

Prophylactic Effects of the Histamine H₁ Receptor Antagonist Epinastine and the Dual Thromboxane A₂ Receptor and Chemoattractant Receptor-Homologous Molecule Expressed on Th2 Cells Antagonist Ramatroban on Allergic Rhinitis Model in Mice

Yuh SUZUKI,^a Toshio INOUE,^b Atsuki YAMAMOTO,^a and Yukio SUGIMOTO^{*,a}

^aDepartment of Medicinal Pharmacology, Division of Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences; 1-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530, Japan; and

^bDepartment of Pharmacotherapy, Nihon Pharmaceutical University; 10281 Ina-machi Komuro, Kitaadachigun, Saitama 362-0806, Japan. Received October 7, 2010; accepted January 13, 2011

The prophylactic use of anti-allergic drugs has been proposed to be effective in the treatment of seasonal allergic rhinitis in humans. However, there is little information regarding the prophylactic effect of thromboxane A₂ (TXA₂) receptor antagonist on allergic rhinitis. Recent studies revealed that a TXA₂ receptor antagonist ramatroban could block the prostaglandin D₂ (PGD₂) receptor and chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2). In the present study, we investigated the prophylactic effects of the histamine H₁ receptor antagonist epinastine and the TXA₂ receptor antagonist ramatroban and seratrodist on mouse models of allergic rhinitis. Female BALB/c mice were sensitized by an intraperitoneal injection of ovalbumin and alum on days 0, 5, 14 and 21. Seven days later, mice were sensitized by intranasal application of ovalbumin thrice a week. Drugs were administered once a day from day 22. The severity of allergic rhinitis was assessed by determining the extent of 2 nasal allergic symptoms (sneezing and nasal rubbing). Histamine sensitivity and eosinophil infiltration into the nasal mucosa were also determined. Epinastine and ramatroban significantly reduced nasal symptoms and the number of eosinophils in the nasal mucosa. Seratrodist showed no effect on nasal symptoms and eosinophil infiltration into the nasal mucosa. In addition, histamine sensitivity was reduced by epinastine and ramatroban. These results indicate that epinastine and ramatroban induce the prophylactic effect on allergic rhinitis.

Key words rhinitis; histamine H₁ receptor; thromboxane A₂ receptor; epinastine; ramatroban; eosinophil

Allergic rhinitis is an inflammatory disease of the nasal mucosa characterized by nasal itching, sneezing, rhinorrhea and nasal obstruction.¹⁾ The pathogenesis of the nasal allergic reaction initially involves the interaction of allergens with a specific immunoglobulin E (IgE) antibody that is bound to the surface of mast cells in the nasal mucosa. As a result, the release of mediators including histamine, leukotrienes (LTs), thromboxane A₂ (TXA₂), platelet activating factor (PAF) and cytokines, which are responsible for allergic signs, may occur.²⁾ Histamine is one of the major mediators of the allergic response and binds to H₁ receptors on nociceptive type-C nerves.³⁾ Stimulation of H₁ receptors leads to the sensation of itching, sneezing, and, *via* reflex stimulation of efferent vagal pathways, glandular secretion and hence anterior rhinorrhea.^{4,5)} Not only histamine, but lipid mediators such as LTs, TXA₂ and PAF are also responsible for nasal obstruction due to oedema of the nasal mucosa membrane.

The TXA₂ is a potent inflammatory mediator, and its receptor expressed in vascular smooth muscle cell and submucosal glands in the human nasal mucosa.⁶⁾ In guinea pig models of allergic rhinitis, it has been demonstrated that the concentration of TXB₂, a stable TXA₂ metabolite, is elevated in nasal cavity lavage fluid.^{7,8)} Accordingly, it is thought that the TXA₂ receptor has an important role in allergic rhinitis.

Pollinosis is seasonal allergic rhinitis due to pollen antigens. The number of patients increases every year, and it appears to be common, occurring in approximately 19 million persons in the United States.⁹⁾ Daily activities and the quality of life are reduced during the pollen season due to rhinoconjunctival symptoms.¹⁰⁾ It has been reported that starting the

treatment for pollinosis with anti-allergic drugs before the initial day of the pollen scattering can relieve the severity of nasal symptoms during the pollen season.^{11,12)} Histamine H₁ receptor antagonists may prevent symptoms of allergic rhinitis while the prophylactic effect of TXA₂ receptor antagonists remains unclear. It is difficult to accurately predict the day when the pollen is scattered, and the quantity of scattered pollen is different by an area and a year. In addition, there is little scientific information on the early treatment. Therefore, it is necessary to study early treatment in an experimental model of allergic rhinitis. In the present study, we investigated the prophylactic effects of the histamine H₁ receptor antagonist epinastine and the TXA₂ receptor antagonist ramatroban and seratrodist in experimental mouse models of allergic rhinitis.

MATERIALS AND METHODS

Animals BALB/c mice (female, 6 weeks of age) were obtained from Japan SLC (Shizuoka, Japan). The animals were housed in an air-conditioned room with controlled temperature (24±2 °C) and humidity (55±15%). Food and water were provided *ad libitum*. All procedures involving the animals were conducted in accordance with the Guidelines for Animal Experiments at the Okayama University Advanced Science Research Centers.

Reagents and Drugs The following reagents were obtained from the sources shown in parentheses: ovalbumin (Grade VII, Sigma, St. Louis, MO, U.S.A.), aluminum hydroxide gel (LSL, Tokyo, Japan) and histamine dihy-

* To whom correspondence should be addressed. e-mail: sugimoto@pharm.okayama-u.ac.jp

drochloride (Sigma) were dissolved in saline. Epinastine hydrochloride (Böehringer Ingelheim, Ingelheim, Germany), ramatroban (Bayer Yakuhin, Osaka, Japan) and seratrodast (Takeda Pharmaceutical Co., Ltd., Osaka, Japan) were suspended in distilled water.

Sensitization Female BALB/c mice were sensitized on days 0, 5, 14 and 21 by the intraperitoneal injection of ovalbumin (1 μ g) in 200 μ l of saline containing aluminum hydroxide gel (100 μ g) as an adjuvant. Then, local sensitization was performed 3 times a week by nasal instillation of 2 μ l of ovalbumin solution (100 mg/ml) into the each nasal cavity using a micropipette from day 28 until day 100.

Evaluation of Nasal Symptoms From day 28 until day 70 once a week, ovalbumin-induced nasal symptoms evaluated. Before the experiment, the animals were put into an observation cage (31 $\times$ 25 $\times$ 18 cm) for 10 min for acclimatization. Immediately after nasal instillation of 2 μ l of ovalbumin solution (100 mg/ml) or histamine solution into the each nasal cavity, the animals were returned to the observation cage (1 animal/cage), and sneezing and nasal rubbing were counted for 30 min, respectively.

Treatment with Drugs Epinastine (10 mg/kg/d), ramatroban (30 mg/kg/d) and seratrodast (30 mg/kg/d) were administered orally once a day from day 22 until day 99. On the day of challenge, each drug was administered orally 1 h before the challenge. However, on the day of evaluation of the nasal symptoms, each drug was administered after the experiment to except the direct inhibitory effect. The daily doses of the drugs were determined according to previous reports that could sufficiently inhibited nasal symptoms and some of allergic pathogenesis in ovalbumin-sensitized rodent models.^{13,14)}

Measurement of Nasal Responsiveness to Histamine To estimate the nasal responsiveness to histamine, nasal symptoms were measured after intranasal instillation of a histamine solution. On day 71, 2 μ l of vehicle (saline) or of increasing doses of histamine in solution (0.1, 1, 10 nmol/mouse) was consecutively inserted into each nasal cavity at 60 min intervals. Sneezing and nasal rubbing were counted for 10 min after the instillation of each dose of histamine.

Assessment of Eosinophil Infiltration Eosinophils were counted 24 h after the challenge on day 100. Mice were anesthetized with diethylether 24 h after the final challenge. The animals were sacrificed by exsanguination, then, their heads were removed. They were fixed in 10% neutral buffered formalin for several days, and decalcified in a 10% ethylenediaminetetraacetic acid (EDTA) solution (pH 7.4) for 1 week. Samples were embedded in paraffin: frontal sections of the nose (4- μ m thick) were stained with hematoxylin and eosin to evaluate the number of eosinophils. All eosinophils that had infiltrated into the mucosa at both sides of the nasal septum were determined microscopically.

Statistical Analysis All experimental data are shown as the mean $\pm$ S.E.M. The data were analyzed using Student's *t*-test or Dunnett's test. A probability value below 0.05 was considered statistically significant.

RESULTS

Prophylactic Effects of Epinastine, Ramatroban and Seratrodast on Nasal Symptoms

Figure 1 shows the pro-

phylactic effects of epinastine, ramatroban and seratrodast on sneezing and nasal rubbing. In the epinastine (10 mg/kg/d)-treated group, the number of both sneezing and nasal rubbing significantly decreased from day 35 until the end of the experimental period. Ramatroban (30 mg/kg/d) also inhibited the increase of nasal symptoms from day 42 until day 70 (with or without statistical significance). However, seratrodast (30 mg/kg/d) did not show a significant suppression of nasal symptoms induced by the antigen in sensitized mice.

Histamine Sensitivity Table 1 shows the effects of epinastine, ramatroban and seratrodast on nasal symptoms induced by histamine. Histamine (1 nmol/mouse) induced a significant increase of nasal symptoms in the control group. In the seratrodast-treated group, histamine (10 nmol/mouse) induced a significant increase of nasal symptoms. However, in the epinastine- and ramatroban-treated group, there was no significant increase in nasal symptoms even at a high dose of

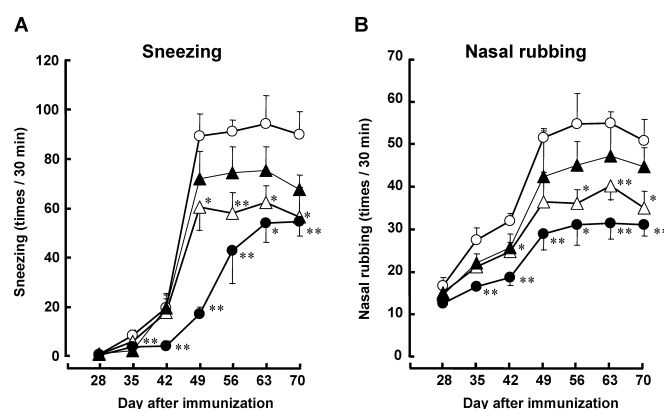


Fig. 1. Prophylactic Effects of Epinastine, Ramatroban and Seratrodast on Sneezing (A) and Nasal Rubbing (B) Induced by Antigen in Sensitized Mice

Epinastine, ramatroban and seratrodast were administered orally once a day from day 22 until day 70. The number of sneezing and nasal rubbing were counted for 30 min immediately after the nasal challenge. Open circles: control. Closed circles: epinastine 10 mg/kg/d. Open triangles: ramatroban 30 mg/kg/d. Closed triangles: seratrodast 30 mg/kg/d. Each point and vertical bar shows the mean $\pm$ S.E.M. of 8 experiments. *, ** Significantly different from control group at $p < 0.05$ and $p < 0.01$, respectively.

Table 1. Histamine Sensitivity

Drugs	Histamine (nmol/mouse)	Sneezing	Nasal rubbing
Control	Saline	13.3 $\pm$ 0.9	21.9 $\pm$ 1.4
	0.1	16.8 $\pm$ 2.9	25.3 $\pm$ 0.9
	1	23.9 $\pm$ 1.2**	30.5 $\pm$ 2.1*
	10	26.6 $\pm$ 3.1**	35.5 $\pm$ 3.3**
Epinastine (10 mg/kg/d)	Saline	8.4 $\pm$ 2.0	10.8 $\pm$ 1.1
	0.1	9.3 $\pm$ 2.0	11.9 $\pm$ 1.6
	1	9.9 $\pm$ 2.0	14.3 $\pm$ 2.1
	10	11.3 $\pm$ 3.2	15.0 $\pm$ 2.1
Ramatroban (30 mg/kg/d)	Saline	12.5 $\pm$ 1.3	20.0 $\pm$ 2.5
	0.1	14.4 $\pm$ 2.8	21.6 $\pm$ 2.4
	1	16.0 $\pm$ 3.5	22.8 $\pm$ 3.2
	10	20.9 $\pm$ 1.3	26.6 $\pm$ 2.6
Seratrodast (30 mg/kg/d)	Saline	13.9 $\pm$ 2.4	20.0 $\pm$ 2.8
	0.1	15.5 $\pm$ 1.0	21.1 $\pm$ 2.7
	1	17.6 $\pm$ 2.0	24.1 $\pm$ 2.3
	10	24.4 $\pm$ 3.2**	30.4 $\pm$ 2.5*

Epinastine, ramatroban and seratrodast were administered orally once a day from day 22 until day 70. On day 71, 2 μ l of vehicle (saline) or of increasing doses of histamine in solution (0.1, 1, 10 nmol/mouse) was consecutively inserted into each nasal cavity at 60 min intervals. Sneezing and nasal rubbing were counted for 10 min after the instillation of each dose of histamine. Each value shows the mean $\pm$ S.E.M. of 8 experiments. *, ** Significantly different from saline-treated group at $p < 0.05$ and 0.01, respectively.

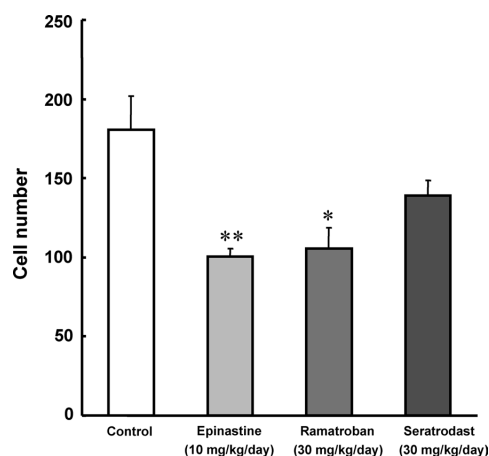


Fig. 2. Effects of Epinastine, Ramatroban and Seratrodast on Nasal Eosinophilia Induced by Antigen in Sensitized Mice

Epinastine, ramatroban and seratrodast were administered orally once a day from day 22 until day 99. Eosinophils were counted 24 h after the challenge on day 100. Each column and vertical bar shows the mean ± S.E.M. of 8 experiments. *, ** Significantly different from control group at $p < 0.05$ and $p < 0.01$, respectively.

histamine (10 nmol/mouse).

Eosinophil Infiltration As shown in Fig. 2, repeated application of epinastine (10 mg/kg/d) and ramatroban (30 mg/kg/d) significantly inhibited the infiltration of eosinophils into the nasal mucosa 24 h after the challenge on day 100. However, repeated application of seratrodast (30 mg/kg/d) showed no significant suppression of eosinophil infiltration into the nasal mucosa.

DISCUSSION

In the present study, we investigated the prophylactic effects of epinastine, ramatroban and seratrodast in an allergic rhinitis model. As a result, epinastine significantly inhibited the increase of nasal symptoms induced by the antigen. On the other hand, seratrodast did not inhibit the increase of nasal symptoms induced by the antigen. It has been reported that seratrodast showed an inhibitory effect on the increase of nasal congestion, but did not suppress the sneezing frequency in the allergic rhinitis model.^{15–17} On the other hand, Yamasaki *et al.* reported that the intranasal application of U-46619, a TXA_2 receptor agonist, caused no sneezing in guinea pigs.¹⁸ This finding is consistent with our result in the present study. Therefore, it is thought that the TXA_2 has an important role on nasal blockage, but has no association with sneezing.

Our study showed that ramatroban significantly inhibited the increase of nasal symptoms induced by the antigen. There is some evidence that ramatroban inhibited the nasal signs.^{19,20} It has been reported that ramatroban showed antagonistic activity not only towards the TXA_2 receptor, but also towards one of the prostaglandin D_2 (PGD_2) receptors, a chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2).^{21,22} CRTH2 contributes to both mast cell-dependent activation of Th2 cells and eosinophils and paracrine activation of Th2 cells, which might occur during immunoglobulin E (IgE)-facilitated antigen presentation.²³ In addition, it was reported that PGD_2 showed a synergistic effect on sneezing and nasal rubbing induced by histamine

via CRTH2.²⁴ Accordingly, it is suggested that ramatroban shows an inhibitory effect on allergic symptoms not only via the TXA_2 , but also via the CRTH2 receptor.

Next, we investigated the effects of these drugs on histamine sensitivity. It has been reported that H_1 receptors and the levels of histidine decarboxylase, histamine synthetase, were elevated in an allergic rhinitis model.^{25,26} Moreover, nasal hyperresponsiveness to histamine gradually developed in response to pollen inhalation in clinical observation²⁷ and in an animal model.²⁸ Our results showed that repeated administration of epinastine and ramatroban significantly inhibited the increase of histamine hypersensitivity in the model of allergic rhinitis. The prophylactic effects of epinastine on seasonal allergic rhinitis in human were investigated, and the results of the present study corresponded with those of these clinical studies. The present study revealed that ramatroban, but not seratrodast, showed a prophylactic effect on experimental allergic rhinitis in mice. This finding suggests that prophylactic treatment with ramatroban is more useful for the control of allergic conditions than therapeutic treatment in allergic rhinitis.

In addition, we investigated the number of eosinophils in the mucosa at both sides of the nasal septum. Allergic rhinitis is characterized by a marked increase in number of eosinophils in the nasal submucosa and epithelium.²⁹ Eosinophils, infiltrating into the tissues from post-capillary venules, degranulate and release PAF, LT and cytotoxic granule proteins such as eosinophil cationic protein (ECP), eosinophil peroxidase (EPO) and major basic protein (MBP), which are presumed to produce epithelial damage.^{30–32} Repeated administration of epinastine and ramatroban significantly inhibited the infiltration of eosinophils into the nasal mucosa. However, repeated administration of seratrodast showed no effect on eosinophil infiltration into the nasal mucosa. It has been reported that epinastine caused an antagonistic effect against PAF,³³ and inhibited the expression of CD11b on eosinophils³⁴ and eosinophil infiltration into the nasal mucosa in an allergic rhinitis model in mice.¹⁸ It was demonstrated that ramatroban inhibited the expression of intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 on endothelial cells by a thromboxane-like prostanoid (TP) receptor antagonism.³⁵ It has also been shown that ramatroban inhibited eosinophil migration by a CRTH2 antagonism.^{36,37} Therefore, epinastine and ramatroban might inhibit eosinophil infiltration into the nasal mucosa by these mechanisms.

de Graaf-in 't Veld *et al.* reported that nasal hyperreactivity is correlated with the percentage of eosinophils in patients with perennial rhinitis.³⁸ Terada *et al.* demonstrated that the administration of ramatroban for 4 weeks to patients with perennial allergic rhinitis resulted in a significant decrease in eosinophil counts and ECP levels in nasal lavage fluid, and nasal reactivity to histamine.³⁹ They also reported that a high correlation was observed between ECP levels and the degree of nasal reactivity. These findings suggest that eosinophils are responsible for the increase of nasal hyperreactivity. Therefore, in the present study, suppression of eosinophilia in nasal mucosa by epinastine and ramatroban resulted in a significant inhibition of nasal symptoms induced by the antigen and histamine. The present study discovered that ramatroban, but not seratrodast, showed a pro-

phylactic effect on experimental allergic rhinitis in mice. This finding suggests that prophylactic treatment with ramatroban is more useful for the control of allergic conditions than therapeutic treatment in allergic rhinitis.

Histamine H₁ receptor antagonists such as chlorpheniramine,⁴⁰⁾ ketotifen⁴¹⁾ and olopatadine²⁸⁾ decrease the number of H₁ receptors in the nasal mucosa. These reports suggest that in the present study, epinastine inhibited eosinophilia at the same level as ramatroban: however the prophylactic effect of epinastine might be stronger than that of ramatroban by this action.

In conclusion, the histamine H₁ receptor antagonist epinastine, the TXA₂ receptor and CRTH2 antagonist ramatroban showed a prophylactic effect on experimental allergic rhinitis in mouse models. However, the TXA₂ receptor antagonist seratrovast did not show a prophylactic effect in the model of allergic rhinitis. Therefore, it was suggested that the CRTH2 antagonism is important for the prophylactic effect. In addition, the prophylactic effect was involved in the suppression of eosinophil infiltration into the nasal mucosa.

REFERENCES

- 1) Kishi K., Saito M., Saito T., Kumemura M., Okamatsu H., Okita M., Takazawa K., *Biosci. Biotechnol. Biochem.*, **69**, 829—832 (2005).
- 2) Gelfand E. W., *J. Allergy Clin. Immunol.*, **114**, S135—S138 (2004).
- 3) Tai C. F., Baraniuk J. N., *Curr. Opin. Allergy Clin. Immunol.*, **2**, 11—19 (2002).
- 4) Howarth P. H., Salagean M., Dokic D., *Allergy*, **55**, 7—16 (2000).
- 5) Sarin S., Undem B., Sanico A., Togias A., *J. Allergy Clin. Immunol.*, **118**, 999—1016 (2006).
- 6) Shirasaki H., Kikuchi M., Seki N., Kanaizumi E., Watanabe K., Himi T., *Prostaglandins Leukot. Essent. Fatty Acids*, **76**, 315—320 (2007).
- 7) Nakanishi Y., Nabe T., Mizutani N., Takenaka H., Kohno S., *Prostaglandins Other Lipid Mediat.*, **74**, 125—137 (2004).
- 8) Yamasaki M., Mizutani N., Sasaki K., Nabe T., Matsumoto T., Ashida Y., Kohno S., *Inflamm. Res.*, **50**, 466—473 (2001).
- 9) Settiple R. A., Charnock D. R., *Clin. Allergy Immunol.*, **19**, 23—34 (2007).
- 10) Okubo K., Gotoh M., Shimada K., Ritsu M., Okuda M., Crawford B., *Int. Arch. Allergy Immunol.*, **136**, 148—154 (2005).
- 11) Kurowski M., Kuna P., Górski P., *Allergy*, **59**, 280—288 (2004).
- 12) Pitsios C., Papadopoulos D., Kompoti E., Manoussakis E., Garriss V., Petalas K., Tassios I., Baibas N., Kontou-Fili K., Saxoni-Papageorgiou P., Papadopoulos N. G., *Ann. Allergy Asthma Immunol.*, **96**, 673—678 (2006).
- 13) Kayasuga R., Sugimoto Y., Watanabe T., Kamei C., *Eur. J. Pharmacol.*, **449**, 287—291 (2002).
- 14) Miyagawa N., Iwasaki H., Kato T., Tanaka M., Shibata T., Wakitani K., *Biol. Pharm. Bull.*, **31**, 2260—2264 (2008).
- 15) Yamasaki M., Sasaki K., Mizutani N., Nabe T., Sakura Y., Matsumoto T., Ashida Y., Kohno S., *Inflamm. Res.*, **50**, 474—482 (2001).
- 16) Kishi Y., Nakano Y., Jiang S., Yatsuzuka R., Kamei C., *Int. Immunopharmacol.*, **7**, 1483—1487 (2007).
- 17) Johnston S. L., Smith S., Harrison J., Ritter W., Howarth P. H., *J. Allergy Clin. Immunol.*, **91**, 903—909 (1993).
- 18) Yamasaki M., Matsumoto T., Fukuda S., Nakayama T., Nagaya H., Ashida Y., *J. Pharmacol. Exp. Ther.*, **280**, 1471—1479 (1997).
- 19) Narita S., Asakura K., Kataura A., *Int. Arch. Allergy Immunol.*, **109**, 161—166 (1996).
- 20) Kayasuga R., Iba Y., Hossen M. A., Watanabe T., Kamei C., *Int. Immunopharmacol.*, **3**, 469—473 (2003).
- 21) Tanaka K., Ogawa K., Sugamura K., Nakamura M., Takano S., Nagata K., *J. Immunol.*, **164**, 2277—2280 (2000).
- 22) Hirai H., Tanaka K., Yoshie O., Ogawa K., Kenmotsu K., Takamori Y., Ichimasa M., Sugamura K., Nakamura M., Takano S., Nagata K., *J. Exp. Med.*, **193**, 255—261 (2001).
- 23) Pettipher R., *Br. J. Pharmacol.*, **153**, S191—S199 (2008).
- 24) Rahman A., Inoue T., Ago J., Ishikawa T., Kamei C., *Eur. J. Pharmacol.*, **554**, 229—234 (2007).
- 25) Mizuguchi H., Hatano M., Matsushita C., Umehara H., Kuroda W., Kitamura Y., Takeda N., Fukui H., *J. Pharmacol. Sci.*, **108**, 480—486 (2008).
- 26) Fukui H., *J. Pharmacol. Sci.*, **106**, 325—331 (2008).
- 27) Kanthawata S., Maturim W., Fooanant S., Manerot M., Trakultivakorn M., *Asian Pac. J. Allergy Immunol.*, **15**, 65—69 (1997).
- 28) Mizutani N., Nabe T., Takenaka H., Kohno S., *J. Pharmacol. Sci.*, **93**, 437—445 (2003).
- 29) Jankowski R., Persoons M., Foliguet B., Coffinet L., Thomas C., Verient-Montaut B., *Rhinology*, **38**, 23—32 (2000).
- 30) Gleich G. J., Loegeing D. A., *Ann. Rev. Immunol.*, **2**, 429—459 (1984).
- 31) Gleich G. J., *J. Allergy Clin. Immunol.*, **105**, 651—663 (2000).
- 32) Baraniuk J. N., *J. Allergy Clin. Immunol.*, **99**, S763—S772 (1997).
- 33) Kamei C., Mio M., Kitazumi K., Tsujimoto S., Yoshida T., Adachi Y., Tasaka K., *Immunopharmacol. Immunotoxicol.*, **14**, 207—218 (1992).
- 34) Matsukura M., Yajima A., Yamazaki F., Yodate T., Yamada H., Tezuka T., *Skin Pharmacol. Appl. Skin Physiol.*, **16**, 405—410 (2003).
- 35) Mitsuhashi M., Tanaka A., Fujisawa C., Kawamoto K., Itakura A., Takaku M., Hironaka T., Sawada S., Matsuda H., *J. Immunol.*, **166**, 617—623 (2001).
- 36) Sugimoto H., Shichijo M., Iino T., Manabe Y., Watanabe A., Shimazaki M., Gantner F., Bacon K. B., *J. Pharmacol. Exp. Ther.*, **305**, 347—352 (2003).
- 37) Shichijo M., Sugimoto H., Nagao K., Inbe H., Encinas J. A., Takeshita K., Bacon K. B., Gantner F., *J. Pharmacol. Exp. Ther.*, **307**, 518—525 (2003).
- 38) de Graaf-in 't Veld C., Garrelds I. M., Koenders S., Gerth van Wijk R., *Clin. Exp. Allergy*, **26**, 903—908 (1996).
- 39) Terada N., Yamakoshi T., Hasegawa M., Tanikawa H., Nagata H., Maesako K., Konno A., *Allergol. Int.*, **47**, 59—67 (1998).
- 40) Murata Y., Miyoshi A., Kitamura Y., Takeda N., Fukui H., *Inflamm. Res.*, **53** (Suppl. 1), S11—S12 (2004).
- 41) Ohkawa C., Ukai K., Miyahara Y., Takeuchi K., Sakakura Y., *Auris. Nasus. Larynx*, **26**, 293—298 (1999).