

Preliminary Study on Japanese Cedar Pollinosis in an Artificial Exposure Chamber (OHIO Chamber)

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ABSTRACT

Background: A pollen exposure chamber (OHIO Chamber) was built in central Tokyo, Japan, in order to study seasonal allergic rhinitis (SAR). Since satisfactory outcomes were obtained from the controlled pollen exposure at the chamber, we conducted preliminary studies in volunteers with SAR.

Methods: Ten volunteers with SAR sensitive to Japanese cedar (JC) pollen were enrolled in this study. In order to investigate the intranasal and intraocular pollen number, volunteers were initially exposed to a low concentration of JC (2500 grains/m³) for at most 1 hour in this chamber. Before and after the exposure, nasal cavities and eyes were washed with 100 ml and 25 ml of saline, respectively. Nasal and eye washing solutions were collected and the number of JC pollen was counted.

After 3 hours the volunteers were subsequently exposed to a moderate concentration of JC (4500 grains/m³) for 2 hours. Subjective nasal and ocular symptoms were recorded and the amount of nasal secretion was measured during the allergen exposure periods.

Results: During the initial exposure, all volunteers except one stayed in the chamber for 1 hour without any nasal or ocular symptoms. The number of pollen in the nose and eyes was 249.2 ± 120.9 and 13.6 ± 13.6 grains, respectively.

During the subsequent 2-hour exposure to JC pollen, nasal and ocular symptoms developed gradually in a time dependent manner in all the volunteers except one.

Conclusions: This is the first clinical study using Japanese cedar pollen under well-controlled conditions in the OHIO chamber in which the induction of allergic symptoms was observed. The OHIO chamber will be useful for studying allergic rhinitis in Japan.

KEY WORDS

allergen exposure, allergy symptoms, artificial exposure chamber, intranasal and intraocular pollen count, Japanese cedar pollen

INTRODUCTION

Japanese cedar (JC) pollinosis is a seasonal allergy, which is unique to Japan, and the causative allergen (JC pollen) is dispersed usually between February and April although the pollen count varies every year. Over 16% of the Japanese population suffer from the allergy during this season¹ and an incredible amount of anti-allergy agent is used, resulting in an under-

mined QOL² and decline in labor productivity.³ This fact indicates that JC pollinosis is a social and economical problem which cannot be ignored.

Allergen exposure tests in natural environments have been conducted as fundamental research on this kind of allergy and also to examine the effectiveness of anti-allergy agents.^{4,5} Studies of this sort, however, are greatly influenced by natural factors such as the amount of pollen, the weather, the temperature, or

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the wind velocity. The biggest problem is that the tests can be done only during the pollen season.

Several allergen exposure units have been built in Europe and the US to evaluate the efficacy of drugs and they are operating safely and effectively.⁶⁻⁸ A pre-defined amount of pollen can be dispersed under stable conditions in these chambers.

Despite the fact that so many Japanese are allergic to JC pollen, this kind of chamber was not available in Japan before 2004. To meet the increased needs for such facilities, the first pollen exposure units in Japan were developed in Wakayama⁹ in the first half of 2005. We built the third allergen exposure chamber, an artificial exposure chamber (AEC; OHIO Chamber), in the center of Tokyo in September, 2005. This chamber not only keeps the temperature and humidity constant, but also automatically cleans and dries the inside of the chamber.

Due to the lack of AECs in Japan, little fundamental data concerning Japanese cedar pollinosis were available up to this point. Since satisfactory outcomes were obtained from the controlled exposure at the OHIO Chamber, we were able to conduct preliminary studies on mildly symptomatic patients, in which we examined the amount of intranasal and intraocular pollen grains and the development of symptoms.

METHODS

SUBJECTS

The subjects of our study were mildly symptomatic adult patients. The inclusion criteria were as follows:

- Subjects must have at least a 2-year history of allergic symptoms during the pollen season, such as sneezing, nasal discharge, nasal obstruction, and itchy eyes.

- Subjects must also have had blood tests within 1.5 years showing positive RAST scores ($\geq$ Class 2) for JC pollen and negative RAST scores ($\leq$ Class 1) for house dust mite.

The exclusion criteria were as follows:

- Subjects with nasal obstruction attributable to a polyp or deformity of the nose.

- Subjects with acute upper respiratory infection(s) such as acute sinusitis, acute pharyngitis, and acute upper respiratory inflammation.

- Subjects with uncontrolled asthma, diabetes, high blood pressure, or eye diseases (glaucoma/cataract).

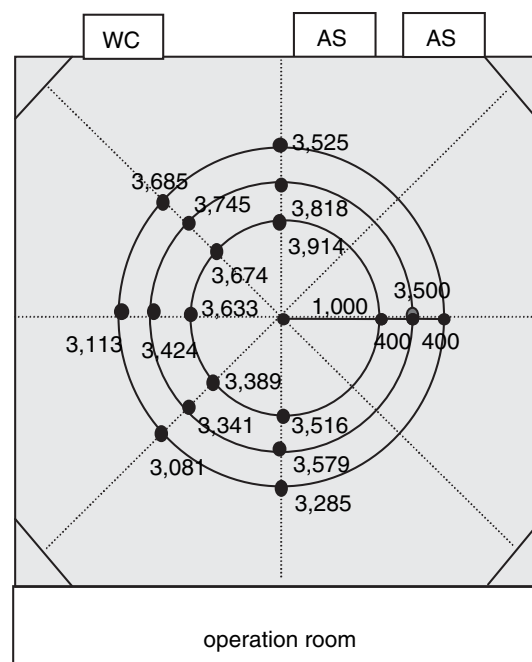
- Subjects who used anti-allergy agent(s) within a week before the start of this study.

- Pregnant women or women who trying to become pregnant.

The study was conducted in accordance with GCP Guidelines and the Declaration of Helsinki. The study was conducted on December 23 (2 months ahead of the start of the pollen season) after having been reviewed and approved by the ethics committee of Shinanozaka Clinic. Informed consent was obtained from



Fig. 1 Appearance of the artificial exposure chamber (OHIO Chamber). Pollen-diffuser in the center (arrow). Blower module in the corner of the room (arrow head)



Target concentration: 3500 grains/m³

WC: toilet

AS: air shower

Fig. 2 Spatial concentration distributions of pollen in the OHIO Chamber at a height of 1.15 m at the target concentration of 3500 grains/m³. The average concentration of pollen was 3500 $\pm$ 419 grains/m³.

all the subjects prior to study entry.

OHIO CHAMBER

The OHIO Chamber was installed in Samoncho Clinic in Yotsuya, the center of Tokyo. Its square measure is 25 m², the height 2.5 m, and the capacity 10 subjects (Fig. 1).

Compressed air transfers pollen grains from an outside dust feeder into the operation room where it is mixed with conditioned air inside the pollen diffuser after which the mixed air jets out upward from the diffuser. The diffuser and the blower modules in

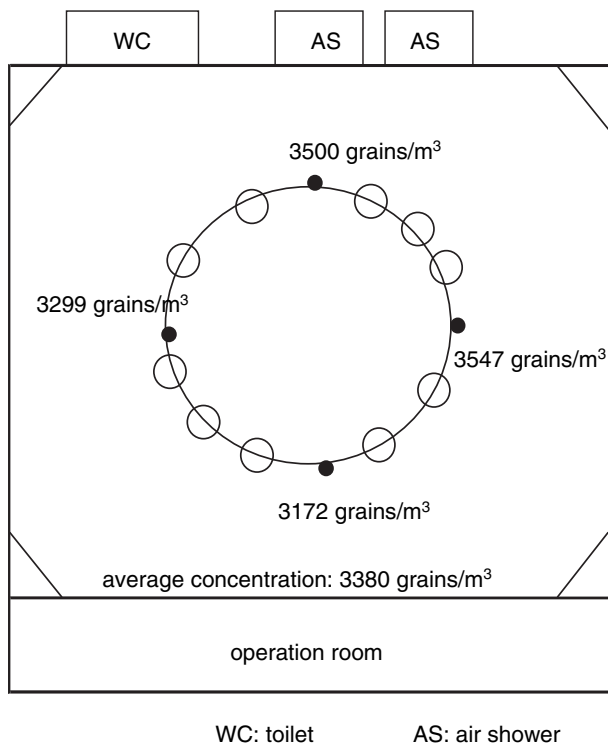


Fig. 3 The pollen concentration at a height of 1.15 m at the four points (●) when the ten volunteers (○) were present in the OHIO chamber. The average concentration was 3380 grains/m³ and uniform distribution of pollen was achieved.

four corners help the pollen grains get distributed evenly. The concentration of pollen grains in the room is measured by a particle counter equipped with a semiconductor laser (KC-20; Rion Co, Tokyo, Japan). This counter can absorb up to 30 L of air per minute and detects particles sized 10–100 μ m.

Spatial distributions of pollen in the unmanned and manned chamber at a height of 1.15 m were measured. The pollen concentration distribution was within a range of $\pm 12\%$ of the target value of 3500 grains/m³ in the unmanned chamber (Fig. 2). The pollen number at each of four points and the average pollen concentration are shown in Figure 3 when 10 subjects were present in the chamber.

The pollen count in this room is stable between 2500 and 120000 grains/m³ (data not shown). Also, the temperature inside the chamber was set at $22 \pm 0.2^\circ\text{C}$ and the humidity was set about 20–55%. The pollen count, temperature, and humidity were monitored every 3 minutes and recorded accordingly.

Before entering the room, subjects were instructed to wear protection gowns so that the pollen grains were not attached to their hair or clothes. When entering or exiting, they also went through an air shower so as to avoid pollen grain contamination outside of the room. During the test, subjects were as-

Table 1 Backgrounds of the subjects.

Subject No.	Sex	Age	RAST score
1	female	37	3
2	female	35	5
3	male	34	4
4	male	45	3
5	male	36	2
6	female	34	4
7	male	41	2
8	female	32	2
9	female	43	3
10	male	48	2

Five men and five women with mild symptoms were enrolled in this study.

This table shows their age and antibody titers (RAST scores) against cedar pollen.

signed to sit on chairs in designated areas of the chamber and in order to ensure the subjects' safety, their behavior was observed closely by staff and physicians situated in the operating room.

STUDY DESIGN

This study consisted of two parts. In the first part, subjects were exposed to low-concentrated JC pollen (2500 grains/m³) in the chamber for up to an hour. They were allowed to exit the room if allergic symptoms developed (either in the nose or in the eyes). Immediately after exiting the room, all the subjects went through an intranasal and intraocular irrigation process using a syringe (Nasaline®; Entpro, Sweden), washing the nasal cavities with 100 ml of saline and the eyes with 25 ml of saline. Then the washing solution from each subject was collected to investigate the number of pollen grains in it.

The subjects were subsequently exposed to moderate-concentrated JC pollen (4500 grains/m³) for 2 hours. Each subject recorded their symptoms (such as sneezing, nasal discharge, nasal obstruction, itchy nose, itchy eyes, and tears) into computers at certain points (after 15, 30, 60, 90, and 120 minutes from the start of exposure).

The symptoms were classified as follows: 1. none, 2. mild, 3. moderate, 4. severe, and 5. very severe. We collected tissue papers with which subjects had blown their noses and measured them by weight. The weight difference between tissue papers before and after use was considered to be the weight of nasal secretion.

The amount of intranasal and intraocular pollen was determined by the following methods. We added 1.25 g of Safranin-O (Wako Jyunyaku Kogyo Inc. Tokyo) to 50 ml of saline and 50 ml of ethanol to create stain solutions, and 5 ml of this stain solution was added to the nasal or ocular lavage fluid. Furthermore, we added 20 drops of Proteinase K (Dako Cy-

Table 2 The number of intraocular and intranasal pollen grains in the first part of the study.

Subject No.#	1	2	3 *	4	5	6	7	8	9	10	Mean $\pm$ SD
Intraocular pollen	2	13	3	6	15	20	12	6	49	10	13.6 $\pm$ 13.6 (grains)
Intranasal pollen	175	175	303	160	198	522	90	260	281	328	249.2 $\pm$ 120.9 (grains)

One subject (*) exited the chamber 10 min earlier since she was about to sneeze.

The remaining subjects were able to stay for an hour without developing symptoms.

As to the subject numbers, see Table 1

Table 3 The number of subjects who showed nose or eye symptoms during 2-hour allergen exposure.

	15	30	60	90	120	(min)
Rhinorrhea	1	3	3	6	8	
Sneezing	0	1	1	4	4	
Nasal obstruction	1	4	6	6	7	
Nasal itching	2	4	4	7	8	
Eye itching	2	5	6	7	6	
Watery eyes	0	2	3	3	3	
Nasal secretion (g/hr)	$\leftarrow$ 0.23 $\pm$ 0.39		$\rightarrow$	$\leftarrow$ 0.73 $\pm$ 0.83		$\rightarrow$

There were increases in the number of subjects who developed nasal and ocular symptoms in a time dependent manner. Six subjects developed nasal obstruction after 60 minutes of exposure, of which 3 subjects marked their symptoms as moderate while 1 subject marked them as severe. Although 8 subjects developed rhinorrhea and nasal itching after 2 hours of exposure to the allergen, only 4 subjects developed sneezing and their symptoms were mild. We measured the amount of nasal secretion from 3 subjects in the first half of the study and from 7 in the latter half of the study. Ocular symptoms also developed as time went by. During the study period, 1 subject showed neither nasal nor ocular symptoms.

tomation Inc, Tokyo, Japan) to nasal lavage fluid in order to reduce its viscosity. After shaking the nasal or ocular lavage fluid for 5 minutes at room temperature, it was put into a suction filtration device using 0.5 μ m filter papers (Advantee Cellulose Nitrate, Toyo Roshi Inc, Tokyo, Japan). Hereafter, the leftover solution was washed once more with saline and the solution was put into a suction filtration device to keep any single grain from being left behind. In this way, all the filter papers were examined under a light microscope ($\times 100$) and the pollen count was recorded.

RESULTS

The subjects consisted of 5 men and 5 women with mild symptoms. Their ages ranged from 32 to 48 years (the average age was 38.5 ± 5.4). Background factors of the subjects are shown in Table 1.

In the first part of this study, the temperature, the humidity, and the concentration of pollen grains in the room were kept within the targeted values. That is, the average number of pollen grains was 2572.8 ± 264.5 grains /m³, the average temperature $22.1 \pm 0.14^\circ\text{C}$, and the average humidity $44.7 \pm 0.48\%$ (Fig. 4). Intranasal pollen counts in the subjects ranged from 90 to 522 and intraocular grains ranged from 2 to 49. No relation was seen between the positions of the subjects within the chamber and the numbers of pollen detected in the nose and eyes. Even though 1

subject had to leave the chamber 10 minutes earlier than the scheduled time because of symptom development, the rest of the subjects were able to stay in the room for an hour without developing symptoms. The average number of intranasal pollen grains and intraocular grains was 249.2 ± 120.9 and 13.6 ± 13.6 , respectively (Table 2).

In the second part of this study, the temperature, the humidity, and the concentration of pollen grains inside the chamber were within targeted values: the average number of pollen grains was 4367 ± 207 grains/m³, the temperature $22.2 \pm 0.35^\circ\text{C}$, and the humidity $44.8 \pm 0.65\%$ (Fig. 5). As time went by, more and more subjects started developing nasal symptoms such as nasal discharge, sneezing, nasal obstruction, and itchy nose. Only 3 subjects showed nasal discharge 60 minutes after the start of exposure, but 6 subjects after 90 minutes and 8 subjects after 120 minutes. Likewise, the number of subjects who developed nasal obstruction increased after 60 minutes of exposure, of which 3 subjects marked their symptoms as moderate while 1 subject marked severe. Although 8 subjects showed nasal itching after 120 minutes, only 4 subjects developed sneezing during exposure and their symptoms were mild. Three subjects blew their nose within 1 hour of the first half of the study, and 7 in the latter half. The amount of nasal secretion in the first half and the latter half of the study was 0.23 ± 0.39 g/hour and 0.73 ± 0.83 g/

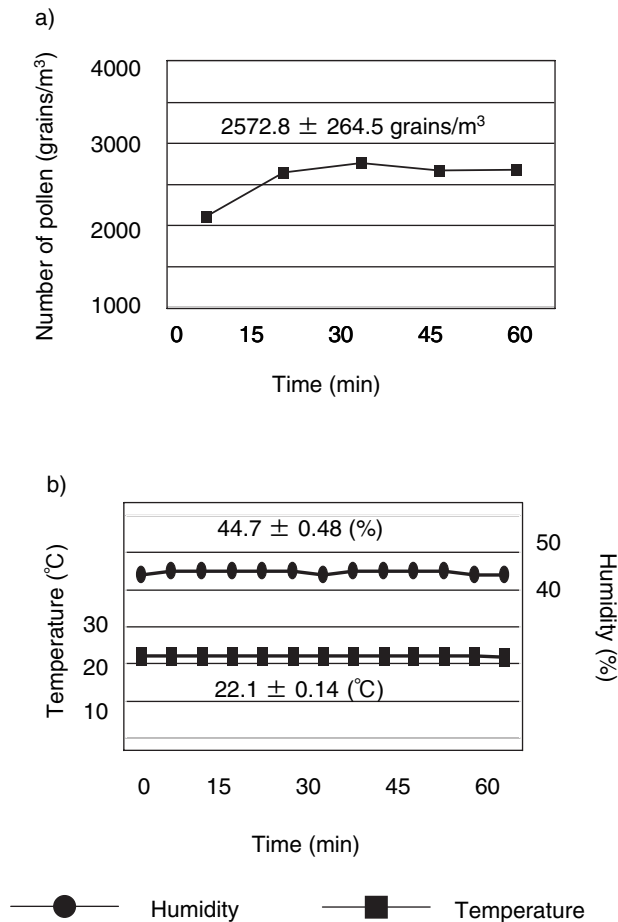


Fig. 4 The pollen count (a) and temperature and humidity (b) in the chamber during the first part of the study. The pollen count at each point shows the average value during 15 minutes. The average number of pollen grains was 2572.8 ± 264.5 grains/m³, the average temperature 22.1 ± 0.14 °C, and the average humidity 44.7 ± 0.48 %.

hour respectively (Table 3).

With regard to ocular symptoms, half of the subjects developed itchy eyes within 30 minutes after entering the chamber, increasing to 7 subjects as time went by. Their symptoms, however, were mild. In addition, only 3 subjects developed epiphora while other 3 subjects experienced neither itchy eyes nor epiphora in the chamber. During the 2-hour exposure to pollen, 1 subject showed no nasal or ocular symptoms. No adverse event was observed in these studies. None of the subjects showed late nasal reaction after returning home.

DISCUSSION

This is a preliminary study showing the amount of intranasal or intraocular pollen as well as allergy symptoms in mildly symptomatic subjects with JC pollinosis under well controlled conditions in an OHIO chamber.

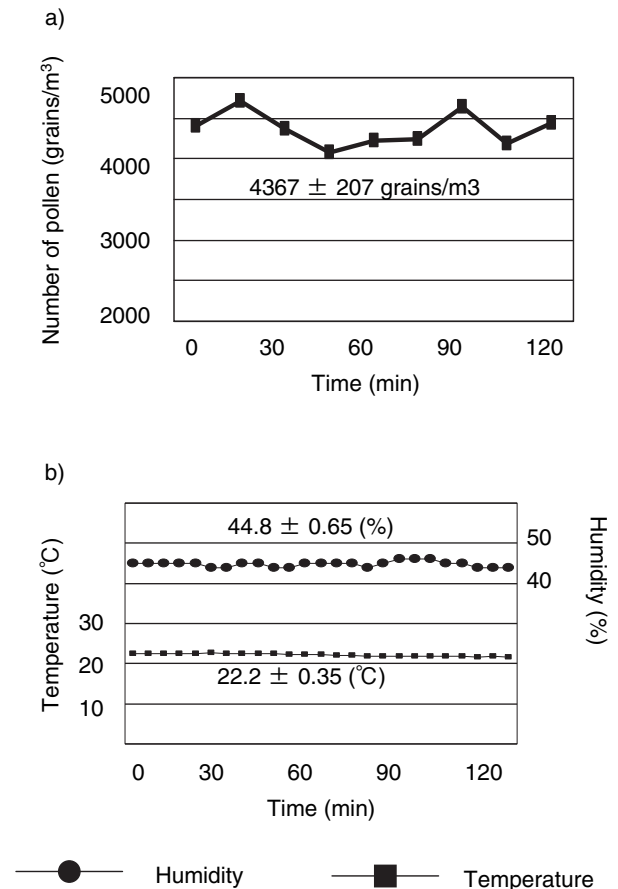


Fig. 5 The pollen count (a) and temperature and humidity (b) in the chamber during the second part of the study. The average number of pollen grains was 4367 ± 207 grains/m³, the average temperature 22.2 ± 0.35 °C, and the average humidity 44.8 ± 0.65 %.

There are several allergen exposure units in Europe and the US⁶⁻⁸ and a lot of useful data are available in the treatment of allergic rhinitis.^{10,11} However it is difficult to simply compare the figures for pollen dispersal between the results obtained from each exposure unit. Each chamber uses a different method to count the pollen grains. For instance, our OHIO chamber uses KC-20 (Rion Co, Tokyo, Japan), a laser particle counter which measures the number of particles between 10 and 100 μ m in diameter. On the other hand, the Japanese Environment Ministry, which has been measuring pollen counts at various stations and providing information during pollen seasons, uses a different kind of laser-counter (KH-3000: Yamato Seisakujo, Japan). We attempted measurements with both KC-20 and KH-3000 and discovered that there is a correlation between the outcomes of the two methods of measurement. We also discovered that KC-20 yields values 5.9 times larger than those obtained using KH-3000 (data not shown).

In the first part of the study, subjects were exposed

to 2500 grains/m³ of JC pollen, which is equivalent to the amount of airborne pollen grains in the early stages of the pollen season. Although their allergic symptoms were reported to be mild, one of the subjects had to leave the room before the scheduled time since the subject was about to sneeze. The other 9 subjects were able to remain in the room for an hour without developing any allergic symptoms. We found that the average number of intranasal and intraocular pollen grains was 249.2 and 13.6, respectively. The subjects sat still during the study for up to 1 hour and did not move around, so their eyes simply received pollen grains, and their shedding tears and blinking cleared the pollen grains. On the other hand, subjects actively inhaled pollen grains through their noses, allowing more pollen to precipitate in the nose and they did neither sneeze nor blow their noses. These could be the possible reason why the number of intranasal pollen grains was much larger than that of the intraocular pollen grains. Gotoh *et al.*¹² conducted a study on the ratio of intranasal to intraocular pollen numbers, which were obtained from healthy volunteers walking in the open air at an ordinary speed for half an hour. Their study showed the almost same result with ours.

In the second part of the study, the concentration of pollen dispersed was increased to 4500 grains/m³. This concentration is equivalent to the amount of airborne pollen grains during the midterm and late stages of the pollen season. Nasal and ocular symptoms gradually developed in a time dependent manner, but these symptoms were mild.

Okuda *et al.*¹³ measured the number of intranasal grains of JC pollinosis patients during pollen seasons over several years. They showed that the average number of JC pollen found in a patient's nose was about 20, although the amount of pollen varies every year. They concluded that 90 to 150 pollen grains were considered to be sufficient to cause symptoms from the dynamic study of pollen in the nose.

In this study, we found that 90 to 500 pollen grains in the nose were not enough to develop nasal symptoms. The only exception was the subject who exited the room in 50 minutes due to sneezing, whose intranasal pollen counted 303. There is a difference in the number of pollen which develops nasal symptoms observed in our study and in the study conducted in a natural environment.¹³ The following could be the reason for the difference; subjects in this study were mildly symptomatic patients with JC pollen; subjects had not received repetitive exposure to JC pollen, because the study was conducted 3 months ahead of the pollen season; and subjects were under psychological pressure since they had never experienced an environmental exposure study.

This is the first study to show the intranasal and intraocular pollen grains and allergic symptoms using the OHIO Chamber. As far as pollinosis is concerned,

however, our data cannot be immediately generalized since the results depend on the amount of pollen, the priming effects of the nasal mucosa, and the severity of the patients' symptoms. We need to evaluate the results of our data carefully. Therefore, further investigations are required to decide an appropriate amount of pollen and exposure time to obtain reproducible results and to secure the safety of the subjects.

REFERENCES

1. Okuda M. Epidemiology of Japanese cedar pollinosis throughout Japan. *Ann. Allergy Asthma Immunol.* 2003;**91**:288-296.
2. Ishikawa T, Soh N. [Evaluation of quality of life (QOL) and efficacy of drug therapy on nasal symptoms and QOL disturbances in the patients with Japanese cedar pollinosis during the season of 2003 in Kyushu and Okinawa districts.]. *Alerugi [Jpn. J. Allergol.]* 2004;**53**:1131-1143 (in Japanese).
3. Kakutani C, Ogino S, Ikeda H, Enomoto T. [Impact of allergic rhinitis on work productivity: a pilot study.]. *Alerugi [Jpn. J. Allergol.]* 2005;**54**:627-635 (in Japanese).
4. Meltzer EO, Weiler JM, Widlitz MD. Comparative outdoor study of the efficacy, onset and duration of action, and safety of cetirizine, loratadine, and placebo for seasonal allergic rhinitis. *J. Allergy Clin. Immunol.* 1996;**97**:617-626.
5. Hyo S, Fujieda S, Kawada R, Kitazawa S, Takenaka H. The efficacy of short-term administration of 3 antihistamines vs placebo under natural exposure to Japanese cedar pollen. *Ann. Allergy Asthma Immunol.* 2005;**94**:457-464.
6. Horak F, Stübner P, Ziegelmayer R *et al.* Controlled comparison of the efficacy and safety of Cetirizine 10 mg o.d. and Fexofenadine 120 mg o.d. in reducing symptoms of seasonal allergic rhinitis. *Int. Arch. Allergy Immunol.* 2001;**125**:73-79.
7. Day JH, Briscoe MP, Welsh A *et al.* Onset of action, efficacy, and safety of a single dose of fexofenadine hydrochloride for ragweed allergy using an environmental exposure unit. *Ann. Allergy Asthma Immunol.* 1997;**79**:533-540.
8. Krug N, Hohlfeld JM, Geldmacher H *et al.* Effect of loteprednol etabonate nasal spray suspension on seasonal allergic rhinitis assessed by allergen challenge in an environmental exposure unit. *Allergy* 2005;**60**:354-359.
9. Enomoto T, Ide T, Ogino S. Development of an environmental exposure unit and effects of cetirizine hydrochloride on relieving the symptoms of cedar pollinosis. *Prog. Med.* 2005;**25**:3141-3149.
10. Day JH, Briscoe M, Widlitz MD. Cetirizine, loratadine, or placebo in subjects with seasonal allergic rhinitis: Effects after controlled ragweed pollen challenge in an environmental exposure unit. *J. Allergy Clin. Immunol.* 1998;**101**:638-645.
11. Horak F, Stübner P, Ziegelmayer R, Ing D, Harris AG. Effect of desloratadine versus placebo on nasal airflow and subjective measures of nasal obstruction in subjects with grass pollen-induced allergic rhinitis in an allergen-exposure unit. *J. Allergy Clin. Immunol.* 2002;**109**:956-961.
12. Gotoh M, Okubo K, Okuda M. Inhibitory effects of face-masks and eyeglasses on invasion of pollen particles in the nose and eye: a clinical study. *Rhinology* 2005;**43**:266-270.
13. Okuda M, Ohkubo K, Gotoh M *et al.* Dynamics of airborne pollen particles from inhalation to allergic reaction in the nose. *Rhinology* 2005;**43**:29-33.