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Double-Masked, Randomized, Placebo-Controlled Clinical Study of the Mast Cell–Stabilizing Effects of Treatment with Olopatadine in the Conjunctival Allergen Challenge Model in Humans

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ABSTRACT

Objective: The purpose of this study was to assess the effects of olopatadine on the release of mast cell–derived mediators after conjunctival allergen challenge (CAC) in humans.

Methods: This was a double-masked, randomized, placebo-controlled clinical trial. Subjects with a clinical history of seasonal allergic conjunctivitis (but no current symptoms or treatment at baseline) were studied. At visit 1, subjects underwent bilateral CAC with increasing doses of allergen every 15 minutes until a significant clinical reaction was obtained, then were evaluated at 15 minutes and 5 hours after CAC. At visit 2 (2 weeks later), subjects were rechallenged to confirm the allergic response. Subjects exhibiting positive reactions at both visits (at both 15 minutes and 5 hours) were randomized and instructed to treat 1 eye with olopatadine and the contralateral eye with placebo (commercially available artificial tears) in a double-masked fashion twice daily for the 5 days immediately preceding visit 3. At visit 3, bilateral CAC was performed with the same dose as at visit 2. Itching and redness were recorded. Tear cytology for inflammatory cell counts (ie, neutrophils, eosinophils, and lymphocytes) was carried out using precolored slides, and cell numbers were counted at 400× magnification. Tear histamine was assessed using radioimmunoassay histamine measurement. Intercellular adhesion molecule (ICAM)-1/CD54 monoclonal antibody was used for immunohistochemical staining of conjunctival epithelial cells obtained by impression cytology.

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Slides were examined by 3 masked investigators and redness was classified on a scale of 0 (absent) to 3 (very intense).

Results: Ten subjects completed the trial. Olopatadine significantly reduced postchallenge itching and redness compared with placebo ($P < 0.01$ and $P < 0.03$, respectively). Olopatadine also reduced the number of neutrophils and the total number of cells at 30 minutes (both $P = 0.015$), and the number of eosinophils ($P < 0.001$), neutrophils ($P < 0.004$), lymphocytes ($P = 0.011$), and total number of cells ($P = 0.001$) at 5 hours postchallenge compared with placebo. Tear histamine levels were significantly lower after challenge in the eyes pretreated with olopatadine compared with placebo (mean [SD], 7 [8] vs 22 [12] nmol/L; $P = 0.04$). Olopatadine significantly reduced tear histamine levels compared with those measured in the same eyes after CAC at visit 2 ($P = 0.001$), whereas placebo did not affect histamine levels. Olopatadine also significantly reduced ICAM-1 expression compared with placebo at 30 minutes and 5 hours postchallenge ($P < 0.03$ and $P < 0.01$, respectively).

Conclusion: In the present study, olopatadine significantly reduced the levels of histamine, cellular infiltrate, and ICAM expression compared with placebo after CAC, suggesting that it reduced the release of mast cell–derived mediators in humans. This inhibition of mediator release correlated with reduction of itching and redness. (*Clin Ther.* 2003;25:2539–2552)

Key words: olopatadine, histamine, mast cell–stabilizing allergic conjunctivitis, conjunctival allergen challenge.

INTRODUCTION

More than 15% of the general US population (and up to 30% in some industrialized countries) has ocular allergy, the most common manifestations of which are seasonal and perennial allergic conjunctivitis.[1] These 2 conditions are characterized by itching, redness, tearing, chemosis, and eyelid swelling,[2] all of which can be attributed to conjunctival mast cell degranulation.[3] The reaction begins once allergens penetrate the tear film and bind to immunoglobulin E (IgE) receptors on the surface of conjunctival mast cells. This process results in mast-cell activation and subsequent degranulation, causing exocytosis of preformed and newly formed proinflammatory and allergic mediators. Of these, histamine plays a major role in eliciting the clinical signs and symptoms of ocular allergy.[4] Activation of neuronal type 1 histamine receptors (H_1) induces itching, whereas activation of vascular endothelial cells through the action of both H_1 and H_2 histamine receptors leads to vasodilation (ie, hyperemia) and transudation of fluid into tissue (ie, eyelid swelling and chemosis). Other mediators released include tryptase, chymase, prostaglandins, leukotrienes, heparin, and vasoactive peptides.[5] In ad-

dition, mast-cell degranulation releases cytokines, which trigger the activation of vascular endothelial cells and the expression of chemokines and adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1)/CD54. In chronic severe allergy such as atopic keratoconjunctivitis (AKC) and vernal keratoconjunctivitis (VKC), these factors lead to a latent recruitment phase that ushers in inflammatory mediators and their eventual infiltration of the conjunctival mucosa.[6,7]

The temporal progression of an allergic reaction can often be delineated by the expression of signs and symptoms and the types of cells present at the site. Because the pathophysiology of many allergic reactions can be similar, the study of one inflammatory condition can provide insight when examining another. Reactions in both the lung and the nose involve a secondary immunologic phase of cellular infiltration after the initial reaction, which is caused by chemoattractant factors released by mast cells. This second phase sometimes prolongs the initial reaction or triggers a second round of signs and symptoms. However, despite many similarities, these early and late reactions are tissue specific, a distinction that must be taken into account when determining clinical significance. Unlike those in nasal and lung tissues, ocular allergies rarely exhibit a second clinical phase. Although in some cases the cellular mechanisms are similar, in allergic conditions, tissue-specific distinctions exist.[8]

In seasonal and perennial conjunctivitis, there is essentially 1 clinical phase. On conjunctival provocation, histamine-induced signs and symptoms are evident within minutes. The itching associated with an early-phase ocular allergic reaction has been shown to peak ~3.5 minutes after provocation,[9] coinciding with mast-cell degranulation.[10] A cellular late-phase reaction is seen only when higher concentrations of allergen are used and only occur in a subset of allergic patients. However, in allergic conjunctivitis, the cellular infiltrate is usually at the subclinical level, meaning that changes at the cellular level (if they occur at all) do not produce clinically visible signs or symptoms.[11] In fact, conjunctival scrapings of patients with mild ocular allergic disease reveal only an infrequent finding of eosinophils.[11,12]

The expression of adhesion molecules such as ICAM-1 and the presence of migratory inflammatory mediators have been documented to follow the early-phase reaction. The presence of ICAM-1 and cellular infiltrate consisting primarily of neutrophils when a late phase was induced via extremely high-level allergen exposure was observed as early as 20 minutes after provocation and extended to 6 hours, at which point lymphocytes and monocytes also became evident among infiltrate cells.[6,13] Unlike findings on the early phase of the ocular allergic reaction, these observations have not been found to correlate with any clinically relevant manifestation of a late-phase response in the vast majority of patients. The induction of inflammatory mediators and cellular infiltration occurs at a subclinical level, except in rare cases (<5% of ocular allergy cases) with more chronic conditions such as AKC or VKC.[14,15]

Several classes of ophthalmic medications are available for the treatment of the early-phase clinical signs and symptoms associated with seasonal and perennial allergic conjunctivitis. Two such classes are antihistamines and mast-cell stabilizers. Antihistamines antagonize the binding of histamine at its receptors, blocking the activation of nerve cells and endothelial cells. Mast-cell stabilizers inhibit the degranulation of mast cells, preventing the cascade of events that triggers the signs and symptoms of the disease. Therapies such as olopatadine, ketotifen, and azelastine, which offer a combination of these 2 mechanisms, have become available commercially. These antiallergy agents claim to exert both mast cell–stabilizing and antihistaminic effects. Mast-cell stabilization has been shown with olopatadine in preclinical research in human conjunctival mast cells,[16] with ketotifen in human conjunctival tissues, and with azelastine in cultured mast cells derived from umbilical cord blood and rat peritoneal mast cells.[17–19]

The conjunctival allergen challenge (CAC) model was designed to reproduce, in a standardized way, the immediate ocular allergic response.[20] It has been shown that a small dose of allergen causes a mild, short-term reaction with spontaneous recovery, whereas in a subset of patients, a large dose can cause an intense and persistent reaction that progresses to cellular recruitment.[21] This modified CAC model has been used to demonstrate the prophylactic effects of a mast-cell stabilizer (lodoxamide[22]) and a topical corticosteroid (desonide phosphate[23]) on the induction of a CAC-induced prolonged ocular allergic reaction.

Because mast-cell degranulation is responsible for the release of inflammatory mediators and the cellular infiltrate that may occur in selected patients, the extent of mast-cell stabilization exhibited by a medication can be determined by the quantification of mast cell–derived mediators in the conjunctiva up to 6 hours after challenge. Olopatadine is the only drug of its category indicated for the treatment of all the signs and symptoms of allergic conjunctivitis, which include itching, tearing, lid swelling, redness, and chemosis.[24] It is a selective H_1 receptor antagonist with mast cell–stabilization properties.[25]

The present study was performed to assess the effects of olopatadine on the release of mast cell–derived mediators after modified CAC in humans. The olopatadine molecule is thought to have multiple mechanisms of action, which we sought to clarify in this study. In addition to a clinical evaluation, the objective parameters of tear histamine levels, tear cytology, and ICAM-1 immunohistochemical expression were analyzed to evaluate the mast cell–stabilizing capability of this drug.

SUBJECTS AND METHODS

Subjects with a clinical history of seasonal allergic conjunctivitis were enrolled in this double-masked, randomized, placebo-controlled clinical trial. Informed written consent was obtained from all subjects. All subjects were asymptomatic and free of any topical or systemic medication, and had positive skin-test results

(wheal diameter >3 mm) or positive specific serum IgE (CAP system, Pharmacia Diagnostics, Uppsala, Sweden). The allergen that induced the greatest response by skin test and was most clinically correlated with seasonal symptoms was chosen for use in the CAC procedure.

Conjunctival challenge was performed according to the standardized procedure described by Abelson et al[20] and modified for the induction of prolonged reaction and cellular infiltration.[21] At visit 1, the allergen dose that induced a positive conjunctival reaction was determined by challenging both eyes with allergen in serial dilutions, increasing the dose every 15 minutes until a significant clinical reaction was obtained (≥3 score for itching and redness on 0–4 scales [0 = none; 4 = severe]). Subjects underwent monitoring 15 minutes postchallenge and then returned 5 hours later for further evaluation. Subjects showing positive signs and symptoms at 15 minutes and at 5 hours returned 2 weeks later for visit 2. At visit 2, a second challenge was conducted with the final dose determined at visit 1 to confirm the prolonged conjunctival reaction. Fifteen minutes was considered the standard time for looking at the early phase of the reaction, and 5 hours was selected as the second time point because this is when the cellular infiltrate peaks in those patients who develop this response after a high-dose challenge.[6,13] Subjects with positive reactions at visits 1 and 2 were randomized and instructed to treat 1 eye with olopatadine and the contralateral eye with placebo (commercially available artificial tears) in a double-masked fashion twice daily for the 5 days immediately preceding visit 3. At visit 3, 15 minutes after the final dose of treatment, subjects underwent bilateral CAC with the same dose as at visit 2.

Slit-lamp examinations were conducted at each visit to note safety parameters, and patients were asked whether they experienced any adverse events. The subject graded itching and the investigator graded redness. Parameters were evaluated in each eye before drug instillation; at baseline before challenge; at 5, 10, 20, and 30 minutes; and at 5 hours postchallenge. Both itching and redness were assessed using the 5-point scale (0–4) described previously.

Tear samples (50 µL) were collected from both eyes with a capillary tube before CAC and within 10 minutes after challenge at visits 2 and 3. Samples were collected from the outer canthus with a microcapillary tube, immediately transferred to a plastic tube, and then centrifuged for 10 minutes at 1000 rpm to separate the cells from the tear fluid. The supernatant tear-fluid samples were immediately frozen and stored at –20°C until analyzed by radioimmunoassay histamine measurement with a commercial kit (Immunotech, Marseille, France) intended for the quantitative determination of histamine levels in biologic samples. This method is based on the competition between the histamine in the sample and the tracer for the binding sites on the antibody-coated tube. Procedures followed the manufacturer's recommendations. The sensitivity of the assay (limit of detection) is 0.2 nM.

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