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ORIGINAL ARTICLE

Efficacy of Epinastine Hydrochloride for Antigen-Provoked Nasal Symptoms in Subjects with Orchard Grass Pollinosis

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ABSTRACT

Background: Among the gramineae species, orchard grass is a typical causative pollen that provokes seasonal rhinitis. The purpose of this study was to examine the protective efficacy of epinastine hydrochloride for signs and symptoms caused by repeated nasal provocation with discs containing orchard grass pollen.

Methods: A single-dose, placebo-controlled, double-blind, crossover clinical study was conducted in subjects with orchard grass pollinosis. The pollen challenge was conducted with the use of provocation discs containing orchard grass pollen.

Results: Epinastine hydrochloride suppressed nasal symptoms caused by nasal provocation tests using orchard grass pollen discs. Among the nasal symptoms, the number of sneezing was significantly inhibited 30 minutes and 60 minutes after the administration of epinastine hydrochloride, as compared with placebo. There were no adverse reactions to the study drugs.

Conclusions: Our results suggest that nasal provocation tests with discs containing orchard grass pollen is a useful method for evaluating the onset of action of antiallergic drugs. As compared with placebo, epinastine hydrochloride decreased early-phase sneezing and the total nasal symptom score after repeated nasal provocations with orchard grass pollen discs.

KEY WORDS

allergic rhinitis, epinastine hydrochloride, nasal provocation, orchard grass

INTRODUCTION

Japanese cedar pollinosis has become a nationwide disease, affecting at least 30 million persons in Japan. Increasing airborne concentrations of Japanese cedar pollen throughout Japan is considered an important reason for such a high prevalence of related allergies.[1-3] Besides cedar and cypress, pollen of other trees, grass, and weeds can evoke various ocular, respiratory, and nasal allergic reactions.

However, in Japan few studies have focused on pollens other than Japanese cedar pollen. The dispersal season of Japanese cedar pollen is from late winter to spring. Even after the season ends, many patients continue to have symptoms of allergic rhinitis.[1] Such symptoms are attributed to allergies to other pollens. Therefore, treatment-related decisions should include an assessment of pollinosis caused by other pollens, as well as Japanese cedar pollinosis.

In 1991 we previously reported the results of immunological studies in 1329 patients with nasal allergy, including sensitization rates, onset rates, and prevalence rates of pollinosis associated with eight kinds of pollen. In that survey we reported that among 792 patients, 24.1% tested positive for antibodies specific to orchard grass pollen,[4] which is consistent with the findings of Practical Guideline for the Management of Allergic Rhinitis in Japan (revised in 2009).[1] Since the antigenicity of grass pollens is very similar to that of other pollens, a definitive diagnosis

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of orchard grass pollinosis is more challenging than that of Japanese cedar pollinosis. It is therefore difficult to pinpoint the season in which orchard grass pollen is dispersed. The occurrence of subclinical symptoms can also preclude the diagnosis of orchard grass pollinosis. Although the prevalence of orchard grass pollinosis remains unclear, the number of patients with this disease has apparently increased. As a leading cause of allergy, grass pollens are thought to come after cedar and cypress pollens. Patients with allergic rhinitis have symptoms such as sneezing, nasal stuffiness, and nasal discharge, negatively affecting their quality of life.

Identifying the causative pollen is important step in the management of allergic rhinitis; treatment effective against the causative pollen is then required. Antiallergic agents are the mainstay of treatment for allergic rhinitis. Our previous studies have shown that among the approved antiallergic agents in Japan, epinastine hydrochloride is highly effective for the prevention and management of seasonal rhinitis induced by Japanese cedar pollen.[5] Since nasal allergic reactions induced by orchard grass pollen are thought to be provoked by the same mechanisms as those induced by Japanese cedar pollen, we tested the hypothesis that epinastine hydrochloride is also effective for allergic rhinitis induced by orchard grass pollen.

We conducted this clinical study to evaluate the inhibitory potency of epinastine hydrochloride for orchard grass pollen-provoked allergic rhinitis. We performed nasal provocation tests using provocation discs containing orchard grass pollen at a concentration of 1/20 (weight/volume). The provocation and observation points were similar to those in our previous study in patients with Japanese cedar pollinosis. The design and results have been reported elsewhere.[5]

METHODS

SUBJECTS

The inclusion criteria required that the subject had a CAP score of ≥ 3 within the past 3 years and positive results on nasal provocation tests[6] with orchard grass pollen at screening. All subjects provided written informed consent in compliance with Good Clinical Practice guidelines for clinical studies.

Volunteers were excluded as subjects if they had a history of allergy or hypersensitivity to the ingredients of epinastine hydrochloride; received treatment with any form of corticosteroids within 1 month before the date of screening; received medication potentially affecting the results of the clinical study within 1 week before the date of screening (e.g., any form of antihistamines, antiallergic agents, or vasoconstrictors); were expected to have a poor response to provocation testing on the basis of the response to previous laser therapy or hyposensitization therapy for orchard grass pollinosis; or had underlying nasal diseases potentially affecting the assessment of the response to nasal provocation, such as acute or chronic rhinitis, nasal polyps, hypertrophic rhinitis, a deviated septum, or sinusitis. We also excluded women who were pregnant, possibly pregnant, or nursing infants, as well as volunteers who were judged not to be eligible for enrollment by the investigators. The enrolled subjects were examined 3 times: at screening before the initiation of the study (Visit 0), Visit 1, and Visit 2. Visit 1 and Visit 2 were separated by a 1-week interval.

Table 1 Medication and provocation schedule

Provocation	Provocation and treatment times (minutes)					
	-65	-60	0	30	60	180
Dummy disc	○					
Provocation-1		○				
Study drug medication			○			
Provocation-2				○		
Provocation-3					○	
Provocation-4						○

This study was conducted between November and December 2009 in accordance with the principles embodied in the Declaration of Helsinki of 1995 (as revised in Edinburgh 2000). The protocol was reviewed and approved by an independent institutional review board of Shinanozaka Clinic (Tokyo, Japan) before study initiation and subject recruitment.

STUDY DESIGN

This was a single-dose, placebo-controlled, double-blind, randomized, crossover study.

A total of 16 subjects with orchard grass pollinosis who met the eligibility criteria were randomly assigned to receive either epinastine hydrochloride 20 mg or a matched placebo at Visit 1.

Before initiation of the provocation study, a dummy disc (containing no pollen) was placed on the nasal inferior turbinates of a subject for 5 minutes to confirm that it caused no provocative signs. Provocations were conducted at 4 time points: 60 minutes before administration of the study drug and 30, 60, and 180 minutes after administration. Nasal provocation discs containing orchard grass pollen were prepared by dipping a round piece of filter paper 3 mm in diameter in a 1 : 20 (W/V) extract of orchard grass pollen. The paper was then lyophilized.

Orchard grass pollen discs were bilaterally placed on the inferior turbinates of a subject. Sixty minutes after the first provocation, the subject received either a 20-mg tablet of epinastine hydrochloride or a matched placebo. The provocation and treatment schedule is shown in Table 1.

Nasal signs and symptoms were evaluated for 4

Table 2 Nasal symptoms score

Score	4	3	2	1	0
Severity	++++	+++	++	+	-
Number of sneezing (times)	≥21	≥11 to ≤20	≥6 to ≤10	≥1 to ≤5	none
Discharge volume (g)	≥2	≥1.5 to <2	≥1 to <1.5	≥0.5 to <1	<0.5
Inferior nasal turbinate mucosal swelling	-	middle turbinate not seen	intermediate between (3) and (1)	to center of middle turbinate	none

time periods: -60 to -55 minutes before treatment, and 30 to 35 minutes, 60 to 65 minutes, and 180 to 185 minutes after treatment. The following nasal symptoms were assessed: number of sneezing, presence or absence of pruritus, and volume of nasal discharge, which was measured by weighing the tissue paper used by the subject. The swelling/color of the inferior turbinate as well as the quality and quantity of nasal secretion in the inferior turbinate were assessed by rhinoscopy.

The nasal mucosa was bilaterally examined and either videotape-recorded or photographed. A videotape recording was taken for 1-minute intervals starting immediately after and 5 minutes after each provocation. One week after Visit 1, 14 of the 16 subjects were re-studied at Visit 2. Subjects were examined in the same manner as the previous visit, except that they received the opposite treatment to that administered at Visit 1.

STUDY DRUGS

Epinastine hydrochloride tablets (with fees paid) and matched placebo tablets were provided by Nippon Boehringer Ingelheim Co., Ltd., Tokyo, Japan. Orchard grass pollen was provided by Allergon AB (with fees paid), Sweden. Test discs containing orchard grass pollen were kindly prepared and provided by Dr. Hiroshi Yasueda, Clinical Research Center for Allergy and Rheumatology, National Hospital Organization, Sagamihara National Hospital.

EVALUATIONS

The efficacy of epinastine hydrochloride against the response to nasal provocation was evaluated on the basis of the changes in nasal symptoms and other variables, evaluated at the predetermined time points.

Four rhinoscopic examinations were conducted 5 minutes after each provocation. Swelling and color of the inferior turbinate mucosa, watery discharge volume, and discharge properties were recorded. Table 2 shows the criteria for scoring nasal symptoms. The total nasal symptom score in this study was based on the number of sneezing, nasal discharge weight, and inferior turbinate mucosal swelling (instead of nasal obstruction) in accordance with Practical Guideline for the Management of Allergic Rhinitis in Japan.[1]

The primary end point of this study was the change in nasal symptoms. The secondary endpoint was the physiological change in nasal findings. To evaluate safety, investigators examined the subjects at Visit 1 and Visit 2. Physical, serum chemical, and 12-lead electrocardiographic examinations were conducted at screening and at the completion of the study.

STATISTICAL ANALYSIS

The number of sneezing and nasal discharge volume (g) at the predetermined time points were expressed as actual values. For the total nasal symptom score, the number of sneezing, nasal discharge volume, and swelling severity of the inferior turbinate mucosa were scored as shown in Table 2. For statistical analysis, changes in the number of sneezing, nasal discharge volume, and total nasal symptom score after treatment were compared with the respective values at the first provocation (-60 minutes before treatment) in each study arm. The Wilcoxon rank sum test with the Bonferroni correction was used for statistical analysis. The proportion of subjects with pruritus of the nasal mucosa was statistically analyzed using Fisher's exact test with the Bonferroni correction. In this study, $p < 0.05$ was considered to indicate statistical significance.

RESULTS

A total of 16 subjects (13 males and 3 females) aged between 21 and 42 years (mean ± SD, 30.75 ± 7.48) were enrolled. The baseline characteristics of subjects are shown in Table 3. The results of single provocation tests at screening are shown in Table 4. Nasal symptoms (number of sneezing, nasal discharge volume, nasal pruritus), nasal provocation test scores, and local nasal findings (swelling and color tone of the inferior nasal turbinate mucosa, watery discharge volume, nasal discharge properties) are presented. Fourteen of the 16 subjects completed the study; two subjects withdrew at Visit 2 because of a common cold, considered unrelated to the study drug by the investigator. Data from 14 and 16 subjects were thus included in efficacy and safety analyses, respectively.

Table 3 Baseline characteristics of subjects (n = 16)

Gender	male	13
	female	3
Age (years)	≥21 to <30	8
	≥30 to <40	5
	≥40 to ≤42	3
	mean ± SD	30.75 ± 7.48
Age at onset (years)	≥7 to <10	3
	≥10 to <20	7
	≥20 to <30	3
	≥30 to <40	1
	=40	1
	unknown	1
	mean ± SD	17.80 ± 9.45
Duration from the onset (years)	≥1 to <10	5
	≥10 to <20	8
	≥20 to ≤29	2
	unknown	1
	mean ± SD	12.80 ± 7.45
CAP (score) †	orchard grass	3.44 ± 0.63
	ragweed	1.31 ± 1.08
	Japanese cedar	3.88 ± 1.36
	Japanese cypress	2.21 ± 1.19
	mites	1.88 ± 1.31
	house dust	2.00 ± 1.21
Co-existing disease	no	12
	yes ‡	4
History of prior allergy	no	16
	yes	0
Prior therapy	no	16
	yes	0

† Values represent means with standard deviation.

‡ All co-existing diseases were seasonal allergic conjunctivitis.

Table 4 Results of single provocation tests at screening (n = 16)

Number of sneezing (times)	none	0
	≥1 to ≤5	10
	≥6 to ≤10	6
	≥11 to ≤20	0
	≥21	0
	min.	2
	max.	10
	mean ± SD	4.25 ± 2.98
Nasal discharge volume (g)	<0.5	1
	≥0.5 to <1	1
	≥1 to <1.5	3
	≥1.5 to < 2	6
	≥2	5
	min.	0.38
	max.	3.17
	mean ± SD	1.71 ± 0.71
Nasal pruritus	no	0
	yes	16
Nasal provocation test score	-	0
	±	0
	+	3
	++	9
	+++	4
Inferior nasal turbinate mucosal swelling	-	2
	+	12
	++	2
	+++	0
Inferior nasal turbinate mucosal color tone	-	4
	+	8
	++	2
	+++	2
Watery discharge volume	-	0
	+	3
	++	5
	+++	8
Nasal discharge properties	-	0
	+	0
	++	0
	+++	16

NASAL SYMPTOMS

The change in the number of sneezing differed significantly between epinastine hydrochloride and placebo at the early time points of 30 minutes and 60 minutes after treatment, but did not differ at 180 minutes (Wilcoxon rank sum test with Bonferroni correction: p = 0.0052, 0.0111 and 0.2502, respectively). The nasal discharge volume decreased slightly, but not significantly at the early time points after treatment with epinastine hydrochloride (p = 0.2674, 0.8104 and 1.0000) as shown in Table 5. The change in the nasal symptom score of the number of sneezing differed significantly between epinastine hydrochloride and placebo at the early time points of 30 minutes and 60 minutes after treatment (Wilcoxon rank sum test with Bonferroni correction: P = 0.0092 and p = 0.0090) as shown in Table 6. The total nasal symptom score also decreased significantly 30 minutes after provocation (p < 0.05) as shown in Figure 1. As for pruritus, Fisher's exact test with the Bonferroni correction indicated a decreasing trend 180 minutes after treatment with epinastine hydrochloride as compared with placebo. However, there were no significant differences between the two drug groups -60, 30, 60, or 180 minutes after administration (p = 1.0000, 1.0000, 1.0000, and 0.1843) as shown in Table 7.

NASAL EXAMINATION BY RHINOSCOPY

Swelling and color tone of the nasal mucosa were

Table 5 Change in nasal signs and symptoms (n = 14)

Symptom	Time points (minutes)	Study drugs	Mean	SD	SE	Quartiles					p value †
						Min.	1st	Median	3rd	Max.	
Number of sneezing (times)	30	epinastine	-4.9	5.3	1.4	-21	-5.0	-3.5	-3.0	0	0.0052
		placebo	0.4	3.9	1.0	-4	-2.0	-1.0	2.0	8	
	60	epinastine	-6.0	4.5	1.2	-15	-9.0	-6.5	-2.0	0	0.0111
		placebo	0.3	5.5	1.5	-11	-4.0	0.5	3.0	10	
	180	epinastine	-4.3	7.0	1.9	-21	-7.0	-3.0	0.0	8	0.2502
		placebo	-0.5	3.9	1.0	-7	-4.0	0.0	2.0	5	
Discharge volume (g)	30	epinastine	-0.871	1.449	0.387	-4.24	-1.780	-0.625	0.000	1.81	0.2674
		placebo	0.256	1.476	0.394	-1.79	-0.660	-0.095	0.890	3.56	
	60	epinastine	-0.331	1.192	0.319	-2.73	-0.960	-0.390	0.160	1.90	0.8104
		placebo	0.204	1.302	0.348	-1.80	-0.450	-0.020	1.070	2.48	
	180	epinastine	-0.249	2.384	0.637	-4.20	-1.480	-0.220	0.420	5.95	1.0000
		placebo	-0.096	1.007	0.269	-1.14	-1.090	-0.390	1.060	1.27	

† Wilcoxon rank sum test with Bonferroni correction.

Table 6 Change in nasal symptom score (n = 14)

Symptom	Time points (minutes)	Study drugs	Mean	SD	SE	Quartiles					p value †
						Min.	1st	Median	3rd	Max.	
Swellings of inferior nasal turbinate	30	epinastine	-0.1	0.6	0.2	-1	0.0	0.0	0.0	1	0.3982
		placebo	0.3	0.6	0.2	-1	0.0	0.0	1.0	1	
	60	epinastine	0.6	1.3	0.4	-2	0.0	1.0	1.0	3	1.0000
		placebo	0.7	0.8	0.2	-1	0.0	1.0	1.0	2	
	180	epinastine	0.4	0.9	0.2	-1	0.0	0.0	1.0	2	0.8556
		placebo	0.7	0.8	0.2	0	0.0	0.5	1.0	2	
Number of sneezing	30	epinastine	-1.0	1.1	0.3	-4	-1.0	-1.0	0.0	0	0.0092
		placebo	0.1	0.8	0.2	-1	0.0	0.0	0.0	2	
	60	epinastine	-1.1	0.9	0.2	-2	-2.0	-1.0	0.0	0	0.0090
		placebo	0.1	0.9	0.2	-1	-1.0	0.0	1.0	2	
	180	epinastine	-0.9	1.3	0.3	-4	-2.0	-1.0	0.0	1	0.0824
		placebo	-0.1	0.5	0.1	-1	0.0	0.0	0.0	1	
Discharge volume	30	epinastine	-0.857	1.351	0.361	-3.00	-2.000	-0.500	0.000	2.00	0.2116
		placebo	0.071	1.542	0.412	-3.00	0.000	0.000	0.000	4.00	
	60	epinastine	-0.071	1.269	0.339	-2.00	-1.000	0.000	0.000	3.00	1.0000
		placebo	0.000	1.468	0.392	-3.00	0.000	0.000	0.000	4.00	
	180	epinastine	-0.429	1.399	0.374	-2.00	-2.000	0.000	1.000	2.00	1.0000
		placebo	-0.214	1.051	0.281	-2.00	-1.000	0.000	0.000	2.00	

† Wilcoxon rank sum test with Bonferroni correction.

evaluated on rhinoscopy at each time point and were classified into 4 severity grades (negative to +++). The quantity and quality of nasal secretion were also assessed at the same time points. There was no difference in the swelling or color tone of the nasal mucosa or in the quantity and quality of nasal secretion between the two groups.

VIDEOTAPE RECORDING ON ANTERIOR RHINOSCOPY

Intranasal images recorded at the predetermined observation time points are shown in Figure 2. The images suggested that epinastine hydrochloride may decrease nasal swelling after provocation as com-

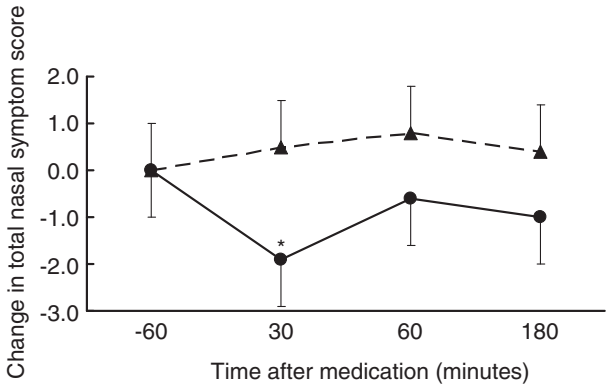

Fig. 1 Change in total nasal symptom score. A total nasal symptom is composed of nasal sneezing score, nasal discharge score, and severity of swelling in inferior turbinate mucosa. ●: epinastine hydrochloride, ▲: placebo, vertical bar on symbols: mean ± SD. * $p < 0.05$.

pared with placebo.

SAFETY

Safety was assessed in all 16 subjects. None of the subjects had any adverse reaction attributed to the study drugs during any part of the study period.

DISCUSSION

Identifying the causative pollen is an important factor in the management of seasonal allergic rhinitis, but weeds and grasses share common antigenic features. In particular, adequate studies of orchard grass pollen are lacking. Studies and analyses of allergens have been carried out globally, whereas antigens that cause pollinosis vary by region.[1]

Choosing an effective treatment is another important factor in the management of seasonal rhinitis. Nasal provocation tests are convenient tools for noninvasively identifying causative pollen antigens in patients with allergic rhinitis. Provocation tests have been widely used to identify allergens because they can be performed throughout the year, regardless of the pollen season. Once the nasal mucosa of a subject is exposed to an antigen, a specific IgE is induced, eliciting allergic symptoms. In patients with cedar pollinosis, provocation tests with nasal discs containing Japanese cedar pollen for 6 consecutive days in a pollen-free season reproduced nasal signs and symptoms similar to those occurring during the cedar pollen season.[7] Recent studies have reported that the use of a pollen-scattering chamber is an effective means of evaluating antiallergic drugs and may cause conditions similar to the actual pollen-dispersing season. This method can elicit symptoms of pollinosis by a specified antigen that evokes the provocative response for individual subjects, but cannot measure the volume of the causative antigen adhering to the

Table 7 Proportion of subjects with pruritus ($n = 14$)

Time points (minutes)	Study drugs	Number of positive	Ratio (%)	p value†
-60	epinastine	12	85.7	1.0000
	placebo	14	100.0	
30	epinastine	11	78.6	1.0000
	placebo	13	92.9	
60	epinastine	11	78.6	1.0000
	placebo	11	78.6	
180	epinastine	6	42.9	0.1843
	placebo	12	85.7	

† Fisher's exact with Bonferroni correction.

nasal mucosa. Another limitation is that the number of scattering systems that can be used in clinical trials is limited.

Nasal provocation tests are useful for the diagnosis of pollinosis, but it is difficult to assess the correlation between the severity of pollinosis in patients during the pollen season and disease severity as evaluated on nasal provocation tests. On the other hand, because allergic rhinitis is a type I allergic reaction, its signs and symptoms depend on the amount of antigen and the responsiveness of the nasal mucosa to the antigen, i.e., the specific hypersensitivity of the individual. We consider it feasible to assess this specific hypersensitivity in individual subjects by means of nasal provocation tests designed to determine the amount of antigen required to produce symptoms or the severity of nasal symptoms developing in response to a given amount of antigen. We previously reported that antihistamines significantly inhibited nasal reactions as compared with placebo on repeated nasal provocation tests using discs containing a specific amount of Japanese cedar antigen.[6] We therefore considered it feasible to perform a placebo-controlled study to evaluate nasal responsiveness to orchard grass pollen. In addition, to ensure that the response to antihistamine treatment on repeated nasal provocation tests was evaluated as reliably as possible, we recruited subjects who had an orchard grass CAP score of +3 or higher as well as two or more nasal symptoms (+ or higher) on a single nasal provocation test using orchard grass antigen discs at screening. We thereby confirmed that the amount of orchard grass antigen in the discs used in this study was above the threshold level in all subjects. Furthermore, the number of sneezing and the quantity of nasal discharge elicited by a single provocation with orchard grass pollen discs at screening were within well-defined ranges. The number of sneezing ranged from a minimum of 2 to a maximum of 10 (mean ± SD, 4.25 ± 2.98). Ten subjects had from 1 to less than 5 sneezings. The quantity of nasal discharge ranged from a minimum of 0.38 g to a maximum of 3.17 g

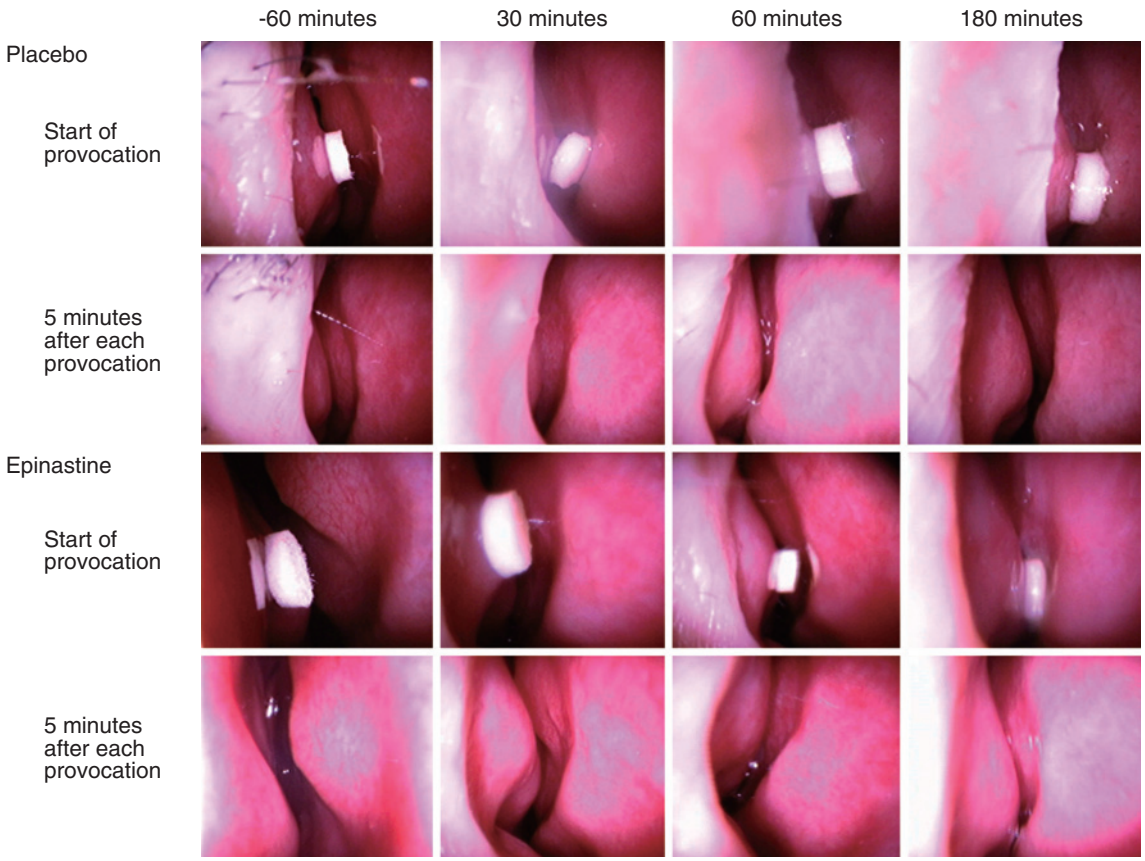

Fig. 2 The appearance of nasal mucosa on rhinoscopy. The nasal mucosa was examined immediately after and 5 minutes after provocations -60, 30, 60 and 180 minutes after treatment with the assigned study drug.

(mean ± SD, 1.71 ± 0.71 g). Nasal discharge was 1.5 to <2.0 g in 6 subjects and 2.0 g to ≤3.17 g in 5 subjects. To minimize the effects of individual differences among the subjects, the changes in the number of sneezing and the quantity of nasal discharge as compared with 60 minutes before administration of the study drug rather than the actual values were used to assess the effects of drug treatment on these variables.

This was a pilot study performed in a small number of subjects. We carried out provocation tests by applying nasal discs containing a fixed amount of pollen to the nasal mucosa and thereby assessed nasal symptoms and rhinoscopic findings. Repeated provocation tests were performed within several hours during the non-pollen-dispersal season. Because the study simulated exposure to pollen many times per day, we could only confirm that the amount of antigen contained in the discs was above the threshold limit.

This present investigation was also a pilot study of discs containing orchard grass antigen. Our results suggested that nasal provocation tests using these discs might be useful for the evaluation of drugs. However, establishment of the usefulness of nasal provocation tests for drug evaluation would require further studies examining correlations between the amount of pollen exposure (amount of pollen contained in orchard grass pollen discs) and nasal reactivity. Differences in individual responsiveness at a fixed amount of antigen should also be studied. Moreover, validation of the usefulness of repeated nasal provocation tests for drug evaluations would also require the establishment of antigen levels, including the threshold value, associated with the onset of nasal symptoms, studies of antigen levels higher and lower than the threshold level in individual patients, and studies of provocation tests performed on consecutive days, simulating pollen dispersal. Since previous studies have reported that eosinophil activation starts 6 hours after antigen induction[8] and that the number of eosinophils in nasal discharge increases significantly 6 to 10 hours after antigen induction,[9] studies should also be performed ≥6 hours after antigen exposure to assess potential effects of eosinophil activation.

The onset of action of epinastine hydrochloride for the suppression of histamine-induced wheal and flare response has been evaluated previously.[10] The skin response was inhibited 30 minutes after the admini-

stration of epinastine hydrochloride. In the present study, antigen induction was initially performed 30 minutes after the administration of epinastine hydrochlorid and the subject was repeatedly exposed to the study antigen. Our previous 5-hour repeated nasal provocation tests using Japanese cedar pollen discs[5] also confirmed that nasal symptoms are suppressed 30 minutes after treatment with epinastine hydrochlorode. However, both of these studies first evaluated drug effectiveness at the predetermined time point of 30 minutes after administration. It thus may be difficult to extrapolate these results and estimate the onset of effectiveness against actual nasal symptoms.

As for the duration of action against provoked nasal symptoms, subjects were observed for up to 180 minutes after administration of epinastine hydrochloride or placebo. Effective blood levels of epinastine hydrochloride for antigen-induced nasal symptoms remain unclear, but the peak drug concentration (Tmax) is reached in 1.9 ± 1.4 hours, and the half-life is 9.2 ± 1.7 hours, suggesting that the duration of action is several hours. In our previous 5-hour, repeated nasal provocation tests using Japanese cedar antigen discs,[5] the number of sneezing and the amount of nasal discharge in the placebo group peaked 60 minutes after administration, tended to decrease at 180 minutes, and returned to a similar level to that after the first provocation at 300 minutes. We therefore considered it appropriate to evaluate nasal reactions for up to 3 hours in repeated nasal provocation tests.

In this clinical study, we expected that epinastine hydrochloride would be effective for sneezing, nasal discharge, and swelling of the nasal mucosa elicited by orchard grass pollen. However, epinastine hydrochloride significantly suppressed sneezing for up to 60 minutes after administration as compared with placebo, but was not effective against nasal discharge, in contrast to the results of our previous study using cedar pollen antigen.[5] This discrepancy was most likely attributed to differences in responsiveness caused by different amounts of provocation antigens. Okamoto *et al.* performed 5 to 6 nasal provocation tests using orchard grass antigen discs at 30-minute intervals.[11] The protocol for that study caused allergic reactions of the eyes and face, as well as nasal symptoms. In contrast, allergic reactions other than nasal symptoms did not occur after repeated provocation tests in any subject in either the epinastine hydrochloride group or placebo group. These differences in responsiveness and symptoms are probably ascribed to the antigen contents of the discs. Assay methods for the principal antigens of orchard grass pollen, Dac g 1 and Dac g 5, have not been established in Japan. We were therefore unable to assay the main antigens in the discs used in this study. It is therefore difficult to comment further on potential reasons for the aforementioned differences in responsiveness and symptoms. Further studies are awaited.

Early onset of effectiveness for allergy is directly link to an improved quality of life (QOL) in patients with allergic rhinitis.[12] To achieve a better QOL, identification of the specific causative antigen is strongly recommended and may contribute to early treatment or prophylaxis during the initial or subsequent phase of allergic rhinitis.

In our previous study using Japanese cedar antigen disc,[5] a priming effect was seen for up to 60 minutes after administration of the study drug in the placebo group. However, we performed nasal provocation tests once daily for 8 days, using house dust mite allergen discs. There was no distinct increase in nasal symptoms.[13] In the present study, we performed 3-hour repeated provocation tests using orchard grass antigen discs in a non-pollen season. There was no increase in sneezing or nasal discharge from after the first provocation in the placebo group. Our results therefore did not shed light on whether or not repeated nasal provocation tests using antigen discs are associated with a priming effect. Factors such as the type and amount of antigen used for provocation or the time intervals or number of days of provocation may thus interact in a complex fashion, leading to differences in the activation of mast cells or eosinophils.

In this study, sneezing was inhibited by epinastine hydrochloride in the early phase, and the effect lasted for up to 60 minutes after administration. Differences in inhibitory latency among studies are poorly understood and require further investigations. Repeated nasal provocations may deplete histamine release from mast cells in the nasal mucosa. Sneezing tends to subside within a few hours. No response was observed 180 minutes after drug administration. We strongly believe that our interventional study will set a milestone for detecting and treating patients with orchard grass pollinosis. Further studies of orchard grass pollinosis should be conducted with the use of nasal provocation discs containing various concentrations of antigen.

In conclusion, this interventional study suggested that nasal provocation tests with discs containing orchard grass pollen are useful for evaluating the onset of action of antiallergic drugs. Epinastine hydrochloride inhibited nasal symptoms as compared with placebo. In particular, early-phase sneezing and total nasal symptom score after repeated nasal provocations by orchard grass pollen were significantly decreased by epinastine hydrochloride.

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