

Noninvasive biological evaluation of response to pranlukast treatment in pediatric patients with Japanese cedar pollinosis

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ABSTRACT

Pranlukast (PLK) is a cysteinyl leukotriene receptor 1 antagonist approved for the treatment of bronchial asthma and allergic rhinitis in Japan. We previously reported that PLK dry syrup (DS) improved the total nasal symptom score, as well as sneezing, nasal discharge, and nasal obstruction scores over placebo. We investigated the efficacy of PLK DS with a noninvasive method in 10- to 15-year-old children with Japanese cedar (JC) pollinosis challenged with pollen allergen using an artificial exposure chamber (OHIO Chamber). Levels of eosinophil cationic protein (ECP) in nasal secretions, nasal obstruction score, and the relationship with nasal obstruction scores were analyzed. The estimated difference of means in ECP levels (PLK DS – placebo) was -22.9 micrograms (95% CI, -45.2 to -0.5), suggesting PLK DS reduced ECP significantly when compared with placebo ($p = 0.0454$). The difference in the least square means for nasal obstruction between the PLK DS and placebo was -0.25 (95% CI, -0.36 to -0.14) with a value of $p < 0.0001$. In addition, a statistically significant, although weak, positive correlation between the nasal obstruction score and nasal ECP levels was observed with placebo treatment (correlation coefficient = 0.2394 ; $p = 0.0428$). Moreover, the inhibition rate of nasal ECP with PLK DS relative to placebo was statistically significant, although weak, positively correlated with the inhibition rate of nasal obstruction (correlation coefficient = 0.3373 ; $p = 0.0219$). PLK DS significantly decreases nasal ECP levels and nasal obstruction score compared with placebo in children with JC pollinosis challenged with pollen allergen. Suppression of mucosal eosinophilic inflammation is one of the pathways by which PLK DS improves pollinosis-induced nasal obstruction.

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Allergic rhinitis (AR) was reported to affect approximately 39% of the entire Japanese population, including adults and children, in a 2008 survey.¹ Typical types of AR in Japan include perennial AR due to house-dust mites and seasonal AR caused by Japanese cedar (JC) pollen. According to the 2008 survey, the prevalence of JC pollinosis was approximately 26%, representing an

approximately 10% increase over the preceding 10 years. Undoubtedly, JC pollinosis is one of the most common types of AR in Japan.¹ JC pollen grains are dispersed in large amounts each year, causing patients severe and bothersome symptoms. The main symptoms of AR are sneezing, nasal discharge, and nasal obstruction. Although these symptoms are not life-threatening, they reduce quality of life,² cause sleep disturbances,³ and interfere with work and learning performance.^{4–7} Nasal obstruction is probably the most annoying and bothersome, because it causes sleep disturbances.^{8–10} In particular, because young children have narrow nasal cavities, they are more prone to stuffy noses caused by AR.

Cysteinyl leukotrienes (CysLTs) C_4 , D_4 , and E_4 are arachidonic acid metabolites expressed in mast cells, eosinophils, and other inflammatory cells after allergen exposure. These are known to modulate the pathophysiology of AR, bronchial asthma, and other allergic diseases. CysLTs are important mediators of allergic and other inflammatory reactions, causing various adverse conditions by binding to CysLT₁ and other receptors.^{11–15} Pranlukast (PLK), a CysLT₁ receptor antagonist, has been shown to be most effective for the treatment of nasal obstruction among the nasal symp-

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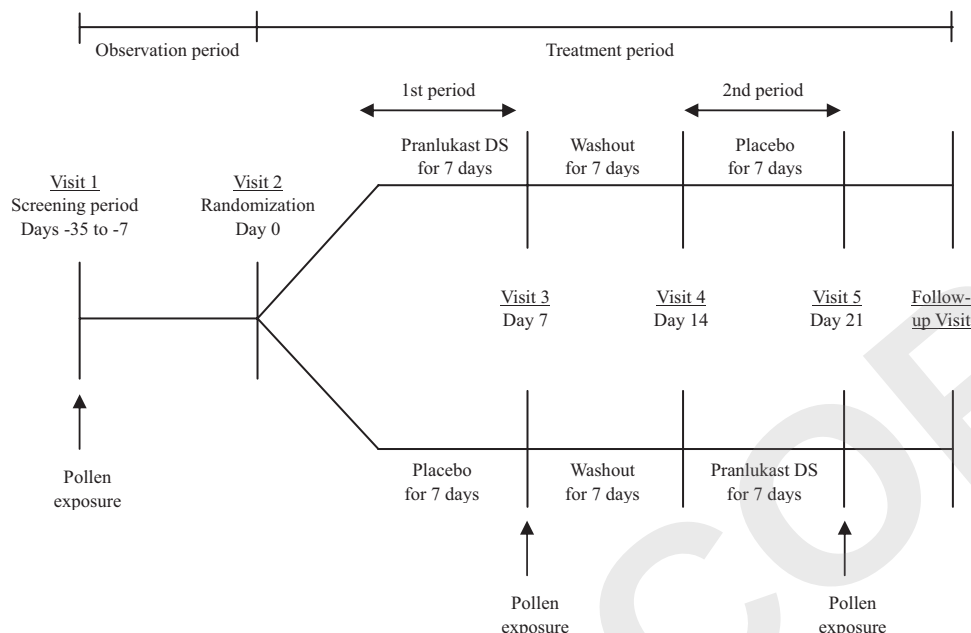


Figure 1. Study schedule. The study periods consisted of observation and treatment periods. The screening exposure in the OHIO Chamber was performed after subjects received placebo treatment for 1 week, to determine whether they would respond adequately to the pollen stimulation and not respond to placebo treatment. One inclusion criterion was that at least one nasal symptom score was at least 1 point worse during exposure than before subjects entered the chamber. Eligible subjects were enrolled in the treatment period and were randomized to receive pranlukast dry syrup (PLK DS) or placebo for 1 week (period 1) followed by a washout period of 7 days, and then crossed over to the other treatment period (period 2) for 7 days. Subjects were exposed to pollen in the OHIO Chamber for 3 hours on the last day of each treatment period (periods 1 and 2).

toms resulting from AR.^{16–18} Eosinophilic infiltration into the nasal mucous membranes is one of the mechanism of nasal obstruction. Leukotriene D₄ (LTD₄) mediates eosinophil migration.¹⁹ The suppression of eosinophil infiltration into the nasal mucosa by PLK has been suggested as one of the major pathways for alleviating nasal mucosal swelling.²⁰ Eosinophil cationic protein (ECP), released from activated eosinophils, is as a specific biological marker for eosinophil infiltration and inflammation.²¹

This study was a randomized, placebo-controlled, double-blind crossover study to investigate the efficacy and safety of PLK dry syrup (DS) in children with JC pollinosis challenged used an artificial exposure chamber (OHIO Chamber). We previously reported that PLK DS resulted in statistically significant improvements in the total nasal symptom score, as well as sneezing, nasal discharge, and nasal obstruction scores, relative to placebo.²² In this article, we investigate the concentration of ECP in nasal secretions and its relationship to the nasal obstruction score.

Nasal lavage is commonly used to examine nasal ECP levels after allergen challenge,^{23–27} but it is thought to affect nasal symptoms. In this pediatric study, nasal discharge collected on facial tissue paper was used to quantitatively determine nasal ECP levels because this noninvasive and convenient sampling

method enabled the monitoring of changes in nasal obstruction over time during exposure to JC pollen.

METHODS

Study Population, Design, and Protocol

This was a randomized, double-blind, placebo-controlled, crossover study in which subjects were exposed to JC pollen in an artificial exposure chamber (OHIO Chamber). The inclusion criteria were as follows: between the ages of 10 and 15 years, allergic symptoms, particularly nasal obstruction, during the pollen season for at least 2 years with a positive multiple antigen simultaneous test (class, ≥ 2) or positive skin test for JC pollen antigen. The exclusion criteria were as follows: acute rhinitis, rhinosinusitis with purulent nasal discharge and allergic symptoms caused by other allergens during the study period. Other detailed criteria on the study population and study design were reported previously.²² The study schedule is represented in Fig. 1, and the evaluation criteria for the severity of nasal symptoms are shown in Tables 1 and 2. The nasal discharge score was assessed in terms of the number of times the subject blew his/her nose, and the nasal secretions collected on the tissue paper used for nose blowing were subjected to nasal ECP measurements.

Table 1 Severity of nasal obstruction

Severity	++++ (score 4)	+++ (score 3)	++ (score 2)	+	– (score 0)
	Completely obstructed	Severe nasal blockage causing prolonged oral breathing	Severe nasal blockage causing occasional oral breathing	Nasal blockage without oral breathing	No symptom

Source: Altered partially from Ref. 1).

The severity of nasal obstruction was judged during each 30-min period during exposure.

Table 2 Severity of sneezing and nasal discharge

Severity	++++ (score 4)	+++ (score 3)	++ (score 2)	+	– (score 0)
Sneezing	≥21 times	20 – 11 times	10 – 6 times	5 – 1 times	Below +
Nasal discharge	≥21 times	20 – 11 times	10 – 6 times	5 – 1 times	Below +

Sneezing: The number of times the subject sneezed during each 30-min period during exposure.

Nasal discharge: The number of times the subject blew his/her nose during each 30-min during exposure.

Collection of Nasal Discharge

In the first and second periods of the study, facial tissues that subjects used to blow their noses during the 180-minute pollen challenge period were collected in polyethylene bags cooled with refrigerant gel packs. After the pollen exposure ended, the polyethylene bags containing collected facial tissues with nasal secretions were then transported by an express courier service in a refrigerated container to Sumika Chemical Analysis Service, Ltd., in Osaka, Japan, for quantitative biochemical analysis. At the laboratory, nasal ECP was extracted from the collected secretion samples and quantitatively analyzed.

Extraction of ECP

The procedure for nasal ECP extraction and quantification is illustrated in Fig. 2. ECP was extracted using 5 mL of Tris-buffered saline with Tween 20 (0.1%) per sheet of tissue paper. Extraction solution was added to each polyethylene bag containing used tissue paper. The bags were then gently kneaded 30 times by hands, and the extraction solution was collected in polypropylene containers by squeezing the tissue paper. Preliminary experiments showed that ECP recovery from tissue paper was reproducible based on an approximate constant recovery rate of 72% on average.

Storage and Measurement of ECP

The extraction solution was stored at –80°C until measurement. Frozen ECP samples (–80°C) were shown to be stable for 60 days, and quantitative determinations were conducted within this period.

ECP extracted from collected samples were quantified using a commercial ELISA kit according to the

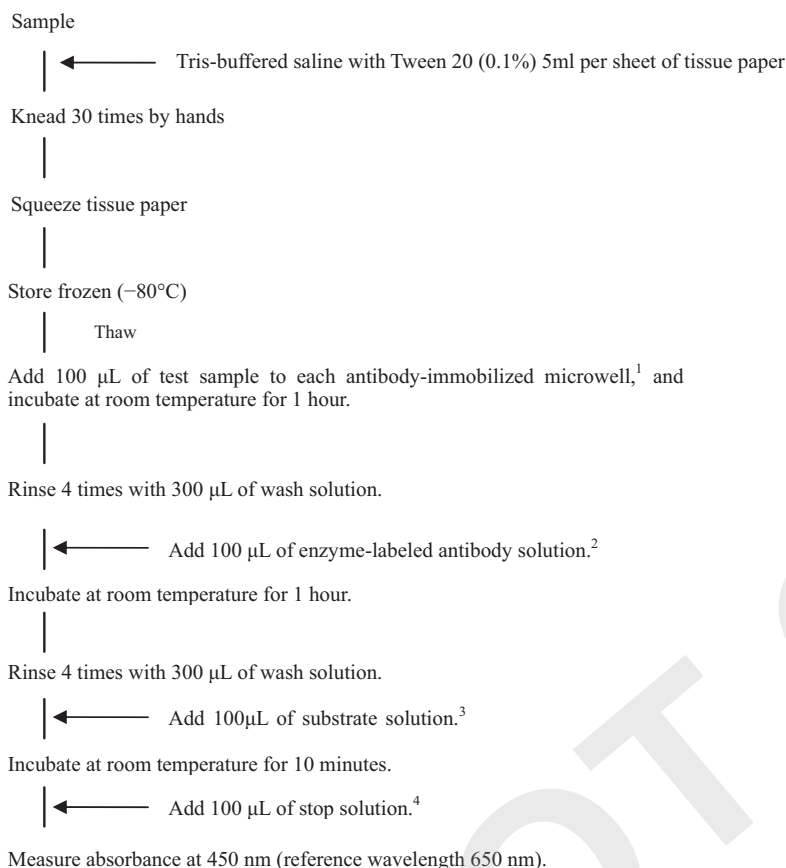
attached instruction (MESACUP ECP Test; Medical & Biologic Laboratories Co., Ltd., Aichi, Japan).

Statistical Analysis

The differences in ECP levels between PLK DS and placebo were tested using the paired *t*-test. Analysis of nasal obstruction was performed using an analysis of covariance model with treatment group, period, and sequence as factors and nasal obstruction score before entering into the OHIO Chamber as covariates in the model, as reported previously.²² The relationship between ECP levels and nasal obstruction scores was evaluated using the Pearson product-moment correlation coefficient. The relationship between inhibition rate of ECP levels ($=100 \times [1 - \{\text{ECP value in PLK DS} / \text{ECP value in placebo}\}]$) that was calculated to measure inhibiting effect of ECP of PLK DS on placebo and the nasal obstruction score were evaluated using with Spearman's rank correlation coefficient. All hypothesis tests were conducted using a two-sided significant level of 0.05. The following data-handling procedures were established before the database was locked: (i) missing ECP values (μg) were imputed as zero for subjects who did not blow their noses and, consequently, had no nasal discharge samples; (ii) samples below the limit of quantification (LOQ; $0.03 \mu\text{g}$) were imputed with one-half the LOQ value; and (iii) nasal discharge samples with possible blood contamination that could interfere with ECP measurement were excluded from further analysis.

RESULTS

The primary efficacy analysis population was the per protocol set, which consisted of 74 subjects (study pop-



- 1: Microplates coated with mouse anti-human ECP monoclonal antibody.
- 2: Peroxidase-labeled rabbit anti-human ECP polyclonal antibody.
- 3: Substrate solution containing 3,3',5,5'-tetramethylbenzidine and hydrogen peroxide.
- 4: Stop solution: 0.5 mol/L sulfuric acid.

Figure 2. Method for measuring eosinophil cationic protein (ECP).

Table 3 Differences in the amount of ECP in nasal discharge by treatment

		<i>n</i>	PLK DS	Placebo
ECP in nasal discharge (µg)	Difference from placebo		71	71
		Mean ± SD	44.0 ± 107.7	66.9 ± 146.7
		Point estimate* (95% CI)	-22.9 (-45.2, -0.5)	
		Paired <i>t</i> -test	<i>p</i> = 0.0454	

*The point estimate was derived as the difference between the mean values calculated using the paired *t*-test.

ECP = eosinophil cationic protein; PLK DS = pranlukast dry syrup.

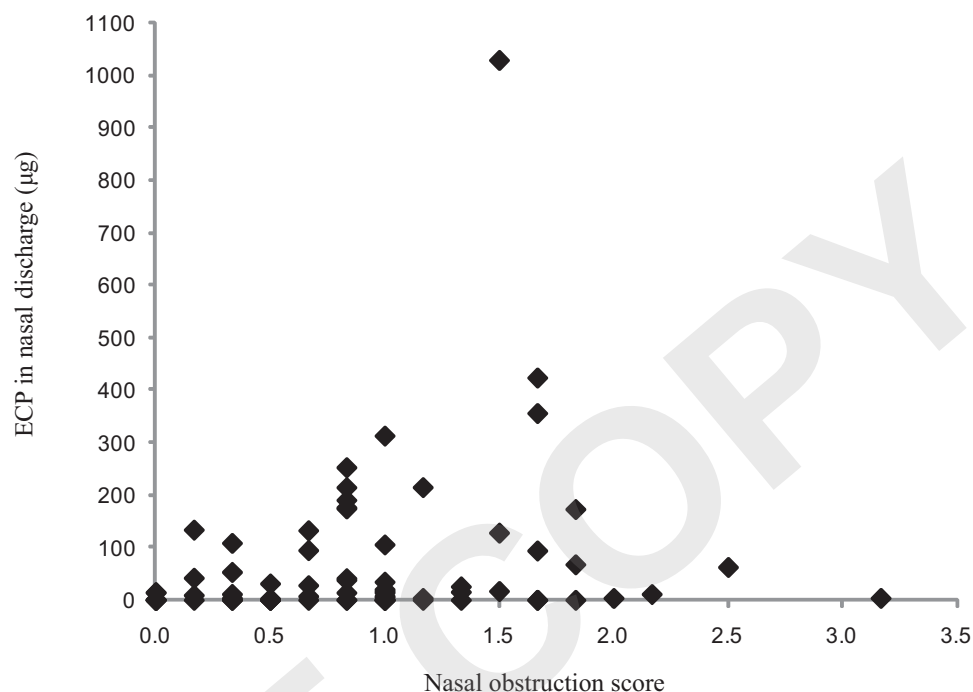
ulation, 84 subjects). From the per protocol set, 148 ECP samples were collected, including 53 samples imputed with 0 because of the absence of nose blowing, 1 sample imputed with one-half the LOQ, and 3 samples excluded from analysis because of possible blood contamination.

The results of paired *t*-test for the difference of ECP (µg) between PLK DS and placebo are shown in Table 3. The estimated difference of means (PLK DS – placebo)

was -22.9 µg (95% CI, -45.2 to -0.5), suggesting significantly lower levels of nasal ECP in the PLK DS (*p* = 0.0454). The difference in the least square means for nasal obstruction between the PLK DS and placebo was -0.25 (95% CI, -0.36 to -0.14) with a value of *p* < 0.0001, as reported previously.²²

To assess possible associations between nasal ECP levels and nasal obstruction, we examined the relationship between nasal ECP levels and nasal obstruction

Figure 3. Relationship between the nasal obstruction score and nasal eosinophil cationic protein (ECP) levels in pediatric seasonal allergic rhinitis (AR) patients treated with placebo. A statistically significant, although weak, positive correlation was observed (correlation coefficient = 0.2394; $p = 0.0428$).



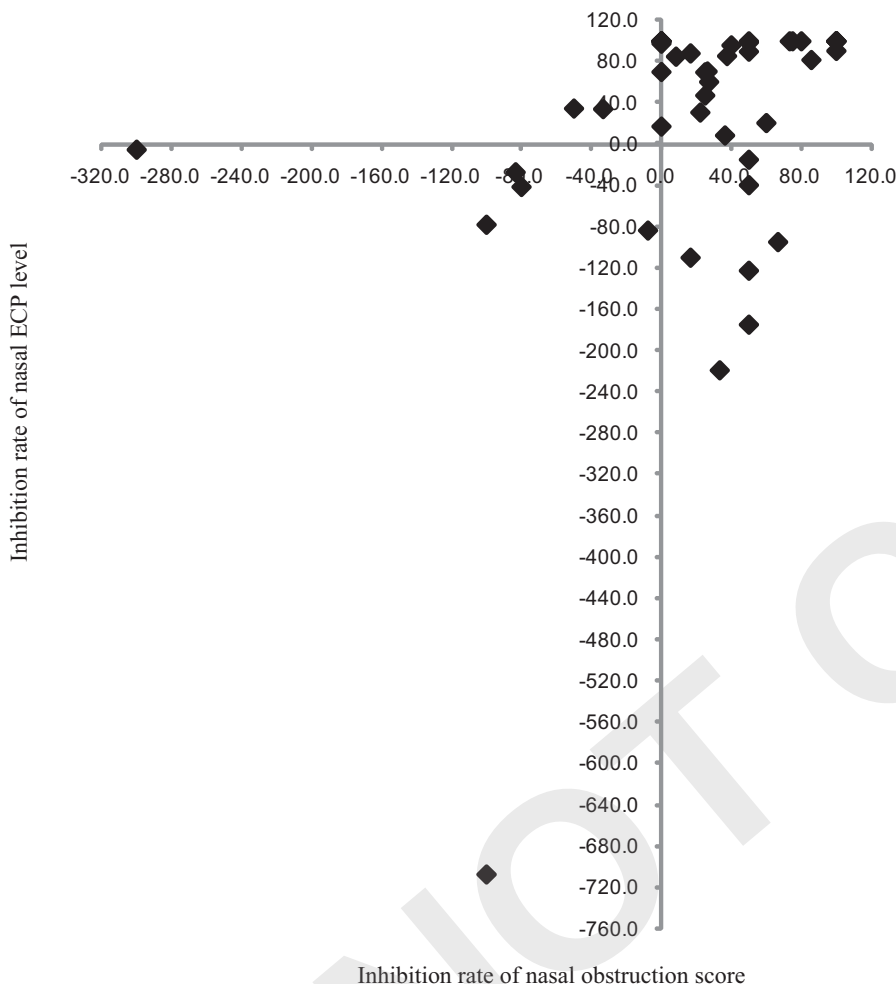


Figure 4. Relationship between the inhibition rate of nasal obstruction score and the inhibition rate of nasal eosinophil cationic protein (ECP) level with pranlukast dry syrup (PLK DS) relative to placebo. A statistically significant, although weak, positive correlation was observed (correlation coefficient = 0.3373; $p = 0.0219$).

enrolled in this study; (ii) this method was thought to yield ECP data without affecting the nasal symptoms that are the primary end point of this study for evaluating the effects of PLK DS as shown in the previous report,²⁰ because nasal lavage could possibly affect the nasal symptoms; and (iii) this method provided measurements of ECP levels in nasal discharges produced during exposure for direct comparison with the nasal obstruction score. As far as we have searched reports in the viewpoint that the sampling method is adaptive and noninvasive for children, no report, conducted with the same method as ours, has been searched. For that reason, we have investigated and confirmed that we can measure ECP levels with reproducibility in nasal discharge samples collected on facial tissue paper used for nose blowing before this study. However, one disadvantage was that no samples were available for patients who did not blow their noses during exposure. In fact, about one-third of the 148 samples included had no nasal discharge because the subjects did not blow their nose once, and ECP levels were imputed with 0 μg before the database locked, based on the predetermined study protocol assumption that no nose

blowing indicates almost no nasal secretion, suggesting the absence of allergen-induced ECP production. In a *post hoc* analysis, to check the affect of this handling on the results, we imputed with 0.015 μg (one-half the LOQ value, the same way as below the LOQ) instead of 0 μg in subjects with no nose blowing, assuming that trace levels of ECP were induced by exposure. However, this change produced no differences (data not shown). Consequently, about one-third of the 148 ECP measurement samples, which had no nasal discharge because of the absence of nose blowing, did not significantly affect the results.

In this study, ECP measurement samples were prepared by kneading the collected facial tissue paper 30 times by hands in a solution containing Tween-20 surfactant. No sonication was performed during sample preparation, because preliminary experiments revealed no recognizable changes in ECP concentration between samples prepared with and without sonication, suggesting that all of the ECPs were thought to be isolated from eosinophils by the current procedure.

Despite the finding that PLK DS suppressed ECP release significantly better than placebo during pollen

exposure, correlations were observed between the suppression of ECP release and the suppression of nasal obstruction after administration of PLK DS. This was probably because of the fact that a variety of factors contribute to the onset of nasal obstruction, including dilatation and increased permeability of the nasal mucosal vessels, in addition to eosinophilic mucosal infiltration.¹

This study examined the effects of PLK DS on nasal symptoms in pediatric patients exposed to pollen allergen for 3 hours, the same as the adult study.⁴⁷ It is reported that after antigen exposure, eosinophil levels in nasal secretions show a mild decrease after an initial peak at hour 3, followed by a second peak starting between hours 7 and 8 and lasting until hour 9.⁴⁸ This suggests the possibility that a higher correlation may be found when the duration of exposure and observation is extended to >3 hours.

In our previous report, PLK DS improved nasal obstruction and other nasal symptoms in pediatric patients with JC pollinosis exposed to the allergen in an artificial exposure chamber.²² The present study demonstrated for the first time that PLK DS significantly decreases nasal ECP levels in children with JC pollinosis challenged with high airborne allergen concentrations. In particular, suppression of mucosal eosinophilic inflammation was suggested as one of the pathways by which PLK DS improves pollinosis-induced nasal obstruction.

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