Evaluation of the Onset and Duration of Effect of Azelastine Eye Drops (0.05%) versus Placebo in Patients with Allergic Conjunctivitis Using an Allergen Challenge Model

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Objective: The trial evaluated the effectiveness of the investigational antihistaminic and antiallergic compound Azelastine Eye Drops (AZE) in the treatment of allergic conjunctivitis using an allergen challenge model.

Design: Randomized, double-blind, placebo-controlled, paired-eye study.

Participants: Adults with a history of allergic conjunctivitis (>2 years) who were asymptomatic throughout the trial, had a positive skin test (cat dander, grass, or ragweed pollen within the last year), and had a positive conjunctival reaction (score 2+ or more for itching and redness in both eyes on a 0–4 scale) during two separate conjunctival provocation tests (CPT) before randomization.

Methods: Eighty patients underwent a 2-week screening period (visits 1 and 2) that included a CPT during visit 1 to establish the allergen threshold dose and a second confirmatory CPT performed at visit 2. Eye symptom assessments for itching (evaluated by patient) and conjunctival redness (evaluated by physician) were performed 5 and 10 minutes after CPT using a 5-point scale (0 = none to 4+ = severe). Qualified patients were randomized to receive one drop of AZE (0.015 mg of azelastine hydrochloride) in one eye and one drop of placebo in the other eye 20 minutes before CPT at visit 3 (onset) and 8 or 10 hours before CPT at visit 4 (duration).

Main Outcome Measures: Individual severity scores for itching (evaluated by patient) and conjunctival redness (evaluated by physician) for each eye at 3, 5, and 10 minutes after CPT at visits 3 and 4 using a 5-point scale (0 = none to 4+ = very severe).

Results: Each of the 80 randomized patients completed the trial. Mean itching and conjunctival redness scores at visit 3 (onset) were significantly lower (P < 0.001) in the AZE-treated eyes than in the placebo-treated eyes. At visit 4 (duration), mean itching and conjunctival redness scores (P ≤ 0.003) for the 8-hour group and mean itching scores (P ≤ 0.001) for the 10-hour group were significantly lower in the AZE-treated eyes than in the placebo-treated eyes. Significant differences in mean tearing and chemosis severity scores were also seen at visit 3 (onset) and visit 4 (duration) in the AZE-treated eyes when compared with the placebo-treated eyes. Treatment with AZE was well tolerated.

Conclusions: Therapy of experimentally induced allergic conjunctivitis with AZE was highly effective, with an onset of action seen within 3 minutes and a duration of effect of at least 8 to 10 hours. Ophthalmology 2000;107:2152–2157 © 2000 by the American Academy of Ophthalmology.

Allergic conjunctivitis is a common condition that affects approximately 25% of the general population in the United States and is usually associated with allergic rhinitis. Allergic conjunctivitis is typical of a mast cell-mediated hypersensitivity reaction whereby the antigen reacts with specific immunoglobulin E antibodies bound to the surface of conjunctival mast cells. This results in the release of mediators (such as histamine) that are responsible for the itching, vasodilation, and edema associated with allergic conjunctivitis.1,2 Current therapies for the signs and symptoms of allergic conjunctivitis include the use of topical and systemic antihistamines, topical mast cell stabilizers, topical vasoconstrictors, and, in severe cases, topical corticosteroids.3 Azelastine Eye Drops (AZE) (ASTA Medica AG, Germany) is a 0.05% ocular solution of azelastine hydrochloride developed as a treatment for allergic conjunctivitis and...
is currently marketed in a number of countries outside the United States for the same indication. Each drop (approximately 0.03 ml) of AZE contains 0.015 mg of azelastine hydrochloride. Unlike some treatments available for allergic conjunctivitis, AZE inhibits multiple components of the allergic response. Investigative work with azelastine hydrochloride demonstrated its ability to inhibit mast cell degranulation, release of histamine, leukotriene biosynthesis and release, inflammatory cell infiltration, intercellular adhesion molecule-1 expression, production of tumor necrosis factor-α and granulocyte colony-stimulating factor by lymphocytes, platelet-activating factor, and eosinophil chemotaxis. Clinical trials with AZE have demonstrated its ability to decrease the number of inflammatory cells (including neutrophils, eosinophils, basophils, and lymphocytes) and to inhibit intercellular adhesion molecule-1 expression after allergen challenge.

In previous studies, AZE has demonstrated significantly greater efficacy than placebo while being generally well tolerated. The current trial was a randomized, single-center, double-blind (matched-pair comparison), placebo-controlled trial designed to evaluate the efficacy (onset and duration of effect) of AZE in asymptomatic patients with allergic conjunctivitis using an allergen challenge model.

**Patients and Methods**

Eligible patients were male and female, 18 to 65 years of age. Diagnostic criteria used to select patients were: a history of allergic conjunctivitis for at least 2 years; positive skin test (prick or puncture) to either cat dander (Felis domesticus), grass pollen (Poa pratensis), or ragweed pollen (Ambrosia artemisiifolia) within the last year; positive conjunctival reaction to an allergen (cat dander, grass, or ragweed pollen) during two separate provocation tests with approximately 7 days between both tests. Patients were to be asymptomatic before allergen challenges at visits 1 and 2 and before randomization at visits 3 and 4 (score of 0 for itching, redness, chemosis, and tearing in both eyes). Females were required to be surgically sterile or postmenopausal (for at least 1 year) or, if of childbearing potential, have been using an effective birth control method and had a negative pregnancy test at visit 1. Patients with a history of retinal detachment, diabetic retinopathy or any retinal disease, bacterial or viral ocular infection, history of prior eye surgery, or a known intolerance to azelastine or other components of the drug product were excluded. Use of contact lenses within 72 hours before visit 1 and during the entire study period was prohibited. The use of investigational drugs, intramuscular, systemic or topical corticosteroids, ocular mydriatics or cholinesterase miotics, ocular anti-infectives, nonocular antihista-minics or antiallergics, tricyclic antidepressants, and MAO inhibitors during the study was prohibited. The use of ocular antiallergics, sympathomimetics, and β-blockers within 24 hours before each visit and ocular miotics (direct acting) and vasoconstrictors within 48 hours before each visit was also prohibited. The protocol was approved by an institutional review board, and all patients signed the institutional review board-approved informed consent before enrollment.

The study included a total of four study visits. After obtaining informed consent, patients underwent a 2-week screening period that included a conjunctival provocation test (CPT) during visit 1 to establish an allergen threshold dose and a secondary confirmatory CPT performed at visit 2 approximately 7 days later. The investigator determined the allergen threshold dose starting with the lowest dose of antigen (2500 allergy units/ml cat dander or 10,000 protein nitrogen units/ml grass or ragweed pollen) and then titrating to the medium (5000 allergy units/ml cat dander or 20,000 protein nitrogen units/ml grass or ragweed pollen) and highest dose (10,000 allergy units/ml cat dander or 40,000 protein nitrogen units/ml grass/ragweed pollen). At 10 minutes intervals until a positive response was achieved. Eye symptom assessments for itching (evaluated by the patient) and conjunctival redness (evaluated by the physician) were evaluated 5 and 10 minutes after CPT using a 5-point scale (from 0 = none to 4+ = very severe). Qualified patients returned approximately 7 days after visit 2 for visit 3, when onset of effect was evaluated. During visit 3, patients were randomized to receive one drop of AZE (0.03 ml containing 0.015 mg of azelastine hydrochloride) in one eye and one drop of placebo (0.03 ml of vehicle) in the other eye 20 minutes before CPT. Patients returned approximately 7 days after visit 3 for visit 4 when duration of effect was evaluated. At this visit, patients were randomized to either the 8- or 10-hour duration group (n = 40 in each subgroup) and were also randomized to receive one drop of AZE in one eye and one drop of placebo in the other eye 8 or 10 hours before CPT. Patients were chronologically randomized according to a single randomization list generated by the sponsor. The AZE vehicle, comprised of benzalkonium chloride, disodium edetate dihydrate, hydroxypropylmethylcellulose, sorbitol solution, sodium hydroxide, and purified water was used as the placebo treatment. The AZE and placebo treatments were packaged in identical bottles and were not distinguishable. For each dosing visit, patients were assigned one bottle containing AZE and one bottle containing placebo. Each bottle was labeled with a standard three-part, double-blind label that identified the study, patient and visit numbers, instructions for use, and storage conditions. During visits 3 and 4, conjunctival redness and chemosis were assessed by the investigator, and itching and tearing were assessed by the patient 3, 5, and 10 minutes after CPT using the same 5-point scale described above. Additional evaluations and assessments performed during visits 3 and 4 included a review of adverse events and concomitant medication use and complete ophthalmic and physical examinations including vital signs (at visit 4 only).

**Outcome Measures**

The main efficacy variables were the individual severity scores for itching and conjunctival redness for each eye at 3, 5, and 10 minutes after CPT at visits 3 and 4. The severity scores for itching and conjunctival redness were used to evaluate onset of effect during visit 3 and duration of effect during visit 4. Secondary efficacy variables included the individual severity scores for tearing and chemosis for each eye (using the same 5-point scale) at 3, 5, and 10 minutes after CPT at visits 3 and 4. Safety variables included adverse event incidences and results of ophthalmic and physical examinations including vital sign measurements.

**Statistical Analysis**

SAS version 6.12 for Windows (SAS Institute Inc., Cary NC) was used for all analyses. All inferential tests were two-sided with a 0.05 level of significance. Assuming a standard deviation of 1.6 for the paired differences of eye symptoms at a given time, a minimum sample size of 34 patients was needed to detect a mean difference of 1 with 95% power at a 0.05 level of significance using the matched-pair t test. Hence, at least 34 completed patients were needed in each of the two subgroups that were to receive the CPT 8 hours and 10 hours after the administration of study drug at visit 4. To allow for a 15% dropout rate, a total of 80 patients were to be randomized at visit 3.
All patients who received study drug were included in the efficacy and safety analyses. For the primary and secondary efficacy variables, inferential comparisons of symptom severity between the AZE and placebo-treated eyes were performed using matched-pair t tests. For the safety analyses, incidences of ocular adverse events were calculated by treatment, whereas incidences of nonocular adverse events were calculated overall.

Results

A total of 80 patients were randomized in this study and were evaluable for the efficacy and safety analyses. All patients received one drop of AZE in one eye and one drop of placebo in the other at both visits 3 and 4. No patients prematurely discontinued participation in the study. The study blind was preserved for all patients. No protocol amendments were implemented during the conduct of the study. A protocol deviation pertaining to the causality of adverse events was made. Specifically, at the time the statistical plan was designed and before database lock and unblinding of the randomization codes, causality of adverse events was changed from not related, possibly related, probably related, or definitely related to possibly, probably, and definitely related.

Table 1 summarizes pertinent patient demographic information.

Most patients were white, female, and had brown or blue eyes. The mean age was 37 years with a range of 18 to 63 years. The mean history of allergic conjunctivitis was 22.6 years, with a range of 3 to 52 years. No clinically significant abnormalities were reported for any of the physical examination or ophthalmic examination parameters assessed at the screening visit.

Most patients were allergic to cat dander (41 patients; 51%), whereas 27 patients (34%) were allergic to grass pollen, and 12 patients (15%) were allergic to ragweed pollen. A total of 55 patients (69%) reached their allergen threshold dose after the low dose of allergen, whereas 18 (23%) required a medium dose, and seven (9%) required a high dose.

Efficacy

The onset of effect of AZE was assessed based on the severity scores of all patients evaluated at visit 3. Onset was defined as the first point after CPT when a statistically significant difference was found between the treatments. Figures 1 and 2 present the mean itching and redness severity scores evaluated at visit 3. Table 2 presents the mean differences (placebo-AZE) in itching and redness scores evaluated at visit 3.

Mean itching and conjunctival redness scores were statistically lower (P < 0.001) in the AZE-treated eyes than in the placebo-treated eyes at 3, 5, and 10 minutes after CPT. Mean differences for itching and conjunctival redness scores ranged from 0.85 to 1.21 and 0.43 to 0.50, respectively. The results of the itching and conjunctival redness scores indicate that AZE has an onset of effect within 3 minutes. Mean tearing and chemosis scores were also statistically lower in the AZE-treated eyes than in the placebo-treated eyes (P ≤ 0.007) at each of the times.

The duration of effect was assessed based on the severity scores at visit 4 for patients who underwent CPT 8 hours or 10 hours after receiving the study drug. Figures 3 and 4 present the mean itching and redness severity scores evaluated at visit 4 for the 8-hour group. Table 3 presents the mean differences in itching and redness severity assessments performed at visit 4 for those patients who received a CPT 8 and 10 hours after receiving treatment.
For patients (n = 40) who had undergone CPT 8 hours after administration of study medication, mean itching and conjunctival redness scores for AZE-treated eyes were significantly lower (P ≤ 0.003) than those for the placebo-treated eyes at 3, 5, and 10 minutes after CPT. Mean differences for itching and conjunctival redness scores ranged from 0.63 to 1.23 and 0.40 to 0.55, respectively. For the AZE-treated eyes, mean tearing scores were significantly lower (P = 0.011) than those for the placebo-treated eyes at the 10-minute time, whereas mean chemosis scores were significantly lower than those for the placebo-treated eyes (P ≤ 0.009) at all times.

For patients (n = 40) who had undergone a CPT 10 hours after administration of study drug, mean itching severity scores for the AZE-treated eyes were significantly lower (P ≤ 0.001) than those for the placebo-treated eyes. Mean conjunctival redness and tearing severity scores were significantly lower than those for the placebo-treated eyes (P ≤ 0.009) at all times.

The results obtained from visit 4 indicate that AZE has a duration of effect for itching and redness of at least 10 hours, although the effect was less pronounced at 10 hours than at 8 hours.

Table 2. Mean Differences and Standard Deviations in Itching and Redness Severity Scores at Visit 3

<table>
<thead>
<tr>
<th>Mean Differences at Each Assessment Timepoint</th>
<th>3 Minutes</th>
<th>5 Minutes</th>
<th>10 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itching</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Placebo-AZE (n = 80)</td>
<td>0.85 (0.99)</td>
<td>1.06 (1.07)</td>
<td>1.21 (1.24)</td>
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<td>P value*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>Redness</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Placebo-AZE (n = 80)</td>
<td>0.43 (0.65)</td>
<td>0.45 (0.74)</td>
<td>0.50 (0.83)</td>
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<tr>
<td>P value</td>
<td>&lt;0.001</td>
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<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*AE = Azelastine Eye Drops.

P value from a matched-pair t-test.

Figure 3. Mean itching severity at visit 4 for the 8-hour group (n = 40).

Table 3. Mean Differences and Standard Deviations in Itching and Redness Severity Scores at Visit 4

<table>
<thead>
<tr>
<th>Mean Differences at Each Assessment Timepoint</th>
<th>3 Minutes</th>
<th>5 Minutes</th>
<th>10 Minutes</th>
</tr>
</thead>
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<tr>
<td>8-hour group</td>
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<td>Itching</td>
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<td></td>
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<tr>
<td>Placebo-AZE (n = 40)</td>
<td>0.63 (0.93)</td>
<td>0.88 (1.07)</td>
<td>1.23 (1.21)</td>
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<td>P value*</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>Redness</td>
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<tr>
<td>Placebo-AZE (n = 40)</td>
<td>0.40 (0.81)</td>
<td>0.55 (0.81)</td>
<td>0.55 (0.96)</td>
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<td>P value*</td>
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<td>&lt;0.001</td>
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</tr>
<tr>
<td>10-hour group</td>
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<td></td>
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</tr>
<tr>
<td>Itching</td>
<td></td>
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<td></td>
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<tr>
<td>Placebo-AZE (n = 40)</td>
<td>0.50 (0.75)</td>
<td>0.60 (1.03)</td>
<td>0.68 (1.02)</td>
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<td>P value*</td>
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<tr>
<td>Redness</td>
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<tr>
<td>Placebo-AZE (n = 40)</td>
<td>0.30 (0.79)</td>
<td>0.25 (0.87)</td>
<td>0.23 (0.83)</td>
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<tr>
<td>P value*</td>
<td>0.021</td>
<td>0.077</td>
<td>0.195</td>
</tr>
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</table>

*AE = Azelastine Eye Drops.

P value from a matched-pair test.

Safety

A total of 62 patients (77.5%) experienced at least one adverse event. The most common adverse event reported was stinging: 37.5% of the patients experienced stinging in the AZE-treated eyes and 11.3% of the patients experienced stinging in placebo-treated eyes. Other commonly reported adverse events included: burning (28.8% for AZE and 12.5% for placebo), taste perversion (20.0% for AZE and 0% for placebo), itching (10.0% for AZE and 11.3% for placebo), tearing (8.8% for AZE and 8.8% for placebo), and cold sensation (7.5% for AZE and 5.0% for placebo). Most of these adverse events were mild and resolved within minutes or hours. Each of these adverse events was considered to be at least possibly associated with the study drug. No serious adverse events were reported. Treatment with AZE or placebo did not result in
Discussion

Allergic conjunctivitis, owing mostly to its high prevalence, results in a significant clinical burden in the United States and worldwide.1 Although serious sequelae arising from allergic conjunctivitis are infrequent, many patients experience substantial discomfort from their symptoms and seek relief with over-the-counter and prescription therapies. Ocular antihistamines provide the mainstay of pharmacotherapy for allergic conjunctivitis.2 For patients who experience predominantly ocular symptoms of seasonal or perennial allergies, ocular antihistamines generally offer faster relief and fewer systemic effects than oral antihistamines.2

Azelastine Eye Drops incorporate the properties of potent H1 receptor antagonism,2 inhibition of preformed mediator release from the mast cell (a ‘mast cell stabilizer’),5–8 and inhibition of a broad array of other mediators (including leukotriene C4, N-alpha-tosyl-arginine methyl ester-esterase (TAME) esterase, and intercellular adhesion molecule-1 expression)13–17 in one compound. The finding that azelastine hydrochloride is a unique antiallergic agent particularly suited for topical treatment of allergic diseases provided the basis for the development of an ocular form of azelastine to treat allergic conjunctivitis. In addition to the present study, several large, multicenter, randomized, placebo-controlled trials have been conducted with AZE during the pollen season with treatment lasting from 2 to 8 weeks. These environmental exposure trials have confirmed the effectiveness and tolerability of ocular azelastine for the treatment of allergic conjunctivitis in the adult and pediatric populations.

Within the last 10 to 20 years, the CPT has been established as a reliable model with which to test the effectiveness of pharmacologic therapies for allergic conjunctivitis.23 This model combines several attributes, including a relatively small requisite sample size, a closely supervised and controlled challenge procedure in which the contralateral eye serves as a control, reproducibility, relatively accurate estimate of duration of effect, and a relatively fast conduct, each of which is important in the study of therapies for allergic conjunctivitis.

In this study using a CPT, AZE was found to inhibit significantly the cardinal symptoms of allergic conjunctivitis—itching and redness—when compared with placebo. As expected, the magnitude of relief was greater for itching than for redness, as is the case with other ocular antihistamines.23 Relief of chemosis and tearing was also highly significant for AZE even though patients were not selected on the basis of a minimum chemosis or tearing score in response to CPT, and thus severity scores after CPT during qualification were generally lower for chemosis and even lower for tearing symptoms as compared with the corresponding scores for itching and redness.

Onset of effect of AZE against all symptoms was seen at 3 minutes, the time of first evaluation after CPT. For itching, a difference of more than 1 is achieved by 5 minutes after CPT. Although not a true ‘onset of effect,’ the time to achieve a significant difference between placebo and active treatments (when a drug administered before CPT as in the current study) is frequently used to define onset in the place of a reversed paradigm (when drug is administered after CPT as soon as symptoms have achieved a minimum level) and is thus more a reflection of the speed of development of allergic symptoms from CPT—and that the previously administered drug inhibits that process—than a reflection of the onset time per se. Without the establishment of a clinically significant difference (e.g., >1), the onset is largely driven by sample size.

Duration of effect for itching and redness was significant at 8- and 10-hour evaluations, although relief was not as great at 10 hours when compared with the 8-hour evaluation. Significant differences were also seen versus placebo at 8 and 10 hours for chemosis and tearing, although the magnitude of these differences was smaller than that seen for itching and redness. These results are consistent with data from other challenge and environmental studies with AZE and with data from studies with azelastine nasal spray, which show a long duration of action of up to 12 hours from a single dose and, therefore, suitability for twice daily dosing.11–13

With regard to safety, adverse events reported in this trial were qualitatively similar to those seen during other trials of azelastine eye drops and were indicative of good tolerability. Notable ocular adverse events that occurred more frequently with AZE than with placebo included mild, transient ocular burning and stinging. Taste perversion was reported by 20% of patients. No patients discontinued use as a result of adverse events. No significant changes were seen in physical or ophthalmic examinations from before to after the study, nor were any serious adverse events reported.

In summary, AZE as studied in this trial provided significant and long-lasting clinical benefit in the relief of the symptoms of allergic conjunctivitis after CPT. Azelastine Eye Drops were generally well tolerated, with symptoms of mild, transient ocular burning and burning and taste perversion being the predominant adverse effects.

Azelastine is unique among the clinically useful antihistaminic and antiallergic compounds in its breadth of activity against the myriad of mediators responsible for allergic inflammation that have been documented in the literature. Some or all of the antiallergic actions of azelastine may contribute to the effectiveness of AZE in treating allergic conjunctivitis shown in this and other studies. Azelastine Eye Drops should prove a potent new weapon among the arsenal of treatment options currently available for allergic conjunctivitis.

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References