

The Effects of Suplatast Tosilate Treatment and Prophylaxis on Lung Histopathology in a Mouse Model of Chronic Asthma

Tuba Tuncel, MD,* Meral Karaman, MD,† Zeynep Arikan Ayyildiz, MD,* Pinar Uysal, MD,* Muge Kiray, MD,† Alper H. Bagriyanik, MD,† Osman Yilmaz, MD,† Ozkan Karaman, MD,* and Nevin Uzun, MD*

Introduction: Suplatast tosilate is a medication that inhibits T_H2 -type cytokines. We aimed to investigate the effects of suplatast tosilate treatment and prophylaxis on lung histopathology and cytokine levels in a mouse model of chronic asthma.

Materials and Methods: Forty-two BALB/c mice were divided into 6 groups: group I (control), group II (vehicle control), group III (dexamethasone), group IV (prophylaxis with suplatast tosilate), group V (treatment with suplatast tosilate), and group VI (prophylaxis and treatment with suplatast tosilate). All of the groups, except for the control and vehicle control groups, were sensitized and challenged with ovalbumin. The mice in the study groups, except those in the group receiving suplatast tosilate for prophylaxis only, were treated with study drugs. After the mice were killed, IL-4, IL-5, and interferon- γ levels were quantified in the lung tissue, which were examined histologically by light and electron microscopy.

Results: There were significant improvements in all histopathological parameters in the group treated with suplatast tosilate compared with the vehicle control group. Similar improvements were observed when the group receiving prophylaxis and treatment with suplatast tosilate was compared with the vehicle control as well. There were no significant differences between the group receiving only prophylaxis with suplatast tosilate and the vehicle control group. Cytokine levels were significantly higher in the vehicle control group when compared with the control group. Although all of the groups had lower cytokine levels than those of the vehicle control group, the differences were not statistically significant.

Conclusions: Treatment with suplatast tosilate was effective in improving all histopathological parameters in a mouse model of chronic asthma. It was observed that the use of prophylactic suplatast tosilate was ineffective and had no additional effects when administered together with treatment.

Key Words: asthma, mice, prophylaxis, suplatast tosilate, treatment

(*J Invest Med* 2014;62: 56–61)

Asthma is the most common chronic disease of childhood.¹ It has a high prevalence in developed countries, and its prevalence is increasing in developing countries.² Anti-inflammatory

drugs continue to be widely used for the treatment of asthma because inflammation is a main characteristic of the disease.³ However, when used in high doses for a long period, especially in children, anti-inflammatory drugs can cause unintended side effects. Because these cause side effects and because these drugs do not completely prevent remodeling, new treatment options are being investigated.^{2,4}

Suplatast tosilate (($\pm$)-[2-[4-(3-ethoxy-2-hydroxypropoxy) phenylcarbonyl] ethyl] dimethylsulfonium *p*-toluenesulfonate) is a dimethyl sulfonium compound.⁵ Both in vivo and in vitro studies have shown that suplatast tosilate inhibits the production of IL-4, IL-5, and IgE and prevents the recruitment of eosinophils.^{6,7} Results from animal and human studies have shown that suplatast tosilate may be effective in the treatment of asthma, with no reported side effects.^{8,9} Because the drug suppresses T_H2 -type cytokines but not T_H1 -type cytokines, it has been hypothesized that it would also be effective in preventing the development of asthma. Positive results were reported in a pilot study in which children at high risk for the development of asthma were given suplatast tosilate.¹⁰

These previous studies suggest that suplatast tosilate is a promising drug for the prophylaxis and the treatment of asthma. However, to our knowledge, there have not been any studies examining the effects of suplatast tosilate treatment or prophylaxis on the histopathological findings of asthma, with the exception of goblet cell hyperplasia. The limited use of suplatast tosilate in human studies may be because it is currently available only in Japan and because there is a lack of the approval for its use in other countries. In this study, we aimed to investigate the effects of treatment and prophylaxis with suplatast tosilate on histopathological findings as well as the levels of IL-4, IL-5, and interferon (IFN)- γ in a mouse model of chronic asthma.

MATERIALS AND METHODS

Experimental Animals

Forty-two specific pathogen-free 6- to 8-week-old male BALB/c mice weighing 20 to 25 g purchased from Dokuz Eylul University, Department of Multidisciplinary Animal Laboratory (Izmir, Turkey), were used in this study. The animals were maintained in hygienic macrolene cages in air-conditioned rooms with a 12-hour light/12-hour dark cycle and were provided ad libitum food and water in a pathogen-free laboratory in the same department. All experimental procedures complied with the requirements of the Dokuz Eylul University Animal Care and Ethics Committee.

Study Groups

The 42 BALB/c mice were divided into 6 groups as follows: group I (control), group II (vehicle control), group III (dexamethasone), group IV (prophylaxis with suplatast tosilate), group V

From the *Division of Allergy, Department of Pediatrics, †Department of Multidisciplinary Laboratory, and ‡Department of Histology and Embryology, Dokuz Eylul University Hospital, Izmir, Turkey.
Received March 8, 2013.

Accepted for publication August 26, 2013.

Reprints: Tuba Tuncel, MD, Division of Allergy, Department of Pediatrics, Dokuz Eylul University Hospital, 35340, Balçova, Izmir, Turkey.
E-mail: ttuncel@yahoo.com.tr.

Supported by the Dokuz Eylul University Scientific Research Foundation Grant.

Copyright © 2013 by The American Federation for Medical Research
ISSN: 1081-5559

DOI: 10.231/JIM.0000000000000012

(treatment with suplatast tosilate), and group VI (prophylaxis and treatment with suplatast tosilate).

Sensitization and Inhalational Exposure

This study was conducted according to a previously described murine model of chronic asthma.¹¹ BALB/c mice were used because of their high sensitivity to ovalbumin.¹² The mice in all of the groups, except for the control group, were sensitized via 2 intraperitoneal injections of ovalbumin (10 μ g/0.1 mL grade V, $\geq$ 98% pure; Sigma, St Louis, MO) along with alum as an adjuvant on day 0 and day 14. Then, beginning on the 21st day of the study, the mice were exposed to an aerosolized ovalbumin solution (2.5% ovalbumin in normal saline) 30 minutes a day, 3 times per week, for 8 weeks in a whole-body inhalation exposure chamber via a SideStream jet nebulizer. More than 80% of the particles generated by this nebulizer had a diameter of 4 μ m or less (and $>$ 40% of the particles had a diameter of $\leq$ 1 μ m). Thus, it was estimated that greater than 90% of the particles would be deposited in the mouse tracheobronchial tree, which would be adequate for the development of the asthma model.¹¹ The particle concentration was maintained between 10 and 20 $\text{mg} \cdot \text{mm}^{-3}$. A schematic of the sensitization and the inhalation exposure is presented in Figure 1.

The mice in the control group were intraperitoneally administered 0.9% NaCl with alum on day 0 and day 14, and beginning on day 21, these mice were exposed to aerosolized 0.9% NaCl for 30 minutes a day, 3 times per week, for 8 weeks. Normal saline was delivered to the whole-body inhalation exposure chamber via aerosolization of compressed air using a SideStream jet nebulizer. The temperature and the relative humidity were maintained at 20°C to 25°C and 40% to 60%, respectively.

Study Drugs

Lyophilized suplatast tosilate (additive-free; Tocris Biosciences, Bristol, United Kingdom) diluted in saline was used throughout this study.

Before sensitization with ovalbumin, the groups receiving prophylaxis with suplatast tosilate were administered the drug (30 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) beginning on day (−1), for a total of 5 days.

During the final 5 days of the development of the asthma model, saline was administered to the vehicle control group, 1 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ of dexamethasone was given to group III, and 30 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ of suplatast tosilate was administered to the groups receiving treatment with suplatast tosilate. The drug administration times are shown in Figure 1.

All drugs were administered via an orogastric route. The doses of dexamethasone and suplatast tosilate, as well as the

prophylactic administration method, were determined from previously reported studies in mice.^{13–15}

Preparation of Lung Homogenates

The animals were killed with an overdose of ketamine 24 hours after the final administration of drugs, after which their lungs were removed and stored at -80°C . For analysis, the frozen lungs were sonicated in a homogenization buffer containing 0.1%, protease inhibitor cocktail (Sigma Aldrich, St Louis, MO), and 0.1 mg/mL of phenylmethanesulfonyl fluoride in phosphate buffered saline, and these homogenates were centrifuged at $15,000 \times g$ for 10 minutes. The supernatants were stored at -80°C .

Cytokine Measurement

Levels of IL-4, IL-5, and IFN- γ in the lung tissue supernatants were quantified with commercially available enzyme-linked immunosorbent assay kits (Bender MedSystems, MedSystems Diagnostics GmbH, Vienna, Australia). Detection levels were 2.0 pg/mL for IL-4, 3.3 pg/mL for IL-5, and 5.3 pg/mL for IFN- γ .

Histopathological Analysis

Histopathological analyses were performed by 2 investigators who were blinded to the study. Photomicrographs were obtained using a high-resolution video camera (Olympus DP71, Japan) attached to an Olympus BX-51 microscope (Olympus Optical, Tokyo, Japan). Histological analysis was performed using the University of Texas Health Sciences Center at San Antonio Image Tool for Windows version 3.00 software.

Tissue specimens were obtained from the midzone of the left lung of each mouse and were fixed in 10% formalin for evaluation by light microscopy. Other 1- to 2-mm³ tissue samples were obtained from adjacent regions and fixed in 2% glutaraldehyde for evaluation by electron microscopy. After fixation, the samples used for light microscopy were embedded in paraffin, and 5- μ m serial sections were prepared. Thirty consecutive sections from each mouse were randomly selected and stained via 1 of 3 different staining processes. The first 10 samples were stained with hematoxylin and eosin, which allowed the general features of the tissue to be examined, including the thickness of the epithelium and the subepithelial smooth muscle layers in the small airways. Measurements were obtained from 4 points in each airway, and because each section contained approximately 2 to 3 airways, nearly 20 or more airways were evaluated in each mouse.¹⁶

The remaining sections were stained with toluidine blue (10 sections) or with periodic acid–Schiff (PAS; 10 sections). Photomicrographs were obtained from 5 random fields from

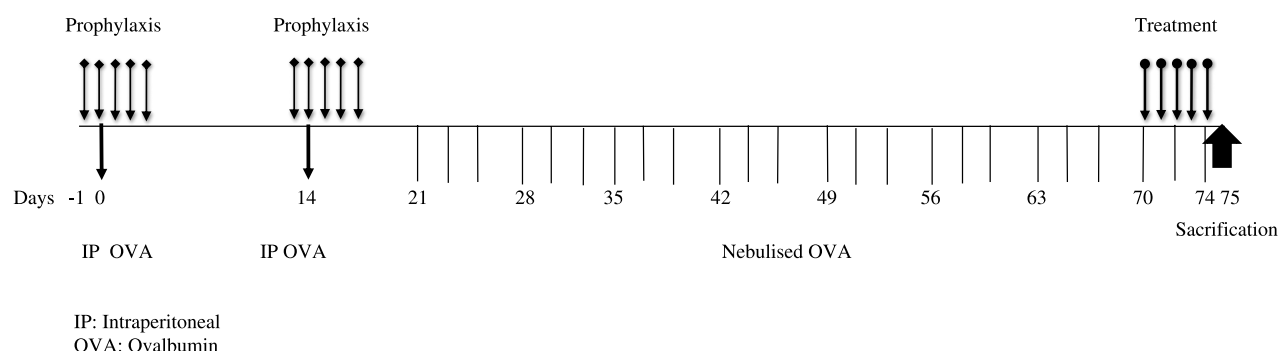


FIGURE 1. Schematic presentation of sensitization, inhalation exposure, and the drug administration times in the murine model of chronic asthma. IP indicates intraperitoneal; OVA, ovalbumin.

each section that was stained with toluidine blue. Mast cells were counted in 8 fields (20,000 μm^2 each) for each mouse. Goblet cells stained with PAS were counted in 3 to 5 randomly selected airways in all 10 sections for each mouse. The circumference of each airway was measured. The number of goblet cells in 100 μm^2 was determined by dividing the total number of goblet cells by the total airway circumference and multiplying the result by 100 for standardization.

For ultrastructural investigation, lung sections were fixed in 2.5% glutaraldehyde for 24 hours. The tissue was postfixed with osmium tetroxide, dehydrated in a graded series of alcohol, and then embedded in Araldite CY212. For each mouse, 5 to 7 thin (60–90 nm) sections were obtained with an ultramicrotome (Leica), stained with uranyl acetate and lead citrate, and examined using a transmission electron microscope (Carl Zeiss Libra 120). After that, 8 to 10 areas were photographed using a Trondle (2048 $\times$ 2048 pixels) digital camera attached to the electron microscope. The basement membrane thickness of the respiratory epithelium was measured at 20 points of equal distance, and the data were recorded in sequence.

Statistical Analysis

The Statistical Package for the Social Sciences version 15 (Statistical Package for the Social Sciences, Inc, Chicago, IL) was used for statistical analysis. Data are presented as mean $\pm$ SE for the 7 animals in each group. Comparisons between all groups were conducted using a one-way analysis of variance (post hoc Bonferroni method). $P < 0.05$ was considered to be statistically significant.

RESULTS

The histological parameters of group II (vehicle control), including the thicknesses of basement membrane and epithelial and subepithelial smooth muscle and the number of goblet and mast cells, were significantly higher than those of group I (control group; $P < 0.05$), indicating that the asthma model was successfully established (Table 1).

These parameters were all significantly lower in group III (dexamethasone), group V (treatment with suplatast tosilate), and group VI (prophylaxis and treatment with suplatast tosilate) when

compared with those of the vehicle control group ($P < 0.05$) (Table 1).

The histopathological parameters, with the exception of the number of goblet cells, were similar between the group receiving dexamethasone and the group receiving treatment with suplatast tosilate ($P = 1.000$), whereas the improvements in the group receiving both prophylaxis and treatment with suplatast tosilate were not statistically different from those in the group receiving treatment with suplatast tosilate alone ($P = 1.000$). Although the histopathological parameters of the group receiving prophylaxis with suplatast tosilate were lower than those of the vehicle control group, the differences were not statistically significant ($P > 0.05$) (Table 1).

The levels of IL-4, IL-5, and IFN- γ in the lung tissue were higher in the vehicle control group than in the control group ($P < 0.05$). Although there was a decrease in the cytokine levels for all of the study groups when compared with those of the vehicle control group, these differences were not statistically significant ($P > 0.05$) (Table 2).

Figure 2 shows the light and electron microscopy images for all of the groups.

Although the airways are normal in the control group, the histopathological findings of asthma can be seen in the airways of the vehicle control group. The improvement of the histopathological findings of asthma in all of the other groups is remarkable.

DISCUSSION

Asthma is a chronic disease that is characterized by reversible airway obstruction, airway inflammation, and remodeling. Asthma involves T_H2 -type inflammation in which IgE, mast cells, lymphocytes, eosinophils, IL-4, IL-5, and other cytokines play major roles.¹ Inflammation causes structural changes, known as airway remodeling, that are characterized by subepithelial fibrosis, thickening of the smooth muscle of the airways, and mucus hypersecretion. These changes are partly irreversible and lead to an increase in the symptoms and the severity of the disease.¹⁷ Airway hyperresponsiveness refers to the narrowing of the airways in response to various stimuli, and it is linked to both inflammation and remodeling. These mechanisms contribute to the narrowing of the airways and cause symptoms such as

TABLE 1. Comparisons of the Histological Characteristics of All Groups

	Group I	Group II	Group III	Group IV	Group V	Group VI
	Control	Vehicle control	Dexamethasone	Prophylaxis	Treatment	Treatment and Prophylaxis
	Mean $\pm$ SE (n = 7)	mean $\pm$ SE (n = 7)	mean $\pm$ SE (n = 7)	mean $\pm$ SE (n = 7)	mean $\pm$ SE (n = 7)	mean $\pm$ SE (n = 7)
Epithelium thickness, μm	13.12 $\pm$ 0.74	22.33 $\pm$ 2.25*	13.42 $\pm$ 0.22†	16.85 $\pm$ 0.71	15.36 $\pm$ 1.15†	14.64 $\pm$ 0.99†‡
Subepithelial smooth muscle thickness, μm	6.52 $\pm$ 0.41	12.36 $\pm$ 1.37*	6.93 $\pm$ 0.26†	9.44 $\pm$ 0.99	7.24 $\pm$ 0.45†§	6.71 $\pm$ 0.53†‡
No. mast cells 20,000 μm^2	0.31 $\pm$ 0.04	1.19 $\pm$ 0.10*	0.70 $\pm$ 0.05†	1.04 $\pm$ 0.09	0.60 $\pm$ 0.03†§	0.89 $\pm$ 0.04†‡
No. goblet cells 100 μm^2	0.30 $\pm$ 0.09	4.41 $\pm$ 0.18*	0.69 $\pm$ 0.09†	3.89 $\pm$ 0.24	1.65 $\pm$ 0.07†	2.75 $\pm$ 0.07†‡
Basement membrane thickness, nm	287.54 $\pm$ 16.47	703.77 $\pm$ 9.09*	415.96 $\pm$ 10.7†	522.57 $\pm$ 20.7	439.11 $\pm$ 25.58†§	513.91 $\pm$ 16.12†‡

* $P < 0.05$ significantly different from group I.

† $P < 0.05$ significantly different from group II.

‡ $P > 0.05$ not significantly different from group V.

§ $P > 0.05$ not significantly different from group III.

TABLE 2. Comparisons of IL-4, IL-5, and IFN- γ Levels

	Group I	Group II	Group III	Group IV	Group V	Group VI
	Control	Vehicle Control	Dexamethasone	Prophylaxis	Treatment	Treatment and Prophylaxis
	mean $\pm$ SE (n = 7)	mean $\pm$ SE (n = 7)	mean $\pm$ SE (n = 7)	mean $\pm$ SE (n = 7)	mean $\pm$ SE (n = 7)	mean $\pm$ SE (n = 7)
IL-4, pg/mL	61.03 $\pm$ 13.68	184.40 $\pm$ 30.15*	127.82 $\pm$ 30.61	170.14 $\pm$ 38.71	136.70 $\pm$ 17.23	134.36 $\pm$ 20.98
IL-5, pg/mL	47.14 $\pm$ 11.77	87.09 $\pm$ 14.59*	82.87 $\pm$ 8.70	98.12 $\pm$ 21.44	84.51 $\pm$ 10.52	67.85 $\pm$ 12.62
IFN- γ , pg/mL	174.91 $\pm$ 24.39	302.40 $\pm$ 34.92*	285.62 $\pm$ 21.74	294.03 $\pm$ 34.55	290.30 $\pm$ 26.81	276.39 $\pm$ 27.88

* $P < 0.05$ when compared with the control group.

wheezing, cough, chest tightness, and breathlessness.¹⁸ The goal of treatment is to ameliorate symptoms by reducing inflammation and preventing remodeling. However, no current treatment can permanently suppress inflammation and prevent remodeling, and therefore, new treatment options continue to be investigated.⁴

In this study, we investigated the effects of suplatast tosilate on the histopathological findings of asthma. We examined the thickness of the epithelia and mast cell counts to assess inflammation, and we determined the thicknesses of the basement membrane and the subepithelial smooth muscle as well as the number of goblet cells to assess the extent of remodeling. As a result of this study, we determined that suplatast tosilate was effective in reducing all of these changes related to inflammation and remodeling.

Previous animal studies with asthma models have shown that suplatast tosilate was effective on goblet cell metaplasia, prevented the recruitment of eosinophils and lymphocytes to the airways, and reduced bronchial hyperreactivity.^{19,20} In studies on humans with asthma, suplatast tosilate reduced eosinophil

counts, goblet cell hyperplasia, and levels of IL-13 and had anti-inflammatory effects as evidenced by improved symptoms, pulmonary function tests, and exhaled nitric oxide levels.^{8,21–23}

Although these studies suggest that suplatast tosilate is effective in the treatment of asthma, to our knowledge, there have not been any studies reporting its effects on the histopathological findings of asthma in humans or animals, with the exception of goblet cell metaplasia. Our study is the first to report that suplatast tosilate is effective in improving all of the histopathological findings related to inflammation and remodeling in asthma.

Recently, the prevention of asthma has gained importance because none of its current treatments are fully effective in improving inflammation and remodeling. Normally, there is a balance between the T_H1 and T_H2 immune response. This balance is disrupted in asthma and allergic diseases, in which the T_H2 -type immune response is dominant.²⁴ Suplatast tosilate has been shown to suppress the T_H2 -type immune response in both in vivo and in vitro studies but does not affect the T_H1 immune response.²⁵ Because of this, we hypothesized that suplatast

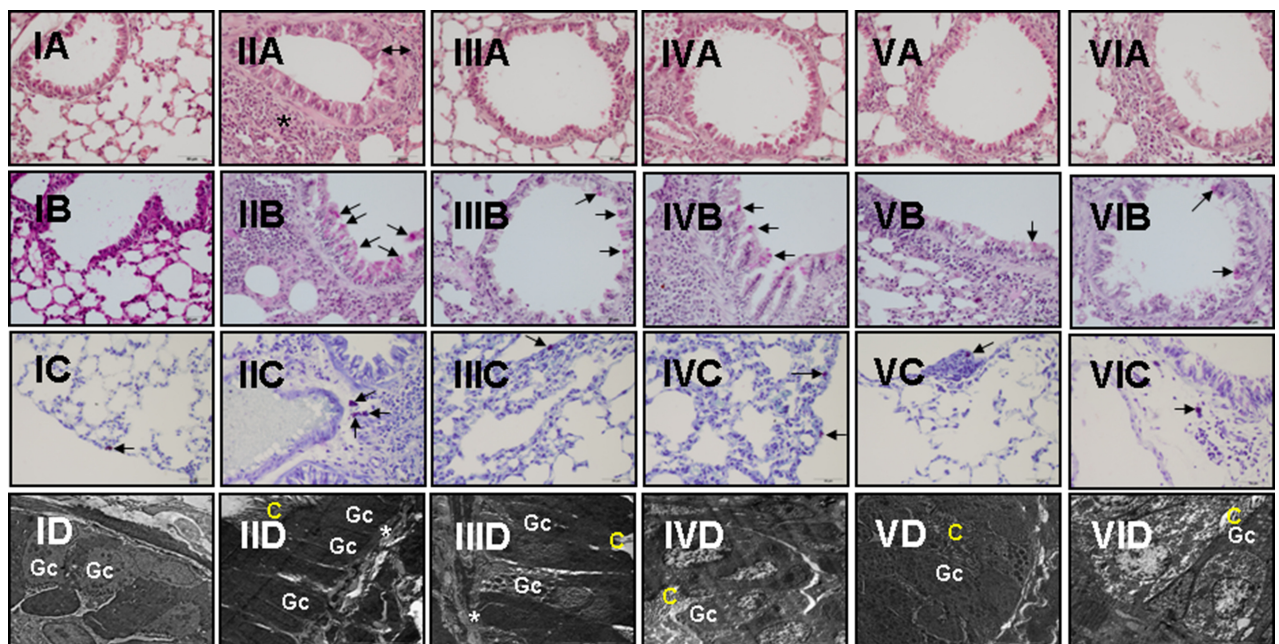


FIGURE 2. Histological views of the airways. I, Group I (control); II, group II (vehicle control); III, group III (dexamethasone); IV, group IV (prophylaxis with suplatast tosilate); V, group V (treatment with suplatast tosilate); VI, group VI (prophylaxis and treatment with suplatast tosilate). A, Hematoxylin and eosin; B, PAS; C, toluidine blue; D, electron microscopy. Hematoxylin and eosin: *peribronchial infiltration; bidirectional arrow, subepithelial smooth muscle (IIA). Periodic acid–Schiff: arrows indicate goblet cells. Toluidine blue: arrows indicate mast cells. Electron microscopy: C indicates cilia; GC, goblet cell; white star, the basement membrane.

tosilate might prevent the development of asthma. To test this hypothesis, we applied suplatast tosilate as a prophylactic before the induction of ovalbumin-induced asthma. We determined that the prophylactic administration of suplatast tosilate does not prevent the development of the histopathological findings of asthma. To our knowledge, there are no other studies reporting the effects of prophylaxis with suplatast tosilate on the histopathological findings of inflammation and remodeling in asthma. In a pilot study in Japan, either suplatast tosilate or ketotifen was administered for 2 years to children with atopic dermatitis and food allergies, both of whom are at high risk for developing asthma, and the effects of these 2 drugs on the development of asthma were investigated. Of the children in the group treated with ketotifen, 65.6% developed asthma, whereas only 20% of the children in the group treated with suplatast tosilate developed the disease. This difference was statistically significant. The number of eosinophils, total IgE, and T_H1/T_H2 cytokine ratios were also significantly different between the 2 groups.¹⁰ The results of this study are contradictory to the results of our study. One reason for the difference in results may be because in the study with the children, the assessments were made while the patients were taking the medication, whereas in our study, assessments were made after the cessation of the drug. To accurately detect the effects of prophylactics, the assessments should be made after the discontinuation of the drug. In addition, the results of this study were based on the clinical development of asthma and not on histological analyses. Previous studies have shown that there is evidence of remodeling in the airways long before the clinical development of asthma.²⁶ Because of these aforementioned reasons, we believe that it was not accurate to conclude that prophylactic suplatast tosilate has positive effects on the development of asthma.

In our study, we followed a previously reported prophylaxis protocol. However, if we had extended the duration of prophylaxis, we may have seen a more profound effect. To our knowledge, there are no reports regarding the appropriate duration of prophylaxis. Because of this, we chose to administer prophylaxis for a duration twice as long as the treatment, which would be equal to 1 year in humans. However, using our protocol, there were no additional benefits of the prophylactic administration of suplatast tosilate to those gained with its treatment.

As a result, further studies regarding suplatast tosilate should include more patients, should study the duration of prophylaxis, should evaluate the results after discontinuation of the prophylactic treatment, and should include histopathological analyses.

We also investigated the effects of suplatast tosilate on T_H2 -type cytokines (IL-4 and IL-5) and on the T_H1 -type cytokines (IFN- γ). We found that cytokine levels in the asthma group were significantly higher than those in the control group. Although we detected a decrease in cytokine levels in all of the study groups as compared with the vehicle control group, this difference was not statistically significant. Earlier reports have shown that suplatast tosilate reduces the levels of T_H2 -type cytokines.^{6,25,27} In this study, although histological findings and cytokine levels were improved, we detected no significant changes in cytokine levels. This may be because we had low sample numbers.

Our study has some limitations. This study was conducted on animals, and therefore, the results cannot be compared directly with those in humans. In addition, although we evaluated the effects of the drugs histopathologically, we neglected to measure airway hyperreactivity. Another limitation is that the effects of suplatast tosilate on the histopathological findings of asthma were not supported by an improvement in cytokine levels.

In conclusion, suplatast tosilate was found to be effective in improving the histopathological parameters in a mouse model of chronic asthma. The use of suplatast tosilate as a prophylactic had no effect on the development of the histopathological findings of asthma, nor did it provide any additional contribution to the treatment before the development of asthma. The results of this study suggest that suplatast tosilate could be effective in the treatment of asthma, but further studies are needed to determine its effectiveness in the prophylaxis of asthma.

REFERENCES

1. From the global strategy for asthma management and prevention. Global Initiative for Asthma (GINA), 2011. Available at: <http://www.ginasthma.org>. Accessed November 3, 2012.
2. Bacharier LB, Boner A, Carlsen KH, et al., for the European Pediatric Asthma Group. Diagnosis and treatment of asthma in childhood: a PRACTALL consensus report. *Allergy*. 2008;63:5–34.
3. Holgate ST, Arshad HS, Roberts GC, et al. A new look at the pathogenesis of asthma. *Clin Sci (Lond)*. 2009;118:439–450.
4. Folli C, Descalzi D, Scordamaglia F, et al. New insights into airway remodelling in asthma and its possible modulation. *Curr Opin Allergy Clin Immunol*. 2008;8:367–375.
5. Koda A, Yanagihara Y, Matsuura N. IPD-1151T: a prototype drug for IgE antibody synthesis modulation. *Agents Actions Suppl*. 1991;34:369–378.
6. Oda N, Minoguchi K, Yokoe T, et al. Effect of suplatast tosilate (IPD-1151T) on cytokine production by allergen-specific human Th1 and Th2 cell lines. *Life Sci*. 1999;65:763–770.
7. Yanagihara Y, Kuniwa M, Ikizawa K, et al. Suppression of IgE production by IPD-1151T (suplatast tosilate), a new dimethylsulfonium agent: (2). Regulation of human IgE response. *Jpn J Pharmacol*. 1993;61:31–39.
8. Zhao GD, Yokoyama A, Kohno N, et al. Effect of suplatast tosilate (IPD-1151T) on a mouse model of asthma: inhibition of eosinophilic inflammation and bronchial hyperresponsiveness. *Int Arch Allergy Immunol*. 2000;121:116–121.
9. Tamaoki J, Kondo M, Sakai N, et al. Effect of suplatast tosilate, a Th2 cytokine inhibitor, on steroid-dependent asthma: a double-blind randomised study. Tokyo Joshi-Idai Asthma Research Group. *Lancet*. 2000;356:273–278.
10. Yoshihara S, Ono M, Yamada Y, et al. Early intervention with suplatast tosilate for prophylaxis of pediatric atopic asthma: a pilot study. *Pediatr Allergy Immunol*. 2009;20:486–492.
11. Temelkovski J, Hogan SP, Shepherd DP, et al. An improved murine model of asthma: selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolised allergen. *Thorax*. 1998;53:849–856.
12. Herz U, Lupp U, Da Palma JC, et al. The relevance of murine animal models to study the development of allergic bronchial asthma. *Immunol Cell Biol*. 1996;74:209–217.
13. Henderson WR Jr, Chiang GK, Tien YT, et al. Reversal of allergen-induced airway remodeling by CysLT1 receptor blockade. *Am J Respir Crit Care*. 2006;173:718–728.
14. Konno S, Asano K, Gonokami Y, et al. Effect of IPD-1151T (Suplatast Tosilate) on airway hyperresponsiveness in mice. *Arerugi*. 1995;44:556–561.
15. Lee Y, Fu C, Chiang B. Administration of interleukin-12 exerts a therapeutic instead of a long-term preventive effect on mite Der p I allergen-induced animal model of airway inflammation. *Immunology*. 1999;97:232–240.

16. Firinci F, Karaman M, Baran Y, et al. Mesenchymal stem cells ameliorate the histopathological changes in a murine model of chronic asthma. *Int Immunopharmacol*. 2011;11:1120–1126.
17. Bousquet J, Jeffery PK, Busse WW, et al. Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med*. 2000;161:1720–1745.
18. Wang L, McParland BE, Paré PD. The functional consequences of structural changes in the airways: implications for airway hyperresponsiveness in asthma. *Chest*. 2003;123(3 Suppl): 356S–362S.
19. Shim JJ, Dabbagh K, Takeyama K, et al. Suplatast tosilate inhibits goblet-cell metaplasia of airway epithelium in sensitized mice. *J Allergy Clin Immunol*. 2000;105:739–745.
20. Asano K, Mizutani T, Shimane T, et al. The inhibitory effect of anti-allergic agent suplatast tosilate (IPD-1151T) on methacholine- and allergen-induced bronchoconstriction in sensitized mice. *Mediators Inflamm*. 2000;9:77–84.
21. Hoshino M, Fujita Y, Saji J, et al. Effect of suplatast tosilate on goblet cell metaplasia in patients with asthma. *Allergy*. 2005;60:1394–1400.
22. Wada M, Nagata S, Kudo T, et al. Effect of suplatast tosilate on antileukotriene non-responders with mild-to-moderate persistent asthma. *Allergol Int*. 2009;58:389–393.
23. Shiga M, Horiguchi T, Kondo R, et al. Long-term monotherapy with suplatast tosilate in patients with mild atopic asthma: a pilot comparison with low-dose inhaled fluticasone. *Asian Pac J Allergy Immunol*. 2011;29:134–142.
24. Macaubas C, Sly PD, Burton P, et al. Regulation of T-helper cell responses to inhalant allergen during early childhood. *Clin Exp Allergy*. 1999;29:1223–1231.
25. Matsumoto K, Hayakawa H, Ide K, et al. Effects of suplatast tosilate on cytokine profile of bronchoalveolar cells in allergic inflammation of the lung. *Respirology*. 2002;7:201–207.
26. Bush A. How early do airway inflammation and remodeling occur? *Allergol Int*. 2008;57:11–19.
27. Yoshihara S, Fukuda H, Arisaka O. Usefulness of suplatast tosilate, a T_H2 cytokine inhibitor based on the T_H1/T_H2 ratio for allergic disease in children: a retrospective study. *Arzneimittelforschung*. 2011; 61:421–424.