

Participation in cysteinyl leukotrienes and thromboxaneA₂ in nasal congestion model in Brown Norway rats

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Abstract

The aim of this study was to investigate the involvement of chemical mediators in a nasal congestion model in Brown Norway (BN) rats. For the above purpose, we studied the effects of pranlukast and zafirlukast (cysteinyl leukotriene (cys-LT) receptor antagonists), seratrodast and ramatroban (thromboxane A₂ (TXA₂) receptor antagonists) on nasal congestion and sneezing induced by toluene 2, 4-diisocyanate (TDI). All of these drugs suppressed the increase of enhanced pause (Penh), the index of nasal congestion, in both early and late phase responses; however, pranlukast, zafirlukast and seratrodast failed to suppress immediate sneezing caused by TDI challenge. These results indicate that cys-LTs and TXA₂ are responsible for the development of both early and late phase nasal congestion. Moreover, these chemical mediators contribute very little to immediate sneezing in a BN rat model of allergic rhinitis.

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1. Introduction

Allergic rhinitis is an inflammatory disease of the nasal mucosa characterized by 3 major symptoms, sneezing, rhinorrhea and nasal congestion. The pathogenesis of the nasal allergic reaction initially involves the interaction of allergens with specific IgE antibody bound to the surface of mast cells and basophils on the nasal mucosa [1,2]. As a result, many symptoms associated with allergic rhinitis are caused by the release of mediators including histamine, cysteinyl leukotrienes (cys-LTs), thromboxanes, prostaglandins and cytokines

[3,4]. Histamine has been recognized as a major mediator in allergic reactions and diseases [5,6], and it is widely accepted that histamine H₁ receptor antagonists are used for the treatment of allergic rhinitis [7,8]. The symptoms of sneezing and rhinorrhea in allergic rhinitis can be attenuated by treatment with histamine H₁ receptor antagonists; however, it has been considered that histamine H₁ receptor antagonists cause no complete inhibition of nasal congestion [9,10]. From these findings, it is reasonable to presume that chemical mediators other than histamine participate in nasal congestion.

Cys-LTs and thromboxane (TX) A₂, cyclooxygenase pathway products, are generated in response to immunological stimulation from various cells, such as mast cells, macrophages and eosinophils [11]. In guinea

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pig models of allergic rhinitis, it has been demonstrated that the concentration of cys-LTs and TXB₂ (a stable TXA₂ metabolite) are elevated in nasal cavity lavage fluid or plasma after antigen challenge [12,13]. These findings suggest that cys-LTs and TXA₂ play an important role in experimental allergic rhinitis of guinea pigs.

In a previous study, we established a nasal congestion model using Brown Norway (BN) rats by intranasal immunization with toluene 2, 4-diisocyanate (TDI) as the antigen [14]. In this model, the animals showed not only immediate sneezing but also nasal congestion in the early and late phases.

In the present study, in order to further clarify the role of cys-LTs and TXA₂ in the development of the symptoms of allergic rhinitis, we investigated the effects of these chemical mediator receptor antagonists on nasal congestion.

2. Materials and Methods

2.1. Animals

Six-week-old male Brown Norway (BN) rats were obtained from Kyudo Co., Ltd., Japan. The animals were housed in an air-conditioned room, maintained at 24±2 °C, with a relative humidity of 55±10%. The rats were given standard laboratory rodent food (Oriental Yeast, Tokyo, Japan) and water ad libitum. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

2.2. Reagents and drugs

The following reagents were obtained from the sources shown in parentheses: TDI (Wako, Tokyo, Japan) and ethyl acetate (Wako). TDI was dissolved in ethyl acetate to a concentration of 10% for sensitization and to a concentration of 5% for challenge. The following drugs were purchased from the companies indicated: pranlukast hydrate (Ono Pharmaceutical Co., Ltd., Osaka, Japan), zafirlukast (AstraZeneca, Osaka, Japan), seratrodist (Takeda Chemical Industries, Ltd., Osaka, Japan) and ramatroban (Bayer Yakuhin, Osaka, Japan). They were suspended in 5% gum arabic and administered orally 1 h before antigen instillation.

2.3. Sensitization and challenge procedures

Sensitization and challenge were performed according to the method described by Tsumuro et al. [14]. In brief, rats were sensitized by intranasal application of 10% TDI solution in ethyl acetate once a day for 5 consecutive days. Two days later, the rats were sensitized with TDI again for 5 days. One week after completion of the sensitization, 5% TDI solution in ethyl acetate was applied to the bilateral nasal cavities to induce nasal allergy-like symptoms.

2.4. Evaluation of TDI-induced nasal congestion and sneezing

Before the experiment, the rats were placed into the animal chamber for 10 min for acclimatization. Nasal congestion was measured in freely moving rats for 6 h after nasal instillation of 5% TDI solution (5 µl/site) using whole body plethysmography-free moving application (Buxco, biosystem XA for windows SFT3410 version 2.7 r4). The enhanced pause (Penh) was used as an index of nasal congestion. Changes in Penh were expressed as the average for 1 h. The increase in Penh 1 and 4 h after the TDI challenge expressed “the early phase” and “the late phase”, respectively. Sneezing was measured in the same animal for 30 min after antigen instillation.

2.5. Effects of drugs on nasal congestion and sneezing

In this study, pranlukast, zafirlukast, seratrodist, and ramatroban were administered orally 1 h before the challenge of 5% TDI at doses of 1, 3 and 10 mg/kg, respectively. In control group, 5% gum arabic solution was given instead of the drugs in sensitized rats. In non-sensitized group, 5% gum arabic solution was also given in non-sensitized rats. 8 rats for each group were tested. Drugs tests were repeated 3 times in the same animals, the drugs were administered at an interval of 7 days.

2.6. Statistical analysis

All experimental data are shown as the means±S.E.M. Statistical analysis was performed using Mann–Whitney *U*-test and Dunnett’s test for comparison with control group and drugs treated groups. A probability value of less than 0.05 was considered significant.

3. Results

3.1. Changes in Penh in TDI challenge

As shown in Fig. 1, in TDI-sensitized rats, Penh was increased significantly, after TDI challenge successively for 6 h.

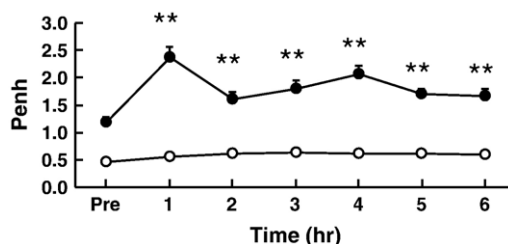


Fig. 1. Comparison of Penh for 6 h between the non-sensitized and sensitized rats. ○: non-sensitized rats. ●: sensitized rats. Each value shows the mean±S.E.M. of 8 experiments. Significant difference was compared with non-sensitized rats group. ***P*<0.01.

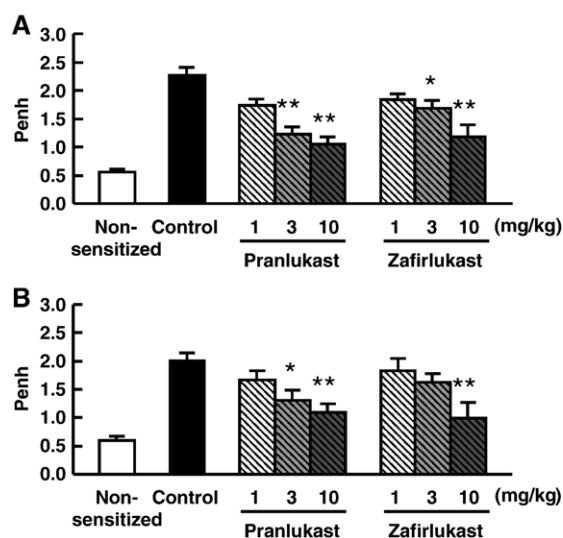


Fig. 2. Effects of cys-LTs receptor antagonists on the increase of Penh in the early (A) and late (B) phase after TDI challenge in sensitized rats. Pranlukast and zafirlukast were administered orally 1 h before antigen instillation. Each column and vertical bar shows the mean±S.E.M. of 8 experiments. Significant difference was compared with control group. * $P<0.05$, ** $P<0.01$.

A time course study showed peaks at 1 and 4 h after the TDI challenge. However, no observable effects were noted in the non-sensitized rats.

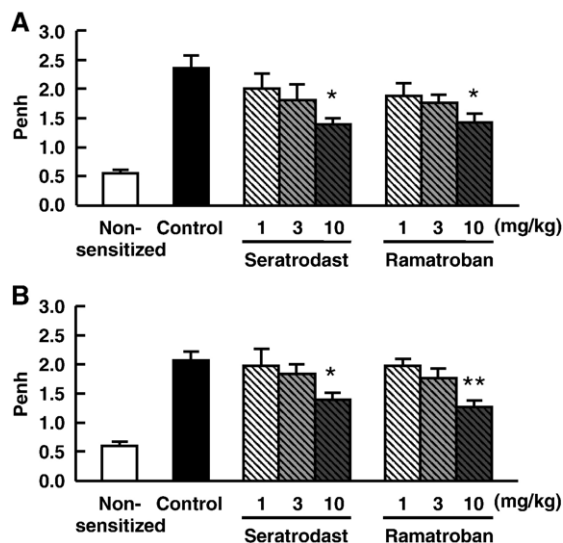


Fig. 3. Effects of TXA₂ receptor antagonists on the increase of Penh in the early (A) and late (B) phase after TDI challenge in sensitized rats. Seratrodast and ramatroban were administered orally 1 h before antigen instillation. Each column and vertical bar shows the mean±S.E.M. of 8 experiments. Significant difference was compared with control group. * $P<0.05$, ** $P<0.01$.

Table 1

Effects of cys-LTs receptor antagonists on sneezing induced by TDI in sensitized rats

Drugs	Dose (mg/kg)	Sneezing
Non-sensitized		10.9±0.7
Control		46.1±3.7
Pranlukast	1	36.9±6.8
	3	36.8±5.2
	10	39.0±8.7
Zafirlukast	1	41.0±3.9
	3	38.8±5.0
	10	37.9±5.8

Drugs were administered orally 1 h before antigen instillation.

Sneezing was measured for 30 min after TDI challenge.

The values shown are the mean±S.E.M. of 8 experiments.

3.2. Effects of cys-LTs receptor antagonists on TDI-induced increase in Penh

The effects of cys-LTs receptor antagonists on Penh in early and late phases are shown in Fig. 2. Pranlukast and zafirlukast caused a dose-related inhibition of the increase of Penh in the early phase. Significant effects were observed at doses of 3 and 10 mg/kg of both drugs. In the late phase, both drugs also dose-dependently inhibited the increase of Penh. Significant effects were observed at doses of 3 and 10 mg/kg of pranlukast and at a dose of 10 mg/kg of zafirlukast.

3.3. Effects of TXA₂ receptor antagonists on TDI-induced increase in Penh

The effects of TXA₂ receptor antagonists on Penh in early and late phases are shown in Fig. 3. Seratrodast and ramatroban caused dose-related inhibition of the increase of Penh in both early and late phases, and significant effects were observed at a dose of 10 mg/kg of both drugs.

Table 2

Effects of TXA₂ receptor antagonists on sneezing induced by TDI in sensitized rats

Drugs	Dose (mg/kg)	Sneezing
Non-sensitized		10.8±0.6
Control		42.4±5.3
Seratrodast	1	39.9±4.2
	3	36.8±4.4
	10	37.4±6.5
Ramatroban	1	38.3±5.7
	3	34.9±4.2
	10	25.3±2.9*

Drugs were administered orally 1 h before antigen instillation.

Sneezing was measured for 30 min after TDI challenge.

The values shown are the mean±S.E.M. of 8 experiments.

*: Significantly different from control group at $P<0.05$.

3.4. Effects of cys-LTs receptor antagonists on TDI-induced increase in sneezing

The effects of cys-LTs receptor antagonists on sneezing after TDI challenge are shown in Table 1. Pranlukast and zafirlukast caused no effect on sneezing, even at a dose of 10 mg/kg.

3.5. Effects of TXA₂ receptor antagonists on TDI-induced increase in sneezing

The effects of TXA₂ receptor antagonists on sneezing after TDI challenge are shown in Table 2. Seratrodast caused no significant inhibition of sneezing even at a dose of 10 mg/kg; however, ramatroban caused a significant inhibition of sneezing at a dose of 10 mg/kg.

4. Discussion

Allergic rhinitis is a type ² allergy caused by antigen binding to IgE antibody on the surface of mucosal mast cells on nasal mucosa. It is well recognized that there are two phases of allergic response named ‘the early phase’, characterized by sneezing, rhinorrhea and nasal congestion and ‘the late phase’, characterized mainly by nasal congestion [1,15]. We previously established an allergic rhinitis model that includes nasal congestion symptoms and sneezing in BN rats [14]. In the present study using the same method as described above, it was found that pranlukast and zafirlukast, cys-LTs receptor antagonists, significantly inhibited both early and late phase nasal congestion. Seratrodast and ramatroban, TXA₂ receptor antagonists, also significantly inhibited both early and late phase nasal congestion. It is well known that cys-LTs increase nasal mucosal blood flow by dilating blood vessels [16], and TXA₂ induce swelling of nasal mucosa by plasma extravasation, which are major causes of nasal congestion [17]. Recent studies show that cys-LTs and TXA₂ are likely released from various inflammation cells, such as mast cells and eosinophils, by antigen–antibody reaction [18], and these mediators then induce an increase in nasal vascular permeability that produces tissue edema in humans and guinea pigs [19–22]. It is obvious that the presence of edema in nasal tissues is an important factor that causes nasal congestion. In addition, Yamasaki et al. [22] reported that intranasal instillation of LTD₄ and U-46619 (a TXA₂ receptor agonist) induced nasal congestion in guinea pigs. From these findings, it seems likely that cys-LTs and TXA₂ are involved in the pathogenesis of nasal congestion.

In the present study, it was also found that pranlukast, zafirlukast and seratrodast had no effects on immediate sneezing in BN rats, even at a high dose. As has been

suggested by clinical observations, immediate sneezing, unlike nasal congestion, appears to be mediated mainly by histamine released immediately after antigen challenge [23]. From these findings, it seems likely that cys-LTs and TXA₂ contribute very little to immediate sneezing. In contrast, ramatroban significantly inhibited the immediate sneezing in this model. Moreover, evidence has indicated that ramatroban inhibited the nasal symptoms induced by antigens in mice and guinea pigs [24,25]. On the other hand, Yamasaki et al. [12] demonstrated that the intranasal application of U-46619 caused no sneezing in guinea pigs, which prompted us to consider that some chemical transmitters other than the TXA₂ receptor may be involved in causing the nasal symptoms induced by antigen. In the recent study, there was some evidence that ramatroban serves as the receptor for prostaglandin D₂, not only acting through TXA₂ receptors, but also through CRTH2 (chemoattractant receptor-homologous molecule expressed on TH2 cells) receptors [26,27]. Furthermore, CRTH2 receptors have been shown to be involved in mast cell-mediated allergic inflammation [28]. Therefore, it can be assumed that ramatroban showed its effect in inhibiting immediate sneezing by antagonizing CRTH2 receptors but not TXA₂ receptors.

In conclusion, the findings of this study clearly indicate that cys-LTs and TXA₂ are responsible for the development of both early and late phase nasal congestion. Moreover, these chemical mediators contribute very little to immediate sneezing in BN rats.

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