Inhibitory Effect of Olopatadine on Antigen-Induced Eosinophil Infiltration and the LFA-1 and Mac-1 Expression in Eosinophils

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ABSTRACT—The inhibitory effect of olopatadine, a new antiallergic drug, on antigen-induced eosinophil infiltration and its mechanisms were examined using the local sensitized rat allergic rhinitis model and isolated IL-5-stimulated rat peritoneal eosinophils. Olopatadine dose-dependently inhibited antigen-induced eosinophil infiltration in the nasal mucosa. Olopatadine dose-dependently repressed the IL-5-induced expressions of CD11a/CD18 (LFA-1) and CD11b/CD18 (Mac-1) on rat peritoneal eosinophils. However, olopatadine had no effect on IL-5-induced CD49d/CD29 (VLA-4) expression. These results suggest that olopatadine may inhibit antigen-induced eosinophil infiltration through repression of LFA-1 and Mac-1 expression.

Keywords: Eosinophil infiltration, Allergic rhinitis, Adhesion molecule

Allergic rhinitis is an inflammatory disorder of the nose characterized by symptoms of sneezing, rhinorrhea and nasal congestion after allergen exposure. In most cases, allergic rhinitis has two phases that are known as the immediate phase and the late phase. The immediate phase is triggered by an IgE-mediated anaphylactic reaction by inhaled allergens (1). In contrast, the late phase reaction is typically referred to as a spontaneous recurrence of symptoms about 12 h after the immediate phase reaction (2). During the late phase, many eosinophils, which can be the source of IL-4, IL-5 (3), leukotriene, and superoxide anion (4), are infiltrated into nasal mucosa. A hallmark of allergic rhinitis is an increased number of eosinophils in the rhinitic nose compared with non-allergic subjects.

Olopatadine (KW-4679), (Z)-11-[[3-dimethylamino)propylidene]-6,11-dihydrodibenz[b,e]oxepin-2-acetic acid monohydrochloride, is a new antiallergic drug. This compound has been shown to be a specific H1 histamine-receptor antagonist with a Ki value of 16 nM (5); and it has been shown to suppress leukotrienes (LTs) and thromboxane (TX) release and platelet-activating factor (PAF) formation by reducing arachidonic acid release from phospholipids in guinea pig eosinophils (6).

In the present study, we examined the effect of olopatadine on antigen-induced eosinophil infiltration into nasal mucosa using the local sensitized rat allergic rhinitis model and investigated the influence of this compound on the expression of adhesion molecules on the surface of eosinophils.

Male Brown Norway (BN) rats weighing 180–230 g were provided by Charles River Japan (Kanagawa). The animals were housed in an air-conditioned room maintained at 25 ± 1°C with humidity of 55 ± 5% and a 12-h light/dark cycle for 1 week before the commencement of experiments. Food and water were provided ad libitum. Ovalbumin (OA; Sigma Chemical Co., St. Louis, MO, USA) was used as the antigen. BN rats were sensitized in our original cages (Fig. 1) that could be circulated with OA (0.2 g/day) all day. In preliminary experiments, we established that the dose of OA used in this study did not elicit any detectable bronchoconstriction (data not shown). Each experimental group consisted of 4 – 6 rats. The rhinitis symptoms (scratching, sneezing, nasal rubor and pituita) were evaluated macroscopically using the following grading codes: −, no change; ±, very slight; +, slight; ++, moderate; ++++, severe, at each period after antigen challenge. For histopathological examination, rats were sacrificed at each period. The nasal tissues were fixed in a buffered 15% formalin solution (pH 7.4) for 7 days and decalcified with 0.5 M EDTA solution for 2 weeks. Peripheral tissue was removed, and the nasal mucosa was embedded in paraffin and thin-sectioned at 4 μm. These sections were stained with a Luna staining using hematoxylin (Wako Pure
Chemical Co., Osaka)-Biebrich Scarlet (Nacalai Tesque, Kyoto) solution. Eosinophils in the nasal septum were counted in the six sections microscopically and results were expressed as an average number of eosinophils per section. Olopatadine (Kyowa Hakko Kogyo Co., Ltd., Tokyo) was dissolved in a distilled water and was orally administrated once a day. Dexamethasone (Orgadrone Infection; Sankyo Co., Ltd., Tokyo) was diluted with a sterilized physiological saline and intradermally subcutaneously administrated once a day.

Eosinophils were collected from abdominal cavities of BN rats. Briefly, a saline-swelled Sephadex G-200 (4,000 beads/mL) (Amersham Pharmacia Biotech AB, Uppsala, Sweden) was intravenously injected into rats on days 0, 2, 5 and 8. Then 1.5 mL of air was injected into the abdominal cavity 24 h after the last Sephadex injection. Forty-eight hours after air injection, rats were anesthetized using diethyl ether and exsanguinated by cutting the carotid arteries. Ten milliliters of phosphate-buffered saline containing 0.25% bovine serum albumin was injected into the abdominal cavity and the fluid was collected. Cell suspension was separated by Percoll (Amersham Pharmacia Biotech AB) density gradient centrifugation, and the eosinophil-rich fraction was collected. The purity was a level greater than 90%. Eosinophils were suspended in RPMI-1640 medium. The cells were stimulated by 10 ng/mL of IL-5 with or without each concentration of olopatadine for 2 h at 37°C. After the cells were labeled using FITC conjugated anti-CD11a antibody (Cosmo Bio, Tokyo), phycoerythrin (PE) conjugated anti-CD11b antibody (Immunootech, Marseile, France), or RED670 conjugated anti-CD49d antibody (Endogen, Woburn, MA, USA), these were analyzed using a flow cytometer. The results were presented as the rate of labeled cells in all cells.

Results were presented as the means ± S.E.M. One-way analysis with Dunnett’s test or a unpaired Student’s t-test was used to determine statistical significance. A statistical significance at $P<0.05$ was used throughout.

When rats were exposed to antigen, rubor was observed from the 2nd day of exposure and reached the maximum on the 5th day. Scratching and nasal pituita were observed from the 5th day. On the other hand, sneezing was almost never observed during the experimental period. The number of infiltrated eosinophils was $3.5 ± 1.3$ cells/section before antigen challenge. The cell number increased from the 1st day ($24.7 ± 1.0$ cells/section) and reached the first peak on the third day ($64.3 ± 4.7$ cells/section). The number of eosinophils first decreased on the 5th day ($15.7 ± 6.3$ cells/section).

![Fig. 1. Photograph of the experimental apparatus for antigen-exposure (a) and the diagram of the apparatus (b).](image)

![Fig. 2. Effects of olopatadine and dexamethasone on ovalbumin (OA)-induced eosinophil infiltration. Brown Norway rats were exposed to antigen for 7 days. Olopatadine (1–10 mg/kg, p.o.) and dexamethasone (0.1 mg/kg, s.c.) were administrated once a day during the experimental period. Each experimental group consisted of 4–6 rats. Each value represents the mean ± S.E.M. *$P<0.05$, **$P<0.01$ vs control; ***$P<0.01$ vs 0 mg/kg of olopatadine.](image)
3.5 cells/section) and reached the second sub-maximum (53.2 ± 2.6 cells/section) on the 7th day. When olopatadine (1 – 10 mg/kg) was orally administered once a day during the exposure to antigen, the number of infiltrated eosinophils on the 7th day was dose-dependently decreased (Fig. 2). The prevention of antigen-induced eosinophil infiltration was significant at 3 and 10 mg/kg. The rhinitis symptoms were not observed in the treatment groups with olopatadine. The significant prevention was also observed by treatment with 0.1 mg/kg (s.c.) of dexamethasone (Fig. 2). On the other hand, the number of infiltrated neutrophils did not change due to antigen challenge and olopatadine treatment. Isolated eosinophils expressed CD11a/CD18 (leukocyte function-associated antigen-1: LFA-1) (29.9 ± 3.5%), CD11b/CD18 (Mac-1) (30.7 ± 3.4%) and CD49d/CD29 (very late antigen-4: VLA-4) (16.4 ± 1.0%) on the cell surface (Fig. 3). When the cells were treated with 10 ng/mL of IL-5, the expressions were significantly increased to 45.1 ± 1.0%, 41.9 ± 6.1% and 24.5 ± 3.5%, respectively (Fig. 3). The treatment with olopatadine (0.1 – 10 μg/mL) dose-dependently repressed the IL-5-induced increase of LFA-1 expression on eosinophils (Fig. 3). The 10 μg/mL of azelastine did not prevent the increase (Fig. 3). Olopatadine, but not azelastine, also repressed the increase of Mac-1 expression (Fig. 3). However, no doses of olopatadine or azelastine modified the increase in VLA-4 expression (Fig. 3).

Leukocytes migration from the circulation into surrounding tissues is a critical stage of the inflammatory process, necessary for the host defense against pathogens. This process involves sequential events that have been described as follows: leukocyte and endothelial cell activation by chemoattractants, rolling of leukocytes on vascular endothelium via selectin interaction with carbohydrate ligands, firm adhesion mediated by interaction between integrins and their ligands, and transendothelial migration (7).

Although many integrins have been shown to play some role in the pathophysiology of allergic diseases, two may be of particular importance: LFA-1 and VLA-4. These molecules have been shown to be central to the adhesion and transendothelial migration of T cells, eosinophils, and other leukocytes (8).

It was reported that oral administration of olopatadine significantly inhibited nasal blockage on guinea pigs (9) and that olopatadine was useful for therapy of allergic dysfunction such as an allergic conjunctivitis in a clinical study (10). The present study demonstrated that olopatadine dose-dependently inhibited antigen-induced eosinophil infiltration into nasal mucosa and repressed IL-5-induced expressions of LFA-1 and Mac-1, not VLA-4, on rat peritoneal eosinophils. Eosinophils adhesion and/or rolling are mediated by some adhesion molecules such as VCAM-1 (11), P-selectin and ICAM-1 (12) on the surface of epithelial cells. It was reported that some antiallergic drugs such as azelastine (13) and cetirizine (14) repressed the expression of adhesion molecules on the epithelial cells. However, the effect of antiallergic drugs on the counter ligands for ICAM-1 and VCAM-1, which are expressed on eosinophils, is not yet reported. In the present study, the repressive effect of olopatadine on IL-5-induced expression of the counter ligands, LFA-1 and Mac-1, is confirmed. However, azelastine, which was reported to down-regulate ICAM-1 (13), could not repress the IL-5-induced expression of LFA-1, Mac-1 and VLA-4. More detailed studies will be required to ascertain the mechanism of repressive action of olopatadine on LFA-1 and Mac-1.

Fig. 3. Effects of olopatadine and azelastine on IL-5 (10 ng/mL)-induced expression of LFA-1, Mac-1 and VLA-4 on rat eosinophils. The results are presented as the percentage of labeled cells in all cells. Each value represents the mean ± S.E.M. of 4 experiments. *P<0.05 vs control; #P<0.05 vs IL-5 alone.
expression.

Olpatadine has been shown to inhibit the production/release of LTB$_4$ and PAF (6). In the present study, olpatadine repressed IL-5-induced expression of LFA-1 and Mac-1 on eosinophils. These actions may be involved in the effectiveness of olpatadine on the inhibitory effect of antigen-induced eosinophil infiltration into the nasal mucosa. Therapy directed at these adhesion molecules might be expected not only to inhibit the accumulation of eosinophils and other leukocytes at inflammatory sites but also to attenuate the activation of cells that is integral to the immune response.

In conclusion, olpatadine, administered orally, inhibited the antigen-induced eosinophil infiltration and repressed IL-5-induced expression of LFA-1 and Mac-1 on eosinophils. Therefore, olpatadine may be also useful for the treatment of the late phase of allergic rhinitis.

REFERENCES

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