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, NG-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
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 Nω-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 Nω-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 × 1010) 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., \(10^{-8} \text{ M}\)) of LTD\(_4\) 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 CysLT\(_1\)-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
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 vasodilatator. In nasal airways, expression of constitutive and inducible NO syntheses 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 syntheses 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**


