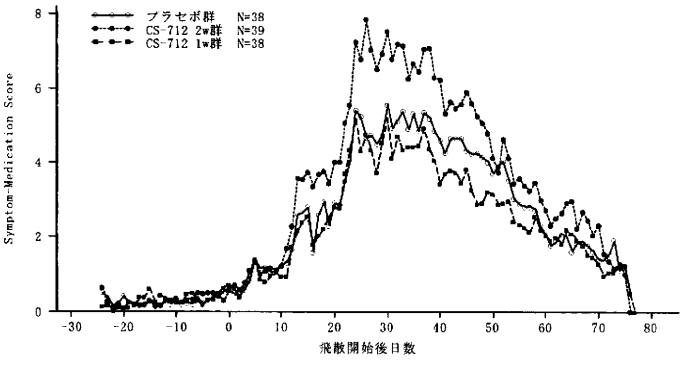
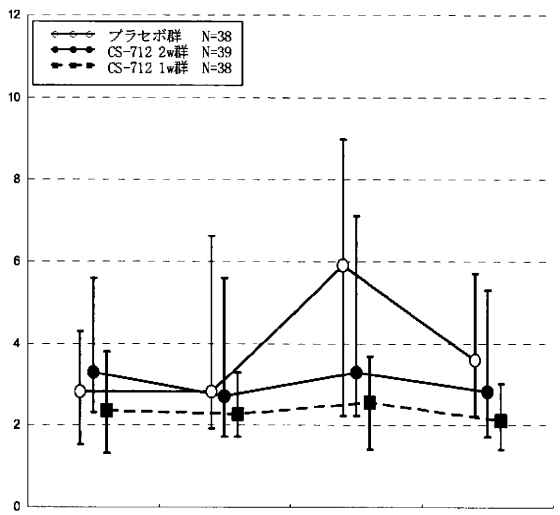


図 1： Symptom-Medication Score の推移（2006 年）



		投与開始時 (2005年10月中旬)	飛散開始前 (2006年1月中旬)	本格飛散時 (2006年3月中旬)	飛散終了時 (2006年4月下旬)
プラセボ群	変化量* (最小値, 最大値)		0.75 (-4.1, 18.0)	1.10 (-2.7, 21.3)	0.70 (-6.3, 10.8)
	P値**		—	—	—
CS-712 2W群	変化量* (最小値, 最大値)		0.00 (-12.0, 7.2)	0.60 (-13.6, 19.3)	-0.10 (-7.1, 6.7)
	P値**		0.0079	0.0260	0.0087
CS-712 1W群	変化量* (最小値, 最大値)		-0.10 (-15.3, 2.2)	-0.05 (-13.7, 8.3)	-0.10 (-4.8, 18.2)
	P値**		0.0018	0.0001	0.0017

図 2 Cry j 1 刺激に対する末梢血リンパ球増殖反応（2006 年）

（*変化量は投与開始前からの変化量、**P 値は CS-712 各群とプラセボ群との比較、Wilcoxon の順位和検定／測定時点毎）

して反応が亢進する傾向が認められたのに対し、CS-712 各群ではその亢進が抑制される傾向が認められたことから、CS-712 がスギ花粉に対する反応性に影響を及ぼした可能性が示唆された。

E. 結論

舌下ペプチド免疫療法は、副作用の発生が極めて少なく、さらに有用性が期待される新規の治療法である。今回の検討では十分な症状改善効果が認められなかったが、投与スケジュールの改良や投与量の設定、治療対象者の選択方法など改良の余地が残されている。対症療法である薬物療法に依存するばかりでなく、疾患を根治させうる免疫療法を新しく開発することは将来のアレルギー治療の課題である。

F. 研究発表

該当項目なし

G. 研究発表

1. 論文発表
2. 学会発表

H. 知的財産権の出願・登録状況

1. 特許取得
   該当項目なし
2. 実用新案登録
   該当項目なし
3. その他
   該当項目なし

Expert Opinion

Clinical pharmacology study of the corticosteroid nasal spray dexamethasone cipecilate (NS-126): examination of the durability of efficacy in the nasal induction test

Minoru Gotoh†, Kimihiro Okubo, Minoru Okuda & Chikuma Hamada
†*Nippon Medical School, Department of Otorhinolaryngology, Tokyo, Japan*

Background: Dexamethasone cipecilate is a corticosteroid nasal spray whose local efficacy durability has been improved by introduction of a liposoluble functional group to its chemical structure. This study was conducted to evaluate the efficacy of once-daily treatment with this drug in patients with perennial allergic rhinitis by a challenge test with house dust antigen (Phase I clinical pharmacology study).

Methods: This study was designed as a randomised placebo-controlled double-blind study in 28 patients with perennial allergic rhinitis. Either 200 μg dexamethasone cipecilate or placebo was administered once daily for 7 days, and the antigen challenge test conducted 23 h after the dose on each day. We evaluated the efficacy primarily through assessment of suppression of immediate nasal symptoms.

Results: When efficacy durability was evaluated by physicians based on a general assessment of the effects of suppression of nasal symptoms, the percentage of patients with efficacy lasting for 24 h differed significantly between the dexamethasone cipecilate group (69.2%, 9 out of 13) and the placebo group (15.4%, 2 out of 13) ($p = 0.015$).

Conclusions: Dexamethasone cipecilate was shown to be a corticosteroid having sustainable local efficacy. The results suggest that once-daily administration of dexamethasone cipecilate is effective in patients with allergic rhinitis, and that its efficacy lasts for 24 h.

Keywords: allergic rhinitis, antigen challenge test, dexamethasone cipecilate, efficacy durability, once daily treatment

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1. Introduction

Allergic rhinitis is a type-I allergic disease of the nasal mucosa, presenting with three major signs: paroxysmal sneezing, water rhinorrhea and nasal congestion. Steroids administered by nasal spraying exhibit effects against these three symptoms to a similar degree, and are also effective against anti-histamine-resistant nasal congestion. They have been used frequently for the treatment of moderate and severe allergic rhinitis.

Dexamethasone cipecilate is a corticosteroid nasal spray expected to exhibit efficacy if used once daily. It possesses a cyclopropanecarboxylate group at the 17-position and cyclohexanecarboxylate at the 21-position of the dexamethasone skeleton (**Figure 1**) [1]. Introduction of these liposoluble substituents is reported to

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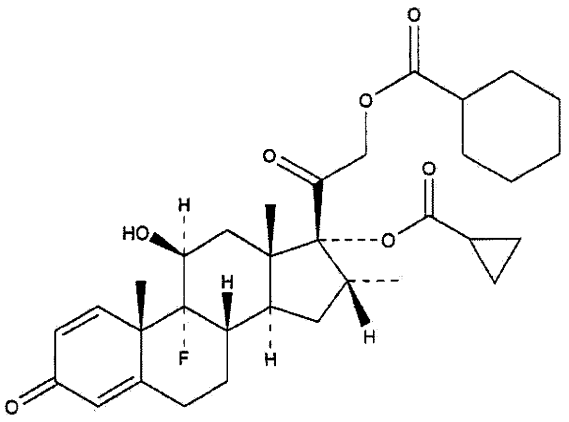


Generic name: Dexamethasone cipecilate (JAN)

Chemical name: 9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3, 20-dione 21-cyclohexanecarboxylate 17-cyclopropanecarboxylate

Figure 1. Chemical structure.

result in the durable efficacy of dexamethasone cipecilate [2]. Dexamethasone cipecilate is a powder formulation, so it is less irritating and is expected to be less likely to cause local adverse reactions of the nose (e.g., nasal irritation, uncomfortable smell) reported for existing liquid-type steroid nasal sprays [3]. To date, a single-dose study of this drug (50, 100, 200 and 400 µg) and a repeated dose study (once daily for 14 days at dose levels of 200 and 400 µg) have been carried out in healthy adult males, demonstrating absence of safety problems [4]. Plasma levels of unchanged drug and its main metabolite were less than the detection limit at all points of measurement, indicating that the level of systemic exposure to this drug is quite low [4].

The present placebo-controlled double-blind study was conducted to evaluate the efficacy and durability of efficacy of dexamethasone cipecilate administered once daily for the treatment of allergic rhinitis by means of nasal challenge test in patients with perennial allergic rhinitis.

2. Methods

2.1 Study design

This Phase I clinical pharmacology study was conducted as a randomised placebo-controlled double-blind parallel group comparison study at the Sekino Clinical Pharmacology Clinic, involving Japanese volunteers during the period from May to August 2001. The protocol for this study was authorised at the Institutional Review Board of Sekino Clinical Pharmacology Clinic. It was implemented in accordance with the Ministerial Ordinance on Good Clinical Practice. Prior to the eligibility assessment, each candidate for this study was informed as to the eligibility assessment and the main study using the leaflet and informed consent form. Subjects gave consent to the study in writing.

2.2 Patients

Male volunteers aged > 20 and < 50 years received the eligibility assessment (Table 1). Volunteers satisfying all of the following requirements were eligible to be enrolled in the study: i) house dust score > 3 in the allergen-specific IgE antibody test (CAP- radioallergosorbent test (RAST) method), and ii) house dust antigen disc positive reaction score > 2 in the nasal challenge test (having all of the three symptoms: i) paroxysmal sneeze or nasal itching sensation, ii) swelling of the mucosa of the inferior nasal turbinate, and iii) watery rhinorrhea.

The following volunteers were excluded from the study: i) control disc-positive reaction score > 1 in the nasal challenge test (having two of the three symptoms listed above), ii) individuals for whom pollen spreading during the study period served as one of the multiple antigens, iii) volunteers receiving antigen-specific immunotherapy, iv) volunteers planning to use a drug possibly affecting evaluation of the study drug efficacy during the period from 7 days before the eligibility test and study drug treatment to the end of treatment, and v) volunteers having nasal disease (acute/chronic rhinitis, nasal polyp, deflected nasal septum, sinusitis or hypertrophic rhinitis) severe enough to hamper efficacy evaluation.

For the period from 7 days before the start of study drug treatment to the end of this study, concomitant use of the following drugs which could affect evaluation was prohibited: anti-histamines, anti-leucotrienes, chemical mediator release inhibitors, steroids, anticholinergics and vasoconstrictors for nasal application.

2.3 Study assessments and procedures

The study drugs used were 200 µg dexamethasone cipecilate capsules and placebo capsules (drug-free capsule

Table 1. Details of the eligibility test.

Test	Description
Subjective and objective symptoms	Interview, auscultation and percussion
Rhinoscopy	Swelling of the inferior nasal turbinate mucosa, čvolume of aqueous secretion
Nasal challenge test	Positive reaction score (control and antigen discs)
Immunoserology	Allergen-specific IgE antibody (CAP-radioallergosorbent test (RAST) method: orchard grass, timothy, ragweed, genus Alnus, Japanese cedar, Japanese cypress, penicillium, cat dandruff, dog epithelium, Dermatophagoides ptreonyssinus, house dust)
Laboratory test	
Hematology	Red blood cell, white blood cell, heamoglobin, heamatocrit, platelet count, differential leucocyte counts (neutrophil, eosinophil, basophil, monocyte, lymphocyte)
Biochemistry	Total protein, total bilirubin, aspartate aminotransferase (glutamyl oxaloacetic transaminase), alanine aminotranferease (glutamyl pyruvic transaminase), alkaline phosphatase, lactate dehydrogenase, blood urea nitrogen, creatinine, total cholesterol, blood glucose, electrolytes (Na^+, K^+, Cl^-)
Urinalysis	Urinary protein, urinary glucose, urobilinogen

indistinguishable by appearance from the 200 µg dexamethasone cipecilate capsule). The indistinguishability in appearance between the study drugs was ensured by a third party before and after the study. Each subject received spraying into both nasal cavities of one 200 µg dexamethasone cipecilate capsule (group A) or one placebo dexamethasone cipecilate capsule (group P) once daily at 20:00 h for 7 days using a specific spraying device which can spray out an equal amount of the study drugs into both nasal cavities at the same time (Figure 2. Jetlizer) [5].

Observations and tests were conducted according to the schedule shown in Table 2. Subjects for whom the positive reaction score in the nasal challenge test on the first day of treatment was > 2 were allocated randomly and received treatment with the test drug or placebo. Interview, rhinoscopy and nasal challenge test were conducted at the start of treatment and 23 h after treatment, measuring the volume of nasal discharge and the resistance of the nasal cavity. The same observations and tests were repeated at 19:00 h on the eighth day of treatment.

Background variables of subjects analysed were birth date, height, body weight, disease type, severity, primary antigen, additional antigen, frequent onset period, age upon onset, duration of sickness, family history of allergy, complications, and prior medication and therapy (over the previous 6 weeks).

Rhinoscopy was carried out immediately before each nasal challenge test and 5 min after installment of the antigen disc for each nasal challenge test. During rhinoscopy, the score of inferior nasal turbinate mucosa swelling (0: absent, 1: visible up to the center of middle nasal turbinate; 2: intermediate between 1 and 3; 3: middle nasal turbinate invisible) and the score of the amount of aqueous secretion (0: absent; 1: superficial attachment; 2: intermediate between 1 and 3; and 3: filling) were rated for the right and left sides separately.

The nasal challenge test was carried out using the standardised antigen disc method for the nose. Two antigen discs (Allergen Disk 'Torii' House Dust, each disc containing 2 – 5 µg protein nitrogen and 7 – 12 µg total nitrogen) were installed on each side, about 1 cm behind the anterior edge of the inferior nasal turbinate. The positive reaction score was rated on a five-grade scale (0: no symptom; 0.5: one symptom; 1: two symptoms; 2: three symptoms; 3: three symptoms and six or more episodes of paroxysmal sneezing) on the basis of the presence of symptoms during the 5-min period after disc installment (I: paroxysmal sneeze or nasal itching sensation; II: swelling of the inferior nasal turbinate mucosa; III: aqueous secretion) and the frequency of paroxysmal sneeing. When the positive reaction score was rated, swelling of the inferior nasal turbinate mucosa was judged to be 'positive' if the rhinoscopy score after antigen disc installment was higher than the score before antigen disc installment in each nasal challenge test. The volume of nasal discharge was measured by weighing the tissue paper with which the nose was blown to collect nasal discharge after each session of rhinoscopy (before and after the nasal challenge test). The airflow resistance of the nasal cavity was measured for the right nasal cavity, the left nasal cavity, and both nasal cavities by the frontal nozzle method (resistance at p = 100 Pa) after removing nasal discharge by blowing before the nasal challenge test and 20 min after each antigen disc installment. If resistance was too high to be measured, it was expressed as > 100 ($Pa/cm^3/sec$). The device used for this measurement was MPR-3100 (Nihon Kohden Corporation).

For evaluation of safety, the interview, auscultation and percussion and laboratory tests listed in Table 1 were carried out. Upon onset of any adverse event in any subject, the causal relationship of the event to the study drug was rated on a three-category scale (1: not related; 2: possibly related; 3: related), taking into account the condition, complications and concomitant drugs of the patients. Adverse events in which causal relationship to the drug were rated as category 2 or 3 were counted as 'adverse drug reactions'.

2.4 Endpoints

Regarding the primary endpoint (24-h efficacy durability after once-daily treatment), durability was rated on a two-category scale (1: durability absent, 2: durability present) on the basis

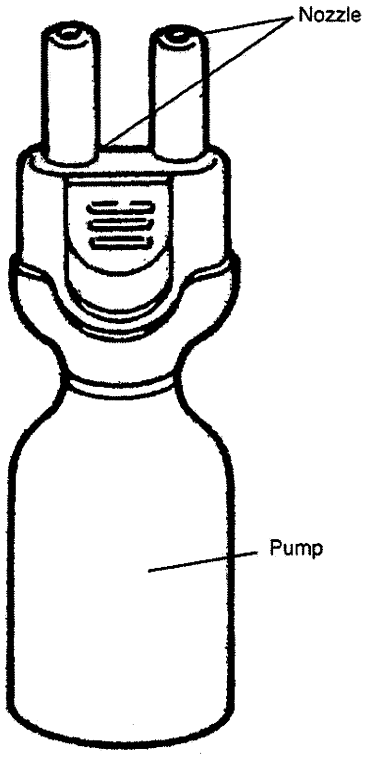


Figure 2. Jetlizer (Unisiajecs). Nozzles of the device are inserted in the both nasal cavities and the study drugs are sprayed into the nasal cavities by pumping the body of the device.

of the 'physician's assessment' after the first to seventh dose of the test drug. If durability was rated as '2: present', the number of doses exhibiting durability and the rationale for that judgment were recorded.

The physician's assessment was made on a two-category scale (1: suppressed; 2: ineffective) on the basis of a general evaluation of the positive reaction score, frequency of paroxysmal sneezing, nasal itching sensation, swelling of the inferior nasal turbinate mucosa, volume of aqueous secretion, volume of nasal discharge and air-flow resistance of the nasal cavity in each nasal challenge test.

A positive reaction score in the nasal challenge test after treatment was compared with that before treatment, and the change in each nasal challenge test rated on a two-grade scale (1: 1 or more improvement in score; 2: score unchanged or worsened). The frequency of paroxysmal sneezing in the nasal challenge test after treatment was compared with that before treatment, and the change at each session of the nasal challenge test rated on a two-grade scale (1: frequency decreased; 2: frequency unchanged or increased). In subjects who had not shown paroxysmal sneezing before treatment, the judgment 'increased frequency' was made if paroxysmal sneezing appeared after treatment. A nasal itching sensation for subjects who had shown a nasal itching sensation in the nasal challenge test before treatment, and a nasal itching sensation in each nasal challenge test was rated on a two-category scale (1: absent; 2: present).

Swelling of the inferior nasal turbinate mucosa was rated on a two-grade scale (1: 1 or more improvement in score; 2: score unchanged or worsened) on the basis of comparison of the score of swelling (the higher of the scores for the right or left side) in each nasal challenge test before and after treatment.

Volume of aqueous secretion evaluated by rhinoscopy was rated on a two-grade scale (1: 1 or more improvement in score; 2: score unchanged or worsened) on the basis of comparison of the score of aqueous secretion volume (the higher of the score on the right or left side) in each nasal challenge test before and after treatment. Volume of nasal discharge weight was rated on a two-category scale (1: volume decreased; 2: no decrease in volume) on the basis of comparison of the volume in each nasal challenge test before and after treatment.

Air-flow resistance of the nasal cavity was rated on a two-category scale (1: resistance decreased; 2: no decrease in resistance) on the basis of comparison of the resistance in both nasal cavities in each nasal challenge test before and after treatment.

2.5 Statistical analyses

This study was designed as an explorative study for evaluation of efficacy durability. Efficacy was evaluated in per protocol set (PPS) population. The target number of subjects was set at 14 for each group based on the prediction that the percentage of responders to treatment 23 h after treatment would be 70% for group A and 10% for group P ($\alpha = 0.05$, $\beta = 0.2$; two-tailed).

The presence/absence of 24-h efficacy durability (the primary endpoint) was compared between group A and group P, and its inter-group difference of proportion was tested by Fisher's exact test. Significance level was set at 5% (two-tailed). Secondary endpoints (physician's assessment at each session of the nasal challenge test, positive reaction score in the nasal challenge test, frequency of paroxysmal sneezing, nasal itching sensation, swelling of the inferior nasal turbinate mucosa, volume of aqueous secretion, volume of nasal discharge, and air-flow resistance of nasal cavity) were tested in the same way as the primary endpoint.

Regarding the frequency of doses needed until achievement of 24-h efficacy durability, the cumulative distribution function was estimated by the Kaplan–Meier method and was compared between the two groups using the generalised Wilcoxon test. Significance level was set at 5% (two-tailed). This study was designed as an explorative study, therefore multiplicity of statistical tests was not taken into account.

3. Results

3.1 Patient characteristics

Of the 51 volunteers who gave consent to the study, 34 were rated as eligible to be included in the study based on the

Table 2. Test schedule.

Time observation	Eligibility test	Start of drug treatment		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8	Last day
		Before	Dose	19:00	Dose	19:00	Dose	19:00	Dose	19:00	Dose	19:00	Dose	19:00	Dose	19:00	9:00 -
Informed consent	↑																
Hospitalization		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interview	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
Auscultation and percussion	↑	↑															↑
Drug treatment (at 20:00)			↑		↑		↑		↑		↑		↑		↑		
Nasal challenge																	
Control disc	↑																
Antigen disc	↑	↑		↑		↑		↑		↑		↑		↑		↑	
Paroxysmal sneezing and nasal itching sensation*	↑ ↑	↑		↑		↑		↑		↑		↑		↑		↑	
Rhinoscopy[‡]	↑ ↑ ↑	↑ ↑		↑↑		↑↑		↑↑		↑↑		↑↑		↑↑		↑↑	
Nasal discharge volume measurement[§]		↑ ↑		↑↑		↑↑		↑↑		↑↑		↑↑		↑↑		↑↑	
Nasal cavity air flow resistance[¶]		↑ ↑		↑		↑		↑		↑		↑		↑		↑	
Allergen-specific IgE antibody test	↑																
Laboratory test	↑	↑															↑

*Frequency of paroxysmal sneezing and presence/absence of nasal itching sensation during the 5-min period after disc installation were observed.

[‡]Rhinoscopy after disc installation is carried out 5 min after installation.

[§]The nose is blown with a tissue paper to collect nasal discharge before and 5 min after disc installation.

[¶]Performed 20 min after disc installation.

eligibility assessment results. Of these 34 subjects, 29 had a positive reaction score > 2 in the pre-treatment nasal challenge test. Treatment with the test drug or placebo was carried out on 28 of these 29 subjects (Figure 3).

Efficacy evaluation involved 26 subjects (13 subjects from group A and 13 from group P), excluding one subject (group A) who cancelled consent to the study and another subject (group P) who discontinued the study before the fourth dose due to acute upper airway inflammation (Figure 3). The latter patient was included in the safety evaluation. Table 3 shows the background variables of the 26 subjects included in the efficacy evaluation (PPS).

3.2 Efficacy assessments

The percentage of subjects in whom 24-h efficacy durability was observed differed significantly between group A (69.2%, 9/13) and group P (15.4%, 2/13) ($p = 0.015$). In group A, the number of doses taken until suppression of antigen challenge among the subjects showing efficacy durability was two in one subject, three in four subjects, four in three subjects and five in one subject. In group P, the number of doses was four in one subject and five in one subject (Figure 4). The cumulative distribution function differed significantly between the two groups ($p = 0.003$, generalised Wilcoxon test).

In the analysis of the physician's assessment at each session of the nasal challenge test, the improvement rate was higher in group A than in group P at the third dose and after. The improvement rate after the fourth and fifth dose differed significantly between group A (61.5% and 76.9%) and group P (7.7% and 30.8%) ($p = 0.011$ and $p = 0.047$, respectively). The improvement rate after the sixth and seventh dose was also higher in group A (61.5% and 69.2%) than in group P (23.1% and 30.8%), although the differences were not statistically significant (Figure 5).

In the positive reaction score at each session of the nasal challenge test, the improvement after the fourth and subsequent doses was > 60% in group A, but was lower (23.1 – 30.8%) in group P. This parameter after the fourth, fifth and seventh doses differed significantly between group A and group P ($p = 0.047$, $p = 0.047$ and $p < 0.001$, respectively) (Figure 5).

The percentage of subjects showing reduction of the frequency of paroxysmal sneezing during the entire treatment period was higher in group A than in group P, and this percentage after the third dose differed significantly between the two groups (group A: 66.7%, group P: 10.0%; $p = 0.020$). After the fourth and subsequent doses, the percentage of subjects showing reduction of the frequency of paroxysmal sneezing was higher in group A than in group P but the difference was not statistically significant (Figure 6). Mean frequency of paroxysmal sneezing decreased markedly after the third and subsequent doses in group A, although it increased in group P (Figure 7).

In the analysis of the presence/absence of a nasal itching sensation, the percentage of subjects free of a nasal itching sensation after the fourth and subsequent doses was higher in group A than in group P, but this difference was not statistically significant (Figure 6).

The improvement rate after the fourth and subsequent doses of swelling of the inferior nasal turbinate mucosa, was higher in group A than in group P, and the improvement rate after the seventh dose differed significantly between the two groups (group A: 76.9%, group P: 23.1%; $p = 0.017$) (Figure 6).

In the analysis of the volume of aqueous secretion by rhinoscopy, the improvement rate after the second and subsequent doses was higher in group A than in group P, and the improvement rate after the fourth dose differed significantly between the two groups (group A: 38.5%, group P: 0.0%; $p = 0.039$) (Figure 6). In the analysis of the volume of nasal discharge weight, the improvement rate after the second and subsequent doses was higher in group A than in group P, and the improvement rate after the fourth dose differed significantly between the two groups (group A: 61.5%, group P: 15.4%; $p = 0.041$), similar to the results on the volume of aqueous secretion. The mean volume of nasal discharge decreased after the third and subsequent doses in group A, whereas it increased after each dose except for the fifth dose in group P (Figure7).

In the analysis of air-flow resistance of the nasal cavity at each point of time, the percentage of subjects showing a reduction of resistance ranged between 46.2 and 76.9% in group A and group P, without significant inter-group difference.

3.3 Adverse events

In group A, adverse events were seen in two subjects. One subject showed elevation in levels of alanine aminotransferase and aspartate transferase and the other subject showed elevation in alanine aminotransferase level. In Group P, adverse events were seen in two subjects. One subject showed upper airway inflammation, increase in white blood cell (WBC) count, increase in neutrophil count, increase in monocyte count, decrease in lymphocyte count, elevation in blood glucose level, and positive urinary glucose; the other subject showed elevation in alanine aminotransferase level.

Adverse events judged to be possibly related to the study drug were seen in one subject each from group A (elevation in levels of alanine aminotransferase and aspartate aminotransferase) and group P (elevation in alanine aminotransferase level). All adverse events were mild or moderate, and recovered or showed a tendency for recovery.

4. Discussion

The nasal challenge test is a useful tool to diagnose allergic rhinitis. It has also been applied to studies of the pathophysiology of allergic rhinitis and evaluation of responses to treatment [6]. This test has been used for evaluation of responses to treatment with steroid nasal sprays [7,8].

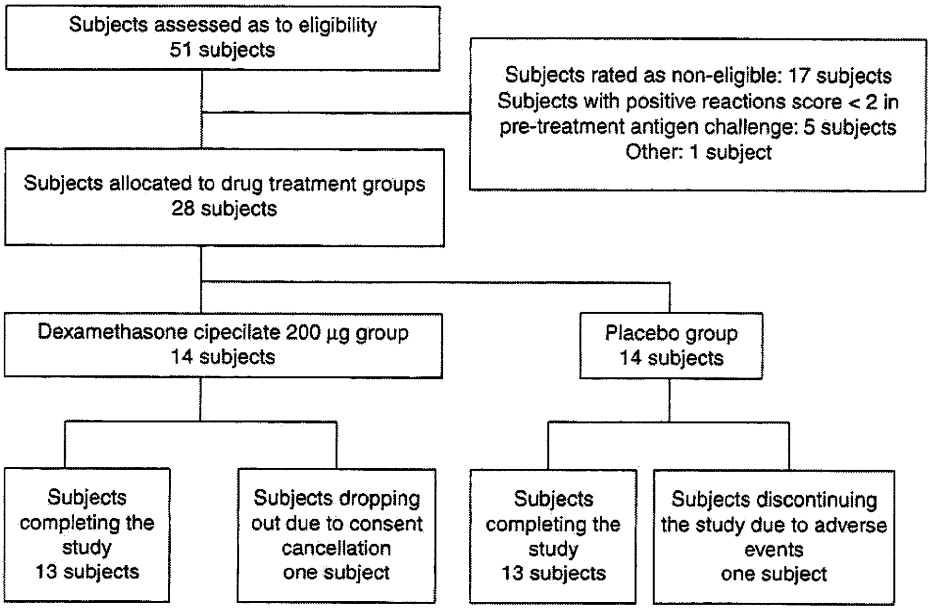


Figure 3. Composition of study group.

The present study was designed to evaluate the 24-h durability of the efficacy of dexamethasone cipecilate. Subjects were treated with the test drug or placebo once daily at 20:00 h for 7 days, and a challenge with antigen carried out 23 h after each dose. In terms of the positive reaction score, frequency of paroxysmal sneezing and volume of aqueous secretion in the nasal challenge test (which are indicators of the suppression of symptoms arising as immediate allergic reactions), the dexamethasone cipecilate 200 µg treatment group showed significant improvement or alleviation as compared with the placebo group. In terms of swelling of the inferior nasal turbinate mucosa (an indicator of nasal congestion), the dexamethasone cipecilate 200 µg group showed significant alleviation after the seventh dose as compared with the placebo group (although the inter-group difference was smaller than that of paroxysmal sneezing or aqueous secretion). Usually, during the nasal challenge test, paroxysmal sneezing and aqueous secretion appear within 5 min and reach a peak 5 – 10 min after the start of exposure to the antigen, whereas nasal congestion appears in several minutes and reaches a peak 30 min after the start of antigen exposure [6]. In the present study, rhinoscopy was carried out 5 min after installment of the antigen disc, so it seems likely that the findings as to swelling of the inferior nasal turbinate mucosa reflect the condition before appearance of antigen-induced symptoms.

Air-flow resistance of the nasal cavity was measured by the anterior method to minimise variances depending on the the skill level of the subject [6], and the resistance on both sides was measured to avoid influence from the nasal cycle. The data on resistance, however, varied greatly among individual subjects, and no marked difference between the dexamethasone cipecilate 200 µg group and the placebo group was noted.

The challenge test during the present study used four antigen discs which was double the dose in clinical use for diagnosis in order to induce symptoms marked enough for evaluation. This design seemed to have allowed us to confirm the superiority of the test drug over placebo in a relatively small study group (13 subjects per group). However, high-dose challenging seems to be a factor responsible for inability to measure air-flow resistance of the nasal cavity (> 100 Pa/cm^3/sec) in some subjects, suggesting that the magnitude of challenge is an important factor when evaluating the efficacy of drugs by the antigen challenge test.

In the analysis of physician's assessment based on a general evaluation of changes in each nasal symptom, antigen challenge was suppressed by ≥ 60% after the fourth and subsequent doses in the dexamethasone cipecilate 200 µg group, and marked efficacy of dexamethasone cipecilate was seen as compared with the placebo. In the present study designed to evaluate efficacy durability through assessment of antigen-challenge suppression, the dexamethasone cipecilate 200 µg group showed significant suppression as compared with the placebo group, and a significant difference between the two groups was revealed in the cumulative distribution function estimated by the Kaplan–Meier method.

Because of their mechanism of action, steroid nasal sprays are thought to exert more potent effects on delayed allergic reactions than on immediate allergic reactions, and they are expected to manifest effects also on immediate allergic reactions if used continuously [9]. In the present study, the nasal challenge test was carried out 23 h after each dose primarily

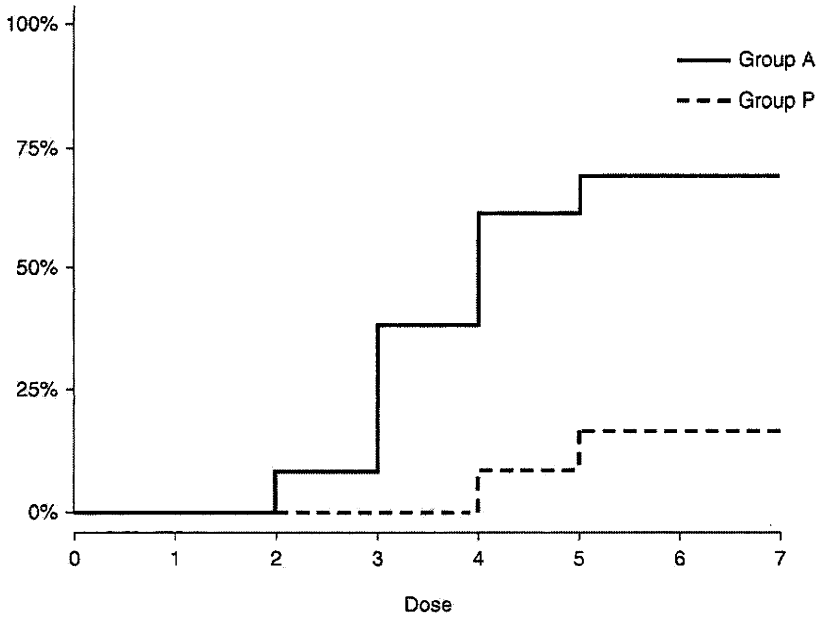
Table 3. Background variables of subjects (PPS).

Group	Group A	Group P	Total
Number of subjects	13	13	26
Disease type			
Paroxysmal sneeze + rhinorrhoea	3	0	3
Paroxysmal sneeze + nasal congestion	0	1	1
Rhinorrhoea + nasal congestion	1	0	1
Paroxysmal sneeze + rhinorrhoea + nasal congestion	9	12	21
Severity			
Mild	5	3	8
Moderate	7	5	12
Severe	1	5	6
Family history of allergy			
Absent	8	7	15
Present	5	6	11
Duration of sickness			
Less than 1 year	0	0	0
Over 1 year and less than 5 years	3	0	3
Over 5 years and less than 10 years	0	1	1
Over 10 years and less than 20 years	7	11	18
Over 20 years	3	1	4
Before drug treatment			
Frequency of paroxysmal sneezing			
0	5	7	12
1 – 5	3	4	7
6 – 10	2	0	2
11 – 15	1	1	2
16 – 20	1	0	1
21 – 25	1	1	2
Nasal itching sensation			
Absent	0	0	0
Present	13	13	26
Score of inferior nasal turbinate mucosa swelling (difference between pre- and post-challenge scores)			
1	11	13	24
2	1	0	1
3	1	0	1
Score of aqueous secretion volume			
1	2	3	5
2	6	2	8
3	5	8	13
Positive reaction score			
2	8	11	19
3	5	2	7

for evaluating suppression of immediate allergic reactions. When dexamethasone cipecilate was administered at 200 µg, it began to exert effects after once-daily administration for 3 – 4 days, and its efficacy seemed to last for 24 h after each dose.

It seems that steroid compounds with higher lipophilicity are taken up into the nasal mucosa more rapidly and at a higher percentage, leading to a higher tendency for retention in the body [10]. Because the cell membrane is liposoluble and the extracellular tissue is water-soluble, compounds with higher lipophilicity are expected to be better taken up into the cell membrane and less likely to move out of cells, resulting in prolonged manifestation of their efficacy. Dexamethasone cipecilate was metabolised to DX-17-CPC which is lacking the 21-position side chain of dexamethasone cipecilate. Dexamethasone cipecilate has an improved lipophilicity thanks to the liposoluble cyclic functional group introduced at the 17- and 21-position side-chains of the steroid skeleton. DX-17-CPC also has high lipophilicity. Both dexamethasone cipecilate and DX-17-CPC contribute to the durability of its efficacy in the nasal cavity [1].

In the analysis of safety, a causal relationship to the study drug was suspected for the adverse event related to liver function (elevation in levels of alanine aminotransferase and



Generalised Wilcoxon test: p = 0.003

		First dose	Second dose	Third dose	Fourth dose	Fifth dose	Sixth and Seventh dose
Group A	Number at risk	13	13	12	8	5	4
	Responders	0	1	4	3	1	0
	Cumulative response rate	0.0%	7.7%	38.5%	61.5%	69.2%	69.2%
Group P	Number at risk	13	13	13	13	12	11
	Responders	0	0	0	1	1	0
	Cumulative response rate	0.0%	0.0%	0.0%	7.7%	15.4%	15.4%

Figure 4. Estimating cumulative distribution function as to suppression of antigen challenge.

aspartate transferase) seen in one subject from the dexamethasone cipecilate group. However, these parameters showed a tendency for returning to pre-treatment baseline levels without necessitating any intervention, and caused no significant clinical problems. The other indicators of liver function showed no abnormal change. The influence from stress arising from hospitalization also seemed to be a factor associated with the abnormalities seen in levels of alanine aminotransferase and aspartate transferase [11].

Steroid nasal sprays in a liquid form contain additive preservatives. Although the concentration of benzalkonium chloride used as a preservative of nasal spray is thought not to have an effect [12,13], it might stimulate the mucosa and to reduce ciliary function [14-18]. This preparation, assuming the form of a powder and free of additives other than lactose, is less likely to cause irritation than liquid preparations, and is expected to reduce the incidence of local nasal adverse reactions especially in long term use.

The effectiveness of steroid nasal sprays for once-daily use has already been demonstrated, and several products in this category have been marketed in Japan, but many of them are in liquid form [19-23]. The preparation we studied can manifest efficacy in a once-daily dose in powder form. This is a promising new steroid nasal spray less likely to cause local nasal adverse reactions.

5. Conclusions

Antigen challenge tests were carried out on 28 patients with perennial allergic rhinitis. Dexamethasone cipecilate was shown to significantly suppress antigen challenge as compared with placebo, and exerted efficacy sustainable for 24 h. When

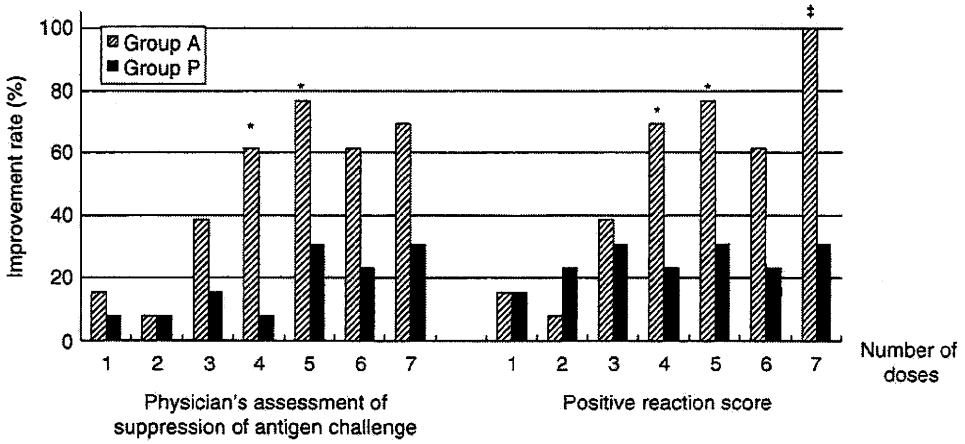


Figure 5. Efficacy assessment with the antigen challenge test.
*$p < 0.05$.
‡$p < 0.01$ (Group A versus Group P, Fisher's exact test).

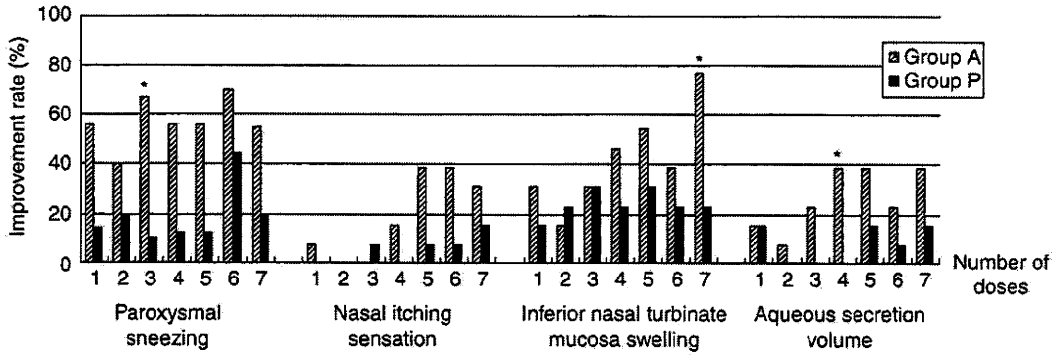


Figure 6. Improvement rate in evaluation of each nasal symptom by the antigen challenge test.
*$p < 0.05$ (Group A versus Group P, Fisher's exact test).

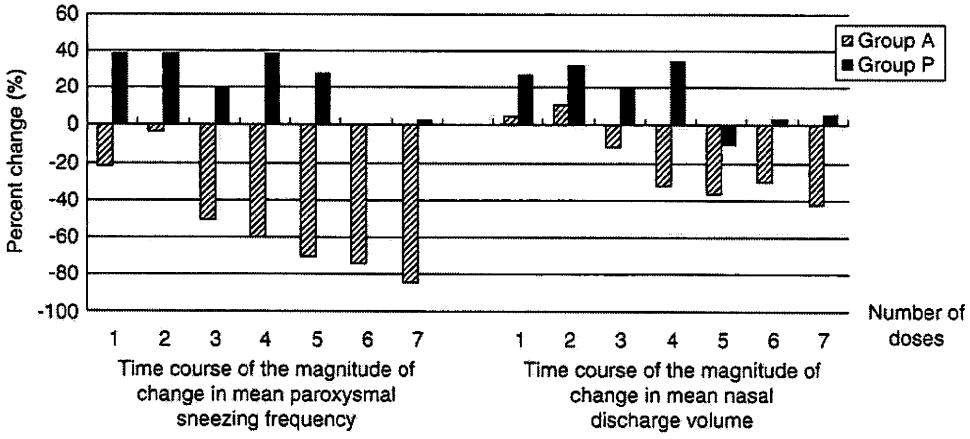


Figure 7. Time course of nasal symptoms in the antigen challenge test.

the cumulative distribution function in relation to the frequency of doses until manifestation of efficacy durability was estimated, it differed significantly between the dexamethasone cipecilate treatment group and the placebo treatment group. The durability of dexamethasone cipecilate efficacy was shown from the third dose onwards.

In terms of the frequency of paroxysmal sneezing, volume of nasal discharge, volume of aqueous secretion and swelling of the inferior nasal turbinate mucosa, the dexamethasone cipecilate treatment group showed significantly higher improvement as compared with the placebo treatment group.

Once-daily treatment with dexamethasone cipecilate at 200 µg was efficacious against allergic rhinitis, and its efficacy seemed to last for 24 h after each dose.

Acknowledgements

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Declaration of interest

This Phase I study was conducted for the purpose of a NDA and was sponsored by Nippon Shinyaku Co. Ltd, the company who develops this product. K Okubo and M Gotoh conducted this study at the Sekino Clinical Pharmacology Clinic. M Okuda served as a medical expert and C Hamada served as a statistical analysis adviser. The authors declare no other conflicts of interest.

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ORIGINAL ARTICLE

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

Minoru Gotoh[1], Kazuhiro Hashiguchi[2] and Kimihiro Okubo[1]

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.

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,

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

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-

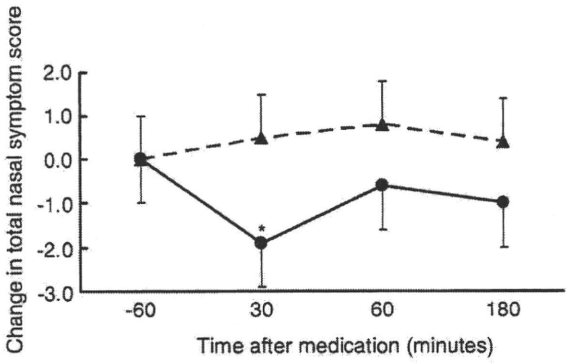

Fig. 1 Change in total nasal symptom score. A total nasal symptom is composed of nasal sneezing score, nasal discharge score, and severity of swelling in inferior turbinate mucosa. ●: epinastine hydrochloride, ▲: placebo, vertical bar on symbols: mean ± SD. * $p < 0.05$.

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

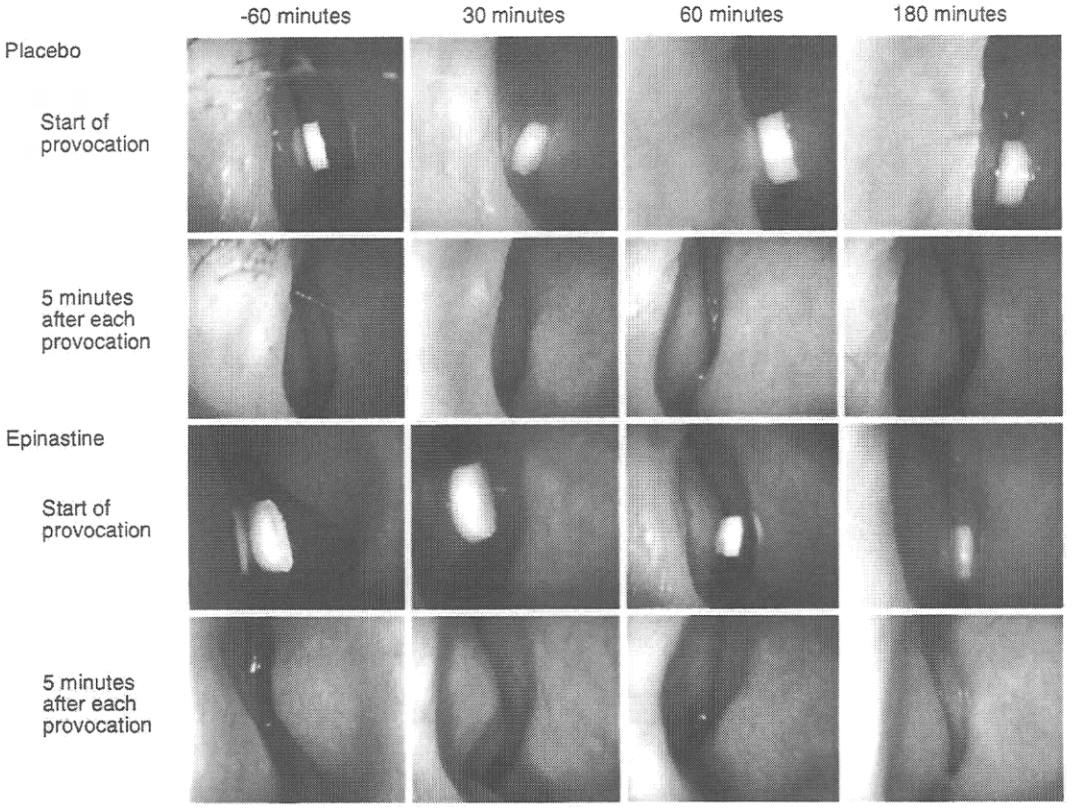

Fig. 2 The appearance of nasal mucosa on rhinoscopy. The nasal mucosa was examined immediately after and 5 minutes after provocations -60, 30, 60 and 180 minutes after treatment with the assigned study drug.

(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-