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Th2 Cytokine Inhibitor Suplatast Tosilate Inhibits Antigen-Induced Mucus Hypersecretion in the Nasal Epithelium of Sensitized Rats

Shino Shimizu, MD; Reiko Hattori, MD; Yuichi Majima, MD; Takeshi Shimizu, MD

Objectives: Th2 cytokines such as interleukin (IL) 4 and IL-13 are potential mediators for mucus hypersecretion in allergic inflammation. To elucidate the functions of Th2 cytokines in allergic rhinitis, we examined the in vivo effects of the Th2 cytokine inhibitor suplatast tosilate on mucus hypersecretion and eosinophil infiltration in rat nasal epithelium.

Methods: We induced hypertrophic and metaplastic changes in goblet cells in the nasal epithelium of ovalbumin-sensitized rats by intranasal challenge with ovalbumin. The effects of orally administered suplatast tosilate on mucus production and eosinophil infiltration were examined.

Results: Suplatast tosilate (30 and 100 mg/kg) dose-dependently inhibited ovalbumin-induced mucus production and eosinophil infiltration. These suppressions of mucus production and eosinophil infiltration were only effective when suplatast tosilate was given in the effector phase; administration in the induction phase resulted in no effect.

Conclusions: These results indicate that Th2 cytokines are important mediators of mucus hypersecretion and eosinophil infiltration in allergic rhinitis. Suplatast tosilate may be useful for the treatment of allergic rhinitis by attenuating the inflammation of the effector phase.

Key Words: allergy, eosinophil, goblet cell, mucus hypersecretion, rat nose, Th2 cytokine.

INTRODUCTION

Hypersecretion of mucus is an important characteristic of allergic inflammation such as allergic rhinitis and bronchial asthma. Ovalbumin (OVA)-sensitized animals have been used to study the pathophysiologic changes in allergic inflammation in the lower airways, including airway hyperresponsiveness, airway eosinophilia, plasma extravasation, bronchocontraction, and mucus hypersecretion. A variety of allergic mediators and inflammatory cells are capable of stimulating mucus synthesis and secretion. Recent studies implicate the Th2 cytokines interleukin (IL) 4 and IL-13 in goblet cell metaplasia in asthma.¹⁻⁷ However, little attention has been paid to the mechanism by which the antigen induces mucus hypersecretion in allergic rhinitis.

Suplatast tosilate is a Th2 cytokine inhibitor that is used for the treatment of patients with asthma and allergic rhinitis in Japan.⁸⁻¹¹ This agent suppresses nasal symptoms such as sneezing, runny nose, and stuffy nose in patients with allergic rhinitis.^{8,9} Suplatast tosilate inhibits the synthesis of Th2 cyto-

kines IL-4, IL-5, and IL-13 in Th2-like cells in vitro and in vivo, and suppresses the IgE synthesis with no direct effect on B cells.¹²⁻¹⁴ Suplatast tosilate has been reported to attenuate antigen-induced goblet cell metaplasia and eosinophil infiltration in the lower airways of sensitized animals.¹⁵⁻¹⁷

In the present study, to elucidate the role of Th2 cytokines in mucus hypersecretion in allergic rhinitis, we induced hypertrophic and metaplastic changes in goblet cells in the nasal epithelium of OVA-sensitized rats by intranasal challenge with OVA. The effects of orally administered suplatast tosilate on mucus hypersecretion and eosinophil infiltration were then examined.

MATERIALS AND METHODS

Sensitization and Intranasal Challenge With OVA. All experiments were approved by the Committee for the Care and Use of Laboratory Animals of Mie University School of Medicine. Sensitization and challenge of rats were performed as previously reported.¹⁸ Fisher-344 rats (6 to 7 weeks old, male)

From the Department of Otorhinolaryngology, Shiga University of Medical Science, Otsu (S. Shimizu, T. Shimizu), and the Department of Otorhinolaryngology-Head and Neck Surgery, Mie University School of Medicine, Tsu (Hattori, Majima), Japan. This study was supported by Grant-in-Aid for General Scientific Research No. 20591999 from the Ministry of Education, Culture, Sports, Science and Technology of Japan, Tokyo.

Correspondence: Takeshi Shimizu, MD, Dept of Otorhinolaryngology, Shiga University of Medical Science, Seta, Tsukinowa, Otsu, Shiga 520-2192, Japan.

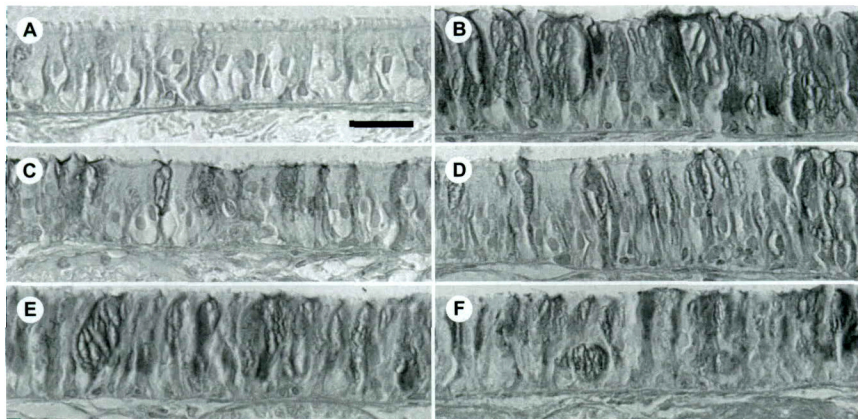


Fig 1. Effects of suplatast tosilate (suplatast) orally administered in both induction phase and effector phase on mucus production in nasal epithelium of ovalbumin (OVA)-sensitized rats. Bar = 30 μ m. **A)** Saline-instilled control. **B)** OVA-challenged rat. **C)** OVA-challenged rat treated with 30 mg/kg suplatast. **D)** OVA-challenged rat treated with 100 mg/kg suplatast. **E)** OVA-challenged rat treated with 100 mg/kg suplatast administered only in induction phase (days 1 to 14). **F)** OVA-challenged rat treated with 100 mg/kg suplatast administered only in effector phase (days 19 to 21). Oral administrations of suplatast inhibited OVA-induced mucus production in rat nasal epithelium. Suppression of mucus production was only effective when suplatast was given in effector phase.

were immunized with an intraperitoneal injection of 0.1 mL saline solution containing 200 μ g of OVA (grade V; Sigma Chemical Co, St Louis, Missouri) and 10 mg of alum on days 1, 2, 3, and 10. Heat-killed *Bordetella pertussis* bacilli (10^{10} in 50 μ L saline solution; Wako Pure Chemical Industries, Ltd, Osaka, Japan) were given by a foot pad injection on day 1 as an adjuvant.

On day 19, the rats were anesthetized with ether, and 0.1 mL of saline solution containing 5 mg of OVA was instilled into both airways of the nasal cavity once a day for 3 days. Sham-challenged rats received 0.1 mL of saline solution in the same manner.

Tissue Preparation. The rats were painlessly killed with an intraperitoneal overdose of sodium pentobarbital at 24 hours after the last intranasal challenge. The head of each rat was removed and fixed in 10% neutral buffered formalin for 3 days, then decalcified in 5% trichloroacetic acid for 7 days. The nasal cavity was transversely sectioned at the level of incisive papilla of the hard palate.¹⁹ The tissue block was embedded in paraffin.

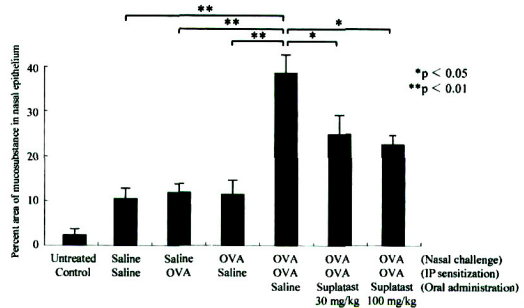
Morphometry. Paraffin sections 5 μ m thick were stained with Alcian blue (pH 2.6)-periodic acid-Schiff stain (AB-PAS) and hematoxylin. The percent area of AB-PAS-stained mucosubstance in the epithelial surface was determined by an image ana-

lyzer (SP500; Olympus, Tokyo, Japan) in nasal epithelium over 2 mm (1 mm each side of the nasal septum $\times 2$) of the basal lamina at the center of the septal cartilage. The infiltrating eosinophils in nasal mucosa were examined by Hansel staining. The number of eosinophils in the nasal septal mucosa was counted over both sides of septal cartilage by means of an oil immersion objective lens (magnification $\times 1,000$).

Treatment With Suplatast Tosilate. Suplatast tosilate ((+)-[2-[4-(3-ethoxy-2-hydroxypropoxy) phenylcarbonyl] ethyl] dimethylsulfonium *p*-toluenesulfonate) was supplied by Taiho Pharmaceutical Co, Ltd (Tokyo). The rats were orally administered 1 mL of saline solution containing 30 or 100 mg/kg of suplatast tosilate at the time of sensitization (induction phase) and challenge (effector phase). For treatment in the induction phase, suplatast tosilate was administered beginning at the same time as the intraperitoneal immunization and continued once a day for 14 days. For treatment in the effector phase, the rats received suplatast tosilate at the same time as the intranasal challenge at days 19, 20, and 21.

Statistics. All data are expressed as mean $\pm$ SD. The difference between variables was analyzed by the Mann-Whitney U test. Probability (p) values of less than 0.05 were considered statistically significant.

Fig 2. Effects of suplatast (30 and 100 mg/kg) on antigen-induced mucus production ($n = 6$). Oral administration of suplatast dose-dependently inhibited antigen-induced mucus production. IP — intraperitoneal.



RESULTS

Intranasal instillation with OVA for 3 consecutive days had induced hypertrophic and metaplastic changes in the goblet cells in the nasal septal epithelium of the OVA-sensitized rats 24 hours after the last challenge. Only a few goblet cells were observed in untreated or saline-instilled controls. The effects of suplatast tosilate on antigen-induced mucus production are shown in Fig 1. Suplatast tosilate orally administered in both the induction phase and the effector phase inhibited OVA-induced mucus production in rat nasal epithelium.

Quantitative changes in the percent area of mucosubstance in this epithelium are described in Fig 2. Intraepithelial mucosubstance was increased significantly by intranasal challenge with OVA in OVA-sensitized rats. This intraepithelial mucus production did not occur in OVA-sensitized rats challenged with saline solution. Oral administration of suplatast tosilate (30 and 100 mg/kg) dose-dependently inhibited OVA-induced mucus production.

Intranasal instillation of OVA in OVA-sensitized rats had induced significant infiltration of eosino-

phils into the nasal septal mucosa 24 hours after the last challenge. Suplatast tosilate significantly inhibited antigen-induced eosinophil infiltration in a dose-dependent manner (Fig 3).

To elucidate the mechanism by which suplatast tosilate inhibited antigen-induced mucus production, we examined the effects of suplatast tosilate administered only in the induction phase or in the effector phase. The suppression of mucus production was only effective when the suplatast tosilate was given in the effector phase (at the same time as OVA challenge for 3 days), whereas administration in the induction phase (at the same time as OVA immunization for 14 days) resulted in no effect (Fig 4). The suppression of eosinophil infiltration was also only effective when the suplatast tosilate was given in the effector phase (Fig 5).

DISCUSSION

In the present study, intranasal instillation with OVA induced hypertrophic and metaplastic changes in goblet cells and mucosal infiltration of eosinophils in the nasal epithelium of OVA-sensitized rats. Oral administration of suplatast tosilate significant-

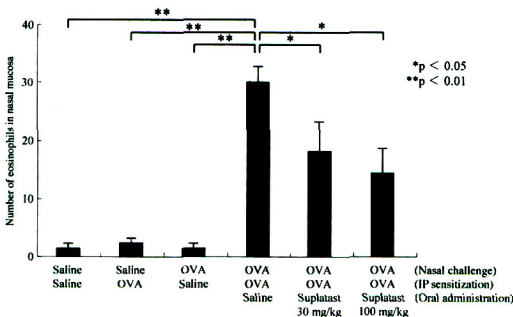


Fig 3. Effects of suplatast (30 and 100 mg/kg) on antigen-induced eosinophil infiltration in nasal epithelium of OVA-sensitized rats ($n = 6$). Intranasal challenge with OVA induced eosinophil infiltration, and suplatast dose-dependently inhibited antigen-induced eosinophil infiltration.

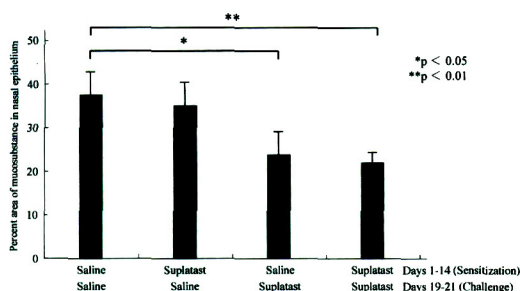


Fig 4. Effects of suplatast (100 mg/kg) administered only in induction phase or in effector phase on OVA-induced mucus production in nasal epithelium of OVA-sensitized rats ($n = 6$). Suppression of antigen-induced mucus production was only effective when suplatast was given at same time as OVA challenge (days 19 to 21); administration at same time as OVA immunization (days 1 to 14) had no effect.

ly inhibited antigen-induced mucus production and eosinophil infiltration in a dose-dependent manner (30 and 100 mg/kg). This is the first report to show the *in vivo* effects of suplatast tosilate on antigen-induced mucus hypersecretion and eosinophil infiltration in the nasal epithelium of sensitized animals.

The Th2 cytokines IL-4 and IL-13 play an important role in the mucus hypersecretion of allergic inflammation in the lower airways. Local administration of IL-4 or IL-13 induces goblet cell metaplasia in the airway epithelium.¹⁻³ Pretreatment with neutralizing IL-4 receptor antibody inhibits antigen-induced goblet cell metaplasia in mice,⁴ whereas IL-4 transgenic mice that specifically express IL-4 in the airways develop goblet cell metaplasia.⁵ Selective neutralization of IL-13 also attenuates antigen-induced mucus production in mice.^{3,6} Interleukin 4 and IL-13 share a receptor component IL-4R α and STAT6 signaling pathway. The importance of the IL-4 signaling pathway in mucus hypersecretion is supported by the findings that IL-4R α -deficient or STAT6-deficient mice sensitized and challenged with antigen undergo abolishment of airway goblet cell metaplasia.^{6,7}

Suplatast tosilate, a Th2 cytokine inhibitor, inhibits the synthesis of IL-4, IL-5, and IL-13 in Th2-

like cells, and suppresses IgE production.¹²⁻¹⁴ This agent suppressed the local production of IL-4, IL-5, and IL-13 in patients with perennial allergic rhinitis⁹ and in a rat model of bronchial asthma.^{15,20} Previous studies demonstrated that suplatast tosilate inhibited antigen-induced goblet cell metaplasia and eosinophil infiltration in the bronchial epithelium of sensitized mice, correlated with the suppression of IL-4 and IL-13 production in the bronchoalveolar fluid.¹⁵ Goblet cell metaplasia in bronchial biopsy specimens taken from asthma patients was suppressed after 3 months' treatment with suplatast tosilate in association with the down-regulation of IL-4- and IL-13-positive cells.¹¹ These findings indicate that the Th2 cytokines IL-4 and IL-13 are important mediators in antigen-induced mucus hypersecretion and that suplatast tosilate inhibited the goblet cell metaplasia through the inhibition of IL-4 and IL-13 production.

The present study demonstrated a similar important role of IL-4 and IL-13 in antigen-induced mucus hypersecretion in the lower and upper airways. We also found that the suppression of mucus production was effective only when suplatast tosilate was given in the effector phase. Administration in the induction phase resulted in no effect. These re-

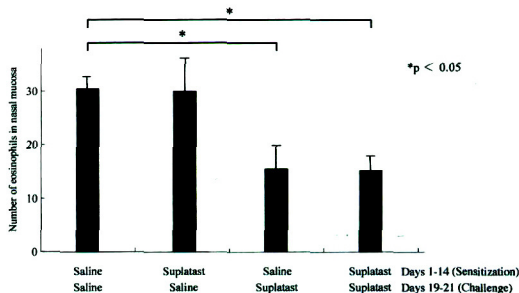


Fig 5. Effects of suplatast (100 mg/kg) administered only in induction phase or in effector phase on OVA-induced eosinophil infiltration in nasal epithelium of OVA-sensitized rats ($n = 6$). Suppression of antigen-induced eosinophil infiltration was only effective when suplatast was given at same time as OVA challenge (days 19 to 21); administration at same time as OVA immunization (days 1 to 14) had no effect.

sults indicate that suplatast tosilate inhibits mucus hypersecretion through the suppression of IL-4 and IL-13 production in the effector phase of allergic inflammation, and not through the inhibition of IgE production in the induction phase.

In our previous study, *cysLT1* antagonist inhibited antigen-induced mucus production and goblet cell degranulation in the nasal epithelium of sensitized rats, indicating that cysteinyl leukotrienes (leukotrienes C₄, D₄, and E₄) are also important mediators for mucus hypersecretion in allergic rhinitis.^{18,21} However, eosinophil infiltration was not affected by the *cysLT1* antagonist, and goblet cell metaplasia and mucus production were not related to the eosinophil infiltration.¹⁴

Both IL-5 and IL-13 are important cytokines for antigen-induced eosinophil infiltration.^{3,6,22} In the present study, suplatast tosilate inhibited antigen-induced eosinophil infiltration in the nasal epithelium of sensitized rats. This finding is consistent with previous studies showing that suplatast tosilate inhibited eosinophil infiltration in lung tissues of sensitized and challenged mice¹⁵⁻¹⁷ and in bronchial biopsy specimens of asthma patients.¹¹ The mech-

anism by which suplatast tosilate inhibits antigen-induced eosinophil infiltration may be explained by the inhibition of IL-5 and IL-13 production in bronchoalveolar fluid, and by the inhibition of IL-5 messenger RNA expression in lung tissue.¹⁶ The present study demonstrates that the inhibition of eosinophil infiltration by suplatast tosilate is also caused by the suppression of Th2 cytokine production in the effector phase of allergic inflammation.

In conclusion, we have induced hypertrophic and metaplastic changes in the nasal epithelial goblet cells of OVA-sensitized rats by intranasal OVA challenge, and we have demonstrated in this model that antigen-induced mucus production and eosinophil infiltration are significantly inhibited by suplatast tosilate. This suppression was only effective when suplatast tosilate was given in the effector phase. These results indicate that Th2 cytokines IL-4, IL-5, and IL-13 are important mediators for antigen-induced mucus hypersecretion and eosinophil infiltration in the nasal epithelium, and that suplatast tosilate has a potential role in the treatment of allergic airway inflammation. It is hoped that such studies will improve the understanding and treatment of mucus hypersecretion in allergic rhinitis.

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