ELSEVIER

European Journal of Pharmacology 449 (2002) 287–291

www.elsevier.com/locate/ejphar

Participation of chemical mediators other than histamine in nasal allergy signs: a study using mice lacking histamine H_1 receptors

Ryoji Kayasuga [a], Yukio Sugimoto [a], Takeshi Watanabe [b], Chiaki Kamei [a,*]

[a] *Department of Pharmacology, Faculty of Pharmaceutical Science, Okayama University, Okayama 700-8530, Japan*
[b] *Department of Molecular Immunology, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-8582, Japan*

Received 25 February 2002; received in revised form 24 June 2002; accepted 25 June 2002

Abstract

The purpose of this study was to investigate the involvement of chemical mediators other than histamine in nasal allergic signs using histamine H_1 receptor-deficient mice. In passively sensitized mice, antigen instillation into the nasal cavity induced significant increases in sneezing and nasal rubbing in wild-type mice, but no such increases were observed in histamine H_1 receptor-deficient mice. In actively sensitized mice, both sneezing and nasal rubbing were also significantly increased in a dose-dependent manner in both wild-type and histamine H_1 receptor-deficient mice. Histamine H_1 receptor antagonists such as cetirizine and epinastine significantly inhibited antigen-induced nasal allergic signs in wild-type mice, although the effects were incomplete. In addition, the thromboxane A_2 receptor antagonist ramatroban also inhibited these responses in wild-type mice. However, the leukotriene receptor antagonist zafirlukast showed no effects in wild-type mice. These results suggested that in the acute allergic model (passive sensitization), only histamine H_1 receptors are related to nasal signs induced by antigen, whereas in the chronic allergic model (active sensitization), both histamine H_1 receptors and thromboxane A_2 receptors were involved in the responses.

Keywords: Nasal allergic sign; Histamine H_1 receptor; Thromboxane A_2; Leukotriene

1. Introduction

In a previous study, we demonstrated a new model of chronic allergic rhinitis showing nasal signs in rats (Sugimoto et al., 2000). Allergic rhinitis is an inflammatory disease of the nasal mucosa characterized by nasal itching, sneezing, rhinorrhea and nasal obstruction. The pathogenesis of the nasal allergic reaction initially involves the interaction of allergens with specific immunoglobulin E antibody bound to the surface of mast cells and basophils on the nasal mucosa. As a result, the release of mediators including histamine, leukotrienes, thromboxanes, platelet activating factor and cytokines, which are responsible for allergic signs, may occur (Baraniuk, 1997; Howarth et al., 2000). Histamine has been recognized as a major mediator in allergic reactions and diseases (Bachert, 1998; White, 1990), and it is widely accepted that histamine H_1 receptor antagonists are important agents in the treatment of nasal signs (Krause, 1994; Buske, 1996). Clinically, however, histamine H_1 receptor antagonists are unable to ameliorate nasal allergic signs completely (Howarth and Holgate, 1984; Meltzer et al., 2000). These findings suggested the importance of chemical mediators other than histamine in allergic rhinitis. However, very little information is available about the participation of chemical mediators in nasal signs in animal models. Therefore, in the present study, we developed a new allergic rhinitis model in mice, and the role of chemical mediators other than histamine in nasal allergic rhinitis was investigated using histamine H_1 receptor-deficient mice.

2. Materials and methods

2.1. Animals

Female histamine H_1 receptor-deficient and wild-type mice weighing 15–22 g were used. Histamine H_1 receptor-deficient mice were generated by homologous recombination as described previously (Inoue et al., 1996) and bred in

* Corresponding author. Tel./fax: +81-86-251-7939.
E-mail address: kamei@pheasant.pharm.okayama-u.ac.jp (C. Kamei).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02005-8

our laboratory. Female wild-type mice (C57BL/6) were obtained from Shimizu Laboratory Supplies, Kyoto. Both strains were housed in a temperature-controlled room at 24 ± 2 °C with 55 ± 15% humidity and were given food and water ad libitum. All procedures involving animals were conducted in accordance with the guidelines of the Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, Okayama University.

2.2. Reagents

Ovalbumin (Sigma, St. Louis, MO, USA), pertussis toxin (Sigma), mouse monoclonal anti-dinitrophenyl antibody (Sigma), dinitrophenyl-ovalbumin (Cosmo Bio, LSL, Tokyo, Japan) and aluminum hydroxide hydrate gel suspension (Cosmo Bio, LSL) were used. These agents were dissolved in saline. Epinastine hydrochloride (Böehringer Ingelheim, KG, Ingelheim, Germany), cetirizine hydrochloride (UCB, Belgium), ramatroban (Bayer Yakuhin, Osaka, Japan) and zafirlukast (AstraZeneca, Osaka, Japan) were also used. These agents were suspended in 5% gum arabic and were administered orally 1 h before antigen instillation.

2.3. Immunization

To prepare immunoglobulin E-dependent passive anaphylaxis in nasal mucosa, 10 μg of mouse monoclonal anti-dinitrophenyl antibody was injected into the tail vein of each mouse. These sensitized mice were used as passively sensitized animals. Mice were given an intraperitoneal injection of ovalbumin (100 μg), aluminum hydroxide gel (1 mg) and pertussis toxin (300 ng) (Oettgen et al., 1994). Five days later, they received a booster injection of 50 μg of ovalbumin alone subcutaneously in the back. From 18 days after the first immunization, daily intranasal sensitization with ovalbumin (50 μg) was performed for a week. These mice were used as actively sensitized animals.

2.4. Titration of passive cutaneous anaphylaxis reaction

The immunoglobulin E titers in the serum were determined by passive cutaneous anaphylaxis reaction. Blood specimens were obtained from the cava abdominalis. The serum samples were stored at −20 °C until use. Serial dilutions of serum obtained from sensitized mice were injected intradermally in a volume of 0.1 ml into the shaved backs of normal rats. After 48 h, the rats were challenged with intravenous injection of 0.2 ml/100 g animal physiological saline containing 1 mg of ovalbumin and 4 mg of Evans blue into the tail vein. After 30 min, the rats were sacrificed, the dorsal back skin was peeled off, and the diameter of the blue spot on the underside of the skin was measured. Histamine was injected as a positive control. The passive cutaneous anaphylaxis titer was expressed as the reciprocal of maximum dilution of the antiserum that gave a positive reaction of more 5 mm in diameter in the dorsal skin.

2.5. Evaluation of nasal signs

Before the experiment, the animals were placed into an observation cage (32 × 22 × 10 cm) for about 10 min for acclimatization. After nasal instillation of 1 μl of dinitrophenyl-ovalbumin solution (0.5–50 μg/site, passively sensitized mice) or ovalbumin solution (0.5–50 μg/site, actively sensitized mice) into the bilateral nasal cavities, the animals were placed into the observation cage (one animal/cage), and sneezing and nasal rubbing were counted for 60 min by the method of Sugimoto et al. (2000).

2.6. Effects of drugs on nasal signs

In this study, test drugs were administered orally 1 h before nasal instillation of ovalbumin (50 μg/site), and sneezing and nasal rubbing induced by ovalbumin were counted for 60 min.

2.7. Statistical analysis

Data are presented as means ± S.E.M. Statistical analysis was performed by one-way analysis of variance with Dunnett's test. A probability value of less than 0.05 was considered significant.

3. Results

3.1. Changes in nasal signs after antigen instillation in passively sensitized mice

In wild-type mice, 5 days after immunization, topical instillation of antigen (dinitrophenyl-ovalbumin) caused

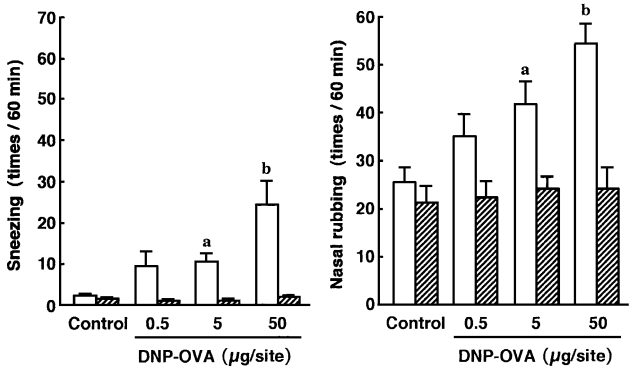

Fig. 1. The numbers of sneezing and nasal rubbing behaviors induced by dinitrophenyl-ovalbumin in passively sensitized wild-type and histamine H_1 receptor-deficient mice. Mice were sensitized by injection of mouse monoclonal anti-dinitrophenyl antibody, and 5 days later, dinitrophenyl-ovalbumin was applied to the bilateral nostrils. Sneezing and nasal rubbing were then counted for 60 min. Wild-type mice (open columns); H_1 receptor-deficient mice (hatched columns). DNP-OVA: dinitrophenyl-ovalbumin. Each column and vertical bar shows the mean ± S.E.M. of eight experiments. [a,b]: Significantly different from control group at $P<0.05$ and $P<0.01$, respectively.

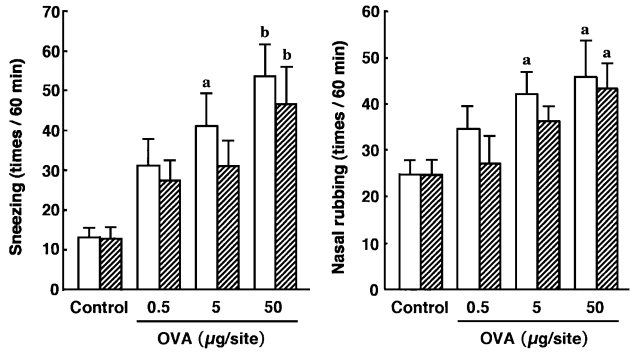

Fig. 2. The numbers of sneezing and nasal rubbing behaviors induced by ovalbumin in actively sensitized wild-type and histamine H_1 receptor-deficient mice. Mice were immunized with ovalbumin, and 25 days later, ovalbumin was applied to the bilateral nostrils. Sneezing and nasal rubbing were then counted for 60 min. Wild-type mice (open columns); H_1 receptor-deficient mice (hatched columns). OVA: ovalbumin. Each column and vertical bar shows the mean ± S.E.M. of 10 experiments. [a,b]: Significantly different from control group at $P<0.05$ and $P<0.01$, respectively.

increases in sneezing and nasal rubbing in a dose-dependent manner, and significant increases in both signs were observed at doses of 5 and 50 μg/site. However, no observable effects were noted even at a dose of 50 μg/site in histamine H_1 receptor-deficient mice (Fig. 1).

3.2. Passive cutaneous anaphylaxis titers

At 25 days after first immunization, the serum immunoglobulin E titers were increased by immunization and no differences were found between wild-type and histamine H_1 receptor-deficient mice (wild-type mice, 1:64 to 1:1024; histamine H_1 receptor-deficient mice, 1:64 to 1:512).

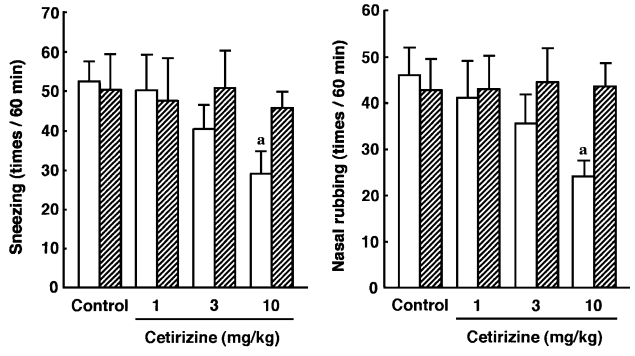

Fig. 3. Effect of cetirizine on nasal signs after antigen challenge in actively sensitized wild-type and histamine H_1 receptor-deficient mice. Mice were immunized with ovalbumin, albumin hydroxide gel and pertussis toxin, and 25 days later ovalbumin was applied to the bilateral nostrils. Sneezing and nasal rubbing were then counted for 60 min. The drug was given orally 1 h before antigen instillation. Wild-type mice (open columns); H_1 receptor-deficient mice (hatched columns). Each column and vertical bar shows the mean ± S.E.M. of 10 experiments. [a]: Significantly different from control group at $P<0.05$.

Table 1
Effects of certain drugs on sneezing and nasal rubbing induced by antigen in actively sensitized wild-type mice

Drugs	Dose (mg/kg, p.o.)	Sneezing	Nasal rubbing
Epinastine	Control	62.9 ± 6.3	39.4 ± 4.8
	1	44.1 ± 8.3	37.4 ± 3.6
	3	41.9 ± 12.3	29.7 ± 1.8
	10	30.7 ± 5.5[a]	21.9 ± 1.5[b]
Ramatroban	Control	54.9 ± 6.9	40.3 ± 5.1
	3	45.3 ± 6.8	31.1 ± 5.5
	10	36.7 ± 6.3	28.9 ± 4.9
	30	33.0 ± 4.1[a]	21.6 ± 4.0[a]
Zafirlukast	Control	56.5 ± 10.1	37.3 ± 3.9
	3	45.4 ± 7.5	30.7 ± 7.5
	10	36.6 ± 7.0	28.6 ± 5.3
	30	35.8 ± 7.8	30.2 ± 6.7

Mice were immunized with ovalbumin, albumin hydroxide gel and pertussis toxin, and 25 days later, ovalbumin was applied to the bilateral nostrils. Sneezing and nasal rubbing were then counted for 60 min. The drugs were given orally 1 h before antigen instillation. Each column and vertical bar shows the mean ± S.E.M. of 10 experiments.
[a] Significantly different from control group at $P<0.05$.
[b] Significantly different from control group at $P<0.01$.

3.3. Changes in nasal signs after instillation of antigen in actively sensitized mice

In both wild-type and histamine H_1 receptor-deficient mice, 25 days after first immunization, topical instillation of antigen (ovalbumin) caused increases in sneezing and nasal rubbing in a dose-dependent manner. In wild-type mice, significant increases in both signs were observed at doses of 5 and 50 μg/site. In histamine H_1 receptor-deficient mice, both signs were significantly induced at a dose of 50 μg/site (Fig. 2).

3.4. Effects of certain drugs on antigen-induced nasal signs

In wild-type mice, the histamine H_1 receptor antagonist cetirizine dose-dependently inhibited sneezing and nasal rubbing induced by ovalbumin, and a significant effect was observed at a dose of 10 mg/kg, but in histamine H_1 receptor-deficient mice, no such inhibition was observed even at a dose of 10 mg/kg (Fig. 3). Epinastine also inhibited both signs induced by ovalbumin, and a significant effect was observed at a dose of 10 mg/kg in wild-type mice (Table 1). In addition, the thromboxane A_2 receptor antagonist ramatroban significantly inhibited both nasal signs at a dose of 30 mg/kg in wild-type mice (Table 1). The cys leukotriene$_1$ receptor antagonist zafirlukast showed no inhibitory effect on the responses even at a dose of 30 mg/kg in wild-type mice (Table 1).

4. Discussion

Allergic rhinitis is a type I allergy caused by antigen binding to immunoglubulin E antibody on the surface of

mucosal mast cells on nasal mucosa and sequent release of chemical mediators by degranulation of mast cells. In the present study, we developed an antigen-induced nasal allergic rhinitis model and investigated the role of chemical mediators other than histamine in nasal signs. In the passively sensitized model, although nasal signs induced by antigen significantly increased in wild-type mice, no such increases were observed in histamine H_1 receptor-deficient mice. Similar results were reported in allergic reactions using histamine H_1 receptor-deficient mice, indicating that vascular permeability in the conjunctiva in allergic conjunctivitis is regulated through histamine H_1 receptors (Nakahara et al., 2000). Therefore, it is reasonable to assume that only histamine H_1 receptors were involved in antigen-induced nasal signs in this acute allergic rhinitis model.

In the present study, sneezing and nasal rubbing caused by antigen were increased in a dose-dependent manner in both wild-type mice and histamine H_1 receptor-deficient mice. At present, we cannot explain why nasal signs were generated in histamine H_1 receptor-deficient mice. However, as described in the text, we found that specific immunoglobulin E antibody level after active sensitization was not significantly different between wild-type and histamine H_1 receptor-deficient mice. This finding suggested that development of sensitization does not involve the stimulation of histamine H_1 receptors. Histamine H_1 receptor antagonists such as cetirizine and epinastine significantly inhibited nasal signs in wild-type mice. Fabre et al. (1995) reported that cetirizine inhibited the release of leukotrienes and prostaglandins from human lung cells. As described in the text, cetirizine showed no inhibitory effect on the nasal responses in histamine H_1 receptor-deficient mice. Therefore, it seems likely that the inhibitory effect of cetirizine on the responses occurs only through histamine H_1 receptors. These findings suggested that histamine H_1 receptors are closely related to antigen-induced nasal signs in this chronic allergic rhinitis model. However, histamine H_1 receptor antagonists did not show complete inhibition of the responses. Therefore, chemical mediators other than histamine were thought to participate in the responses induced by antigen.

To investigate the roles of chemical mediators in nasal allergic signs, we studied the effects of chemical mediator receptor antagonists such as ramatroban and zafirlukast in wild-type mice. The thromboxane A_2 receptor antagonist ramatroban significantly inhibited antigen-induced nasal signs in wild-type mice. Similar findings were also reported by Narita et al. (1996), who showed that ramatroban significantly inhibited sneezing and nasal scratching induced by antigen in a guinea pig allergic rhinitis model. In addition, the level of thromboxane B_2 (stable breakdown product of thromboxane A_2) in nasal cavity lavage fluid was reported to increase 20 min after antigen challenge in guinea pigs (Yamasaki et al., 2001). These findings suggested that thromboxane A_2 was responsible for early phase responses of nasal signs. In contrast, the leukotriene receptor antagonist zafirlukast did not show significant effects on nasal signs in wild-type mice. Clinically, Donnelly et al. (1995) showed that sneezing and rhinorrhea were significantly improved by zafirlukast as compared with placebo. This finding suggested that leukotrienes are important mediators of allergic rhinitis signs. However, Fujita et al. (1999) reported that pranlukast caused no inhibition of antigen-induced sneezing in a guinea pig model. Similar findings were also reported by Pullerits et al. (1999) and Meltzer et al. (2000), who showed that montelukast and zafirlukast caused no inhibition of nasal clinical signs. These results were consistent with those of the present study. Therefore, it is reasonable to assume that thromboxane A_2 receptors were also involved in antigen-induced sneezing and nasal rubbing.

In conclusion, in the acute allergic model (passive sensitization), only histamine H_1 receptors are related to nasal signs induced by antigen, whereas in the chronic allergic model (active sensitization), both histamine H_1 receptors and thromboxane A_2 receptors were involved in the responses.

References

Bachert, C., 1998. Histamine—a major role in allergy? Clin. Exp. Allergy 28 (S6), 15–19.

Baraniuk, J.N., 1997. Pathogenesis of allergic rhiniris. J. Allergy Clin. Immunol. 99, S763–S772.

Buske, L.M.D., 1996. Clinical comparison of histamine H_1-receptor antagonist drugs. J. Allergy Clin. Immunol. 98, S307–S318.

Donnelly, A.L., Glass, M., Minkwitz, M.C., Casale, T.B., 1995. The leukotriene D_4-receptor antagonist, ICI 204,219, relieves symptoms of acute seasonal allergic rhinitis. Am. J. Respir. Crit. Care Med. 151, 1734–1739.

Fabre, J.M., Marty-Ane, C., Alauzen, M., Souques, F., Bousquet, J., Campbell, A.M., 1995. Pharmacologic heterogeneity of human lung and colon cells: effect of terfenadine and cetirizine. Allergy 50, 362–365.

Fujita, M., Yonetomi, Y., Shimouchi, K., Takeda, H., Aze, Y., Kawabata, K., Ohno, H., 1999. Involvement of cysteinyl leukotrienes in biphasic increase of nasal airway resistance of antigen-induced rhinitis in guinea pigs. Eur. J. Pharmacol. 369, 349–356.

Howarth, P.H., Holgate, S.T., 1984. Comparative trial of two non-sedative H_1 antihistamines, terfenadine and astemizole, for hay fever. Thorax 39, 668–672.

Howarth, P.H., Salagean, M., Dokic, D., 2000. Allergic rhinitis: not purely a histamine-related disease. Allergy 55, 7–16.

Inoue, I., Yanai, K., Kitamura, D., Taniuchi, I., Kobayashi, T., Niimura, K., Watanabe, T., Watanabe, T., 1996. Impaired locomotor activity and exploratory behavior in mice lacking histamine H_1 receptors. Proc. Natl. Acad. Sci. U. S. A. 93, 13316–13320.

Krause, H.F., 1994. Therapeutic advances in the management of allergic rhinitis and urticaria. Otolaryngol. Head Neck Surg. 111, 364–372.

Meltzer, E.O., Malmstrom, K., Lu, S., Prenner, B.M., Wei, L.X., Weinstein, S.F., Wolfe, J.D., Reiss, T.F., 2000. Concomitant montelukast and loratadine as treatment for seasonal allergic rhinitis: a randomized, placebo-controlled clinical trial. J. Allergy Clin. Immunol. 105, 917–922.

Nakahara, H., Izushi, K., Sugimoto, Y., Watanabe, T., Kamei, C., 2000. Vascular permeability in allergic conjunctivitis in mice lacking histamine H_1 receptors. Eur. J. Pharmacol. 409, 313–317.

Narita, S., Asakura, K., Kataura, A., 1996. Effects of thromboxane A_2 receptor antagonist (Bay u 3405) on nasal symptoms after antigen challenge in sensitized guinea pigs. Int. Arch. Allergy Immunol. 109, 161–166.

Oettgen, H.C., Martin, T.R., Wynshaw-Boris, A., Deng, C., Drazen, J.M., Leder, P., 1994. Active anaphylaxis in IgE-deficient mice. Nature 370, 367–370.

Pullerits, T., Praks, L., Skoogh, B.-E., Ani, R., Lotvall, J., 1999. Randomized placebo-controlled study comparing a leukotriene receptor antagonist and a nasal glucocorticoid in seasonal allergic rhinitis. Am. J. Respir. Crit. Care Med. 159, 1814–1818.

Sugimoto, Y., Kawamoto, E., Chen, Z., Kamei, C., 2000. A new model of allergic rhinitis in rats by topical sensitization and evaluation of H_1-receptor antagonists. Immunopharmacology 48, 1–7.

White, M.V., 1990. The role of histamine in allergic diseases. J. Allergy Clin. Immunol. 86, 599–605.

Yamasaki, M., Mizutani, N., Sasaki, K., Nabe, T., Matsumoto, T., Ashida, Y., Kohno, S., 2001. Involvement of thromboxane A_2 and peptide leukotrienes in early and late phase nasal blockage in a guinea pig model of allergic rhinitis. Inflamm. Res. 50, 466–473.