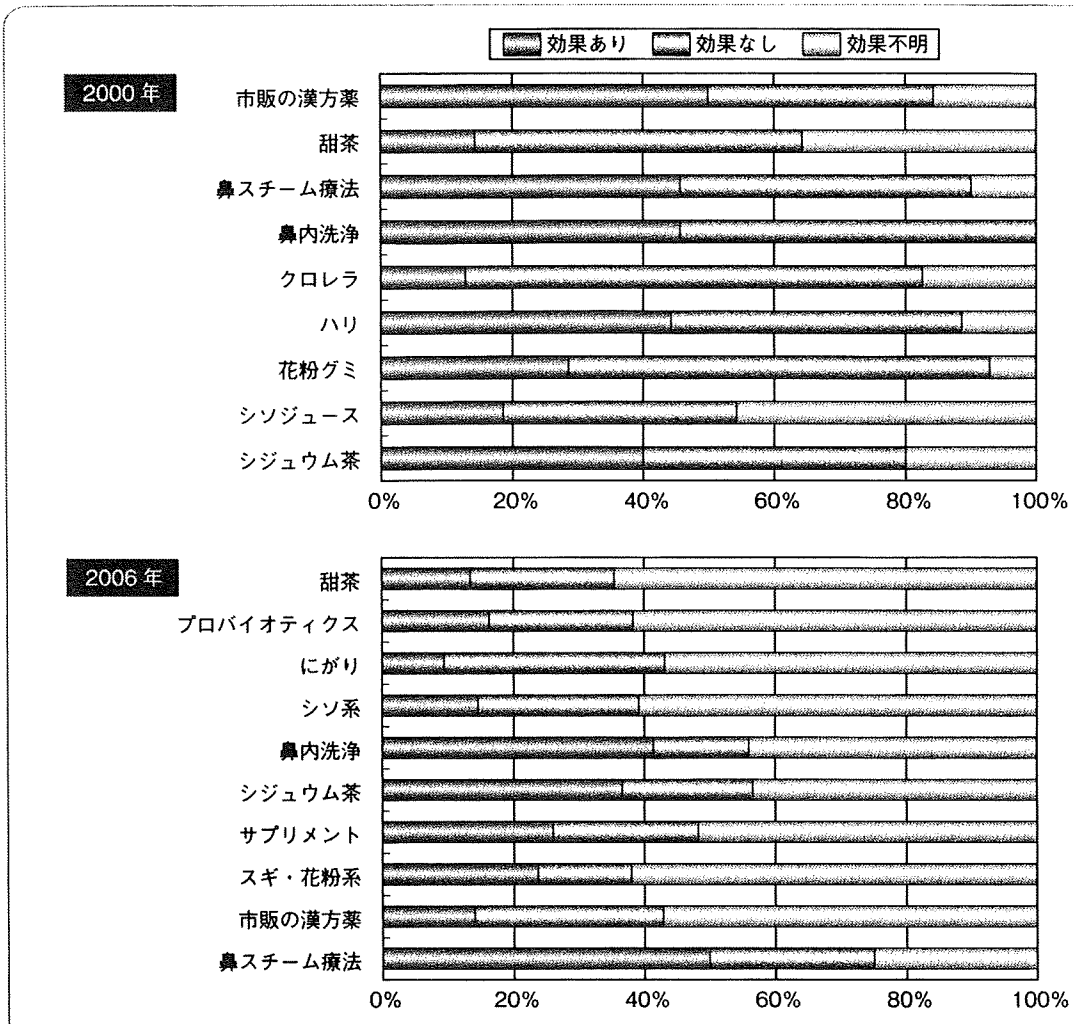

図 民間療法に対する患者評価（文献2より引用）

milk fermented with Lactobacillus acidophilus strain L-92 on symptoms of Japanese cedar pollen allergy : a randomized placebo-controlled trial. *Biosci Biotechnol Biochem* **69** : 1652-1660, 2005

5) Fujiwara D, Wakabayashi H, Watanabe H, *et al.* : A double-blind trial of *Lactobacillus paracasei* strain KW3110 administration for immunomodulation in patients with pollen allergy. *Allergology International* **54** : 143-149, 2005

KEY WORD

プロバイオティクス：宿主の腸内細菌叢のバランスを変えることにより，宿主に保険効果をもたらす，生きた微生物（を含む食品）のことです．乳酸菌，ビフィズス菌などが含まれ，アレルギーの発症抑制や症状緩和作用について現在研究されています．

ADVICE

民間療法はあくまでも補助的に活用すべきものですから，自分に合って手軽に健康増進を図れるもののほうが体にも心にも良い結果をもたらすのではないでしょうか．

Original Paper

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2009;149:141–149
DOI: 10.1159/000189197

Received: March 31, 2008
Accepted after revision: July 23, 2008
Published online: January 6, 2009

Validation Study of the OHIO Chamber in Patients with Japanese Cedar Pollinosis

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Key Words

Allergen exposure · Clinical trial · Japanese cedar pollen · Japanese patients · OHIO Chamber · Seasonal allergic rhinitis · Validation study

Abstract

Background: An artificial exposure chamber (OHIO Chamber), which allows dispersal of a fixed concentration of Japanese cedar (JC) pollen under stable conditions, was constructed. This study was conducted to identify the exposure conditions assuring validity of the clinical tests conducted using this chamber. ***Methods:*** Twenty-four adult patients with JC pollinosis were exposed to different concentrations of JC pollen: 0 (only during the summer period), 4,000, 8,000 and 12,000 grains/m^3, and the nasal and ocular symptoms were self-assessed during a 4-hour period of exposure. The amount of nasal discharge was measured and the sneezing frequency was recorded. This study was conducted twice during the summer and winter periods, i.e. non-pollen seasons. The reproducibility of the symptoms between the two seasons was assessed. ***Results:*** None of the subjects developed any symptom at the pollen concentration of 0 grains/m^3. No significant differences in the time to the onset of symptoms were found between the summer and winter study, regardless of the pollen concentration. There were no significant differences between the summer and winter study in the total symptom score and total nasal symptom score at any pollen concentration, suggesting the very favorable reproducibility of symptoms. ***Conclusions:*** Efficient and reproducible results are obtained in patients exposed to JC pollen in the OHIO Chamber. The results suggest the conditions of JC pollen exposure have scientific validity and the OHIO Chamber has the potential to contribute significantly to basic and clinical studies of JC pollinosis.

Introduction

Japanese cedar (JC) pollinosis is a representative disease of seasonal allergic rhinitis in Japan. The incidence of JC pollinosis has steadily increased over the last 30 years, and currently the disease has been reported to occur at a prevalence of >16% in the Japanese population [1]. The age at onset of JC pollinosis is declining, and ap-

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parently the symptoms also increase in severity annually. Thus, JC pollinosis can undermine the quality of life, interfere with the activities of daily living and cause a decline in labor productivity, thus posing an important socioeconomic problem [2].

A large number of antiallergic drugs exerting different mechanisms of action have been developed and used for the treatment of JC pollinosis in the clinical setting. There have been several clinical studies on the clinical effects and safety of various drugs administered during the pollen season, i.e. between February and April [3–5]. However, the number of pollen grains dispersed varies each year and among districts, and the temperature and climate are also not constant, making it difficult to reliably compare the results of evaluations of the efficacy and safety of drugs between different years. To overcome these drawbacks, an allergen exposure unit that allows exposure to a fixed number of pollen grains in a stable environment has increasingly been employed for such studies. The use of such a chamber allows studies to be conducted under the same climatic conditions as those prevalent during the pollen season at any time of the year, which is particularly useful for the study of seasonal allergic rhinitis.

There are several allergen exposure facilities in Europe, the US and other countries, e.g. the Vienna Challenge Chamber in Austria [6], the Environmental Exposure Unit in Canada [7, 8] and Germany [9], and the allergen exposure unit in the US [10]. Clinical efficacies and onset and duration of action of antiallergic drugs have been investigated in patients with allergic rhinitis at each facility.

Owing to the large number of patients with JC pollinosis in Japan, the need was felt for the development of such an environmental exposure unit for studying JC pollinosis. Therefore, we established an environmental exposure unit (OHIO Chamber) designed to allow dispersal of JC pollen in Tokyo [11], which is the third unit established in this country after those in Wakayama [12] and Osaka [13].

It is considered necessary to establish some exposure conditions and the scientific validity necessary for the judgment of drug efficacy in such a chamber. A consensus report on the Chamber, which has recently been issued, has also shown the importance of a validation study [14]. Since there are few data concerning the conditions of exposure to JC pollen and it is difficult to compare the respective exposure chambers, we conducted a basic and systematic evaluation of the OHIO Chamber.

In the present study, the reactivity and safety of several JC pollen exposure levels were investigated in the patients during the summer and winter periods, i.e. non-pollen seasons. The results established the validity of such testing conducted in the OHIO Chamber.

Patients and Methods

Patients

Adult patients with JC pollinosis were studied. In these patients, the severity of rhinitis could be judged from diaries recording symptoms in the pollen dispersal season.

The inclusion criteria were as follows: a history of symptoms of JC pollinosis for at least 2 years, a positive RAST for JC pollen antigen (CAP-RAST class ≥ 2) and a positive provocation test with a JC pollen disc, which is a paper disc measuring 5 mm in diameter containing a defined amount of JC pollen extract (kindly supplied by Sagamihara Hospital).

The exclusion criteria were as follows: a history of nasal and/or ocular diseases prior to entry into the Chamber, a history of treatment with steroid injections within 6 months prior to entry into the Chamber, a history of treatment with oral, inhalational or topical steroids and/or antihistamines within 4 weeks prior to entry into the Chamber, evidence of upper and/or lower respiratory tract inflammation within 2 weeks prior to entry into the Chamber, asthma, or a past history of anaphylaxis; pregnant, possibly pregnant and lactating women as well as women who intend to become pregnant within the proposed study period, and patients judged to be unsuitable for participation in the study for any reason by the physicians in charge of the study.

The study was conducted in accordance with Good Clinical Practice Guidelines and the Declaration of Helsinki. It was performed during the summer and winter periods of 2006, after it was reviewed and granted prior approval by the institutional review board of the Shinanozaka Clinic. Written informed consent was obtained from all of the participants prior to their entry into the study.

Study Design

This study was a randomized, double-blind, cross-over trial. It was conducted twice: during the summer (July) and during the winter (November), i.e. non-pollen seasons.

With regard to the target pollen dispersal concentrations in the Chamber, four different concentrations were set: none (target pollen exposure level, 0 grain/m^3; only in summer), and low (4,000 grains/m^3), moderate (8,000 grains/m^3) and high concentrations (12,000 grains/m^3). The patients were exposed to each of the dispersal concentrations in the Chamber for 4 h at intervals of at least 7 days.

The person in charge of allocation prepared a correspondence table with a table of random numbers that showed the correspondence of each group to the target pollen dispersal levels. Only the person and a technician who managed the control of the pollen count were aware of the target pollen dispersal concentration. The correspondence table, which included the pollen dispersal concentration, was managed by them until the end of the study. The person who managed the control of the pollen grain count started

the pollen dispersal after confirming that all the subjects had entered the Chamber and sat down. The person then dispersed the appropriate number of JC pollen grains on the basis of the correspondence table.

The pollen dispersal concentration in the Chamber was recorded every 3 min during the study period.

Assessment of Efficacy

Nasal and ocular symptoms and safety were evaluated in the patients, who were instructed to grade and record the severity of their nasal (sneezing, nasal discharge, nasal obstruction and itchy nose) and ocular symptoms (epiphora and itchy eyes) at regular intervals (immediately before entering the Chamber and at 15-min intervals thereafter) according to the following scale: 0 = none (no symptoms); 1 = mild (symptoms present but easily tolerated); 2 = moderate (awareness of symptoms; bothersome, but tolerable); 3 = severe (definite awareness of symptoms; difficult to tolerate, but does not interfere with the activities of daily living), and 4 = very severe (difficult to tolerate and interferes with the activities of daily living). The mean of the sum of the scores for the four nasal and two ocular symptoms was calculated as the total symptom score (TSS), the mean of the sum of the scores for the nasal symptoms as the total nasal symptom score (TNSS), and the mean of the sum of the scores for the ocular symptoms as the total non-nasal symptom score (TNNSS).

The interval from the time of entry of a patient into the Chamber to the occurrence of the first nasal symptom (any of the 4 nasal symptoms) or the first ocular symptom was designated as 'time to occurrence of symptoms', and the time was recorded for each patient.

The patients were instructed to blow their noses with tissue paper given in advance to each of them. The tissue paper used was recovered in plastic bags at regular intervals (every 30 min). The difference in the weight of the tissue paper measured before and after use was calculated to express the amount of nasal discharge in each subject. Each participant actually counted and recorded the frequency of sneezes at regular intervals him/herself.

Statistical Analysis

The results of the time to the occurrence of symptoms after being exposed to one of the three target dispersal concentrations were compared by the Kruskal-Wallis test in both the summer and winter seasons. In the tests conducted during the summer and winter seasons, the cumulative incidence of the symptoms observed at each pollen dispersal level was analyzed by the Kaplan-Meier method, and the difference in the time to symptom occurrence and the incidence of symptoms at each pollen dispersal level between the summer and winter studies were analyzed by the log-rank test. Significant differences in TSS and TNSS at each point of observation and each concentration level compared to baseline were analyzed by the Wilcoxon signed-rank test. To evaluate the reproducibility of the nasal and ocular symptoms between the summer and winter studies, the results of TSS, TNSS, the total amount of nasal discharge and the sum of the number of sneezes during the summer and winter periods for the same target pollen dispersal concentration were compared by Spearman's rank-order correlation coefficient test. The correlation coefficient and the p value for each result were determined. Differences with p values <0.05 were regarded as significant.

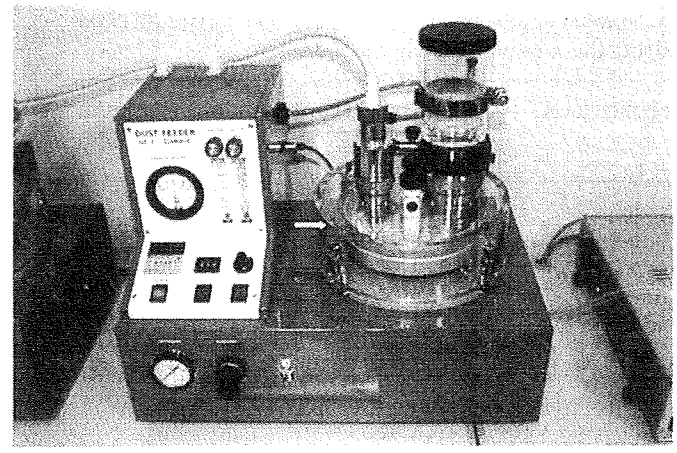

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Fig. 1. Pollen supply system: dust feeder and pollen grains on a turntable (→) are shown.

OHIO Chamber

The OHIO Chamber is a chamber measuring 5 × 5 m (25 m^2) with a height of 2.5 m and a capacity of 12 subjects at the maximum, which allows dispersal of JC pollen at constant target pollen dispersal concentrations. 'OHIO' of the OHIO Chamber is short for the facility, and is a combination of initials of the family names of four doctors (Okubo, Hashiguchi, Ishikawa and Okuda), who were involved in the design and development of this facility. The outside air, passed through activated charcoal and HEPA filters, served as conditioned air, with a ventilation frequency of 20 times per hour.

Stable production of pollen as an aerosol with high pollen concentration and homogeneous dispersion of the aerosol at high concentrations in the indoor air are needed to maintain the stability of the pollen concentrations in the Chamber. The following method was adopted for pollen production: Pollen grains on the turntable (rotating at a constant speed) were aspirated with an ejector using compressed air (dust feeder), and an aerosol with high pollen concentrations was thereby supplied (fig. 1). The aerosol was diluted with the outside air and dispersed in the Chamber. Air flow generator systems aimed at circulating the indoor air were set in the four corners of the Chamber, which allowed even distribution of the pollen at the target pollen dispersal concentration in the Chamber [11]. The target pollen dispersal concentrations were determined by means of the laser particle counter KC-20 (Rion, Tokyo, Japan), which allows real-time determination of the pollen concentration on the basis of the scattered light of laser illuminant.

Results

Patient Characteristics

This study included 24 patients (8 males and 16 females; mean age 38.5 ± 9.9 years) who were assigned to one of three groups, each consisting of 8 subjects. Assess-

Table 1. Number of pollen particles dispersed at the time of exposure during the summer and winter studies

Target pollen level	Actual pollen concentration particles/m³	
	summer	winter
0 grains/m³		
Group A	331 ± 126	
Group B	343 ± 91	ND
Group C	147 ± 50	
4,000 grains/m³		
Group A	4,183 ± 348	4,978 ± 1,811[a] (4,374 ± 427)
Group B	4,199 ± 360	4,517 ± 260
Group C	3,435 ± 356	4,107 ± 293
8,000 grains/m³		
Group A	6,808 ± 602	8,039 ± 655
Group B	6,939 ± 697	8,150 ± 572
Group C	7,642 ± 601	7,974 ± 784
12,000 grains/m³		
Group A	10,303 ± 797	11,808 ± 1,002
Group B	10,615 ± 662	12,377 ± 827
Group C	12,494 ± 566	12,201 ± 942

The subjects were divided into three groups (groups A–C) and were exposed to pollen dispersal. The pollen concentrations were counted by a laser particle counter every 3 min. The number of pollen particles counted in each group was expressed as means ± SD.

[a] The number of pollen particles 150 min after the start of exposure to the pollen was 11,166. This finding was attributed to aspiration of a large amount of pollen into the sensor tube when a subject accidentally hit the sensor tube in the Chamber. It was thus clarified that this high concentration was not due to any technical problem in the method used for the pollen dispersal. The figures in parentheses indicate means ± SD calculated after excluding the abnormally large value at 150 min.

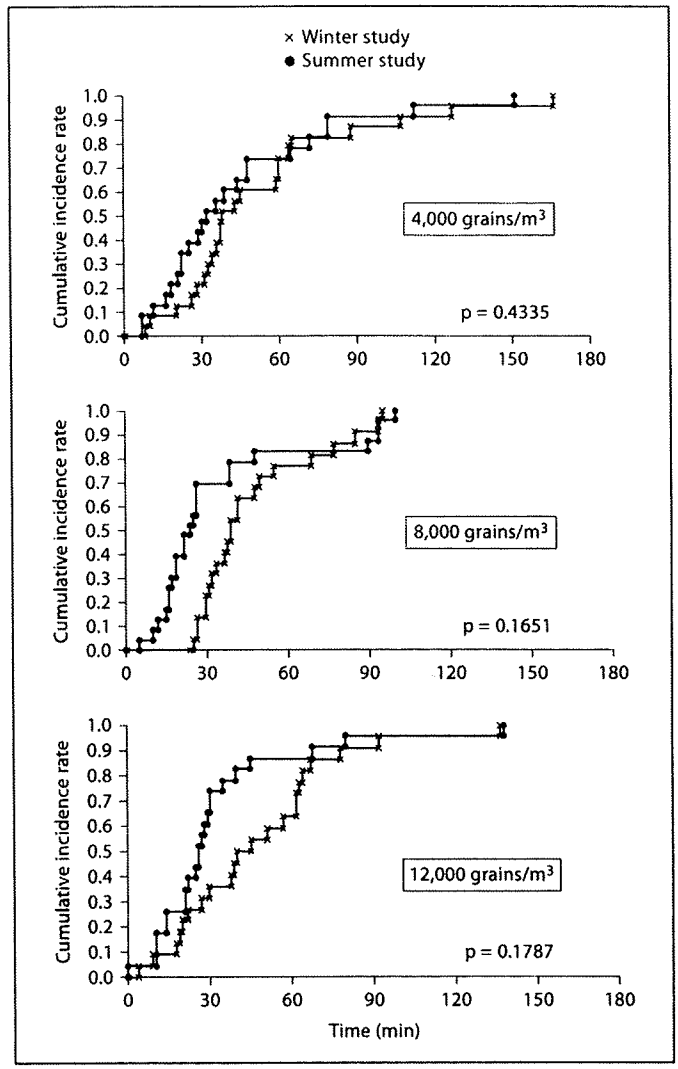

Fig. 2. Comparison of the time to symptom occurrence at the same target pollen dispersal concentration between summer (●) and winter (x) studies using Kaplan-Meier's method. No significant seasonal effect was found at each pollen concentration ($p > 0.05$; log-rank test).

ment of the severity of their symptoms from the records maintained in symptom diaries by individual subjects during the pollen dispersal season revealed that symptoms were mild in 4 subjects, moderate in 8 subjects and severe in 12 subjects. In the summer study, 1 subject could not undergo the study at the target pollen dispersal concentration of 12,000 grains/m³ because of poor physical condition. None of the subjects had any allergic symptoms at the start of the study.

Pollen Dispersal Concentrations, and Temperature and Humidity in the Chamber

Assessment of the target pollen dispersal concentration and the number of pollen grains actually dispersed at the time of exposure during the summer and winter studies revealed that the number of pollen grains actually dispersed was within 10–15% of the target pollen dispersal concentration. In the winter study conducted with the target concentration set at 4,000 grains/m³, an excessively large value of the pollen concentration was recognized 150 min after the start of pollen exposure. This large value was attributed to the aspiration of a massive amount of pollen into the sensor tube in the Chamber when a subject accidentally hit the sensor tube. Thus, it was clarified that the abnormal value was not attributable to any technical failure in the method of pollen dispersal in the Chamber (table 1).

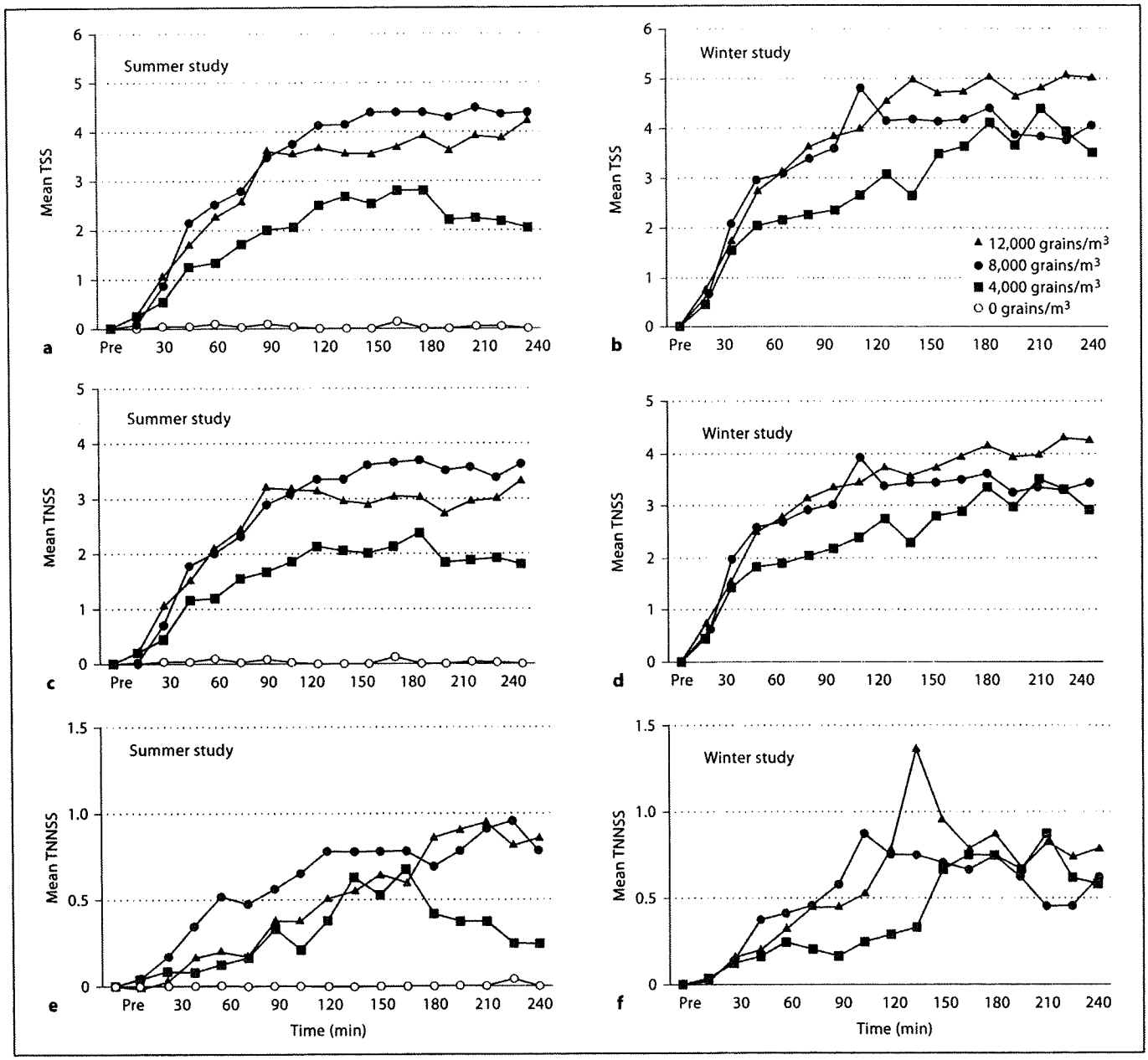

Fig. 3. Mean total symptom scores (TSS) at different pollen concentrations in the summer (**a**) and winter studies (**b**) are shown. None of the subjects developed any symptoms at the pollen concentration of 0 grains/m^3 (open circle). TSS increased in a dose-dependent manner for all pollen dispersal concentrations. TNSS and TNNSS at the three different pollen dispersal concentrations in the summer (**c**, **e**) and winter studies (**d**, **f**) are shown. There were no significant differences in the TSS and TNSS between each of the three pollen exposure concentrations both in summer and winter studies. At each concentration, TSS and TNSS were significantly increased compared to baseline from 30 min after exposure.

The mean temperature and humidity values in the Chamber determined at 3-minute intervals in each study were adopted as the values for the analysis. Room temperature was 22.1 ± 0.1°C and relative humidity was 44.4 ± 1.0%, being close within the range of target values.

Time to Symptom Occurrence

With regard to the time to symptom occurrence at each pollen dispersal concentration in the Chamber (table 2), none of the subjects developed any symptoms at the target pollen dispersal concentration of 0 grains/m^3.

Table 2. Time to the occurrence of first symptoms at each pollen exposure level

Exposure level	Summer study, min	Winter study, min
0 grains/m^3	NA	ND
4,000 grains/m^3	53.1 ± 37.9	44.0 ± 35.4
8,000 grains/m^3	40.9 ± 22.7	35.0 ± 25.3
12,000 grains/m^3	59.0 ± 53.4	34.1 ± 28.9

Data are given as means ± SD. NA = Not available; ND = not done.

Table 3. Correlation coefficients between the summer and the winter studies for the TSS, TNSS, the amount of nasal discharge and the frequency of sneezing

Pollen concentration	Correlation coefficient[a]	p value
TSS		
4,000 grains/m^3	0.659	<0.001
8,000 grains/m^3	0.678	<0.001
12,000 grains/m^3	0.757	<0.001
TNSS		
4,000 grains/m^3	0.631	<0.001
8,000 grains/m^3	0.685	<0.001
12,000 grains/m^3	0.749	<0.001
Amount of nasal discharge		
4,000 grains/m^3	0.538	<0.001
8,000 grains/m^3	0.408	<0.001
12,000 grains/m^3	0.589	<0.001
Frequency of sneezes		
4,000 grains/m^3	0.062	0.226
8,000 grains/m^3	0.261	<0.001
12,000 grains/m^3	0.112	0.032

There are correlations in the TSS, TNSS and the amount of nasal discharge, suggesting favorable reproducibility of symptoms at each pollen concentration.

[a] Spearman's rank-order correlation coefficient test.

There were no significant differences in the time to the occurrence of symptoms among the three other target pollen dispersal concentrations examined (4,000, 8,000 and 12,000 grains/m^3) either in the summer or the winter study ($p = 0.297$ and 0.390, respectively; Kruskal-Wallis test). For the same pollen dispersal concentration, there was also no significant difference in the time to symptom occurrence between the summer and the winter study (Kaplan-Meier estimates and log-rank test; fig. 2).

Symptoms Observed at the Various Pollen Dispersal Concentrations and Reproducibility of the Symptoms

At the target pollen dispersal concentration of 0 grains/m^3 (placebo), none of the participants developed any nasal or ocular symptoms. Therefore, no attempt was made to study this target pollen dispersal concentration in the winter study.

The severity of symptoms increased in the subjects as the target pollen dispersal concentration increased. The tendency during the winter study was similar to that during the summer study; at all the target pollen dispersal concentrations examined, the symptom scores began to increase almost immediately from 30 min after the start of exposure. The TSS reached a plateau 90–120 min after the start of the study (fig. 3a, b).

The time course of changes in the TNSS showed the same tendency as that of the TSS in both the summer and the winter studies (fig 3c, d). Changes in the TNNSS were slower compared to those of the nasal symptoms, and in most cases reached a plateau approximately 120 min after the start of pollen dispersal in the patients exposed to target concentrations of 8,000 and 12,000 grains/m^3, and about 150–165 min after the start of pollen dispersal in the patients exposed to the target concentration of 4,000 grains/m^3 (fig. 3e, f). We analyzed whether or not the symptom scores (TSS and TNSS) increased significantly compared with the baseline scores for each pollen dispersal concentration, and in the summer and winter studies, significant increases in the symptom scores (TSS and TNSS) were observed for all the pollen exposure concentrations examined from 30 min after the start of exposure (Wilcoxon signed-rank test: $p < 0.05$).

The total amount of nasal discharge determined in each subject every 60 min after the start of the pollen exposure increased in a time-dependent manner, reaching a plateau approximately 120 min after the patients had entered the Chamber (fig. 4a). The frequency of sneezing, which was measured at ~30-min intervals after the start of exposure, started to increase from 30 min after the start of exposure but remained mostly unchanged after 60 min (fig. 4b).

The correlation coefficients between the summer and the winter studies were high for the TSS, TNSS and the amount of nasal discharge at each pollen dispersal concentration examined. There were no significant differences in the TSS and TNSS between the summer and the winter studies at the pollen dispersal concentrations examined (table 3). The results suggested favorable reproducibility of symptoms at each of the pollen dispersal

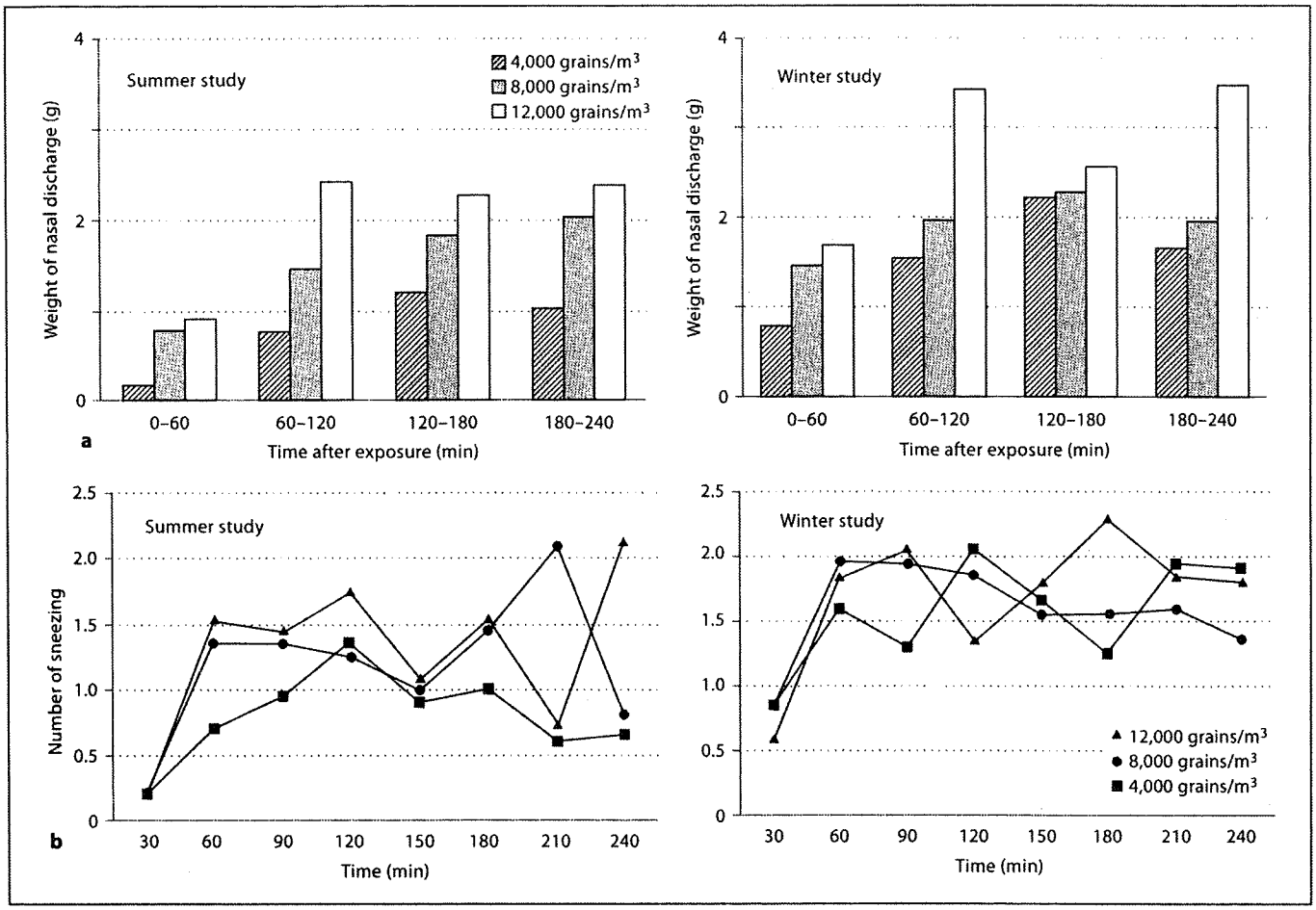

Fig. 4. a The amount of nasal discharge measured every 60 min at three different pollen dispersal concentrations in the summer and winter studies. For the same pollen dispersal concentration, the amount of nasal discharge did not significantly differ between the summer and winter studies, indicating that there were correlations in the results (Spearman's rank-order correlation coefficient test). **b** The frequency of sneezing recorded every 30 min at three different pollen dispersal concentrations did not significantly differ between the summer and winter studies.

concentration examined, whereas there was no correlation in the frequency of sneezing between the summer and winter studies.

Discussion

This study was designed to identify the validity of tests conducted in patients exposed to JC pollen in the OHIO Chamber. The results of the exposure tests conducted twice during non-pollen seasons in patients with JC pollinosis demonstrated that symptoms characteristic of pollinosis manifested consistently and that the severity of the symptoms for a given JC pollen grain dispersal concentration were reproducible.

The number of JC pollen grains dispersed annually has been reported to be in the range of 500–2,000 grains/m^3 (measured by a different type of laser counter, KH-3000, Ministry of the Environment, Japan) in the suburban districts of Tokyo, in which the number of JC pollen grains dispersed is usually large. These numbers are equivalent to the range of 3,000–12,000 determined by the KC-20 leaser counter used in the OHIO Chamber system [11]. Based on these findings, the pollen dispersal concentrations used in this study (maximum of 12,000 grains/m^3) are considered to be valid and not too high. Therefore, we selected four different pollen dispersal concentrations, namely 0, 4,000, 8,000 and 12,000 grains/m^3, aiming to assess the validity of the pollen dispersal concentrations. With regard to the duration of exposure,

the results of the validation study reported by Krug et al. [9] were used as reference values. They conducted a 4-hour exposure test and showed that the manifestation of the symptoms in their subjects reached a plateau after approximately 2 h of exposure. Based on these results, observation of the symptoms for at least 2 h was considered necessary, and a test exposure time of 4 h was adopted.

The pollen dispersal concentration in the OHIO Chamber was measured using a laser particle counter (KC-20), allowing real-time determination of the pollen dispersal concentration. The results of the pollen dispersal concentrations are transmitted instantaneously to the operator in the operating room outside the Chamber, allowing a constant pollen concentration to be maintained in the Chamber. In the present test, the difference between the actual numbers of pollen grains dispersed and the target pollen dispersal concentrations in the Chamber was within ~10% during both the summer and winter periods, which is acceptable. At the target pollen dispersal concentration of zero, however, 150–350 particles were detected in the Chamber, which were approximately 10 μm in size. Taking into consideration the approximately 30-μm size of JC pollen grains, these particles detected in the Chamber were not considered to be JC pollen, but rather particles brought into the Chamber when the subjects entered the Chamber. Krug et al. [9] showed similar results in their validation study, in which they used not only a laser particle counter, but also a system of two rotating rod samplers to count pollen numbers light-microscopically.

Chamber temperature and humidity could be kept constant within a range of ±0.1°C and ±1.0%, respectively, in both the summer and winter, being also satisfactory.

The volunteers were exposed to three distinct pollen dispersal concentrations during the summer and winter periods aiming to assess the reproducibility of the symptoms developing in response to pollen exposure in the OHIO Chamber. One patient with mild symptoms of JC pollinosis showed marked reaction only to the pollen dispersal concentration of 4,000 grains/m^3 in the winter study. This patient showed no or few symptoms at the higher pollen dispersal concentrations. One possible reason for this is that the low temperature of the outside air increased this patient's nasal hypersensitivity [15]. The other patients showed reproducible symptoms during the summer and winter studies.

The results of the present study indicate the reproducibility of the symptoms at all the pollen dispersal concentrations examined. Krug et al. [9] exposed the subjects to the same pollen dispersal concentration 5 times to observe the reproducibility of the symptoms. We conducted validation tests in different seasons in patients who were not informed of the pollen dispersal concentrations in order to avoid the influence of their experiences and recollections on the self-assessment.

Our assessment methods included TSS evaluation, which is a subjective method, as well as the determination of the amount of nasal discharge and the frequency of sneezing, which serve as objective indicators. Recent studies conducted in the exposure chambers in Austria [16] and Germany [17] have also mainly used TNSS to evaluate patient symptoms before and after treatment with antihistamines. Our TSS and TNSS levels were lower than those in reports on the efficacy of antihistamines for allergic rhinitis. One of the reasons for this finding could be that subjects with varying degrees of symptom severity were enrolled in our study, since the aim of this study was not to determine the efficacy of antiallergic drugs. In other words, there were 4 patients with very mild symptoms following pollen exposure in the natural environment. TNSS was as low as 1 even after exposure to extremely high pollen concentrations in these 4 patients. On the other hand, while examining the efficacy of antiallergic drugs using the OHIO Chamber in the future, it would be necessary to select the subjects who will show an increase in TNSS with increasing pollen exposure.

A correlation between allergic rhinitis and asthma has been established previously [18]. Due to their diameter (~ 30 μm), JC pollen grains are retained in the nasal cavity and not considered to invade the lower airways. Therefore, the development of asthma as a result of JC pollen inhalation is quite improbable. In the past 2 decades, while we have experienced many years with massive dispersal of JC pollen, there have been no or few reports on the occurrence of asthma during these massive JC pollen dispersals. Krug et al. [9] and Horak et al. [19] have demonstrated that there were no changes in the respiratory function of subjects in studies using pollen exposure chambers. In this study, none of the volunteers in the OHIO Chamber developed lower airway symptoms during the pollen exposure according to their subjective and objective symptoms.

In conclusion, we have shown the reproducibility of the severity of symptoms in the same patients in the OHIO Chamber during the summer and winter studies, both of which are non-JC pollen dispersal seasons. Reproducibility was recognized regardless of the severity of the symptoms in the subjects. Relatively stable symptoms occurred at concentrations of at least 4,000 grains/m^3 in the present study. In particular, exposure to 8,000 grains/

m^3 yielded relatively high TNSS. The symptoms reached a plateau approximately 90–120 min after the start of exposure, indicating that the optimum amount and duration of exposure for testing in the OHIO chamber are at least 8,000 grains/m^3 and 120 min, respectively. The results also demonstrated the reactivity and safety of several JC pollen exposure levels in the OHIO chamber.

This study also allowed the assessment of the efficacy of antiallergic drugs for JC pollinosis patients and the effects of medications administered before the JC pollen dispersal season suppressing the manifestation of symptoms. The present study indicates that the exposure chamber has the potential to contribute significantly to basic and clinical studies of JC pollinosis.

Acknowledgments

We wish to express our thanks to Prof. em. Minoru Okuda (Nippon Medical School) for his advice in the preparation of this article, Mr. Kazuhiro Habe (Tokyo Research Center of Clinical Pharmacology) for the construction of the OHIO Chamber, Mr. Kazufusa Ito (Tokyo Clinical Contract Research Organization) for analyzing the data and to Ms. Kyoko Matsuda and Ms. Satoko Shimizu for managing the tests during the summer and winter periods.

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*Allergology International. 2008;57:***-****
DOI: 10.2332/allergolint.O-07-514

ORIGINAL ARTICLE

A Randomized Double-Blind Comparative Study of Sublingual Immunotherapy for Cedar Pollinosis

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ABSTRACT

Background: Seasonal allergic rhinitis (SAR) induced by Japanese cedar pollen is a substantial problem in Japan. Sublingual immuno-therapy (SLIT) is safer than conventional antigen-specific immunotherapy, the only treatment modality by which complete cure of the disease can be expected. We investigated the safety and efficacy of SLIT in the treatment of cedar pollinosis patients compared to placebo.

Methods: A randomized, placebo-controlled, double-blind study was conducted in 61 cedar pollinosis patients. Increasing doses of standardized Japanese cedar extract or placebo were administered sublingually in intervals ranging from daily to once a week after six weeks. The primary efficacy variable was the mean of the daily total symptom scores (TSS) during the pollen dispersing period. Secondary efficacy variables included the QOL scores and related variables.

Results: Primary efficacy variable scores were significantly lower for some days in the SLIT group than in the placebo group ($P < .01$ or $P < .05$). Secondary efficacy for the QOL score in SLIT group was almost of half of placebo group. There was no significant difference in the overall incidence of side effects between the SLIT group and the placebo group.

Conclusions: SLIT was effective and safe in the treatment of cedar pollinosis.

KEY WORDS

Japanese cedar, placebo-controlled study, QOL, seasonal allergic rhinitis

INTRODUCTION

In agreement with the results of worldwide epidemiological assessments, the number of patients with allergic rhinitis such as Japanese cedar (JC) pollinosis in Japan is increasing.[1] Okuda considers that the current prevalence of allergic rhinitis is 16%, but many researchers predict that the rate will still increase.[2] Pollinosis is a typical type I allergy in which allergic conjunctivitis and allergic rhinitis develop. In spite of its refractory nature, pollinosis deteriorates patient QOL only in severe cases; however, it greatly affects the patient's life in general in that they must keep working even if the condition is severe.[3] Many of the patients with cedar pollinosis have also been sensitized to cypress pollen which disperses after cedar pollen. Consequently, symptoms of cedar pollinosis are followed by those of cypress pollinosis; patient symptoms last, though they are seasonal, for as long as 4 months (from February to May).

Pharmacological therapy prescribed by general practitioners is common for the treatment of the disease. Both oral medications and topical medication, however, are symptomatic treatment; they do not cure the disease or remain effective until the following year.[4] Antigen-specific subcutaneous immuno-

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Received 3 September 2007. Accepted for publication 4 February 2008.

therapy (SCIT) is the only treatment modality by which complete cure of the disease can be expected.[5] WHO position paper stipulates the use of standardized antigen and the concentration of the antigen to be maintained.[6] The efficacy of the therapy has been proven in placebo-controlled, double-blind comparative studies using pollen, house dust mite, and animal protein.[6,7] In Japan, it is customary to start the administration of causative antigen extract by subcutaneous injection at the threshold of skin reaction or its 10-fold diluted concentration, and to increase the dose gradually.[4] Treatment with SCIT requires special attention because it may cause, as a side effect, anaphylactic shock, which prevents the therapy from becoming popular in Japan.[8] In order to reduce the possibility of this side effect, immunotherapy is administered by other routes (sublingual, intranasal, oral, and transbronchial) in Europe and the United States, and has achieved desired outcomes.[9-11] Especially, sublingual immunotherapy (SLIT) has become popular in Europe considerably, and there are many reports supporting the effectiveness of the therapy.[11-13] As for side effects due to SLIT, there are no reports of anaphylactic shock, but oral itching, skin reaction (such as urticaria), and mild asthma-like attacks have been reported.[13] Since cedar pollinosis greatly deteriorates patient QOL, many physicians and patients will opt for immunotherapy if it is proven to be safe. We conducted a randomized, placebo-controlled double-blind comparative study to investigate whether SLIT reported in Europe and the United States is effective for the treatment of JC pollinosis and whether it can be performed safely.

STUDY DESIGN

This multi-centre, double-blind, randomized, placebo-controlled, parallel-group study was conducted in six centers across Japan between October 2004 and April 2005. The study protocol was approved by the appropriate local ethics committees, and the study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided written informed consent prior to participation.

SUBJECTS

Patients with JC pollinosis were enrolled in this study if they had a RAST score of 2 against JC or above and pollinosis symptoms during the cedar pollen dispersal period at least in the past 2 years and if they had visited any of the following medical institutions: Department of Otorhinolaryngology, Nippon Medical School; Department of Otorhinolaryngology, University of Fukui; Department of Otorhinolaryngology, Head and Neck Surgery, Okayama University; Department of Otorhinolaryngology, Dokkyo University School of Medicine; Department of Otorhinolaryngology, University of Yamanashi; and Department of Otorhinolaryngology, Head and Neck Surgery, Chiba University. Patients who had nose diseases (perennial allergic rhinitis, nasal septum deviation, or sinusitis) which may interfere with accurate symptom assessment were excluded from the study. Patients receiving treatment for conditions such as severe cardiac disease and malignant tumor were also excluded. As a result, a total of 61 patients were blindly randomized either to the active group or the placebo group in the ratio of 2 active to 1 placebo.

METHODS

The study was initiated in October, 2004. Patients were assigned and randomized to either the active group or the placebo group. Cedar antigen extract (active group) at concentrations of 2 to 2000 JAU/ml diluted with diluent (made by Torii Pharmaceutical Co., Ltd.) and diluent alone (placebo group) were used in eye drop containers (made by Hirakata Plastic).

Administration of the antigen extract was started at 2 JAU/ml, which is considered a sufficiently safe level, and was increased to the final maintenance concentration of 2000 JAU/ml. Active drug was administered as follows: 1 drop (about 50 μl) to 20 drops (about 1 ml) of prepared extract was dropped onto bits of bread (about 1.5 cm × 1.5 cm × 1.5 cm), which were held sublingually for 2 minutes and then expectorated. The treatment schedule was as follows: antigen extract was administered sublingually daily from Week 1 to Week 4; 20 drops of the antigen extract 2000 JAU/ml were administered two days per week in Week 5, once per week in Week 6 and thereafter throughout the season (Table 1).

Patients experiencing pollinosis symptoms in cedar and cypress pollen dispersal periods received symptomatic treatment with medications such as antihistamines on an as needed basis; such patients were asked to record the date of treatment in their allergy diary.

ENDPOINTS

The patients were instructed to fill in their allergy diary from February 22, 2005 to April 6, 2005, the period when cedar and cypress pollen dispersed in 2005, and they were also asked to fill in QOL questionnaire once a month during the same period. Symptoms recorded in the allergy diary (sneezing, runny nose, nasal congestion, and interference with daily life), the total nasal symptom scores calculated based on each symptom, sneezing, runny nose, nasal congestion (none; 0, mild; 1, moderate; 2, severe; 3), and symptom medication scores (antihistamine; 1, topical steroid; 2, general steroid; 3) were calculated. The Japanese Allergic Rhinitis QOL Standard Questionnaire No.1 (JRQLQ No1) was used for the assessment of the QOL of patients with allergic rhinitis (Fig. 1). Nasal and Ocular symptom scores, QOL-

Table 1 Allergen administration schedule (Increasing dosing)

	Week 1 (2 JAU/ml)	Week 2 (20 JAU/ml)	Week 3 (200 JAU/ml)	Week 4 (2000 JAU/ml)	Week 5 (2000 JAU/ml)
Day 1	1 drop	1 drop	1 drop	1 drop	20 drops
Day 2	2 drops	2 drops	2 drops	2 drops	
Day 3	3 drops	3 drops	3 drops	4 drops	
Day 4	4 drops	4 drops	4 drops	8 drops	
Day 5	6 drops	6 drops	6 drops	12 drops	20 drops
Day 6	8 drops	8 drops	8 drops	18 drops	
Day 7	10 drops	10 drops	10 drops	20 drops	

Initial dose of SLIT for JC pollinosis was 1 drop of 2 JAU/ml of standardized JC allergen, and the administrating dose is increased up to 20 drops of 2000 JAU/ml at 4th week, the maintenance dose.

Japanese Rhino-conjunctivitis Quality of Life Questionnaire (JRQLQ No.1)

To patients with allergic rhinitis (including pollinosis)
These days, the aim of medical treatment is not just to cure disease but also to give patients a better quality of life. The purpose of this survey is to determine to what extent your rhinitis interferes with your life and whether it would be improved by treatment. As with all medical treatment, the information you provide in this survey will remain strictly confidential.

You may find some of the following questions difficult to answer, but just answer to the best of your ability.

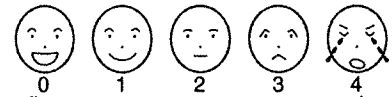
/ Tick the box that best describes the severity of the worst nasal and eye symptoms you have experienced in the past 1–2weeks.

Nasal and eye symptoms	0. No	1. Mild	2. Moderate	3. Severe	4. Very severe
Runny nose	☐	☐	☐	☐	☐
Sneezing	☐	☐	☐	☐	☐
Blocked nose (nasal congestion)	☐	☐	☐	☐	☐
Itchy nose	☐	☐	☐	☐	☐
Itchy eyes	☐	☐	☐	☐	☐
Watery eyes	☐	☐	☐	☐	☐

// Tick the box that best describes the worst extent to which the symptoms in / above have interfered with your quality of life in the past 1–2 weeks. If any of the items listed under Quality of life below definitely do not relate to the symptoms in / (nose, eyes), then there is no need to tick a box for that particular item.

Quality of life	0. No	1.Yes, slightly	2.Yes, moderately	3.Yes, severe	4.Yes, very severe
1. Reduced at work/home	☐	☐	☐	☐	☐
2. Poor moral concentration	☐	☐	☐	☐	☐
3. Reduced thinking power	☐	☐	☐	☐	☐
4. Impaired reading book/newspaper	☐	☐	☐	☐	☐
5. Reduced memory loss	☐	☐	☐	☐	☐
6. Limitation of out of life (e.g. sport, picnics)	☐	☐	☐	☐	☐
7. Limitation going out	☐	☐	☐	☐	☐
8. Hesitation friends or relatives	☐	☐	☐	☐	☐
9. Reduced contact with friends or others by telephone or conversation	☐	☐	☐	☐	☐
10. Not an easy to be around	☐	☐	☐	☐	☐
11. Impaired sleeping	☐	☐	☐	☐	☐
12. Tiredness	☐	☐	☐	☐	☐
13. Fatigue	☐	☐	☐	☐	☐
14. Frustration	☐	☐	☐	☐	☐
15. Irritability	☐	☐	☐	☐	☐
16. Depression	☐	☐	☐	☐	☐
17. Unhappiness	☐	☐	☐	☐	☐

/// Please circle the number of the face that best describes your general state (including your symptoms, life and emotion) in the past 1–2 weeks.



Do not fill out the following

To be completed by physician

Patient's name: Medical record to: Age: yr Sex: M F

Name of medical institution: Physician's name: Date:

Diagnosis:
SAR (Antigen) Treatment [prevention,drug,immunology,therapy,operation]
PAR (Antigen) Treatment [prevention,drug,immunology,therapy,operation]
Non-Allergy: Disease: ( ) Treatment: ( )

QOL score: None 0 , Mild 1 , Moderate 2 , Severe 3 , Very severe 4
Total QOL score
Score by QOL category 1–5 points daily life 6–7 points out-door
8–10 points social 11 points sleep
12–13 points body 14–17 points psyco-life
Please write the names of drugs used it possible

Score: None: 0 points Mild: 1 point Moderate: 2 points
Severe: 3 points Very Severe: 4 points

Fig. 1 Japanese Allergic Rhinitis QOL Standard Questionnaire No.1 (JRQLQ No1).

related questionnaire scores, and the overall face scale were calculated and statistically analyzed. In other words, the QOL deterioration score was calculated by subtracting QOL-related questionnaire scores recorded in February (i.e. at baseline) from the scores recorded in the middle of March to April, when the largest amount of pollen dispersal was observed.

STATISTICAL ANALYSIS

Symptom scores, total symptom scores, and symptom medication scores calculated from the allergy diary in the placebo group and the active group were analyzed by non-paired t-test and the Chi-squared test

Table 2 The background of the subjects

Items	Placebo $n = 22$	Active $n = 37$	p value (*)
Age	40.14±15.30	40.65±15.14	0.901
Sex			
Male	7 (31.8%)	18 (48.6%)	
Female	15 (68.2%)	19 (51.4%)	
Nasal and eye symptoms	0.62±0.54	0.43±0.35	0.169
QOL-related questionnaire	0.25±0.29	0.21±0.25	0.568
Usual daily activities	0.13±0.25	0.11±0.32	0.762
Outdoor activities	0.14±0.48	0.21±0.46	0.630
Social functioning	0.05±0.22	0.08±0.23	0.648
Sleep disturbance	0.14±0.36	0.09±0.29	0.537
Physical problems	0.17±0.33	0.24±0.46	0.510
Emotional function	0.12±0.23	0.11±0.32	0.953
Overall face scale	1.14±0.73	1.09±0.74	0.780

Fig. 2 The changing the number of cedar and cypress pollen dispersals in 2005.

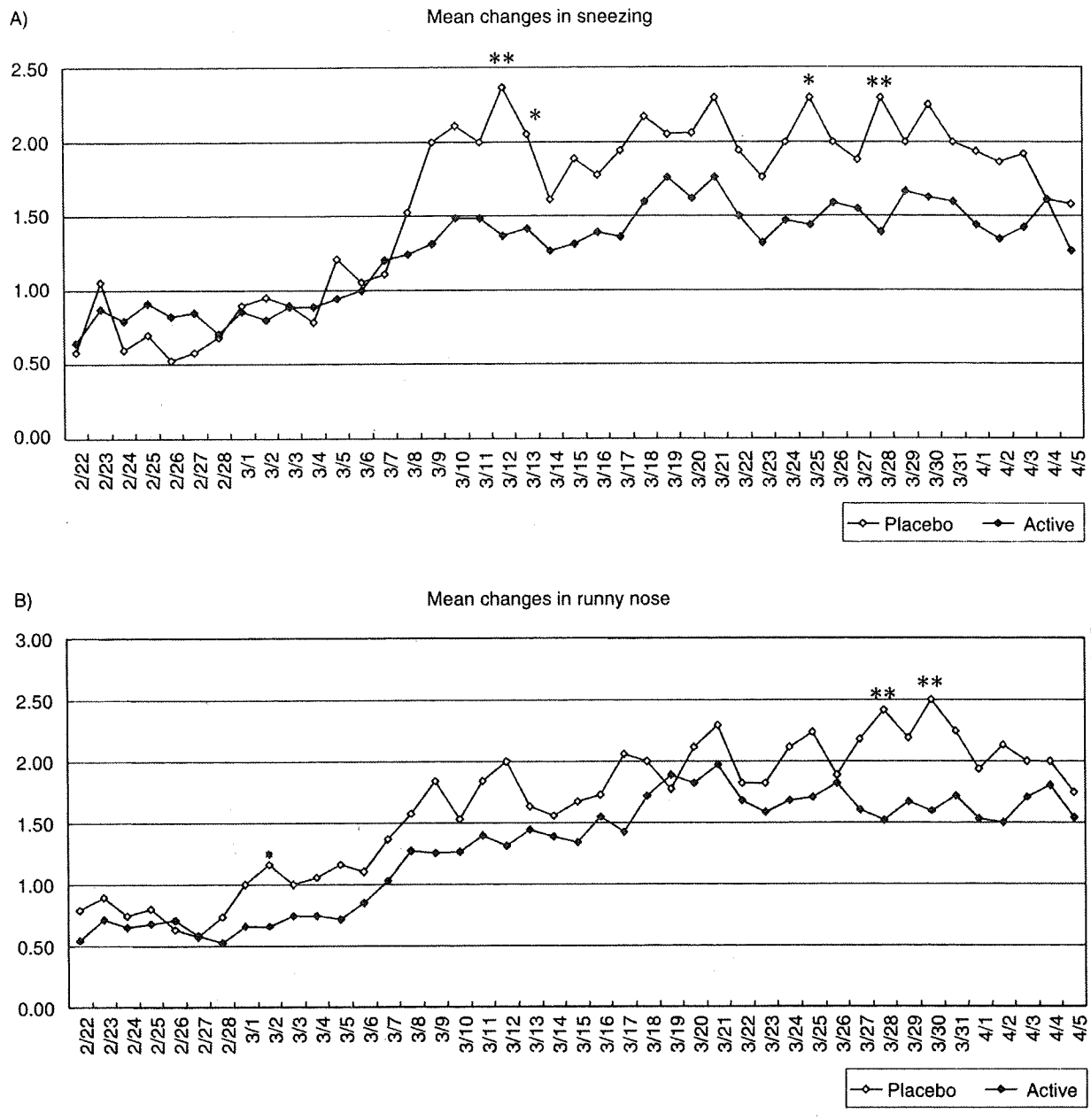
using SPSS 11.0J. QOL-related questionnaire score of the 2 groups were compared using analysis of covariance (ANCOVA).

RESULTS

Of the 61 randomized patients, there were 2 dropouts for those whose treatment was unknown; there were 37 patients in the active group and 22 patients in the placebo group. In the analysis of allergic symptoms, 2 patients whose outcome was available only in the form of a diary were excluded, and the results of 36 patients in the active group and 21 patients in the placebo group were analyzed. In the analysis of QOL, 3 patients were excluded because baseline assessment was unavailable, and the results of 35 patients in the active group and 21 patients in the placebo group were analyzed.

As shown in Table 2, no difference was observed between the two groups in terms of patient characteristics (sex was analyzed by the Chi-squared test, and other items were analyzed by t-test).

In 2005, the number of cedar and cypress pollen dispersals observed was the largest during the 10-year period since 1995. According to the data of the Chiyoda ward—the area nearest to the Nippon Medical School—announced by the Tokyo Metropolitan Government, the first pollen dispersal was observed on February 22, which was about the same time as in the past years, and an average of 10,625 pollens per square centimeter by the Durham method were ob-

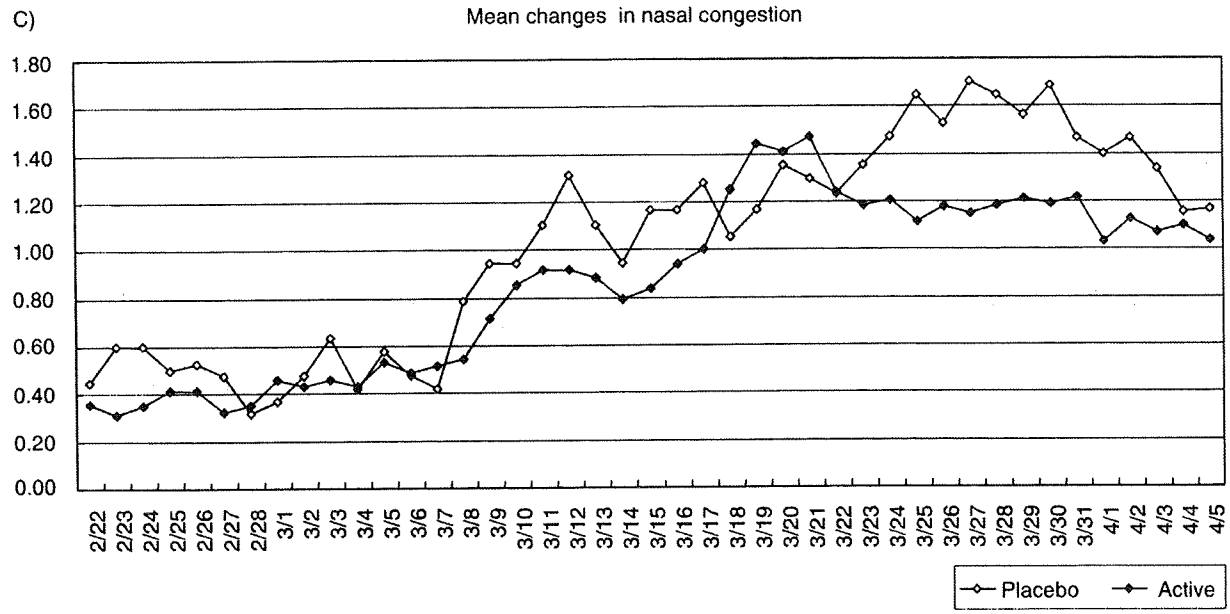


served during the season (Fig. 2). The number of the cedar and cypress pollens by the same method observed in each institution was 3424, 2383, 16002, 5859 and 7752 for University of Fukui, Okayama University, Dokkyo University, University of Yamanashi and Chiba University respectively, and these pollen numbers were also largest dispersing during the last ten years at any place.

Symptom scores for sneezing (Fig. 3A) and runny nose (Fig. 3B) in the active group were significantly better than those in the placebo group on 4 days and 2 days, respectively, but no difference was observed between the active group and the placebo group in terms of nasal congestion (Fig. 3C). Between the 2 groups, there was no difference in the number of medications used during the season (Fig. 3D).

The active group had a significantly lower total symptom score (Fig. 4A) and symptom medication score (Fig. 4B) on 4 days during the season. Overall, better outcomes were observed in the active group during the latter half of the season (i.e. from the end of March to the beginning of April), which roughly overlaps the period when the largest amount of cedar and cypress pollen was dispersed.

In the placebo group, the nasal and ocular symptom score was 1.15, the QOL-related questionnaire score was 1.10, and the overall face scale score was 1.24; in the active group, the nasal and ocular symptom score was 0.92, the QOL-related questionnaire score was 0.58, and the overall face scale score was 1.03: the deterioration score in the QOL-related questionnaire in the active group was only about half the score in the placebo group (Fig. 5A). In each domain of QOL question items, deterioration in usual daily activities, outdoor activities, social functioning, sleep problems, general physical problems, and emotional function in the active group was only about half the score in the placebo group as well. The p-values for the above domains were 0.089, 0.086, 0.067, 0.060, 0.083 and 0.046; a significant difference was observed



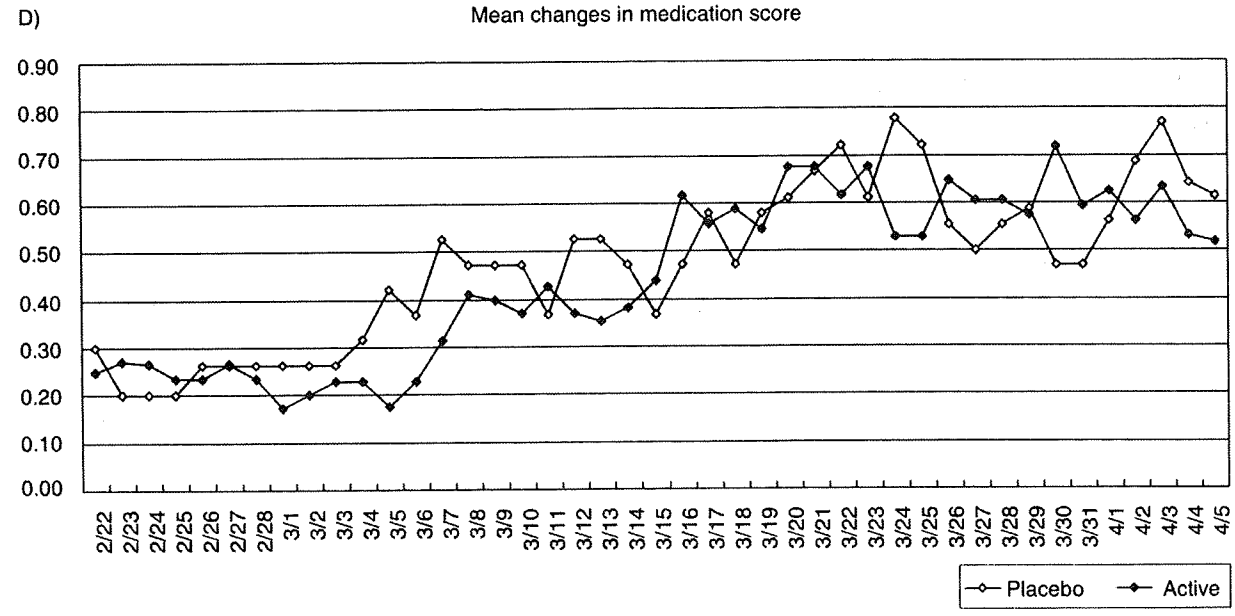


Fig. 3 The mean changes in each symptom in the season of 2005. **A)** Mean changes in sneezing. **B)** Mean changes in runny nose. **C)** Mean changes in nasal congestion. **D)** Mean changes in medication score. The open square indicates the placebo group, the filled square indicates the active group. Significant difference was evaluated as * $p < 0.05$; ** $p < 0.01$.

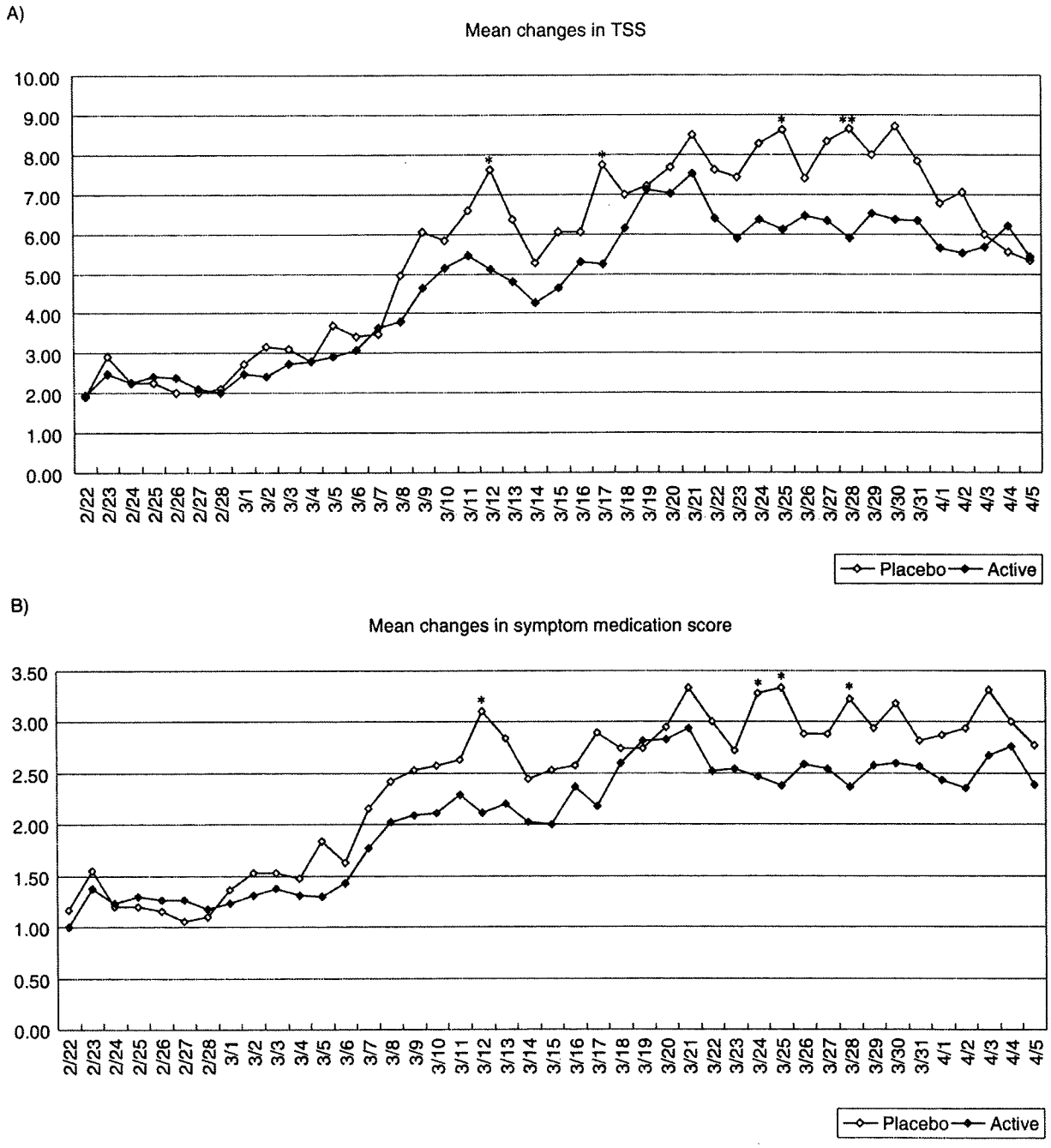


Fig. 4 The mean changes in **A)** the total symptom score (TSS) and **B)** symptom medication score in the season of 2005. Significant difference was evaluated as * $p < 0.05$; ** $p < 0.01$.

only in emotional function (Fig. 5B).

SIDE EFFECTS

No systemic side effect occurred during SLIT. Local side effects occurred in six volunteers in the active group. Mild mouth itching was exhibited in all six volunteers in increasing dose up to 2000 JAU 1 ml, however this itching was diminished for two or three times just after allergen administration. All six volunteers finished this study totally without any change of this protocol.

DISCUSSION

Approximately 16% of the Japanese population are af-

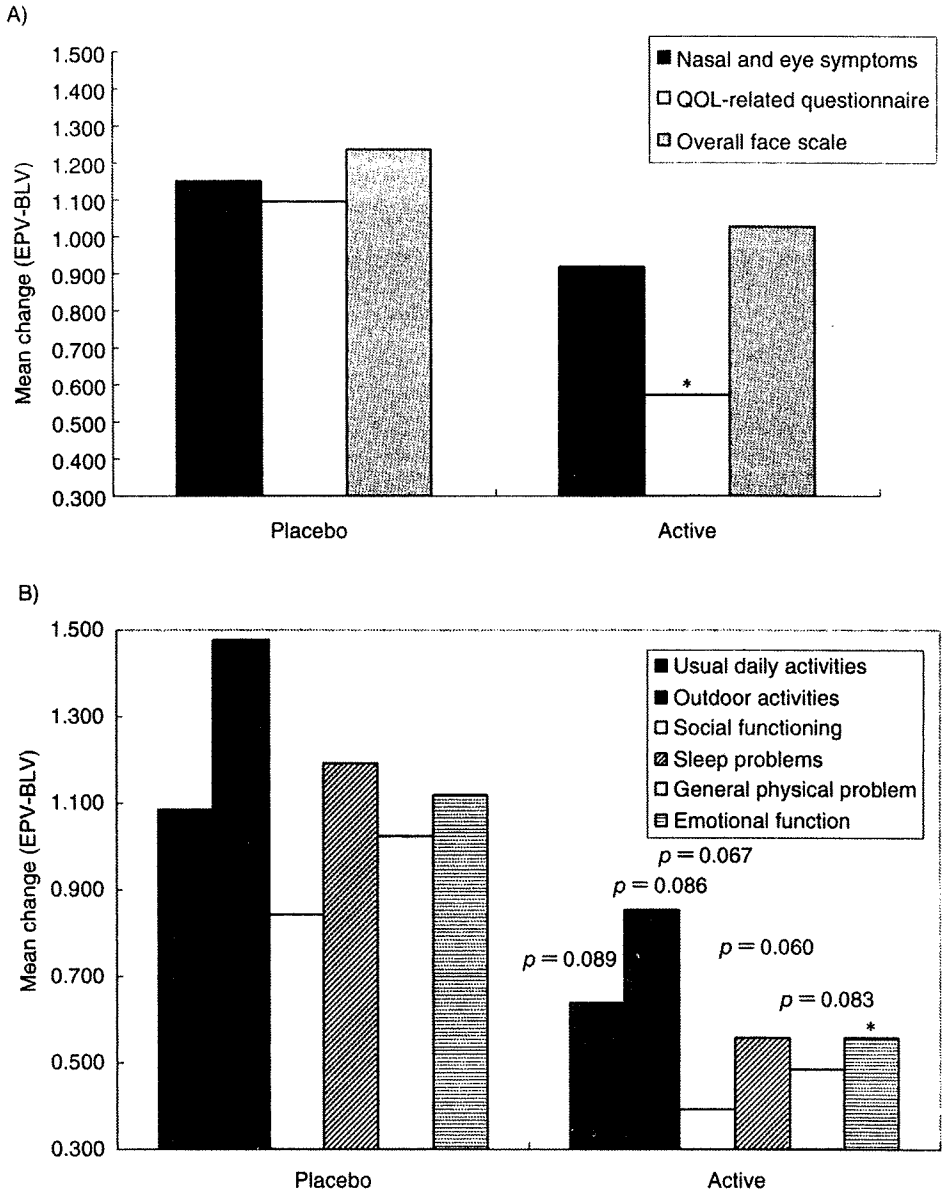


Fig. 5 The mean changes of **A)** QOL scores (nasal and eye symptoms, QOL related questionnaire, overall face scale) and **B)** each domain of QOL question items deterioration (in usual daily activities, outdoor activities, social functioning, sleep problems, general physical problems, and emotional function) from baseline data of February to peak data of peak pollen scattering period. Difference between placebo and active indicates * $p < 0.05$ (analysis of covariance, ANCOVA). Placebo: $n = 21$, Active: $n = 35$.

fected by Japanese cedar pollinosis[2] and the proportion of severe status patients is higher than with grass or ragweed pollinosis, which are the representative conditions in other countries, and the symptoms persist for about 3 months, becoming a social issue. When the amount of pollen increases, patients show more severe symptoms, and the number of severe status patients is greatest in mid-March (late season) when the pollen count reaches its peak. Substantial antigen exposure enhances the antigen-antibody reaction in the airways (airway hypersensitivity), which is the mechanism involved in severe pollinosis, and SCIT may control the exacerbation of the symptoms in the latter half of the cedar pollen season by inhibiting antigen-related enhancement of nasal mucosal hypersensitivity.

As shown in the WHO position paper, the effects of immunotherapy in the treatment of pollinosis have been substantiated in many double-blind comparative studies.[6] However, the therapy tends to be avoided in Japan because of factors such as the current high cost, the complicated procedure involved, and possible side effects. In Japan, owing to these disadvantages and the fact that the department of allergy has not been widely established in medical institutions, pharmacological therapy is the mainstream modality for the treatment of pollinosis. Still, immunotherapy is an important modality for the complete cure of allergic diseases.

The efficacy of our SLIT was not demonstrated based on patient allergy diaries. However, the quality of life (QOL) score was approximately 1/2 of that in the placebo group, with a significant difference. In addition, a *P*-value corresponding to a significant difference was obtained in each QOL domain. In the mental health domain, there was a significant difference. Assessment using the Japanese guidelines differs from that in other countries; even a single sneeze is regarded as (+). In other countries, 4 grades (none, mild, moderate, and severe) are employed for assessment, and the presence or absence of symptoms is not evaluated. For this reason, the usefulness of SLIT may not have been demonstrated based on diaries. However, the QOL is evaluated via self-assessment, which is consistent with the system for the self-reporting of symptoms in other countries (none to severe). Therefore, QOL assessment of SLIT was favorable, and was consistent with the reduction rates in other countries. According to the JRQLQ criteria, the reduction rate for nasal/ocular symptoms was 22%, consistent with the evaluation of SLIT in other countries. In the future, the JRQLQ criteria, which were designed in reference to overseas self-assessment, may be essential for evaluating drug efficacy and such a novel treatment. This finding is suggestive of the fact that the QOL questionnaire developed in Japan is of good quality,[14] and that SLIT is effective for preventing QOL deterioration in patients with pollinosis rather than for lowering their symptom score. Placebo effects of SLIT may be present. However, it was evaluated in 2005, when the amount of scattered pollen was highest over the past 10 years. In addition, considering that the study involved a placebo-controlled design, we can conclude that SLIT was effective for cedar pollinosis in Japan. In evaluating the treatment response, we cannot rule out the influence of Japanese cypress pollen scattering. However, in a study excluding Japanese cypress pollen-positive reacting patients, the efficacy of SLIT and reduction rate for symptoms were also similar (unpublished data). This maybe caused by the combination of a large amount of JC and a small amount of cypress that was dispersed in 2005. These types of pollinosis should be regarded as JC/Japanese cypress pollinosis, as their seasons are sequential in the near future. In addition, a Japanese cypress pollen antigen for immunotherapy must be prepared. It should be considered that symptoms of cedar/Japanese cypress pollinosis in April are associated with cedar pollen scattering-related nasal mucosal/conjunctival inflammation, not with Japanese cypress pollen scattering alone.

Less side effects including problematic anaphylaxis are noted in SLIT although the side effects observed cannot be theoretically complete anaphylactic shock when comparing the therapy administered via injection with sublingual route.[15] Similar to the oral allergy syndrome (OAS), which is the focus of public attention, the development of symptoms such as strange feelings, oral itching, and swelling were feared because the antigen remains in the oral cavity; however, itching was the only reaction observed so far. The results obtained from the study of tentative SLIT, which was performed exclusively in the Department of Otorhinolaryngology, Nippon Medical School, were roughly consistent with the results of similar studies conducted every year thereafter, including the results of the study in 2005,[16] In our study of SLIT for the treatment of cedar pollinosis, symptom medication score was consistently lower than that of the pharmacological therapy group throughout the pollen dispersal season. The finding indicates that patients receiving SLIT tend to use fewer drugs, which is consistent with the results of a double-blind comparative study using a placebo,[17] SLIT, which is as effective as pharmacological therapy and decreases the amount of drug use, is considered advantageous also in the current medical economy in Japan.

The mechanism of action for SLIT, or for conventional SCIT, is still unclear, but for SCIT, reduction of effector cells[18,19] and blocking antibody[20-23] have been the conventional theories. Recently, however, it has become widely accepted that immunotherapy may modify the T cell response to natural allergen because of T cell anergy and/or immune deviation.[24-27] For SLIT in particular, allergen administered to the oral mucosa accumulates in the submandibular lymph node, in which the immune response occurs[28] and peaks at approximately 2 hours after administration.[29] In our investigation, an increase in the Stimulatory Index in PBMC during the early phase of SLIT conducted in 1999 shows at least that systemic immune induction was caused by sublingually administered antigen.[30] In SLIT, it is intended to cause fewer side effects than SCIT injection by decreasing systemic effects. However, it has become clear that the therapy also leads to systemic immune induction, which is greatly different from conventional topical immunotherapy administered intranasally or orally.

In the present study, SLIT both inhibited the exacerbation of symptoms in the latter half of the season