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Original Article

Effects of orally administered olopatadine hydrochloride on the ocular allergic reaction in rats

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Abstract

Background: Olopatadine hydrochloride is an anti-allergic agent with histamine H_1 receptor antagonistic action. We investigated the effects of olopatadine on passive anaphylaxis reaction- and compound 48/80-induced conjunctivitis in rats.

Methods: Allergic conjunctivitis was induced in rats passively sensitized by injection of rat anti-ovalbumin (anti-OVA) serum into the upper subconjunctiva of the right eye, followed by intravenous administration of the antigen and Evans blue dye. After 30 min, the amount of dye leaking into the conjunctiva was measured. Non-allergic conjunctivitis was induced in rats by injection of compound 48/80. Olopatadine or other reference compounds were orally given 1 h before the challenge.

Results: The amount of dye leaking into the conjunctiva following passive anaphylaxis was significantly inhibited by oral administration of 0.01–1 mg/kg olopatadine and the ID_{50} value was 0.093 mg/kg. In the control group, pathological examination revealed edema and lymphocyte infiltration in the conjunctiva and the palpebral skin. Olopatadine, at 0.03 and 0.3 mg/kg, reduced the grade of these pathological findings. The other antiallergic drugs (1 mg/kg loratadine, 0.3 mg/kg epinastine, 0.3 mg/kg cetiridine, 1 mg/kg ebastine, 30 mg/kg fexofenadine and 3 mg/kg chlorpheniramine), when administered orally, inhibited the passive anaphylaxis reaction-induced vascular hyperpermeability of the conjunctiva, as was the case with olopatadine hydrochloride. Subconjunctival administration of compound 48/80 induced vascular hyperpermeability, which is presumably mediated by histamine release. Oral administration of 0.1 and 1 mg/kg olopatadine significantly inhibited the amount of dye leakage.

Conclusion: Orally administered olopatadine is expected to improve allergic conjunctivitis.

Key words: conjunctivitis, olopatadine, rats.

Introduction

Patients with allergic conjunctivitis suffer from itching, redness, edema and tearing, accompanied by vascular hyperpermeability in the conjunctiva. Allergic conjunctivitis often occurs concomitantly with rhinitis in subjects with seasonal allergy.[1] Of the various mediators, histamine plays a crucial role and, thus, antihistamine drugs are used as first-line therapy for ocular and nasal allergy.

Topical therapy has been the preferred treatment for ocular allergic disease. Eye drops can be applied easily and seldom lead to systemic side-effects. Moreover, the physical presence of the drops themselves will have a washout effect, helping to remove the inflammatory mediators and, thereby, lessening some of the symptoms. In contrast, topical treatment may also have some disadvantages. Although topical steroids are effective in reducing the influx of inflammatory cells, they have little

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effect on mast cell mediator secretion and take several days to achieve their maximal effect. Oral histamine H_1 receptor antagonists have been shown to be effective against the allergy,[2] although they are not always useful because they sometimes exhibit some systemic side-effects, such as sedation.

Olopatadine hydrochloride (Allelock®; Kyowa Hakko Kogyo, Shizuoka, Japan) is an anti-allergic agent with histamine H_1 receptor antagonistic action[3,4] that is indicated for the treatment of the signs and symptoms of allergic rhinitis, chronic urticaria, eczema dermatitis, prurigo, pruritis cutaneous, psoriasis vulgaris and erythema exsudativum multiforme. Olopatadine exhibits potent antihistamine activity *in vivo* following its systemic administration.[5] In addition, a previous *in vitro* study has demonstrated that olopatadine inhibits the release of chemical mediators, such as thromboxane B_2, peptide leukotriene and tachykinin.[6–8] Moreover, olopatadine inhibits the anti-IgE antibody induced release of tumor necrosis factor (TNF)-α from human conjunctival mast cells and, furthermore, decreases the anti-IgE mast cell supernatant-induced upregulation of intercellular adhesion molecule-1 (ICAM-1) on human conjunctival epithelial cells.[9,10] Olopatadine ophthalmic solution 0.1% (w/v; Patanol®; Alcon Laboratories, Fort Woth, TX, USA) has been approved for the treatment of the signs and symptoms of allergic conjunctivitis.

Anti-allergic drugs are widely used for the treatment of allergic conjunctivitis and there is considerable literature about the clinical efficacy of anti-allergic drugs. In contrast, little work has been published on the effects of anti-allergic drugs in experimental allergic conjunctivitis. The present study was conducted to clarify the effects of orally administered olopatadine on the passive anaphylaxis reaction-induced vascular hyperpermeability of the conjunctiva in a rat model of allergic conjunctivitis. We also examined the effects of the other anti-allergic drugs (loratadine, epinastine, fexofenadine, cetiridine, ebastine and the classic antihistamine chlorpheniramine). Furthermore, we examined the effect of olopatadine on the vascular hyperpermeability induced by compound 48/80.

METHODS

Animals

Male Sprague-Dawley rats (5 weeks old) were purchased from Charles River Japan (Kanagawa, Japan). Experiments were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, and the experimental protocol was approved by the Committee for Animal Experiments in Kyowa Hakko Kogyo. Rats were kept in a specific pathogen-free animal facility at a temperature of 22–24°C, humidity of 50–60% and a 12 h light/dark cycle.

Materials

Olopatadine hydrochloride, loratadine, epinastine hydrochloride, fexofenadine hydrochloride, cetirizine hydrochloride and ebastine were synthesized or extracted from commercially available tablets in our institute. Sodium cromoglicate ophthalmic solution 2% w/v (Intal®; disodium cromoglycate (DCSG)) was purchased from Fujisawa Pharmaceutical (Osaka, Japan). Chlorpheniramine maleate, ovalbumin (OVA; albumin, chicken egg) and compound 48/80 were purchased from Sigma Chemical (St Louis, MO, USA). Evans blue was purchased from Aldrich (Milwaukee, WI, USA). *Bordetella pertussis* inactive microorganism suspension and formamide were purchased from Wako Pure Chemical (Osaka, Japan). Olopatadine, epinastine, cetirizine and chlorpheniramine were dissolved in distilled water. Loratadine, fexofenadine and ebastine were suspended in 0.5% w/v methylcellulose solution. All drugs were administered orally at a volume of 1 mL/100 g bodyweight.

Antiserum

Rat antiserum to OVA was obtained according to the method of Stotland and Share.[11] Rats were sensitized by injection of 0.6 mL saline containing OVA (1.5 mg), alum (12 mg) and 6×10^9 cells *B. pertussis* into the four footpads. Five days later, the sensitization was boosted by an intramuscular injection of 0.05 mL saline containing OVA (0.5 mg). A further 5 days later, whole blood was collected from the abdominal aorta and serum was stored at –80°C. The passive cutaneous anaphylaxis titer in rats was 1 : 64.

Passive anaphylaxis reaction-induced vascular hyperpermeability in the rat conjunctiva

Rats were passively sensitized by diluted rat anti-OVA serum (1 : 16) injected subconjunctivally into the right eye. Forty-eight hours after passive sensitization, 5 mg OVA dissolved in 1 mL of 0.5% w/v Evans blue solution was injected intravenously. Thirty minutes after the challenge, rats were killed and the eyeballs and conjunctiva

were removed and immersed in formamide at 45°C overnight. The amount of dye in the extracted solution was determined with a spectrophotometer (THERMO max™; Molecular Devices, Sunnyvale, CA, USA) at 625 nm. Olopatadine and reference drugs were administered orally 1 h before antigen challenge. Disodium cromoglycate (10 μL; 0.2 mg/site) was applied topically in the eye 20 min before challenge. Vehicle (distilled water or 0.5% w/v methylcellulose solution) was administered orally 1 h before the challenge as a control.

Histopathology

A passive anaphylaxis reaction was produced in the rat conjunctiva, as noted above. The effects of 0.03 and 0.3 mg/kg olopatadine were examined. Thirty minutes after challenge, rats were killed. The eyelids and eyeballs were removed and fixed in neutral-buffered formalin and then embedded in paraffin wax. Tissue sections for light microscopic observation were stained with hematoxylin and eosin. Preparations were observed under a light microscope (BX60; Olympus Optical, Tokyo, Japan). Pathological examination revealed edema and lymphocyte infiltration in the conjunctiva and the palpebral skin.

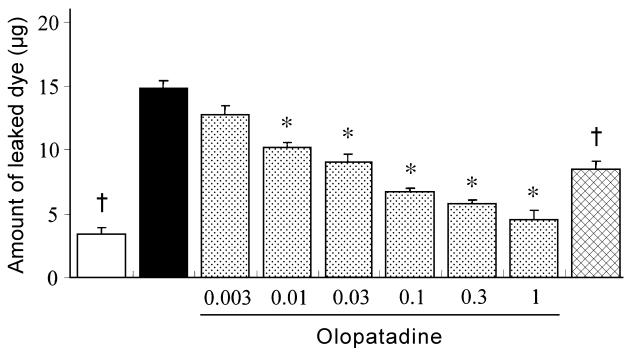

Fig. 1 Effects of orally administered olopatadine on passive anaphylaxis reaction-induced vascular hyperpermeability of the conjunctiva in rats. Animals were passively sensitized by the rat anti-ovalbumin (OVA) serum or treated with saline (□). Forty-eight hours after sensitization, a mixture of OVA and Evans blue dye was injected intravenously. Thirty minutes after the challenge, each conjunctiva was isolated and the pigment was extracted and quantified photometrically. Olopatadine was administered orally 1 h before the challenge. Disodium cromoglycate (10 μ; ⊠) was applied topically to the eye 20 min before challenge. Distilled water was administered orally 1 h before the challenge as a control (■). Data are the mean ± SEM ($n = 6$). *$P < 0.001$ compared with control (Dunnett's test); †$P < 0.001$ compared with control (Student's *t*-test).

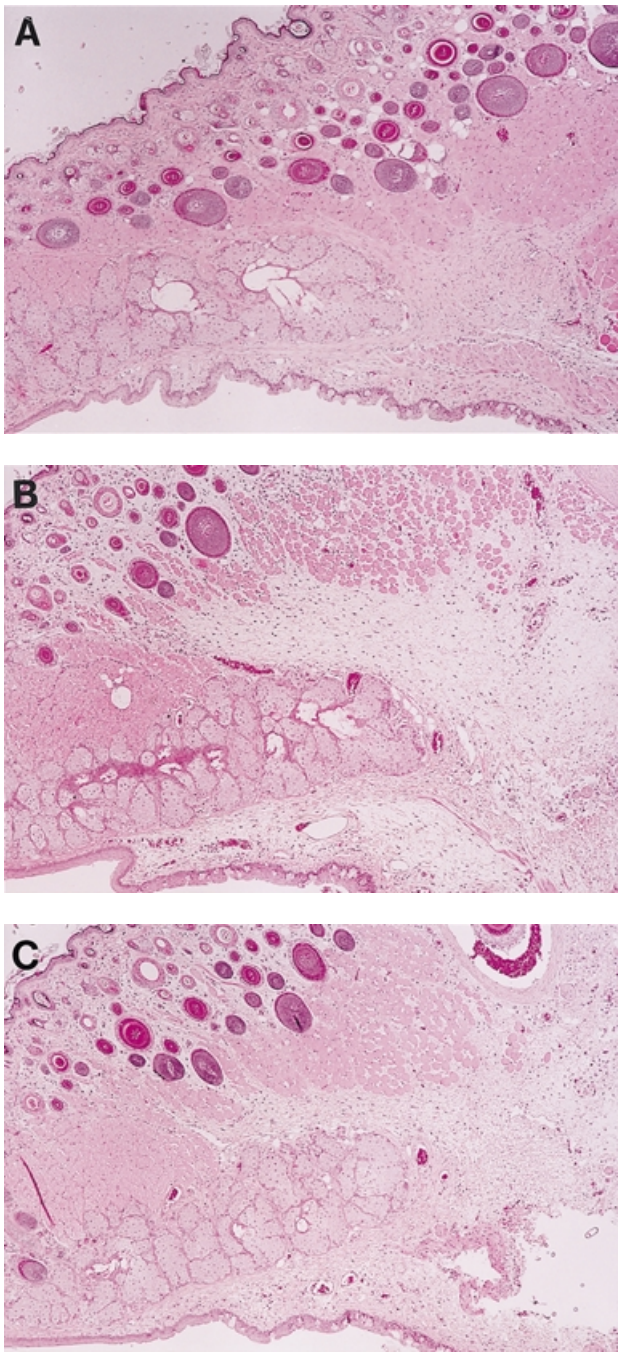

Fig. 2 Photographs showing the effects of orally administered olopatadine on passive anaphylaxis reaction-induced conjunctivitis in rats. Animals were passively sensitized by rat anti-ovalbumin (OVA) or treated with saline (non-sensitized). Forty-eight hours after sensitization, 0.03 mg/kg olopatadine or distilled water (control) was administered orally and then OVA solution was injected intravenously. Thirty minutes after the challenge, the conjunctiva and palpebral skin were isolated. Tissue sections were stained with hematoxylin and eosin. (a) Non-sensitized, (b) control and (c) 0.03 mg/kg olopatadine. (Original magnification ×60.)

Table 1 Effects of orally administered olopatadine on the morphology of the conjunctiva and palpebral skin in rats with passive anaphylaxis reaction-induced conjunctivitis

Site	Finding	Grade	No. animals			
			Non-sensitized	Control	Olopatadine	
Conjunctiva	Edema in the submucosa	–	6	1	5	6
		±		1	1	
		+		4		
		++				
		+++				
Palpebral skin	Edema in the dermis	–	6	1	4	6
		±			2	
		+		3		
		++		2		
		+++				

Animals were passively sensitized by rat anti-ovalbumin (OVA) or treated with saline (non-sensitized). Forty-eight hours after sensitization, 0.03 or 0.3 mg/kg olopatadine or distilled water (control) was administered orally and then OVA solution was injected intravenously. Thirty minutes after the challenge, the conjunctiva and palpebral skin were isolated. Tissue sections were stained with hematoxylin and eosin and graded as follows: –, no remarkable abnormality; ±, very slight change; +, slight change; ++, moderate change; and +++, severe change. Six animals were examined for each group.

The edema was graded as follows: –, no remarkable abnormality; ±, very slight changes; +, slight changes; ++, moderate changes; and +++, severe changes.

Compound 48/80-induced vascular hyperpermeability in the rat conjunctiva

Rats were injected intravenously with 1 mL of 1% w/v Evans blue dye. Immediately thereafter, each animal was injected subconjunctivally with compound 48/80 (50 μg/50 μL) in the right eye. Thirty minutes after the injection of compound 48/80, animals were killed and hyperpermeability responses were quantitated as noted above. Olopatadine was administered orally 1 h before the injection of compound 48/80. Disodium cromoglycate (10 μL) was applied topically to the eye 20 min before the injection of compound 48/80. Vehicle (distilled water) was administered orally 1 h before the injection and served as a control.

Data analysis

Data are presented as the mean ± SEM. The *F*-test, followed by the Aspin–Welch test or Student's *t*-test, was used for analysis of differences between two groups. Multiple comparisons among treatment groups were assessed by one-way analysis of variance, followed by Dunnett's test for ad hoc comparisons. $P < 0.05$ was considered statistically significant. The ID_{50} value was calculated by the probit method.

RESULTS

Effects of orally administered olopatadine on the passive anaphylaxis reaction-induced conjunctivitis

As shown in Fig. 1, the amount of dye leaking into the conjunctiva was significantly increased in the control group compared with the non-sensitized group. The amount of dye leaking into the conjunctiva in the control and non-sensitized groups was 14.8 ± 0.6 and 3.4 ± 0.5 μg, respectively. Orally administered olopatadine (0.01–1 mg/kg) significantly inhibited dye leakage and ID_{50} was 0.093 mg/kg. At 0.2 mg/site, DSCG significantly inhibited dye leakage by 43.2%.

Pathological examination revealed edema and lymphocyte infiltration in the conjunctiva and the palpebral skin in the control group (Fig. 2; Table 1). Olopatadine (0.03 mg/kg) reduced both edema and lymphocyte infiltration. No swelling or lymphocyte migration was observed in rats treated with 0.3 mg/kg olopatadine.

Effects of orally administered loratadine, epinastine, fexofenadine, cetirizine and ebastine on the passive anaphylaxis reaction-induced vascular hyperpermeability of the conjunctiva

In another series of experiments, we examined the effects of the anti-allergic drugs loratadine, epinastine,

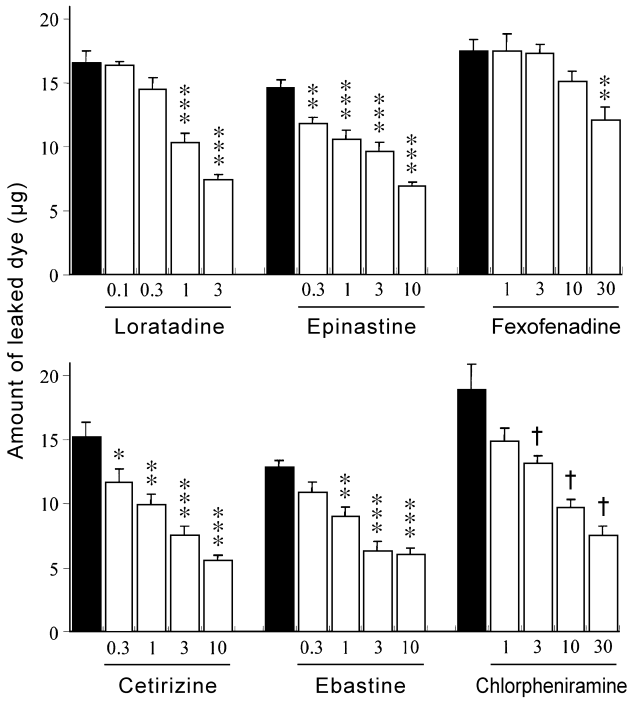

Fig. 3 Effects of loratadine, epinastine, fexofenadine, cetirizine, ebastine and chlorpheniramine on passive anaphylaxis reaction-induced vascular hyperpermeability of the conjunctivitis in rats. Animals were passively sensitized by the rat anti-ovalbumin (OVA) serum. Forty-eight hours after sensitization, a mixture of OVA and Evans blue dye was injected intravenously. Thirty minutes after the challenge, each conjunctiva was isolated and the pigment was extracted and quantified photometrically. Various drugs and distilled water or 5% w/v methycellulose (■) were administered orally 1 h before the challenge. Data are the mean ± SEM ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control (Dunnett's test); $^{\dagger}P < 0.05$ compared with control (Steel test).

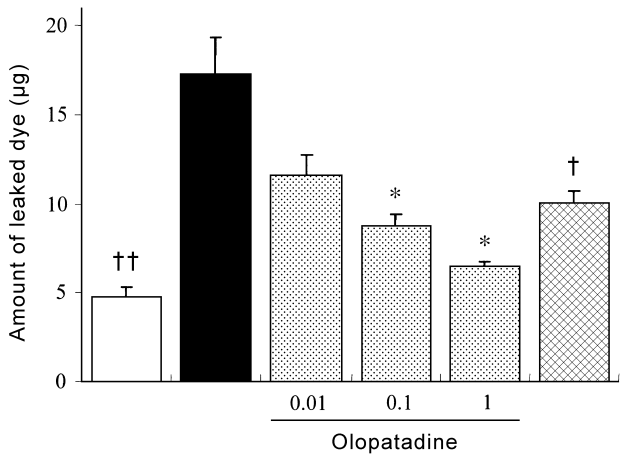

Fig. 4 Effects of orally administered olopatadine on compound 48/80-induced vascular hyperpermeability of the conjunctiva in rats. Animals were treated with 50 μL of 0.1% w/v compound 48/80 or saline (□) and Evans blue dye was injected intravenously. Thirty minutes after the injection, each conjunctiva was isolated and the dye was extracted and quantified photometrically. Various doses of olopatadine were administered orally 1 h before the challenge. Disodium cromoglycate (10 μL; ⊠) was applied topically in both eyes 20 min before the challenge. Distilled water was orally administered 1 h before the challenge as a control (■). Data are the mean ± SEM ($n = 6$). $^{*}P < 0.05$ compared with control (Steel test); $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ compared with control (Aspin-Welch test).

fexofenadine, cetiridine, ebastine and the classic antihistaminic chlorpheniramine on the passive anaphylaxis-induced vascular hyperpermeability of the conjunctiva. As shown in Fig. 3, the amount of dye leaking into the conjunctiva was significantly increased in the control group compared with the non-sensitized group. Loratadine (at doses of 1 mg/kg and more) significantly inhibited dye leakage and the ID_{50} for loratadine was 2.1 mg/kg. Epinastine (at doses of 0.3 mg/kg and more) significantly inhibited dye leakage and the ID_{50} for epinastine was 9.7 mg/kg. Cetiridine (at doses of 0.3 mg/kg and more) significantly inhibited dye leakage and the ID_{50} for cetridine was 3.3 mg/kg. Ebastine (at doses of 1 mg/kg and more) significantly inhibited dye leakage and the ID_{50} for ebastine was 5.2 mg/kg. Fexofenadine (30 mg/kg) significantly inhibited dye leakage. Chlorpheniramine (at doses of 3 mg/kg and more) significantly inhibited dye leakage and the ID_{50} for chlorpheniramine was 12.4 mg/kg.

Effects of orally administered olopatadine on the compound 48/80-induced vascular hyperpermeability of the conjunctiva

As an index of antihistamine activity, we determined the effect of orally administered olopatadine on the vascular hyperpermeability response to compound 48/80. As shown in Fig. 4, injection of compound 48/80 significantly increased the amount of dye leaking into the conjunctiva compared with the amount of dye leaking out following saline injection. The amount of dye leaking in the control and non-sensitized groups was 17.3 ± 2.0

and 4.7 ± 0.6 μg, respectively. Olopatadine (0.1 and 1 mg/kg) significantly inhibited dye leakage by 49.7 and 63.0%, respectively. At 0.2 mg/site, DSCG also significantly inhibited dye leakage by 42.2%.

Discussion

The present study demonstrated that orally administered olopatadine inhibited the amount of dye leaking into the conjunctiva following passive anaphylaxis or compound 48/80 in rats. Our present results are in accordance with previous observations that olopatadine inhibits antigen- or histamine-induced experimental conjunctivitis in guinea pigs, as was examined using a scoring system.[12] Similarly, the other histamine H_1 receptor antagonists also inhibited the passive anaphylaxis-induced dye leakage into the conjunctiva, although the potencies were somewhat less than that of olopatadine. Moreover, the present pathological examination clarified that olopatadine reduced the grade of edema in the conjunctiva and palpebral skin, as well as lymphocyte infiltration. The present results suggest that orally administered olopatadine is effective for the treatment of conjunctivitis.

Allergic conjunctivitis frequently occurs concomitantly with rhinitis in subjects with seasonal allergy. Currently, anti-allergic drugs with histamine H_1 antagonistic action are prescribed for ocular and nasal allergic diseases. Olopatadine ophthalmic solution (eye drops) has been shown to be effective for the treatment of the signs and symptoms of allergic conjunctivitis.[13,14] Indeed, although topical therapy is the preferred treatment for ocular allergy, it cannot adequately control the signs and symptoms associated with multiple target organ hypersensitivity, including allergic rhinitis. Thus, systemic anti-allergic therapy may be of additional benefit in patients receiving a local therapy. The results of the present study indicate amelioration of the conjunctivitis by orally administered olopatadine, suggesting that the dose of this agent may be effective against conjunctivitis occurring concomitantly with rhinitis.

In the present study, we used a rat model of allergic conjunctivitis[15,16] and conjunctival vascular hyperpermeability[17] that is sensitive to histamine H_1 antagonists. Thus, it is likely that the effectiveness of olopatadine in the conjunctivitis model used in the present study involves histamine H_1 receptor blockade, because this drug exhibits potent histamine H_1 antagonistic activity.[2–5] Moreover, the inhibitory effect of olopatadine on histamine release may have played a role, because this drug has been shown to inhibit histamine release from mast cells.[18] Accordingly, DSCG ophthalmic solution, an inhibitor of histamine release, also possessed an inhibitory effect on vascular hyperpermeability. Moreover, in the present study, olopatadine inhibited compound 48/80-induced vascular hyperpermeability. Compound 48/80 is known to cause histamine release from rat conjunctival mast cells,[19,20] which possibly contributes to vascular hyperpermeability. These observations suggest that the amelioration by olopatadine of the conjunctivitis is mainly mediated by its suppressive effect on histamine action (i.e. H_1 rreceptor antagonistic action and/or an inhibitory action on histamine release).

In addition to the suppressive effect on histamine action, some other mechanisms may be involved in the amelioration of the conjunctivitis by olopatadine. This notion is supported by the clinical observation that olopatadine ophthalmic solution showed some advantage over the histamine H_1 receptor antagonist ketotifen.[13,14] It has been reported that the levels of histamine and substance P in tears of patients with allergic conjunctivitis are elevated.[21,22] Thus, the increased level of substance P, in addition to histamine, in tears may contribute to the pathogenesis and severity of ocular allergic diseases. Accordingly, the inhibition of tachykinin release by olopatadine[8] may be involved in the inhibition f conjunctivitis by this drug. In contrast, olopatadine inhibited the release of TNF-α from human conjunctival mast cells and, furthermore, it decreased the upregulation of ICAM-1 on human conjunctival epithelial cells,[9,10] suggesting that the reduced TNF-α release and ICAM-1 upregulation may also have played roles in the efficacy of olopatadine. Taken together, the results indicate that it is likely that olopatadine modulates the ocular allergic reaction via interference with multiple signaling pathways in the conjunctiva. Further studies are needed to elucidate the effect of olopatadine on various chemical mediators in the conjunctiva and tears.

In conclusion, orally administered olopatadine was demonstrated to inhibit the passive anaphylaxis reaction- or compound 48/80-induced vascular permeability, probably mainly via its histamine suppressive action. Olopatadine was more potent than the other anti-allergic drugs in the passive anaphylaxis reaction-induced vascular hyperpermeability. Thus, it is expected that oral administration of olopatadine is effective for the treatment of conjunctivitis.

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