

ORIGINAL ARTICLE

Effect of Ketotifen Fumarate, Olopatadine, and Levocabastine on Ocular Active Anaphylaxis in the Guinea Pig and Ocular Immediate Hypersensitivity in the Albino Rat

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ABSTRACT *Purpose:* To study the effects of ketotifen fumarate, olopatadine, and levocabastine on ocular active anaphylaxis in guinea pigs and on ocular immediate hypersensitivity in albino rats. *Methods:* Clinical grading scores and Evans blue dye leakage to eyelids and to eyeballs were assessed in five treatment groups ($n = 10$): ketotifen fumarate 0.025%, olopatadine 0.1%, levocabastine 0.05%, negative control, and positive control. *Results:* At 20 minutes after challenge, edema scores for ketotifen-treated guinea pigs were statistically significantly lower than those for levocabastine or olopatadine. Active treatment significantly reduced vascular leakage in both models. Ketotifen significantly reduced vascular leakage in eyelids compared with the other drugs. In guinea pigs, vascular leakage in eyeballs was significantly reduced with ketotifen fumarate compared with olopatadine and levocabastine. *Conclusions:* In the guinea pig model, ketotifen was more effective than olopatadine and levocabastine at reducing conjunctival edema and vascular permeability in eyelids and eyeballs. In the rat model, ketotifen was more effective at reducing vascular permeability in eyelids than olopatadine and levocabastine.

KEYWORDS Animal study; ketotifen fumarate; ocular immediate hypersensitivity; olopatadine; seasonal allergic conjunctivitis

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INTRODUCTION

Seasonal allergic conjunctivitis (SAC) produces a range of ocular symptoms that vary from itching and redness to swelling, excessive lacrimation, and mucous discharge.^{1,2} It is the most common form of ocular allergy and has an estimated prevalence between 5 and 22%, depending on the geographical area studied.³ Although vision is not impaired by SAC, the distressing signs and

symptoms may cause extreme discomfort that can affect quality of life.²

It is believed that ocular allergic anaphylaxis in people affected by SAC is triggered by the interaction between allergen, antibody, and mast cell. Formation of this immune complex results in mast-cell degranulation and the release of chemical mediators.⁴ One such mediator is histamine, which increases conjunctival vascular permeability and stimulates vasodilation. Pharmacologic approaches to the treatment of SAC have included H₁-receptor blockade, mast-cell stabilization, and non-steroidal anti-inflammatory drugs. Drugs that combine multiple pharmacologic mechanisms have been developed because they have the potential to enhance the quality and duration of symptom relief.⁵ In contrast to levocabastine, a highly specific histamine H₁-receptor antagonist, olopatadine and ketotifen fumarate exhibit extended antiallergic activity. Olopatadine and ketotifen possess H₁-receptor blocking and mast cell-stabilizing activity.⁶ They further inhibit the upregulation of ICAM-1 expression^{7,8} and inhibit TNF- α release from conjunctival mast cells⁹ and peripheral blood mononuclear cells.¹⁰ Reduced cytokine release from mast cells diminishes the potentiating release of chemoattractants and the infiltration of inflammatory cells. Ketotifen, indeed, prevents late-phase anaphylactic events such as platelet-activating factor (PAF)-mediated reactions¹¹ as well as eosinophil chemotaxis and activation.¹²

This paper reports the effects of ketotifen fumarate (Zaditen Ophtha[®], 0.025% ophthalmic solution), olopatadine (Patanol[®], 0.1% ophthalmic solution), and levocabastine (Levophta[®], 0.05% ophthalmic suspension) in two different animal models. Ocular active anaphylaxis was studied in guinea pigs and ocular immediate hypersensitivity in albino rats. Lid edema was assessed and plasma exudation to eyelids and to eyeballs was quantified. This method gives a reliable measure of the edematous processes linked with the allergic reaction, i.e. vascular permeability accompanied by lid swelling and chemosis.

MATERIALS AND METHODS

The animals were treated in accordance with the Association for Research in Vision and Ophthalmology resolution on the use of animals in research and all experiments adhered to International Good Laboratory Practice Guidelines.

Animals

In the guinea pig model, 50 male animals (Charles River, St Aubin-Les-Elbeuf, France) weighing 300–350 g were maintained under standard conditions of temperature, relative humidity, and light, with free access to food and water. Standard conditions were also maintained for the 50 male albino rats (IFFA CREDO, L'Arbresle, France) weighing between 200 and 300 g that were used in the study.

Study Design

Both models were controlled and masked. In each, the animals were randomly assigned to one of five treatment groups ($n = 10$): ketotifen fumarate 0.025% (Zaditen Ophtha[®], Novartis Ophthalmics AG, Hettlingen, Switzerland), olopatadine 0.1% (Patanol[®], Alcon Laboratories, Inc., Fort Worth, TX, USA), levocabastine 0.05% (Levophta[®], Laboratoires Chauvin, Montpellier, France), a negative control (NaCl 0.9%, Unilarm[®], CIBA Vision Faure) without antigen or compound 48/80 instillation, and a positive control (NaCl 0.9%, Unilarm[®], CIBA Vision Faure, Annonay, France) with antigen or 48/80 instillation.

Sensitization and Allergy Challenge

In the guinea pig model, the animals were sensitized 14 days before the induction of ocular active anaphylaxis by two simultaneous intraperitoneal 500 μ l injections of 100 μ g egg albumin (Sigma Chimie, St Quentin Fallavier Cedex, France) in combination with 20 mg alum (Sigma Chimie) in 1 ml phosphate-buffered saline (PBS) at pH 7.4.

For both animal models, test compounds were administered by instillation into the right conjunctival sac at time points of 2 days, 1 day, 3 hours, 2 hours, 1 hour, 40 minutes, and 10 minutes before induction of the allergy challenge. Instillations performed 1 and 2 days before induction were given four times per day at 3-hourly intervals starting at 9 a.m. At each instillation, a single administration of 10 μ l was given to the rats and 30 μ l to the guinea pigs.

Fifteen minutes before the induction of an immune response, 16 mg/kg of a tracer substance (Evans blue dye, Sigma Chimie; 40 mg/ml in sterile saline) was administered by intracardiac injection. In guinea pigs, ocular active anaphylaxis was then induced by a single topical administration of egg albumin (100 mg/ml in

TABLE 1 Clinical grading scale of edema.

Grade	Description
0	No swelling
1	Slight swelling
2	Obvious swelling with partial eversion of lids
3	Swelling with lids almost half closed
4	Swelling with lids more than half closed

PBS, pH 7.4). In rats, ocular immediate hypersensitivity was induced by a single topical administration of compound 48/80 (1 mg in 10 μ l PBS, pH 7.4)

Clinical Grading Score

In both models, clinical grading of edema was performed 10 and 20 minutes after the induction of allergy, based on quantitative evaluation using a clinical grading scale (Table 1). The animals were subsequently anesthetized using pentobarbital (60 mg/kg) and sacrificed with an overdose of pentobarbital. Before death, blood samples were obtained by cardiac puncture and transferred to heparinized tubes; eyelids and eyeballs were removed after death and the wet weights determined.

Vascular Permeability Evaluation

Edema was quantified in both models by the determination of plasma exudation of Evans blue dye to eyelids and eyeballs. Samples were lyophilized for 24 hours and the Evans blue dye content in the blood, eyelids, and eyeballs determined using a spectrophotometric method. (When Evans blue dye is extravasated, it is absorbed by plasma proteins and stains edematous tissues, indicating an increase in water and protein vascular permeability.) The dye was extracted from the samples by immersion in a solution of acetone and 0.5% sodium sulfate (7/3; v/v) for 24 hours at room temperature. After centrifugation at 1800 rpm for 10 minutes, the optical density of the supernatant was determined at 620 nm and quantified according to a standard curve. The blood-eyelid and blood-eyeball permeability indices were determined as the ratio between the amount of dye in the eyelid or eyeball and that in the total blood. Permeability indices were calculated from the wet tissues.

Statistical Analysis

For both models, data for blood-eyelid and blood-eyeball permeability indices were ranked and then

subjected to the Kruskal-Wallis ANOVA and the Student-Newman-Keuls tests. In the guinea pig model, comparison of clinical scores for edema was performed using stepwise discriminant function analysis and least significant difference (LSD) test. Statistical analysis was conducted using Statistica™ Mac Software and a probability of $p < 0.05$ was considered significant.

RESULTS

Clinical Grading Score

Guinea Pigs

There was no statistical difference between the negative control NC (saline, no challenge) and ketotifen fumarate ($p = 1.00$), olopatadine ($p = 1.00$), or levocabastine ($p = 0.413$) in the mean clinical grading scores or edema in treated animals at 10 minutes after induction of allergic conjunctivitis, indicating a maximal protective effect of the drugs (Fig. 1A). The positive control PC (saline, ovalbumin challenge) was significantly different ($p < 0.001$) from the negative control and the three drugs. At 20 minutes, the scores for animals treated with any of the three drugs were significantly lower than for the positive control (at least $p < 0.05$), indicating a continued protective effect by the drugs. However, scores ($\pm$ SD) for animals treated with ketotifen fumarate (0.0 ± 0.0) were statistically significantly lower than for animals that received levocabastine (0.50 ± 0.53 ; $p < 0.05$) or olopatadine (0.60 ± 0.52 ; $p < 0.01$) (Fig. 1B).

Albino Rats

The mean clinical grading scores for edema in all groups were 0 at both 10 and 20 minutes after induction of allergic conjunctivitis in rats.

Vascular Permeability Evaluation

Blood-eyelid permeability

Blood-eyelid permeability results are shown in Figure 2A. For both animal models, the Kruskal-Wallis ANOVA by ranks analysis indicated that there was a statistical difference in blood-eyelid permeability among the treatment groups ($p < 0.001$).

Guinea Pigs

Blood-eyelid permeability (mean $\pm$ SD) ranged from $5.02 \pm 1.15\%$ in the negative control to $61.43 \pm 20.87\%$ in the positive control (Fig. 2A). All treatments reduced

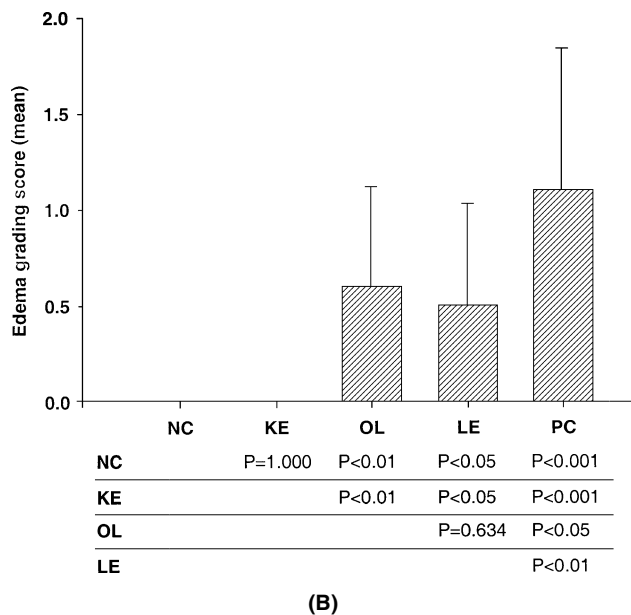
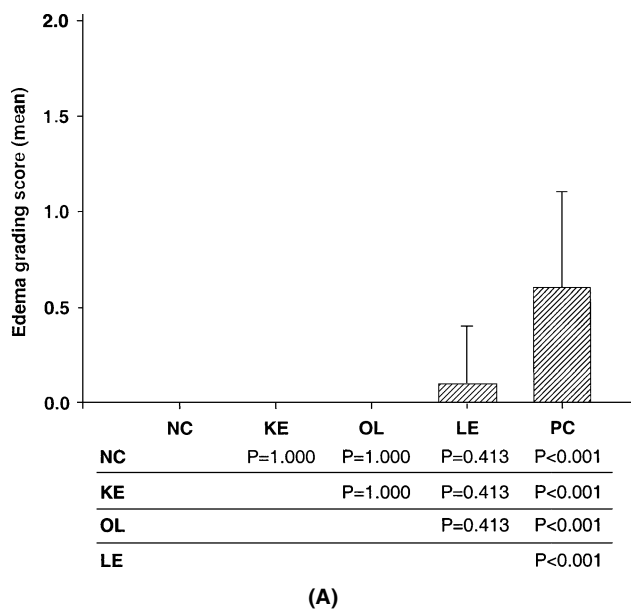


FIGURE 1 Clinical evaluation scores for edema recorded at (A) 10 minutes and (B) 20 minutes after allergic conjunctivitis was induced by the active anaphylaxis reaction with egg-albumin sensitization and challenge in the guinea pig. The animals were previously treated with ketotifen fumarate 0.025% (KE), olopatadine 0.1% (OL), or levocabastine 0.05% (LE). Negative (NC, without challenge) and positive (PC, with challenge) control groups received unpreserved saline. Each point is the mean $\pm$ SD of 10 values. Lower clinical evaluation scores represent a better outcome. The tables below the graphs show the statistical difference (Newman-Keuls test) between each of the test compounds. Note that the albino rats were not included in this figure because the score values for all test compounds were 0.

vascular leakage compared with the positive control. Ketotifen fumarate significantly reduced leakage compared with olopatadine ($p < 0.001$) and levocabastine ($p < 0.01$).

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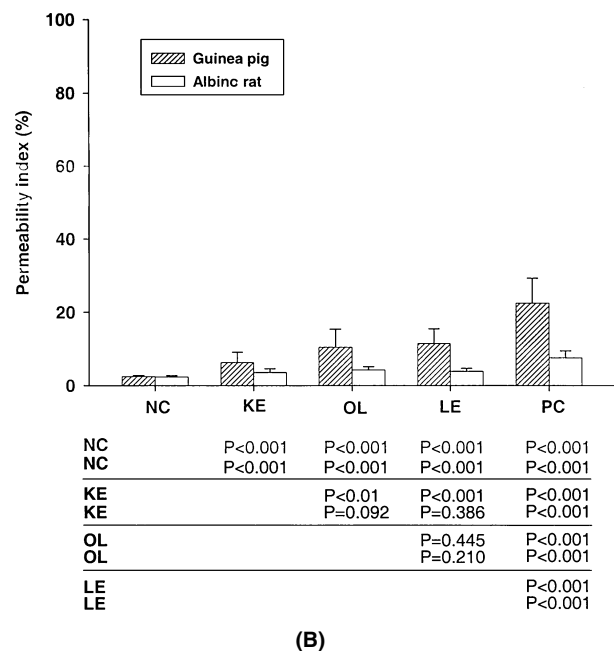
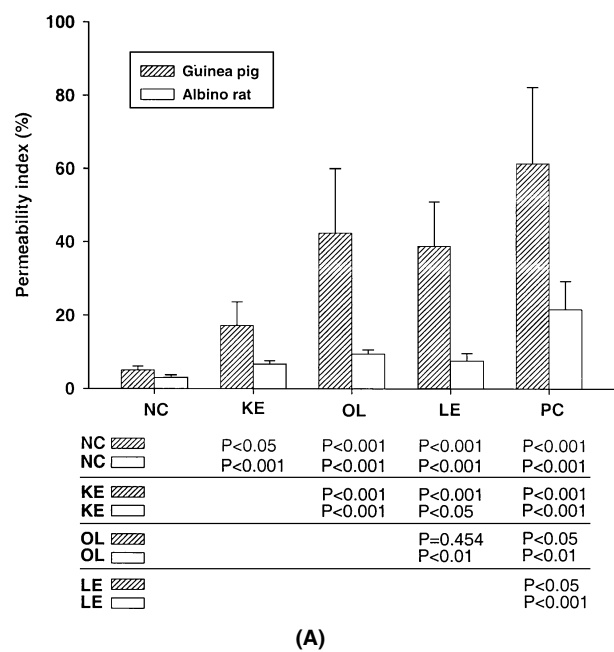


FIGURE 2 (A) Blood-eyelid and (B) blood-eyeball permeability determined 20 minutes after allergic conjunctivitis was induced by the active anaphylaxis reaction with egg-albumin sensitization and challenge in guinea pigs and rats after induction with 48/80. The animals were previously treated with ketotifen fumarate 0.025% (KE), olopatadine 0.1% (OL), or levocabastine 0.05% (LE). Negative (NC, with no challenge) and positive (PC, with challenge) control groups received unpreserved saline. Each point is the mean $\pm$ SD of 10 values. Lower scores represent a better outcome. The tables below the graphs show the statistical difference (Newman-Keuls test) between each of the test compounds.

Albino Rats

Blood-eyelid permeability ranged from $2.99 \pm 0.64\%$ in the negative control to $21.58 \pm 7.68\%$ in the positive control. Vascular leakage was reduced in all treatment

groups compared with the positive control. Leakage was also significantly reduced with ketotifen fumarate compared with olopatadine ($p < 0.001$) and levocabastine ($p < 0.05$) (Fig. 2A).

Blood-eyeball permeability

Blood-eyeball permeability results are shown in Figure 2B. In both animal models, Kruskal-Wallis ANOVA by ranks analysis indicated that there was a statistical difference among treatment groups ($p < 0.001$).

Guinea Pigs

Blood-eyeball permeability ranged from $2.44 \pm 0.33\%$ in the negative control to $22.39 \pm 6.92\%$ in the positive control. All treatments reduced leakage compared with the positive control. Ketotifen fumarate was found to significantly reduce vascular leakage compared with olopatadine ($p < 0.005$) and levocabastine ($p < 0.001$).

Albino Rats

The range of the permeability was lower than in guinea pigs: $2.32 \pm 0.41\%$ in the negative control to $7.57 \pm 1.90\%$ in the positive control. Leakage was also significantly reduced in all treatment groups compared with the positive control. There was no statistically significant difference between ketotifen fumarate and olopatadine ($p = 0.092$) or levocabastine ($p = 0.386$).

DISCUSSION

Guinea pig and rat models are used for studies on allergic conjunctivitis because they both closely resemble human physiology and their ocular vascular permeability is comparable.^{13,14} Effects on ocular active anaphylaxis in the guinea pig have been studied for several antiallergic agents, including levocabastine, lodoxamide, cromoglycate, and ketorolac.^{4,15} Similarly, many agents have been studied for their effects on ocular immediate hypersensitivity in rats.^{4,14} Both active anaphylaxis reaction with ovalbumin immunization and challenge in guinea pigs and ocular immediate hypersensitivity after instillation of compound 48/80 in rats have been validated as models of the human ocular allergic reaction.^{4,16} In addition, Evans blue dye extravasation has been used as a reliable and objective parameter of increased vascular permeability.¹⁴

In the guinea pig model of ocular active anaphylaxis, the three active drugs had significantly better outcomes for clinical gradings, eyelid permeability, and eyeball permeability than the positive control. However, there were differences among the three active drugs, with ketotifen fumarate producing significantly better outcomes for vascular permeability of both the eyelids and eyeballs than levocabastine or olopatadine.

The rat model, used to study ocular immediate hypersensitivity, appeared to be less sensitive than the guinea pig model despite the fact that it produced similar results. Although it was not possible to detect any effect when looking at the clinical gradings, an extravasal permeability could be determined in eyelids as well as in eyeballs. The indices were much lower than in the guinea pig model. However, the eyelid- and eyeball-permeability data indicated significantly better outcomes for the three active drugs than for the positive control. Also, ketotifen fumarate produced significantly better outcomes than levocabastine or olopatadine with regard to vascular permeability in eyelids.

The absence of clinical edema symptoms in the rat immediate-sensitivity model was surprising. It can be argued that the guinea pig model, which is expected to more closely mimic the complex anaphylactic processes involving many mediators (e.g., prostaglandins, leukotrienes, cytokines), would elicit a more intense allergic reaction than the rat model, which relies on histamine release only. However, clinical signs to the used dose of compound 48/80 are usually well detectable.⁴ So, the most evident explanation for the absence of a clinical response in this case is that the reaction to compound 48/80 was so weak that it could only be detected by biochemical means.

The different mechanisms of action of the active drugs possibly account for the observed differences in efficacy. Levocabastine is a histamine H₁-receptor antagonist. Olopatadine is a mast-cell stabilizer as well as an antihistamine. Ketotifen fumarate not only blocks H₁ receptors and stabilizes mast cells, but also prevents the recruitment of eosinophils, which play a central role in the more chronic and severe forms of allergic inflammation processes, such as vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC). The multiple mechanisms of action of ketotifen would be expected to provide rapid and prolonged relief of symptoms in a patient.

CONCLUSION

The results of this study suggest that, in a guinea pig model of SAC, ketotifen fumarate is more effective at reducing conjunctival edema and vascular permeability in eyelids and eyeballs than olopatadine and levocabastine. In the rat model, ketotifen was more effective at reducing vascular permeability in eyelids than olopatadine and levocabastine. There was no difference in the efficacy of the three active drugs in rat eyeballs.

The results of these animal models suggest that ketotifen may provide greater relief from the assessed symptoms of SAC than olopatadine and levocabastine. Nevertheless, results from clinical trials are necessary to establish to what extent these findings are applicable to the clinical setting.

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