

Involvements of cysteinyl leukotrienes and nitric oxide in antigen-induced venodilatation of nasal mucosa in sensitized rats *in vivo*

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Abstract

To determine an *in vivo* venodilatation of nasal mucosa, which is thought to be one of the causes of nasal obstruction in allergic rhinitis, venous diameters of nasal septa were directly measured in anesthetized rats. An application of antigen to nasal mucosa of sensitized rats caused an increase in diameters of mucosal veins, that is, venodilatation: the maximal response (about 20% increase in diameters) was observed at 55 min after antigen challenge. The antigen-induced increase in venous diameter of nasal mucosa was significantly inhibited by pretreatment with a cysteinyl leukotrienes (CysLTs) receptor antagonist, SR2640, and a nitric oxide (NO) synthase inhibitor, *N*^G-monomethyl-L-arginine, indicating that CysLTs and NO might be involved in the venodilatation of nasal mucosa induced by antigen challenge. Blocking the action of CysLTs and NO might be therefore useful for the therapy of nasal obstruction in allergic rhinitis.

Key words: allergic rhinitis, nasal obstruction, nasal mucosal vein, nitric oxide, cysteinyl leukotrienes

Introduction

Allergic rhinitis is characterized by sneezing, rhinorrhea and nasal obstruction. The nasal obstruction associated with allergic rhinitis is probably due to a dilatation of plexus cavernosum and mucosal congestion by an increase in nasal microvascular permeability although the exact mechanism(s) remains to be clear. The nasal obstruction is thought to decline the quality of life (QOL) in patients with allergic rhinitis. However, there is little effective medication for the nasal obstruction in allergic rhinitis.

In early phase of allergic reaction, a lot of mediators, including cysteinyl leukotrienes (CysLTs: LTC₄, LTD₄ and LTE₄) and nitric oxide (NO), are released following the antigen-antibody reaction. CysLTs were also detected in nasal washings following antigen challenge in subjects with allergic rhinitis (Shaw *et al.*, 1985; Miadonna *et al.*, 1999) and have an ability to

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induce nasal obstruction in a guinea pig model of allergic rhinitis (Mizutani *et al.*, 2001). The nasal obstruction induced by LTD₄ was inhibited by pranlukast, a CysLT₁-receptor antagonist, and N^ω-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase (Mizutani *et al.*, 2001), suggesting an important role of NO released by an activation of CysLT₁-receptors. One of the functions of NO is the relaxation of vascular smooth muscle cells, regulating blood flow through vessels (Moncada *et al.*, 1991; Bredt and Snyder, 1994). Thus, NO may also play an important role in nasal obstruction in allergic rhinitis. In fact, an involvement of NO in antigen-induced nasal obstruction has been reported in sensitized guinea pigs (Imai *et al.*, 2001).

In the present study, to show an *in vivo* venodilatation induced by antigen exposure in sensitized rats, venous diameters in nasal mucosae were directly measured in anesthetized animals. Furthermore, the effects of 2-(3-[2-quinolylmethoxy] phenylamino) benzoic acid (SR2640), a selective CysLT₁-receptor antagonist (Frolund *et al.*, 1991) that has reportedly higher affinity to CysLT₁-receptors than a clinical medication pranlukast (Lynch *et al.*, 1999; Alexander *et al.*, 2007), and N^G-monomethyl-L-arginine (L-NMMA), an NO synthase inhibitor, on the antigen-induced venodilatation were also determined to show involvements of CysLTs and NO in the relaxing response.

Methods

Animals and antigen sensitization

Male Wistar rats (6 weeks of age, specific pathogen-free, 170–190 g; Charles River Japan, Inc.) were sensitized with 2,4-dinitrophenylated *Ascaris suum* antigen (DNP-Asc) by the method described in the previous paper (Misawa and Chiba, 1993). In brief, animals were sensitized with DNP-Asc (2 mg protein, *s.c.*) together with killed *Bordetella pertussis* (2×10^{10}) as an adjuvant and were boosted by DNP-Asc (0.5 mg protein, *i.m.*) 5 days later. Eight days after the first immunization, the following experiments were carried out. All experiments were approved by the Animal Care Committee at the Hoshi University (Tokyo, Japan).

Measurement of changes in diameter of nasal mucosal veins

Venous diameter of nasal septal mucosa in anesthetized rats was measured by the method of previous paper (Chiba *et al.*, 2006). In brief, rats were anesthetized with urethane (2 g/kg, *i.p.*) and nasal mucosa was exposed surgically. Then the nasal septal mucosa was observed under a stereoscopic microscope (SZX9; Olympus Optical Co., Ltd., Tokyo, Japan) at a magnification of 40×. A digital camera (Camedia C-4040 Zoom; Olympus Optical Co., Ltd.) was equipped with the microscope to obtain photographs of nasal septum blood vessels. After an equilibration period, a photograph was taken for measurement of baseline venous diameter (50–80 μm) and then pre-warmed 20 μL of DNP-Asc (6 mg protein/mL) was applied to the exposed mucosal surface of nasal septum directly. After the application of DNP-Asc, the nasal mucosa was photographed continuously at 15-sec interval for the first 5 min and thereafter at 5-min interval for 60 min to examine the time-course change in the diameter of veins. In some experiments, pre-warmed 20 μL of SR2640 (10⁻⁸ M) or L-NMMA (10⁻⁵ M) was pretreated to the exposed mucosal surface 10 min before the application of DNP-Asc. Our preliminary studies showed that both SR2640 and L-

NMMA completely inhibited the venodilatation induced by a 50% effective concentration (*i.e.*, 10^{-8} M) of LTD₄ under these condition (not shown). The diameter of veins was measured using Image-Pro Plus (Media Cybernetics, San Diego, CA, USA) application with a computer and expressed as percentage of the baseline venous diameter. Three randomly selected veins were analyzed in each animal and the average was determined as N=1.

Statistical analyses

All the data are expressed as the mean with S.E. Statistical significance of difference was determined by one-way or two-way analysis of variance (ANOVA).

Results and discussion

The nasal obstruction is one of the characteristics of allergic rhinitis. In patients with seasonal allergic rhinitis, nasal obstructive symptom has been induced by nasal allergen challenge (Wang *et al.*, 1997). Application of allergen to nasal cavity also caused a significant increase in nasal airway resistance in patients with seasonal allergic rhinitis (Maniscalco *et al.*, 2001). One possible mechanism of the nasal obstruction is thought to be a dilatation of veins in nasal mucosa, although the exact mechanism(s) is not fully understood. In the current study, we tried to demonstrate a direct evidence of venodilatation induced by antigen in nasal mucosae of the sensitized rats.

An increase in the antigen-specific IgE was demonstrated in rats by the sensitization procedure used in the current study (Misawa and Chiba, 1993; Tada and Okumura, 1971). Antigen application to nasal mucosae of the sensitized rats caused a gradual increase in diameters of mucosal veins, *i.e.*, venodilatation (Fig. 1A, Fig. 1B, *closed circles*). The maximal response (about 20% increase in diameters) was observed at 55 min after the antigen challenge. The effect of antigen observed in this group of animals was statistically significant when the time-course curve was analyzed by one-way ANOVA ($P<0.001$). In contrast, the nasal antigen challenge had no significant effect in the non-sensitized naive control rats (Fig. 1B, *open circles*; $P=0.979$ by one-way ANOVA). When the time-course curve of the sensitized rats (Fig. 1B, *closed circles*) was compared with that of the non-sensitized naive control animals (Fig. 1B, *open circles*), a significant difference in the changes in venous diameters was also observed between the groups ($P<0.001$ by two-way ANOVA). These findings indicate that the antigen-induced venodilatation observed in the sensitized animals are mediated by a specific antigen-antibody reaction, at least at the onset of the response. This is the first study, to our knowledge, which directly showed the *in vivo* venodilatation induced by antigen challenge in rat nasal mucosa. It has been believed that dilatation of nasal blood vessels largely contributes to the increase in upper airway resistance (Mizutani *et al.*, 2001), an index of airway obstruction.

The current study also demonstrated an inhibitory effect of a CysLT₁-receptor antagonist, SR2640 (Frolund *et al.*, 1991), on the antigen-induced venodilatation in the sensitized rats: the antigen-induced increase in venous diameters was significantly inhibited by pretreatment with SR2640 (Fig. 1B, *closed circles vs. closed squares*; $P<0.001$ by two-way ANOVA). In patients with seasonal allergic rhinitis, nasal allergen challenge caused a significant increase in CysLTs in

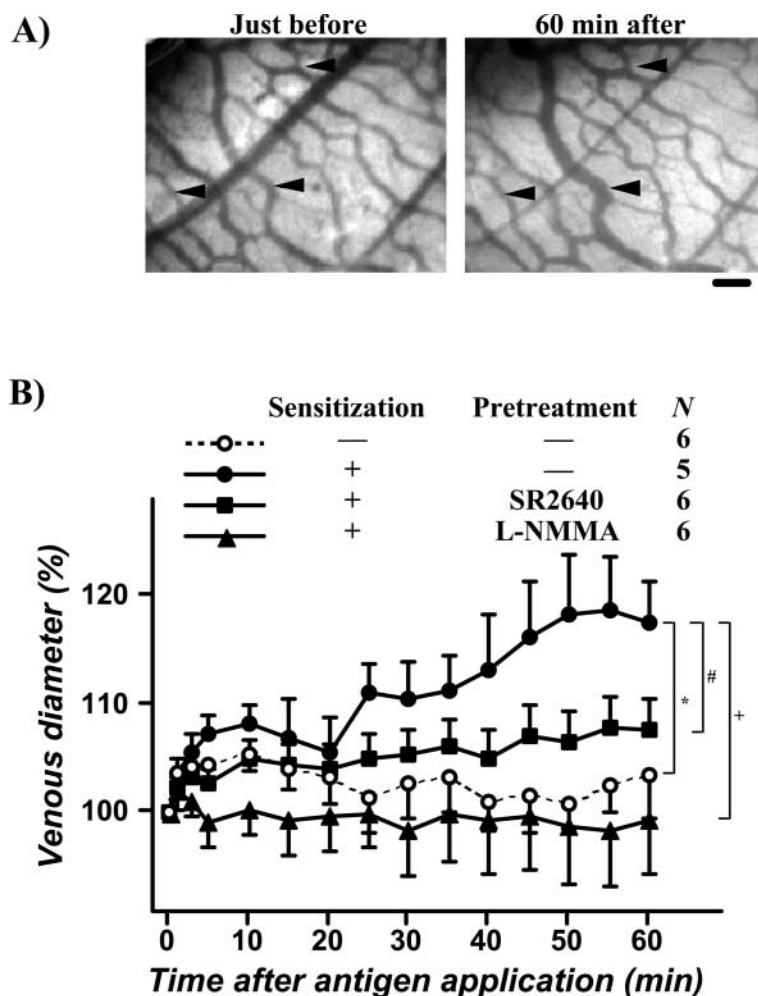


Fig. 1. (A) Typical photographs of mucosal veins obtained just before (*left*) and 60 min after (*right*) the application of DNP-Asc antigen (6 mg protein/mL, 20 μ L) in sensitized rats. The bar indicates the length of 300 μ m. The venous diameters were measured for three randomly selected vessels (*arrowheads*) in each animal. (B) Time-course changes in venous diameters of nasal mucosae induced by DNP-Asc antigen in non-sensitized naive control (*open circles*) and sensitized rats (*closed circles*). Effects of pretreatment with SR2640 (10^{-8} M, 20 μ L; a cysteinyl leukotrienes receptor antagonist; *closed squares*) and *N*^G-monomethyl-L-arginine (L-NMMA; 10^{-5} M, 20 μ L; an NO synthase inhibitor; *closed triangles*) on the antigen-induced increase in venous diameter in the sensitized rats are also shown. Each point represents the mean $\pm$ S.E.M. from 5–6 different animals. Antigen application to the sensitized rats caused a significant increase in venous diameters (*open circles* vs. *closed circles*; *, $P < 0.001$ by two-way ANOVA). The antigen-induced increase in venous diameters in the sensitized animals was significantly inhibited by pretreatment with SR2640 (*closed circles* vs. *closed squares*; #, $P < 0.001$ by two-way ANOVA) or L-NMMA (*closed circles* vs. *closed triangles*; *, $P < 0.001$ by two-way ANOVA).

nasal secretions associated with the nasal obstruction (Wang *et al.*, 1995; 1997). There is increasing evidence that CysLT₁-receptor antagonists are effective against nasal obstruction in patients with allergic rhinitis (Jiang, 2006). A CysLT₁-receptor antagonist, pranlukast, is now

clinically used for treatment of allergic rhinitis in Japan. On the other hand, an involvement of CysLTs in the nasal obstruction has also been suggested in a guinea pig model of allergic rhinitis (Mizutani *et al.*, 2001; Yamasaki *et al.*, 2001). Antigen inhalation to sensitized guinea pigs caused an increase in CysLTs in nasal cavity lavage fluids (Yamasaki *et al.*, 2001). The increase in upper airway resistance, an index of nasal obstruction, induced by antigen challenge in sensitized guinea pigs was inhibited by treatment with pranlukast (Mizutani *et al.*, 2001; Yamasaki *et al.*, 2001). Moreover, LTD₄ has an ability to induce a relaxation of nasal vasculature both *in vivo* (Mizutani *et al.*, 2001) and *in vitro* (Chiba *et al.*, 2006). It is thus possible that the released CysLTs following the antigen-antibody reaction causes a relaxation of nasal venous network, resulting in an induction of the nasal obstruction.

One of the important findings of the present study is the inhibitory effect of an NO synthase inhibitor, L-NMMA, on the antigen-induced venodilatation: the increase in venous diameters induced by antigen challenge in the sensitized rats was blocked by the pretreatment with L-NMMA (Fig. 1B, *closed triangles*). The results indicate an involvement of NO in the venodilatation induced by antigen challenge. NO is known as a potent vasodilator. In nasal airways, expression of constitutive and inducible NO synthases has been demonstrated in normal subjects and animals (Chiba *et al.*, 2006; Kawamoto *et al.*, 1998; 1999; Oh *et al.*, 2003). Immunohistochemical studies also demonstrated the localization of constitutive NO synthases in endothelial and epithelial cells in nasal mucosa (Kawamoto *et al.*, 1998; 1999). We have previously reported that the relaxation of vasculature induced by LTD₄ was inhibited by L-NMMA in isolated guinea pig nasal mucosae (Chiba *et al.*, 2006). Similarly, the *in vivo* nasal obstruction induced by LTD₄ was inhibited by L-NAME in guinea pigs (Mizutani *et al.*, 2001). Thus, CysLTs released by the antigen-antibody reaction might cause the generation of NO in endothelial/epithelial cells of nasal mucosa, resulting in the venodilatation. The stronger inhibitory effect of L-NMMA than SR2640 (Fig. 1, *closed triangles vs. closed squares*; $P < 0.01$ by two-way ANOVA) might also indicate an involvement of mediators other than CysLTs in the release of NO.

In conclusion, the current study clearly showed evidence that nasal antigen challenge to the sensitized rats causes a venodilatation of nasal mucosa *in vivo*. The antigen-induced venodilatation was inhibited by a CysLT₁-receptor antagonist and an NO synthase inhibitor, indicating that CysLTs and NO might be involved in the pathogenesis of nasal obstruction in allergic rhinitis.

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References

- Alexander, S.P.H., Mathie, A. and Peters, J.A. (2007). 7TM receptors: leukotriene. *Br. J. Pharmacol.* **150** (Suppl. 1): S48–S48.
- Bredt, D.S. and Snyder, S.H. (1994). Nitric oxide: a physiologic messenger molecule. *Annu. Rev. Biochem.* **63**: 175–195.
- Chiba, Y., Matsuo, K., Sakai, H., Abe, K. and Misawa, M. (2006). Effects of 1400W, a potent selective inducible NOS inhibitor, on histamine- and leukotriene D₄-induced relaxation of isolated guinea

- pig nasal mucosa. *Nitric Oxide* **15**: 142–147.
- Chiba, Y., Oshita, M., Matsuo, K., Sakai, H. and Misawa, M. (2006). Comparison of norepinephrine responsiveness of mucosal veins *in vivo* with that of isolated mucosal tissue *in vitro* in guinea pig nasal mucosa. *Am. J. Rhinol.* **20**: 349–352.
- Frolund, L., Madsen, F. and Nielsen, J. (1991). Reproducibility of leukotriene D₄ inhalation challenge in asthmatics. Effect of a novel leukotriene D₄/E₄-antagonist (SR 2640) on leukotriene D₄-induced bronchoconstriction. *Allergy* **46**: 355–361.
- Imai, A., Nabe, T., Mizutani, N., Sakurai, H., Takenaka, H. and Kohno, S. (2001). Involvement of nitric oxide in pollen-induced biphasic nasal blockage in sensitised guinea pigs. *Eur. J. Pharmacol.* **423**: 63–70.
- Jiang, R.S. (2006). Efficacy of a leukotriene receptor antagonist in the treatment of perennial allergic rhinitis. *J. Otolaryngol.* **35**: 117–121.
- Kawamoto, H., Takeno, S. and Yajin, K. (1999). Increased expression of inducible nitric oxide synthase in nasal epithelial cells in patients with allergic rhinitis. *Laryngoscope* **109**: 2015–2020.
- Kawamoto, H., Takumida, M., Takeno, S., Watanabe, H., Fukushima, N. and Yajin, K. (1998). Localization of nitric oxide synthase in human nasal mucosa with nasal allergy. *Acta Otolaryngol. Suppl.* **539**: 65–70.
- Lynch, K.R., O'Neill, G.P., Liu, Q., Im, D.S., Sawyer, N., Metters, K.M., Coulombe, N., Abramovitz, M., Figueroa, D.J., Zeng, Z., Connolly, B.M., Bai, C., Austin, C.P., Chateaneuf, A., Stocco, R., Greig, G.M., Kargman, S., Hooks, S.B., Hosfield, E., Williams, D.L. Jr., Ford-Hutchinson, A.W., Caskey, C.T. and Evans, J.F. (1999). Characterization of the human cysteinyl leukotriene CysLT₁ receptor. *Nature* **399**: 789–793.
- Maniscalco, M., Sofia, M., Carratu, L. and Higenbottam, T. (2001). Effect of nitric oxide inhibition on nasal airway resistance after nasal allergen challenge in allergic rhinitis. *Eur. J. Clin. Invest.* **31**: 462–466.
- Miadonna, A., Milazzo, N., Gibelli, S., Salmaso, C., Lorini, M. and Tedeschi, A. (1999). Nasal response to a single antigen challenge in patients with allergic rhinitis—inflammatory cell recruitment persists up to 48 hours. *Clin. Exp. Allergy* **29**: 941–949.
- Misawa, M. and Chiba, Y. (1993). Repeated antigenic challenge-induced airway hyperresponsiveness and airway inflammation in actively sensitized rats. *Jpn. J. Pharmacol.* **61**: 41–50.
- Mizutani, N., Nabe, T., Imai, A., Sakurai, H., Takenaka, H. and Kohno, S. (2001). Markedly increased nasal blockage by intranasal leukotriene D₄ in an experimental allergic rhinitis model: contribution of dilated mucosal blood vessels. *Jpn. J. Pharmacol.* **86**: 170–182.
- Moncada, S., Palmer, R.M. and Higgs, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* **43**: 109–142.
- Oh, S.J., Min, Y.G., Kim, J.W., Lee, S.J. and Jarin, P.R. (2003). Expression of nitric oxide synthases in nasal mucosa from a mouse model of allergic rhinitis. *Ann. Otol. Rhinol. Laryngol.* **112**: 899–903.
- Shaw, R.J., Fitzharris, P., Cromwell, O., Wardlaw, A.J. and Kay, A.B. (1985). Allergen-induced release of sulphidopeptide leukotrienes (SRS-A) and LTB₄ in allergic rhinitis. *Allergy* **40**: 1–6.
- Tada, T. and Okumura, K. (1971). Regulation of homocytotropic antibody formation in the rat. I. Feedback regulation by passively administered antibody. *J. Immunol.* **106**: 1002–1011.
- Wang, D., Clement, P., Smits, J. and Derde, M.P. (1995). Concentrations of chemical mediators in nasal secretions after nasal allergen challenges in atopic patients. *Eur. Arch. Otorhinolaryngol.* **252** (Suppl. 1): S40–S43.
- Wang, D., Smits, J., Waterschoot, S. and Clement, P. (1997). An approach to the understanding of the nasal early-phase reaction induced by nasal allergen challenge. *Allergy* **52**: 162–167.
- Yamasaki, M., Mizutani, N., Sasaki, K., Nabe, T., Matsumoto, T., Ashida, Y. and Kohno, S. (2001). Involvement of thromboxane A₂ and peptide leukotrienes in early and late phase nasal blockage in a guinea pig model of allergic rhinitis. *Inflamm. Res.* **50**: 466–473.