

Participation of chemical mediators in the development of experimental allergic conjunctivitis in rats

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Abstract

In the present study, we investigated the participation of chemical mediators in the development of experimental allergic conjunctivitis in rats. Cetirizine (a histamine H_1 receptor antagonist), ramatroban (a thromboxane A_2 (TXA₂) receptor antagonist) and zafirlukast (a cysteinyl leukotrienes (cys-LTs) receptor antagonist) were orally administered from day 14 to day 42 during repeated topical antigen challenge. An increase in reactivity to antigen- and histamine-induced eye scratching behavior was observed by topical sensitization in sensitized rats. Although increased reactivity to antigen was not influenced by cetirizine, ramatroban and zafirlukast, increased reactivity to histamine was significantly inhibited by ramatroban. The development of conjunctival edema was also observed for topical sensitization. Cetirizine caused no inhibition of the development of conjunctival edema, but ramatroban and zafirlukast inhibited the development of conjunctival edema. In addition, the number of eosinophils in the conjunctiva was increased by topical sensitization. Cetirizine had no significant effect on the increase in the number of eosinophils. However, ramatroban and zafirlukast were effective in inhibiting an increase in the number of eosinophils induced by topical sensitization.

These results indicate that TXA₂ is involved in increased histamine reactivity, and TXA₂ and cys-LTs are associated with not only the conjunctival edema but also eosinophil infiltration during the development of experimental allergic conjunctivitis in rats. © 2004 Elsevier B.V. All rights reserved.

Keywords: Experimental allergic conjunctivitis; Histamine reactivity; Conjunctival edema; Eosinophil infiltration; Thromboxane A_2 ; Cysteinyl leukotrienes

1. Introduction

It is well known that intense itching and swelling of the conjunctiva were observed with acute and perennial allergic conjunctivitis [1]. The infiltration of eosinophils into the conjunctiva can also be detected similar to other allergic diseases [2]. In an animal study, we developed an allergic conjunctivitis model

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after the topical application of antigen to sensitized rats [3]. The rats exhibited severe itching, edema, infiltration of eosinophils into the conjunctiva and increased reactivity to histamine.

Several cells including mast cells and chemical mediators play a crucial role in the immunopathogenesis of ocular allergy, which involves a cascade of chemical mediators and reactions [4,5]. Mast cells are activated by antigen–antibody reactions, and the IgE-mediated activation of mast cells leads to the release of chemical mediators such as histamine, thromboxane A₂ (TXA₂) and cysteinyl leukotrienes (cys-LTs) [6,7]. These mediators cause several symptoms associated with allergic conjunctivitis. For example, itching is induced not only by histamine but also by TXA₂ in an allergic conjunctivitis model in rats [3]. TXA₂ and cys-LTs are responsible for the infiltration of eosinophils into the mucosa [8,9]. However, literature on the participation of chemical mediators in the development of an allergic conjunctivitis is scarce. Therefore, the present study was undertaken to clarify the participation of chemical mediators in the development of an allergic conjunctivitis using a rat allergic conjunctivitis model as previously reported [3].

2. Materials and methods

2.1. Animals

Six-week-old male Wistar rats were obtained from Japan SLC, Shizuoka, Japan. The animals were housed in an air-conditioned room maintained at 24±2 °C with relative humidity of 55±15%, and were given standard laboratory rodent chow (Oriental Yeast, Tokyo, Japan) and water ad libitum. All procedures involving the animals were conducted in accordance with the guidelines of the Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, Okayama University.

2.2. Reagents

The following reagents were obtained from the sources shown in parentheses: ovalbumin (OVA, Grade VII; crystallized and lyophilized, essentially salt-free, Sigma, St. Louis, MO, USA), aluminum

hydroxide hydrate gel (alum, LSL, Tokyo, Japan) and histamine dihydrochloride (Nacalai Tesque, Kyoto, Japan). Inactive suspension *Bordetella pertussis* was kindly provided by the Kitasato Institute Research Center for Biology, Saitama, Japan. The following drugs were purchased from the companies indicated: ramatroban (Baynas[®], Bayer Yakuhin, Osaka, Japan), zafirlukast (Accolate[®], AstraZeneca, Osaka, Japan) and cetirizine hydrochloride (Zyrtec[®], UCB, Tokyo, Japan). They were suspended in 5% gum arabic and orally administered at 10 mg/kg 1 h before topical sensitization.

2.3. Sensitization

The rats were sensitized by injection with 0.6 ml of physiological saline containing OVA (1 mg), alum (2 mg) and 10¹⁰ killed *B. pertussis* cells into all four footpads on the first day. Five days later, they were boosted by subcutaneous injection with 1 ml of physiological saline containing OVA (0.5 mg) in 10 sites on the back. Then, local sensitization was performed every day from day 14 to day 42 by instilling OVA in physiological saline (10 mg/ml, 5 µl) into the bilateral eyes using a micropipette.

2.4. Evaluation of eye scratching behavior

Before the experiment, the animals were placed into an observation cage (32×22×10 cm) for about 10 min for acclimatization. After the instillation of 5 µl/site of OVA dissolved in physiological saline solution (10 mg/ml) into the bilateral eyes, they were placed into the observation cage (one animal/cage), and the number of instances of eye scratching behavior was counted for 20 min. Eye scratching behavior was defined as fore-limb movements that occurred two times or more and was directed at the ocular surface.

2.5. Histological examination

Twenty-four hours after challenge on days 14, 21, 28, 35 and 42, the rats were anesthetized with diethylether, bled, and the conjunctiva were removed. They were then fixed with 10% neutral buffered formalin for 2 days. The samples were embedded in paraffin, and 4-µm-thick frontal sec-

tions were stained by Luna's method to evaluate the number of eosinophils present. The number of eosinophils that had infiltrated the conjunctival mucosa was microscopically determined. Then, the other 4- μ m-thick frontal conjunctiva sections were stained by hematoxylin and eosin to evaluate conjunctival edema.

2.6. Statistical analysis

All data are represented as the means $\pm$ S.E.M. Statistical analysis was performed using by one-way analysis of variance (ANOVA) with the Dunnett's test. A probability value of less than 0.05 was considered to be statistically significant. ED₅₀ values were calculated by the Probit method.

3. Results

3.1. Changes in reactivity to antigen after topical sensitization

To investigate the reactivity changes for antigen on eye scratching behavior, topical application of OVA was performed before (day 14) and after (days 21, 28, 35 and 42) immunization. After application, the number of instances of eye scratching behavior was counted for 20 min and rats showing more than 10 times were considered to be positive responders.

Table 1

Changes in ED₅₀ values for antigen-induced eye scratching behavior due to cetirizine, ramatroban and zafirlukast administrations in sensitized rats

Group	Days after sensitization				
	14	21	28	35	42
Control	608	3.09	2.76	3.27	2.60
Cetirizine	610	3.25	2.64	2.66	2.62
Ramatroban	598	2.55	3.83	3.59	4.17
Zafirlukast	604	2.35	2.52	2.84	2.82

The numerals indicate the ED₅₀ value (μ g/site). Drugs were orally administered at 10 mg/kg 1 h before topical sensitization.

Antigen was applied to the bilateral eyes, and the number of instances of eye scratching behavior was counted for 20 min. Rats exhibiting eye scratching behavior more than 10 times were considered to be positive responders. The ED₅₀ was calculated by the Probit method.

Table 2

Changes in ED₅₀ values for histamine-induced eye scratching behavior due to cetirizine, ramatroban and zafirlukast administrations in sensitized rats

Group	Days after sensitization				
	14	21	28	35	42
Control	10.3	7.46	3.30	3.19	2.90
Cetirizine	11.2	7.35	3.61	3.38	3.23
Ramatroban	9.72	9.75	12.0	10.2	9.78
Zafirlukast	9.98	7.05	3.55	3.27	3.74

The numerals indicate the ED₅₀ value (μ mol/site). Drugs were orally administered at 10 mg/kg 1 h before topical sensitization.

Histamine was applied to the bilateral eyes, and the number of instances of eye scratching behavior was counted for 20 min. Rats exhibiting eye scratching behavior more than 10 times were considered to be positive responders. The ED₅₀ was calculated by the Probit method.

Table 1 shows the changes in reactivity to antigen-induced eye scratching behavior. For the control group, reactivity to antigen on days 21, 28, 35 and

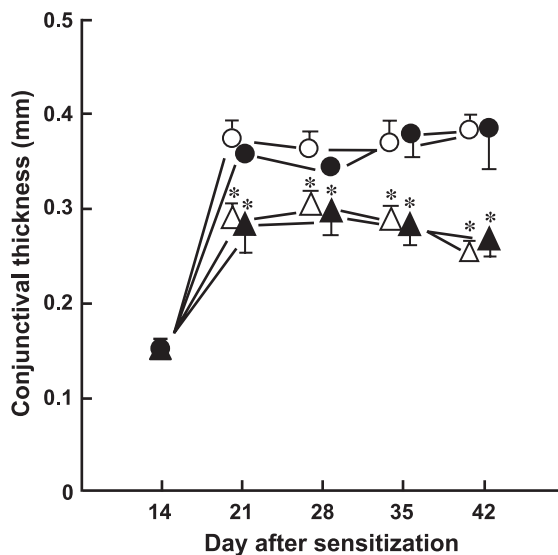


Fig. 1. Changes in conjunctival edema after repeated administration of cetirizine, ramatroban and zafirlukast in sensitized rats. The drugs were orally administered at 10 mg/kg 1 h before topical sensitization. Twenty-four hours after the final antigen challenge, the rats were sacrificed and their conjunctiva were removed. Conjunctival specimens were fixed and stained by hematoxylin and eosin. The results are the following: open circle, control; closed circle, cetirizine; open triangle, ramatroban; and closed triangle, zafirlukast. Each point and vertical bar shows the means $\pm$ S.E.M. of five experiments. *: Significantly different from the control group at $P < 0.05$.

42 increased by 195–234 times compared with before topical immunization (day 14). Similar results were obtained even when cetirizine, ramatroban and zafirlukast were repeatedly administered from day 14 to day 42.

3.2. Changes in reactivity to histamine after topical sensitization

The experimental procedures were similar for using antigen. For the control group, reactivity to histamine-induced eye scratching behavior increased by 1.4–3.6 times compared with before topical immunization. Similar results were obtained for the cetirizine and zafirlukast administration groups. However, for the ramatroban-treated group, a similar ED₅₀ was obtained compared with before topical immunization, indicating that increased reactivity to histamine was inhibited by ramatroban (Table 2).

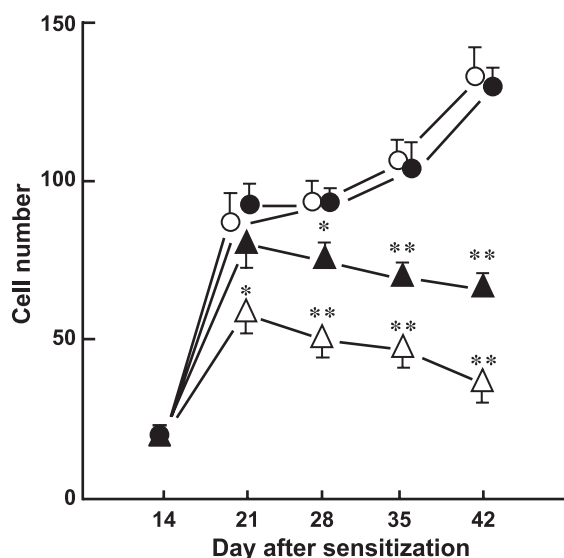


Fig. 2. Changes in the number of conjunctival eosinophils after repeated administration of cetirizine, ramatroban and zafirlukast in sensitized rats. The drugs were orally administered at 10 mg/kg 1 h before topical sensitization. Twenty-four hours after the final antigen challenge, the rats were sacrificed and their conjunctiva were removed. Conjunctival specimens were fixed and stained by Luna's method, and the numbers of eosinophils were counted in whole specimens. The results are the following: open circle, control; closed circle, cetirizine; open triangle, ramatroban; and closed triangle, zafirlukast. Each point and vertical bar shows the means $\pm$ S.E.M. of five experiments. *, **: Significantly different from the control group at $P<0.05$ and $P<0.01$, respectively.

3.3. Changes in conjunctival edema after topical sensitization

Fig. 1 shows the changes in conjunctival edema that occurred after topical sensitization. For the control group, conjunctival edema gradually increased from day 21 to day 42 compared with day 14. Similar result was observed for the cetirizine-treated group. However, for the ramatroban and zafirlukast groups, conjunctival edema was significantly inhibited compared with the control group from day 21 to day 42.

3.4. Changes in conjunctival eosinophil numbers after topical sensitization

Changes in the number of eosinophils in the conjunctival mucosa of rats sensitized with antigen are shown in Fig. 2. For the control group, the number of conjunctival eosinophils gradually increased from day 21 to day 42. A similar result was observed for the cetirizine treated group. However, for the ramatroban and zafirlukast groups, the number of conjunctival eosinophils significantly decreased compared with the control group. The effect on the ramatroban treated group was more potent than that of the zafirlukast group.

4. Discussion

Reactivity to antigen- and histamine-induced eye scratching behavior increased after topical sensitization. In addition, although an increase in reactivity to antigen in the present allergic conjunctivitis model was not influenced by any of the drugs used, the increase in reactivity to histamine was significantly inhibited by repeated administration of the TXA₂ antagonist ramatroban. Tohda et al. [10] also reported that increased histamine reactivity is inhibited by ramatroban in a study on asthmatic histamine hyper-responsiveness in sensitized guinea pigs. Gleich and Loegering [11] proposed an intimate relationship between eosinophils and hypersensitivity to stimulants including histamine.

The number of conjunctival eosinophils was increased by topical sensitization, and repeated administration of ramatroban and the cys-LT antagonist zafirlukast resulted in a significant inhibition of

eosinophil infiltration from the conjunctival mucosa. Eosinophils play a crucial role in the pathophysiology of various allergic diseases such as atopic dermatitis [12], allergic asthma [13] and allergic rhinitis [14]. Yamasaki et al. [15] reported that seratrodist (a TXA₂ antagonist) and pranlukast (a peptide leukotriene antagonist) inhibited eosinophil infiltration into the nasal mucosa in guinea pigs. Kayasuga et al. [16] also showed that ramatroban and zafirlukast significantly inhibit eosinophil infiltration into the nasal mucosa in mice. From these findings, we believe that TXA₂ and cys-LTs are closely correlated with the infiltration of eosinophils into the rat conjunctiva mucosa similar to that for nasal mucosa. TXA₂ and cys-LTs are likely released from mast cells by antigen–antibody reaction, and these mediators then induce the infiltration of eosinophils into the conjunctiva mucosa through receptors. After this, infiltrated eosinophils are degranulated and release injury substances such as major basic protein (MBP) and eosinophil cationic protein (ECP). Epithelial cells are then harmed mainly by MBP and ECP and inflammation including edema is generated, resulting in increased histamine reactivity [11].

We found that the development of conjunctival edema also occurs in our allergic conjunctivitis rat model [3]. In addition, the development of edema induced by topical sensitization was inhibited by repeated administration of ramatroban and zafirlukast. Many studies have investigated the relationship between allergic edema and TXA₂ or cys-LTs. The sulfidopeptide LTs can facilitate plasma leakage and edema formation as well as cellular diapedesis [17]. Therefore, TXA₂ and cys-LTs may be closely related to aggravation of the conjunctival edema.

From these results, we conclude that TXA₂ and/or cys-LTs is responsible for the development of experimental allergic conjunctivitis in rats.

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