoms. Thus, the annual pollen season is an agonizing period for patients. No study has reported symptoms and their clinical courses after conjunctival provocation with purified cedar pollen allergen Cry j1 as well as suppression of these allergen-induced ocular symptoms by antihistamine eye drops.

Methods: Nine patients with Japanese cedar pollinosis who had no nasal or ocular symptoms were included in the present study, after obtaining informed consent in writing. 1) Purified cedar pollen allergen Cry j1 was instilled in the left eye and phosphate-buffered saline (PBS) in the right eye as a control. 2) Levocabastine hydrochloride ophthalmic suspension and ketotifen fumarate ophthalmic solution were respectively instilled in the left and right eyes, which were then challenged with the allergen. Ocular symptoms after provocation with the allergen were recorded through the clinical course.

Results: Pollen allergen-induced ocular symptoms were itching and hyperemia of the palpebral conjunctiva, and itching lasted for more than 5 hours. Moreover, preadministration of antihistamine eye drops suppressed the increases in the ocular symptom scores, eliminating itching within 1 hour. Allergen provoked not only ocular symptoms but also nasal symptoms in 77.8% of patients.

Conclusions: Preadministration of antihistamine eye drops suppressed the symptoms induced by the allergen, which suggests that this is an effective early therapy for Japanese cedar pollinosis, if it is started before the pollen season. However, self-protection by patients using a mask may not be effective enough to suppress nasal symptoms during the pollen season, requiring them to additionally wear glasses to avoid exposure to the allergen.

KEY WORDS

allergen provocation test, allergic conjunctivitis, antihistamine eye drops, Japanese cedar pollinosis, ketotifen fumarate, levocabastine hydrochloride

INTRODUCTION

Approximately 16.2% of the Japanese population suffer from cedar pollinosis, which is one of the most common seasonal allergic diseases in Japan.¹ Because various symptoms including ocular, laryngo-pharyngeal, and skin symptoms are simultaneously manifested in addition to nasal symptoms, the annual pollen season is agonizing for patients.²

Anti-allergic or steroid eye drops are major therapies for allergic conjunctivitis. However, steroid eye drops, with excellent anti-inflammatory action, should be used with care because of adverse drug reactions after continuous use, such as glaucoma. Therefore, anti-allergic eye drops are selected as a first-line therapy for allergic conjunctivitis. Anti-allergic eye drops are classified into two types according to their action mechanisms; suppression of the release of chemical

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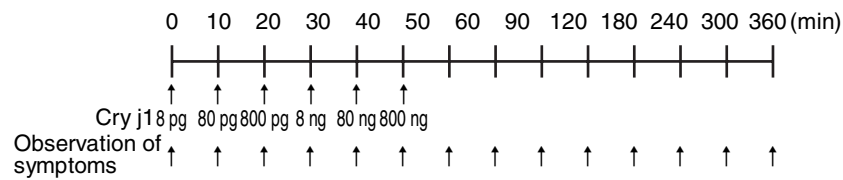
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1. Investigation of the allergen threshold dose for conjunctival symptoms



2. Comparing effect of the antihistamine eye drops

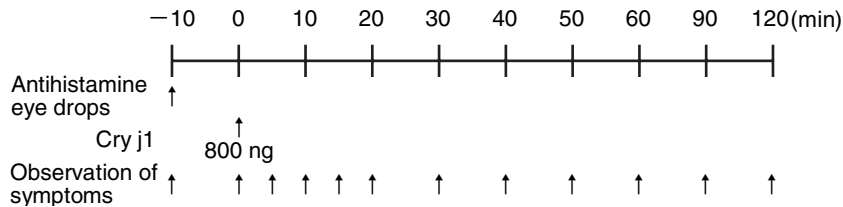


Fig. 1 Protocol of the conjunctival provocation test

Table 1 Subjective symptom scores for allergic conjunctivitis.

Symptoms	Score
Itching	0: none (no itching)
	1: mild (mild continuous itching that does not require rubbing)
	2: moderate (itching that requires rubbing)
	3: severe (subject cannot resist urge to rub eyes)
Tearing	0: none (no tear formation)
	1: mild (slight tear formation)
	2: moderate (marked tear formation necessitating frequent wiping of the eyes)
	3: severe (tears rolling down cheeks)

mediators and blockage of histamine H₁-receptors. Major symptoms of allergic conjunctivitis such as itching, hyperemia, and edema are mediated by histamine H₁-receptors, and therefore, antihistamine eye drops are expected to be “fast-acting.” Levocabastine hydrochloride is a potent and specific antagonist to histamine H₁-receptors.³ In our previous study on the nasal provocation test with Japanese cedar allergen, a levocabastine hydrochloride nasal spray was found effective in suppressing nasal symptoms.⁴ In addition, some studies on conjunctival provocation with levocabastine hydrochloride ophthalmic suspension controlled by placebo⁵ or sodium cromoglycate eye drops,⁶ reported that levocabastine hydrochloride rapidly suppressed ocular symptoms.

In the present study, Cry j1, which is the purified allergen of Japanese cedar pollen, was newly used for performing the conjunctival provocation test in patients with Japanese cedar pollinosis during its asymptomatic period, so as to investigate the induced

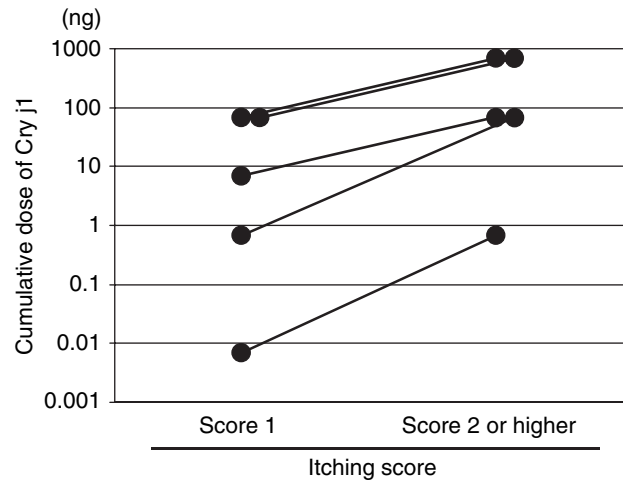


Fig. 2 Relationship between itching score and cumulative dose of Cry j1.

symptoms and their clinical courses as well as suppression of these allergen-induced ocular symptoms by antihistamine eye drops.

METHODS

SUBJECTS

Nine adult patients with Japanese cedar pollinosis who had no nasal or ocular symptoms were included in the present study from October to November. They were given an explanation about the study before participating, and written informed consent was obtained from all of the patients. All of the patients had a history of ocular symptoms during the pollen season, confirmed by means of either a positive skin prick test or serum Japanese cedar pollen specific

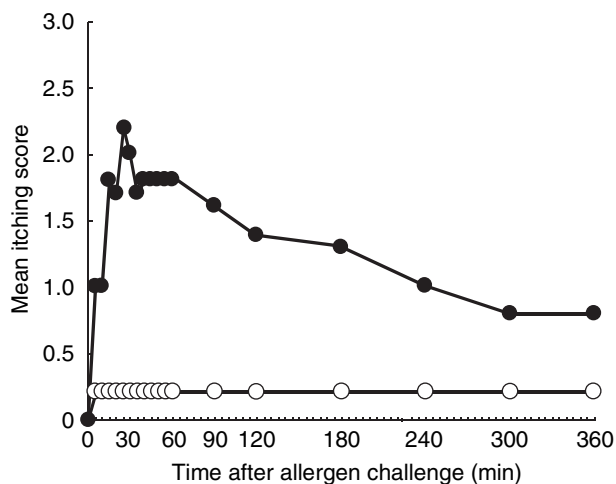


Fig. 3 Time course of mean itching score in the conjunctival provocation test. Cry j1 (●) and PBS (○) was instilled in the left and right eye, respectively ($n = 5$).

IgE antibody level (class 2 or higher).

METHODS

Purified cedar pollen allergen Cry j1 was purchased from Seikagaku Corp. (Tokyo, Japan). The allergen Cry j1 was diluted with sterile phosphate-buffered saline (PBS) to concentrations of 10 pg, 100 pg, 1 ng, 10 ng, 100 ng, and 1 μ g per 50 μ l immediately before use for the conjunctival provocation test. Levocabastine hydrochloride ophthalmic suspension (Livostin® Eye Drops 0.025%) was purchased from Nippon Shinyaku Co., Ltd. (Kyoto, Japan) and ketotifen fumarate ophthalmic solution (Zaditen® ophthalmic solution) was purchased from Novartis Pharma K.K. (Tokyo, Japan). This study was a single-masked comparative study. A clinical evaluation was performed mainly on the patients' subjective symptoms with a view of evaluating the patients' quality of life (QOL).

THRESHOLD DOSE OF PURIFIED CEDAR POLLEN ALLERGEN CRY J1 FOR MANIFESTATION OF OCULAR SYMPTOMS

Five male patients with Japanese cedar pollinosis (mean age 40.6 years) were instilled with 40 μ l of the allergen solution in the left eye and 40 μ l of sterile PBS in the right eye. According to the dosing regimen shown in Figure 1, instillation of the allergen started with a low dose (8 pg), and the affected eye was observed for 10 minutes after every instillation. Until ocular symptoms of score 2 or higher were induced, the concentration of allergen was stepwise increased (80 pg, 800 pg, 8 ng, 80 ng, and 800 ng) in the left eye, while PBS was instilled in the right eye. The severity of itching and tearing was given a score of 3 for severe symptoms, 2 for moderate symptoms, 1 for mild symptoms, and 0 for no symptoms (Table

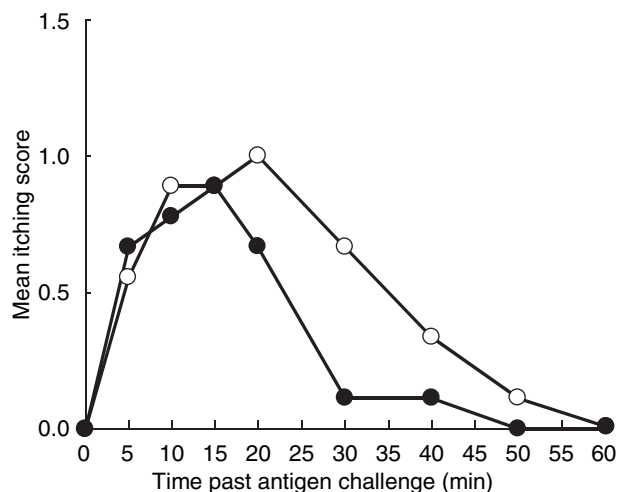


Fig. 4 Time course of mean itching score in the conjunctival provocation test after antihistamine eye drops were administered. Levocabastine hydrochloride (●) and ketotifen fumarate (○) was instilled in the left and right eye, respectively ($n = 9$). There was no significant difference between the two antihistamine eye drops.

1).

All of the patients were followed up to record symptoms for 6 hours after provocation.

COMPARISON OF PREVENTIVE EFFECTS AGAINST ALLERGEN-INDUCED SYMPTOMS BETWEEN ANTIHISTAMINE EYE DROPS

Two drops each of levocabastine hydrochloride ophthalmic suspension and ketotifen fumarate ophthalmic solution were instilled in the left and right eye, respectively, in patients with Japanese cedar pollinosis (7 male and 2 female patients: mean age 39.2 years, including 5 patients for investigating the threshold dose). According to the dosing regimen shown in Figure 1, the allergen dose was investigated and set at 800 ng for all patients because moderate symptoms scored 2 or higher were observed in all patients at 800 ng in the threshold dose setting study. Then, 800 ng of allergen each was instilled in both eyes 10 minutes after instillation with the respective eye drops, and the eyes were observed through the clinical course.

STATISTICAL ANALYSIS

The data were statistically analyzed by the Wilcoxon's matched-pair signed-rank test method.

RESULTS

THRESHOLD DOSE FOR INDUCING OCULAR SYMPTOMS

The dose of allergen was increased stepwise, and the actual exposure to the allergen is represented by the cumulative dose. Figure 2 shows the cumulative dose

Table 2 Other subjective symptoms observed in the conjunctival provocation test.

Case No.	Nasal itching	sneezing	nasal mucus	nasal congestion	aural itching	headache
1	○	○	○	○	—	—
2	○	—	○	—	—	—
3	○	—	—	—	○	—
4	○	—	—	—	—	—
5	○	—	—	—	—	—
6	○	—	—	—	—	—
7	○	—	—	—	—	—
8	—	—	—	—	—	○
9	—	—	—	—	—	—

of allergen at which the itching severity was scored as 1 and 2 or higher. While there were individual differences in the provoking dose of allergen, patients all showed moderate symptoms scored 2 or higher at the cumulative dose of 800 ng. On the basis of these dose-finding study results, the dose of Cry j1 allergen was set at 800 ng in the comparative study of antihistamine eye drops.

CHANGES IN THE ITCHING SCORE OVER TIME IN THE CONJUNCTIVAL PROVOCATION TEST

Figure 3 shows the time course of the mean itching score in the conjunctival provocation test. The itching severity after instillation with PBS was kept and scored as low as 0.2 without any changes. In contrast, the mean score in the allergen-induced eye reached 1.0 at 5 minutes, 1.5 at 15 minutes, and a maximum of 2.2 at 25 minutes. At 4 hours the score was reduced to 1.0, although the itching was retained for a duration of 6 hours or more. In the present test, tearing occurred in no patients.

COMPARISON OF PREVENTIVE EFFECTS AGAINST INDUCTION OF OCULAR SYMPTOMS BY ALLERGEN BETWEEN THE ANTIHISTAMINE EYE DROPS

Preadministration with antihistamine eye drops lowered the mean highest score of itching to 0.9 at 15 minutes for levocabastine hydrochloride and 1.0 at 20 minutes for ketotifen fumarate, and thereafter, the symptom disappeared within 60 minutes after the provocation (Fig. 4). In the conjunctival provocation test, the symptom score of itching reached a maximum of 2.2 with the itching persisting for more than 6 hours (Fig. 3). Therefore, the present study indicated that preadministration of antihistamine eye drops apparently suppressed the onset of the symptoms. Although no significant difference in the ocular symptom-suppressing efficacy was seen between these antihistamine eye drops, the mean score 0.1 at 30 minutes of levocabastine hydrochloride was lower than the mean score 0.7 of ketotifen fumarate ophthalmic solution. In the present study, no adverse

events were observed, except that one patient complained of irritation at administration with levocabastine hydrochloride ophthalmic suspension and so did 5 patients with ketotifen fumarate ophthalmic solution.

Symptoms other than ocular symptoms were observed in the present conjunctival provocation test (Table 2). Nasal itching was the most frequent, and occurred in 77.8% of patients. Other symptoms included sneezing, nasal discharge, aural itching, and headache.

DISCUSSION

Our previous questionnaire survey in patients with Japanese cedar pollinosis who visited otolaryngologists revealed that nasal symptoms developed in 99.8% of the patients, with ocular symptoms in 90.5%, laryngopharyngeal symptoms in 52.6%, and skin symptoms in 24.8%, and that allergic conjunctivitis is a complication in the majority of patients.² This means that treatment of allergic conjunctivitis may benefit patients with Japanese cedar pollinosis. Allergic conjunctivitis is nowadays treated by antiallergy or steroid eye drops in most cases, but continuous use of steroid eye drops is associated with adverse drug reactions such as glaucoma, even though they are excellent in anti-inflammatory action. Therefore, use of such eye drops is limited only to a short term under appropriate follow-up of the symptoms. In contrast, antiallergy eye drops are widely used as a primary drug with less adverse drug reactions. Antihistamine eye drops are expected to be fast-acting, because they block histamine H₁-receptors involved in the main complaints of allergic conjunctivitis such as itching, hyperemia, and edema. Eye drops of ketotifen fumarate and levocabastine hydrochloride were developed as such products in Japan, and are now clinically used. Levocabastine hydrochloride, a long acting, highly potent and selective histamine H₁-receptor antagonist was developed as a topical antihistaminic drug for nasal and ophthalmic use. Levocabastine hydrochloride has been demonstrated to be potent and long-acting antihistaminic activity

against compound 48/80-induced shock in rats, compared with other antihistaminic drugs.³ It has also been reported that levocabastine hydrochloride is fast- and long-acting as compared with ketotifen fumarate in a rat model of allergic conjunctivitis.⁷

In our present study on the conjunctival provocation test with purified cedar pollen allergen Cry j1, the induced symptoms and their changes were observed over time to investigate suppression of the allergen-induced symptoms by antihistamine eye drops. Conjunctival provocation tests were performed by a method to instill the allergen in the conjunctival sac to induce antigen-antibody reactions, which is used for definite diagnosis of allergic conjunctivitis.^{8,9} In addition, these tests are used to evaluate pharmacological efficacy, and have been reported for provocation by cedar pollen allergen.¹⁰⁻¹⁵ Various methods were attempted, whereas we established the method for conjunctival provocation tests with purified cedar pollen allergen Cry j1. In this study, the clinical allergic reactions were observed in the same manner as the conventional method.

The allergen-induced major ocular symptoms were itching and hyperemia of the palpebral conjunctiva in comparison between Japanese cedar pollen antigen and placebo. All of the patients showed the moderate itching symptom (score 2 or higher) when the allergen dose was accumulated to 800 ng. While the placebo-instilled eye showed no symptomatic changes with the mean score being as low as 0.2, the allergen-induced eye showed ocular symptoms, the mean score of which was 1.0 at 5 minutes after the provocation and a maximum of 2.2 at 25 minutes after the provocation (Fig. 3). The threshold dose showed individual differences in the induction of ocular symptoms, as previously reported in the nasal provocation test.⁴ Usually, moderate or severe ocular symptoms persist for more than 6 hours, and are likely to affect the patients' QOL. From this viewpoint, suppression of the onset of ocular symptoms is beneficial for patients. Preadministration of levocabastine hydrochloride or ketotifen fumarate effectively suppressed induction of symptoms by allergens, eliminating the ocular itching symptom within 1 hour. Although there were no significant differences between the two pretreatment groups, levocabastine hydrochloride ophthalmic suspension appeared to remit the symptoms earlier at 30 minutes or so. A combination of eye drops and nasal spray of levocabastine hydrochloride has been reported to effectively suppress seasonal allergic symptoms due to white birch pollen, being useful as an early therapy,¹⁶ and the present study results suggested that levocabastine hydrochloride is expected to be beneficial for patients as an early therapy for Japanese cedar pollinosis. In the present study, neither of antihistamine eye drops caused adverse events, but one patient complained of irritation at administration with

levocabastine hydrochloride ophthalmic suspension and so did 5 patients at instillation with ketotifen fumarate ophthalmic solution. This difference in induction of irritation was likely to be attributable to the difference in pH of both eye drops: ketotifen fumarate ophthalmic solution, acidic with a pH of 4.8 to 5.8; levocabastine hydrochloride ophthalmic suspension, almost neutral with a pH of 6.0 to 8.0.

From the above results, we conclude that continuous exposure to a small quantity of pollen allergen drifting before the day when the cedar pollen dispersion starts may elevate the sensitivity to the allergen, and therefore, use of antihistamine eye drops as an early therapy before drift of the pollen is an effective therapy to suppress substantial manifestation of ocular symptoms. In addition, as the allergen stimulated the nasal mucosa through the nasolacrimal canal and induced nasal symptoms in the present conjunctival provocation test, self-protection from allergic reactions using a mask may not be effective enough during the pollen season, requiring patients to additionally wear glasses to avoid exposure to the allergen.

REFERENCES

1. Baba K, Nakamura A. [Epidemiology of pollinosis and perennial allergic rhinitis in Japan.] *Allergology* 2003;**15**: 84-91 (in Japanese).
2. Dake Y, Enomoto T, Sakoda T *et al.* [Preventive care for Japanese cedar pollinosis.] *ORL Tokyo* 2003;**46**:51-56 (in Japanese).
3. Dechant KL, Goa KL. Levocabastine. A review of its pharmacological properties and therapeutic potential as a topical antihistamine in allergic rhinitis and conjunctivitis. *Drugs* 1991;**41**:202-224.
4. Shibano A, Dake Y, Enomoto T *et al.* [Effect of levocabastine hydrochloride on a nasal provocation test with Japanese cedar Pollen allergens.] *Jpn. J. Allergol.* 2004; **53**:325 (in Japanese).
5. Takamura E, Nomura K, Fukagawa K *et al.* [Effect of 0.025% levocabastine hydrochloride eyedrops for seasonal allergic conjunctivitis on ocular provocation test.] *Nippon Ganka Gakkai Zasshi* 2000;**104** (Suppl 1):144 (in Japanese).
6. Abelson MB, George MA, Smith LM. Evaluation of 0.05% levocabastine versus 4% sodium cromoglycate in the allergen challenge model. *Ophthalmology* 1995;**102**:310-316.
7. Minami K, Sugimoto Y, Kamei C. [Effect of Levocabastine Hydrochloride on Allergic Conjunctivitis in Rats.] *Atarashii Ganka* 2002;**19**:787-791 (in Japanese).
8. Ishizaki M. [Conjunctival provocation test.] *Clin. Immunol.* 1988;**20**:198-202 (in Japanese).
9. Sakashita M. [Provocation Test in Conjunctival Allergy.] *Atarashii Ganka* 1990;**7**:353-357 (in Japanese).
10. Sakuma Y, Mita H. [Inhibitory effect of Intal (Disodium Cromoglycate) eye drops on ocular provocation for Japanese cedar pollinosis.] *Jpn. J. Allergol.* 1988;**37**:1178-1182 (in Japanese).
11. Sakuma Y, Mita H. [Inhibitory effect of AA-673 eye drops on ocular provocation for Japanese cedar pollinosis.] *Jpn. J. Allergol.* 1989;**38**:93-97 (in Japanese).
12. Sakuma Y, Mita H. [Topical ketotifen suppressed ocular provocation for Japanese cedar pollinosis.] *Rinsho Ganka*

- 1989;**43**:1251-1254 (in Japanese).
- 13.** Sakuma Y, Mita H. [Inhibition of ocular provocation by pemirolast eyedrops in Japanese cedar pollinosis.] *Rinsho Ganka* 1990;**44**:1437-1440 (in Japanese).
- 14.** Sakuma Y, Mita H. [Suppression of ocular reactions in cedar pollinosis by tranilast ophthalmic solution.] *Rinsho Ganka* 1993;**47**:1935-1938 (in Japanese).
- 15.** Sakuma Y. [Inhibitory effects of tranilast eyedrops on the release of chemical Mediators.] *Atarashii Ganka* 1996;**13**:621-623 (in Japanese).
- 16.** Bende M, Pipkorn U. Topical levocabastine, a selective H1 antagonist, in seasonal allergic rhinoconjunctivitis. *Allergy* 1987;**42**:512-515.