



Comparative efficacy of topical antihistamines in an animal model of early phase allergic conjunctivitis

M. Eugenia Sanchis-Merino^a, Javier A. Montero^{b,c,*},
Jose M. Ruiz-Moreno^{c,d}, Alejandra E. Rodriguez^c, Silvia Pastor^c

^a Allergy Unit, Pio del Rio Horteja University Hospital, Valladolid, Spain

^b Ophthalmology Unit, Pio del Rio Horteja University Hospital, Valladolid, Spain

^c Vissum, Alicante Institute of Ophthalmology, Alicante, Spain

^d Department of Ophthalmology, Albacete Medical School, Castilla-La Mancha University, Albacete, Spain

Received 27 November 2007; accepted in revised form 18 February 2008

Abstract

In order to compare the relative efficacy of topical antihistamines with balanced saline solution (BSS) and benzalkonium chloride (BC) in the early phase of allergic conjunctivitis in an animal model of ocular anaphylaxis, 96 male guinea pigs were sensitized with intraperitoneal egg albumin (EA) and aluminum hydroxide. Seventy-six animals were used for determination of Evans blue (EB) extravasation and 20 for clinical evaluation of the allergic response (redness, edema, discharge and itch-scratch response). Eighteen days after sensitization the animals were topically challenged by conjunctival instillation of EA and treated 15 min before and 15 min after challenge with commercially available drugs (ketotifen, ketotifen single dose units [SDU], olopatadine, azelastine, spaglumic acid and emedastine) and controls (BSS and BC). The animals used for EB quantification were anesthetized and received an intravenous injection of EB simultaneously to the topical challenge. The ocular extravasation of the colorant was determined by 620 nm absorbance spectrophotometry. The animals used for clinical evaluation were observed for clinical signs of the allergic reaction. EB ocular extravasation was significantly lower in the eyes treated by spaglumic acid and emedastine. The clinical scoring was consistent with EB extravasation, though the difference was not statistically significant. Spaglumic acid and emedastine seem to be the most useful drugs to reduce EB extravasation and allergic signs in an animal model of early phase allergic conjunctivitis. © 2008 Elsevier Ltd. All rights reserved.

Keywords: animal model; allergic conjunctivitis; azelastine; benzalkonium chloride; emedastine; ketotifen; olopatadine; spaglumic acid

1. Introduction

Allergic conjunctivitis is a common condition which may affect between 9.7 (Vazquez Nava et al., 2002) and 18.2% (Austin et al., 1999; Hesselmar et al., 2001) of the population, as is evidenced by the frequent purchase of over-the-counter antiallergic drops (Bielory, 2007). Seasonal allergic

conjunctivitis (SAC) is the most frequent form of allergic conjunctivitis. SAC is an immediate type I hypersensitivity reaction to environmental antigens, usually derived from pollen from different plants. In SAC the allergen comes into direct contact with the nasal mucosa and conjunctiva and interacts with IgE bound to tissue mast cells resulting in the release of mediators and clinical manifestations.

Topical antihistamines are among the most frequently used therapies to treat SAC and presently several different drugs have become available. However, direct comparison on the effects of these drugs to determine their comparative efficacy has been performed to a limited extent, and in most of the series reported the drugs were used as provided by the manufacturer and not in their commercial formulation (Calonge

* Corresponding author. Garcia Morato 35, 9. 47006, Valladolid, Spain. Tel.: +34 620 152 735; fax: +34 983 351 931.

E-mail addresses: msanchis1@hotmail.com (M.E. Sanchis-Merino), msm02va@wanadoo.es, javmonmor@hotmail.com (J.A. Montero), jm.ruiz@umh.es (J.M. Ruiz-Moreno), alejandra@vissum.com (A.E. Rodriguez), silvia@vissum.com (S. Pastor).

et al., 1990, 1996; Merayo-Llodes et al., 1996; Bayer et al., 2003; Calonge et al., 2003; Greiner and Udell, 2005; Shoji et al., 2005).

The aim of this study is to compare the relative efficacy of the commercial formulation of topical antihistamines with balanced saline solution (BSS) and benzalkonium chloride (BC) in the early phase of allergic conjunctivitis in an animal model of ocular anaphylaxis.

2. Material and methods

Ninety-six male Dunkin Hartley guinea pigs weighing 350–450 g (Charles River Laboratories, Spain) were sensitized by intraperitoneal injection of 100 µg egg albumin (EA) and 20 mg aluminum hydroxide in 1 mL phosphate buffer saline (PBS) (all from Sigma Laboratories, Spain).

Animals were used according to the EC Directive 86/609/EEC for animal experiments and the Association of Research in Vision and Ophthalmology guidelines for use of animals in experimental procedures. The work was carried out according to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals.

Seventy-six animals were used for the quantification of Evans blue (EB) extravasation and 20 were used for clinical evaluation of the allergic response.

The pH and osmolarity of the commercial forms of the drugs used were determined.

A pharmacokinetic study was not performed since previous studies have reported almost undetectable levels of circulating drugs after topical administration (EMA, 2007).

2.1. Allergic challenge and quantification of Evans blue extravasation

Eighteen days after sensitization general anaesthesia was induced by the intramuscular injection of 5 mg/kg xylazine chlorhydrate 2% (Bayer, Barcelona, Spain), and 40 mg/kg ketamin chlorhydrate (Merial, Spain) in 76 animals. Additionally, local subcutaneous anaesthesia to the cervical area was performed with 1% mepivacaine (B Braun Medical, Spain).

The animals were topically challenged by the instillation of 30 µL EA (100 µg/mL) in PBS pH 7.4 in the lower conjunctival cul-de-sacs, and treated 15 min before and 15 min after the challenge with 30 µL of the commercially available formulation of ketotifen 0.025% (eye drops and single dose units [SDU], Novartis, Spain), olopatadine 0.1% (Alcon Laboratories, United Kingdom), azelastine 0.05% (Asta Medica, Spain), emedastine 0.05% (Alcon Cusi, Spain) and spaglumic acid (Thea Laboratories, Spain). BC 0.01% (Bial, Spain) and BSS (BSS, Alcon Cusi, Spain) were used as treatment controls. The right and the left eyes of each animal were randomly assigned to different drugs, and the drugs were administered by a masked investigator.

The experiment was repeated four times in small groups of four to five eyes for each drug to prevent bias ($n = 18$ eyes per drug).

Two further control groups were established. One positive control group (two animals, four eyes) was formed by sensitized animals undergoing egg albumin challenge with no topical treatment; and one negative control group (two animals, four eyes) formed by sensitized animals which were not challenged and were not treated with any drug.

The right jugular vein was exposed and EB (Sigma Laboratories, Spain) was intravenously injected (1.25 mg/100 g body weight in a volume of 0.2 mL PBS) simultaneously to the topical challenge. The animals were sacrificed 120 min after the topical challenge by an intramuscular overdose of sodium pentobarbital (Especialidades Veterinarias, Spain) while still under general anaesthesia, followed by cervical dislocation. The eyelids and eyeballs were completely removed and immersed in an extracting solution of 10 mL acetone and sodium sulphate (70/30 v/v). The tubes were frequently and vigorously shaken during 24 h at room temperature. Afterwards, the tubes were centrifuged at 3000 rpm during 15 min and the supernatant was collected.

Vascular permeability and EB extravasation were evaluated in terms of absorbance. The optical density of the supernatant was measured by spectrophotometry at 620 nm, and the results were quantified according to a standard curve.

2.2. Allergic challenge and clinical evaluation

Sixteen animals were topically challenged by the instillation of 30 µL EA (100 µg/mL) in PBS pH 7.4 in the lower conjunctival cul-de-sacs. The animals were divided in eight groups of two and treated 15 min before and 15 min after the challenge with 30 µL of ketotifen SDU, ketotifen, olopatadine, azelastine, emedastine, spaglumic acid, BC or BSS, respectively. Both eyes from each animal received the same treatment, and the pair of animals receiving the same treatment was kept in the same cage for observation and scoring of the allergic signs.

Two further pairs of animals were used as positive ($n = 4$ eyes) and negative ($n = 4$ eyes) controls as described for EB extravasation.

The itch-scratch response (ICR), conjunctival redness, eyelid edema and discharge were evaluated as previously described (Yanni et al., 1994) by two masked observers immediately after challenge, at 30 min and at 90 min (Table 1). Afterwards the animals were sedated by the intramuscular injection of 5 mg/kg xylazine chlorhydrate 2% and 40 mg/kg ketamin chlorhydrate and sacrificed by an intramuscular overdose of sodium pentobarbital followed by cervical dislocation.

2.3. Statistics

A descriptive statistic analysis was performed and the data were evaluated. Kruskal–Wallis test was used to compare samples with non-normal distribution. P values under 0.05 were considered significant.

Table 1
Clinical scoring of allergic signs (adapted from Yanni et al., 1994)

Conjunctival redness	Normal conjunctiva	0
	Pink conjunctiva	1
	Red conjunctiva	2
	Dark red conjunctiva with petechiae	3
Eyelid edema	No edema	0
	Lower lid edema	1
	Upper and lower lid edema, lids partially closed	2
	Swollen, everted eyelids, lids at least half closed	3
	Swelling of both lids and side of face	4
Discharge	No discharge	0
	Glazed, glassy appearance	1
	Moist lids and surrounding hair	2
	Moist lids and surrounding hair, thicker mucous-like	3
Itch scratch response	Uninterrupted clusters of rapid hind limb movements directed to the ocular surface during 60 min	
	None or one cluster of scratching	0
	Two to five clusters of scratching	1
	Six to ten clusters of scratching	2
	More than 11 clusters of scratching	3

3. Results

3.1. Evans blue extravasation

Table 2 and Fig. 1 show the mean, median and interquartile range absorbance of extravasated EB in the treated animals and the control groups.

A first evaluation of the absorbance of all the eyes treated with one specific drug compared with the absorbance of all the eyes treated with other drugs (inter animal comparison) did not show statistically significant differences ($p = 0.22$, Kruskal–Wallis test). However, a trend favoring the efficacy of spaglumic acid and emedastine was found (Fig. 1).

Table 2
Absorbance of extravasated Evans blue after conjunctival challenge

	<i>n</i> (eyes)	Mean	SD ^a	Median	Interquartile range (25–75%)
BSS ^b	18	0.0766	0.0382	0.0627	0.0513–0.0845
BC ^c	18	0.0745	0.0377	0.0805	0.0435–0.0980
Ketotifen	18	0.0743	0.0451	0.0705	0.0340–0.1085
Ketotifen SDU ^d	18	0.0837	0.0318	0.0830	0.0635–0.1025
Olopatadine	18	0.0814	0.0386	0.0710	0.0545–0.1190
Azelastine	18	0.0805	0.0499	0.0630	0.0365–0.0985
Spaglumic acid	18	0.0491	0.0182	0.0530	0.0285–0.0670
Emedastine	18	0.0616	0.0334	0.0525	0.0322–0.0887
Positive Control ^e	4	0.0797	0.0072	0.0695	0.0745–0.0848
Negative Control ^f	4	0.0469	0.0038	0.0420	0.0442–0.0495

^a SD: Standard deviation.

^b BSS: Balanced saline solution.

^c BC: Benzalkonium chloride.

^d SDU: Single Dose Unit.

^e Positive control: egg albumin (EA) challenge with no drug.

^f Negative control: no EA challenge, no drug (statistically significant difference between positive and negative controls, $p = 0.01$, Kruskal–Wallis test).

The difference between positive and negative controls was statistically significant ($p = 0.01$, Kruskal–Wallis test).

A second analysis comparing the data for each specific animal, i.e. the right vs the left eye treated with different drugs (intra animal comparison), showed statistically significant differences for spaglumic acid vs emedastine and BSS ($p = 0.017$ and 0.02 respectively, Kruskal–Wallis test), and emedastine vs BC ($p = 0.05$, Kruskal–Wallis test) (Fig. 2). The differences were not statistically significant for the other intra animal comparisons.

3.2. Clinical scoring

Table 3 shows the clinical scoring of allergic signs (ICR, conjunctival redness, eyelid edema and discharge).

Considering all the clinical signs, the differences were statistically significant comparing the positive control group vs the negative control group and those animals treated by emedastine and spaglumic acid ($p = 0.0005$, Kruskal–Wallis test) (Fig. 3).

The clinical score of discharge and redness in the negative control group and the animals treated by spaglumic acid and emedastine was significantly lower than the positive control group ($p = 0.0005$, Kruskal–Wallis test).

The conjunctival edema score in the negative control group was significantly lower than the positive control group ($p = 0.002$, Kruskal–Wallis test). All the treated eyes showed less edema than the positive control group, though the differences were not statistically significant.

3.3. pH and osmolarity

The pH and osmolarity of BSS, BC and the commercial presentations of the drugs are described in Table 4.

4. Discussion

Animal models have been frequently used to determine in vivo efficacy of antihistamines (Calonge et al., 1990, 1996;

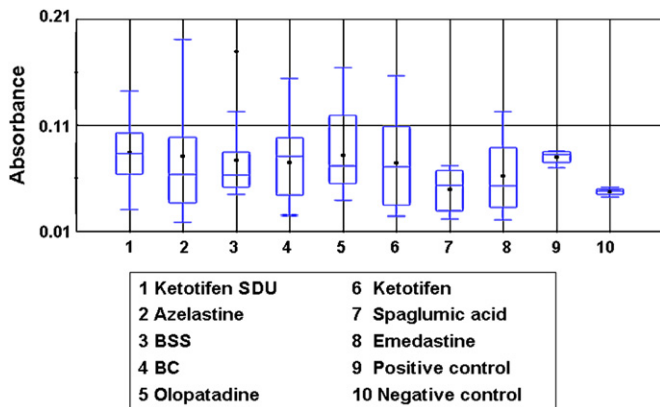


Fig. 1. Inter-animal comparison. Mean, median and interquartile range absorbance of extravasated Evans blue in the treated animals and the control groups. The eyes were randomized to be treated by ketotifen single dose units (SDU), azelastine, balanced saline solution (BSS), benzalkonium chloride (BC) 0.01%, olopatadine, ketotifen, spaglumic acid or emedastine.

model of ocular active anaphylaxis still presents several advantages in the evaluation of topical treatment. The guinea pig model resembles human allergic conjunctivitis and has been validated as model of the human ocular allergic reaction (Khosravi et al., 1995; Calonge et al., 1996, 2003) with the additional advantage that the conjunctiva is very similar to human, permitting the penetration of the allergen (Calonge et al., 1990).

The rationale for the use of animal models in the evaluation of topical antihistamines has been to avoid the high effect of placebo in clinical trials, the variation of symptoms depending on the presence of atmospheric allergens and the limitations derived from scoring of symptoms (Bayer et al., 2003). Several papers have been previously published reporting the comparative effect of topical antihistamines in animal models, including levocabastine, lodoxamide, cromoglycate, nedocromil, ketotifen and olopatadine (Kamei et al., 1991; Yanni et al., 1994; Khosravi et al., 1995; Calonge et al., 1996; Goldschmidt and Luyckx, 1996; Yanni et al., 1996; Schoch, 2003, 2005), most of them with financial support from or directly performed by interested pharmaceutical companies (Yanni et al., 1994; Khosravi et al., 1995; Calonge et al., 1996; Yanni et al., 1996; Schoch, 2003, 2005). Furthermore, the drugs used in most of the previously published papers

Merayo-Llodes et al., 1996; Bayer et al., 2003; Calonge et al., 2003; Greiner and Udell, 2005; Shoji et al., 2005). Even though presently murine models of allergic conjunctivitis may be preferred for the investigation of the immunological basis of the diseases (Groneberg et al., 2003), the guinea pig

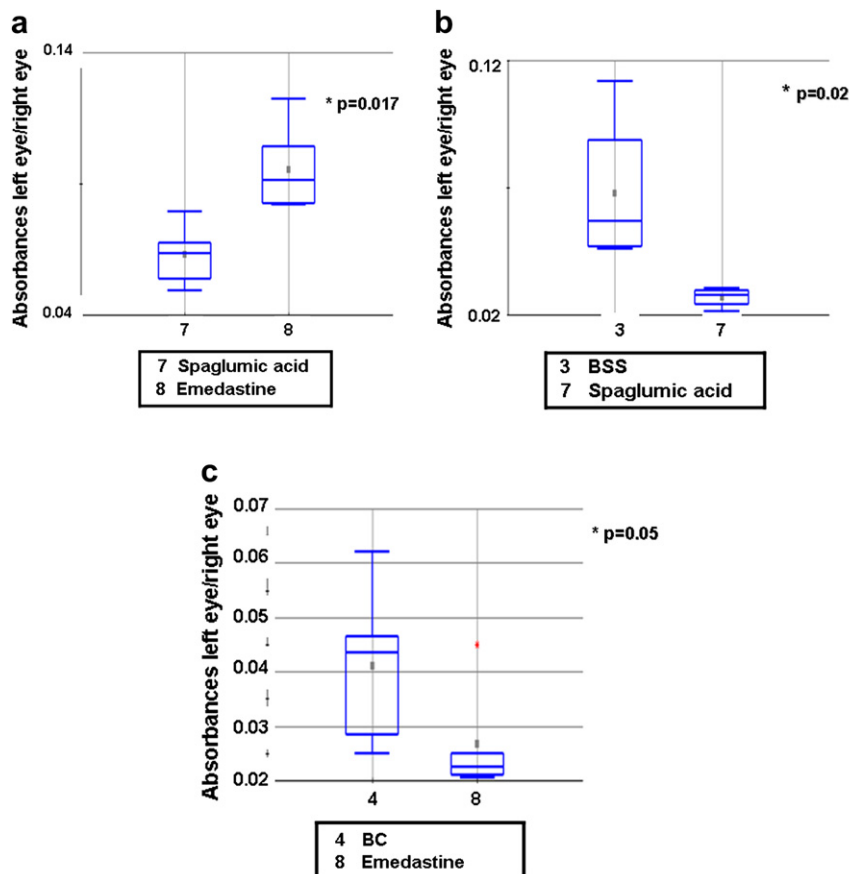


Fig. 2. Intra-animal comparison. Comparative absorbance of extravasated Evans blue in the right (RE) vs the left eye (LE) in each group of animals after conjunctival challenge with egg albumin. Only the statistically significant differences are shown. a: Spaglumic acid (RE) vs emedastine (LE). b: Balanced saline solution (BSS) (RE) vs spaglumic acid (LE). c: Benzalkonium chloride (BC) (RE) vs emedastine (LE).

Table 3
Average clinical scoring of allergic signs

	Conjunctival redness			Edema			Discharge			ISR ^a	Total
	T0 ^b	T30 ^c	T90 ^d	T0	T30	T90	T0	T30	T90		
BSS ^e	0	1.5	1	0	1	1	0	1.5	1	1	8
BC ^f	0	1	1	0	1	1	0	1	2	2	9
Ketotifen	0	1	2	0	1.5	2	0	1.5	2	1	11
Ketotifen SDU ^g	0	1	2	0	1	2.5	0	2	2	1	11.5
Olopatadine	0	1	1	0	1.5	2	0	1.5	2	1	10
Azelastine	0	1	1	0	1	1	0	1	0	2	7
Spaglumic acid	0	1	0	0	0	0	0	0	0	0	1
Emedastine	0	1	0	0	0	0	0	0	0.5	0	1.5
Positive control ^h	0	2	3	0	1.5	2	0	2.5	2.5	1	14.5
Negative control ⁱ	0	1	0	0	0	0	0	0	0	0	1

$p = 0.0005$ for the total score, considering the positive control vs the negative control and animals treated by emedastine and spaglumic acid (Kruskal–Wallis test). $p = 0.0005$ for discharge and redness, considering the positive control vs the negative control and animals treated by spaglumic acid and emedastine (Kruskal–Wallis test). $p = 0.002$ for conjunctival edema, considering the positive control vs the negative control (Kruskal–Wallis test).

^a ISR: Itch-scratch response.

^b T0: Immediately after challenge.

^c T30: 30 min after challenge.

^d T90: 90 min after challenge.

^e BSS: Balanced saline solution.

^f BC: Benzalkonium chloride.

^g SDU: Single Dose Unit.

^h Positive control: egg albumin (EA) challenge with no drug.

ⁱ Negative control: no EA challenge, no drug.

were supplied by the manufacturer and were not in the commercially available forms. The presence of preservatives (BC 0.01% in most of the commercial forms such as emedastine, ketotifen, olopatadine, azelastine, spaglumic acid) except for ketotifen SDU (preservative free, since single dose unit presentation prevents the risk for contamination), lead us to include two groups treated with ketotifen (eyedrops and SDU), and two control treatment groups (one treated with BSS and one with BC) in order to determine the possible role of BC.

BC has been reported to be a weak allergen (Fuchs et al., 1993) which may play a role after repeated instillation of drops as occurs in glaucoma patients (Costagliola et al., 2001), though the damage to the ocular surface may be toxic rather than allergic (Herreras et al., 1992). Our results suggest that the presence or absence of BC does not seem to play any role in the extravasation of EB or in the clinical score in this animal model of allergic conjunctivitis. However, the small size of the sample does not allow drawing conclusions from this difference.

A great dispersion of the EB extravasation data was found in our series. However, even though the differences in EB extravasation were not statistically significant, there was a trend favouring spaglumic acid and emedastine. This trend was also seen in the clinical scores in all the clinical signs considered.

The dispersion of the extravasation data lead us to consider that each animal might show a different level of sensitization, even though they were inbred and the same sensitizing procedure had been used in all of them. Consequently, each animal might be considered a unique element of the experiment, and the results achieved in one eye might only be compared with those of the fellow eye of the same animal. Comparing the results of one eye of each animal with the fellow eye, the

results were statistically significant favouring spaglumic acid and emedastine.

We may consider that general anesthesia may have affected the results in the animals studied by EB extravasation. However, all the drugs were used under the same conditions, as has been previously reported for other studies measuring EB extravasation, reducing the influence of anesthesia on the results. Furthermore, performance of this procedure in unanaesthetized animals not only would be inadmissible, but pain would influence the results in a higher degree.

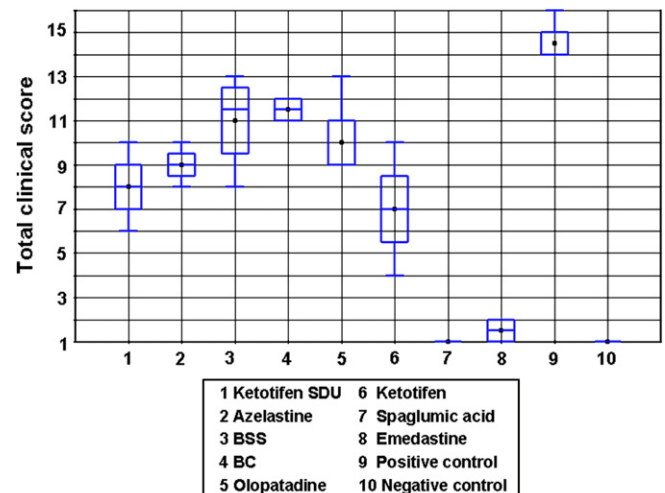


Fig. 3. Mean, median and interquartile range of clinical scoring in the treated animals and the control groups. The score of itch-scratch response (ISR), conjunctival redness, eyelid edema and discharge was evaluated as described in Table 1 by two masked observers immediately after challenge, at 30 min and at 90 min. The total score is shown in the graphic.

Table 4
pH and osmolarity of the controls and the commercial antihistamines

	pH	Osmolarity (mOsm/L)
BSS ^a	7.30	318
BC ^b	7.15	310
Ketotifen	6.12	250
Ketotifen SDU ^c	5.86	247
Olopatadine	6.99	296
Azelastine	5.67	281
Spaglumic acid	7.24	454
Emedastine	7.53	294

^a BSS: Balanced saline solution.

^b BC: Benzalkonium chloride.

^c SDU: Single Dose Unit.

EB extravasation and clinical scoring were lower among eyes treated with drugs formulated with a more physiological pH such as olopatadine (pH 6.99), spaglumic acid (pH 7.24) and emedastine (pH 7.53). Azelastine (pH 5.67) was among the drugs with higher EB extravasation, even higher than the controls BSS and BC. Low pH in some of these drugs such as azelastine and ketotifen may be related with the transient stinging and burning reported on instillation of topical ocular allergy drugs (Joseph Titi, 1996).

Osmolarity did not seem to influence the results. In our series, the highest osmolarity (454 mOsm/L) was found in spaglumic acid drops. Hyperosmolar solutions such as 10–50% glucose (505–2525 mOsm/L) have been reported to induce a non-specific immediate inflammatory response (Bonini et al., 2007).

Previous papers have reported on the comparative effect of topical antihistamines in experimental and clinical trials. Purello et al. have reported on the comparative efficacy of spaglumic acid and lodoxamide on patients with seasonal allergic conjunctivitis (Purello D'Ambrosio et al., 1997). These authors found a greater efficacy of lodoxamide inhibiting the conjunctival response and for a longer duration than spaglumic acid. Emedastine has been thoroughly tested in clinical studies showing greater antihistaminic effects than levocabastine (Netland et al., 2000; Secchi et al., 2000). Clinical studies comparing ketotifen and emedastine have proved similar antiallergic effects to treat SAC after single-dose administration (D'Arienzo et al., 2002; Horak et al., 2003).

Animal studies on the rat and guinea pig model have shown that ketotifen was more effective than olopatadine (Schoch, 2005). Other clinical studies agreed with these results (Ganz et al., 2003), did not show differences between both drugs (Avunduk et al., 2005), or favoured olopatadine (Aguilar, 2000; Artal et al., 2000; Leonardi and Abelson, 2003). Similarly, olopatadine has been reported to be more effective than azelastine (Spangler et al., 2001).

According to our results, most of the topical antihistamines tested did not prove to be superior to other drugs or to BSS or BC. EB extravasation and clinical scores were lower for BC, BSS and all the topical drugs tested, compared with the positive controls. Only those eyes treated by spaglumic acid and

emedastine had a significantly lower EB extravasation and clinical scores than those treated with other drops.

Topical BSS seems to be useful in the early phase of the allergic reaction. This effect may be attributed to the removal of allergens and inflammatory mediators from the ocular surface, which can also be attributed to any other drop.

Our results have not shown differences among the eyes treated with drops containing BC (ketotifen, olopatadine, azelastine, spaglumic acid, emedastine and the BC control) and those treated with preservative free drops (BSS, ketotifen SDU). Furthermore, most of the tested drugs did not seem to significantly reduce EB extravasation compared with BSS or BC. However, we have only tested the effect of these drugs to treat the early phase of the allergic reaction. Further experiments on animal models are needed to study the efficacy of topical antihistamines in the late phase of the allergic reaction and after repeated instillation of these drops.

Acknowledgments

This paper was presented in part at the EAACI meeting, Gothenburg 2007.

This paper has received financial support from the Junta de Castilla y Leon (Biomedical Research Projects), Spain.

The authors have no financial interests in the procedures and drugs mentioned in this paper.

References

- Aguilar, A.J., 2000. Comparative study of clinical efficacy and tolerance in seasonal allergic conjunctivitis management with 0.1% olopatadine hydrochloride versus 0.05% ketotifen fumarate. *Acta Ophthalmol. Scand.* 78 (Suppl. 230), 52–55.
- Artal, M.N., Luna, J.D., Discepolo, M., 2000. A forced choice comfort study of olopatadine hydrochloride 0.1% versus ketotifen fumarate 0.05%. *Acta Ophthalmol. Scand.* 78 (Suppl. 230), 64–65.
- Austin, J.B., Kaur, B., Anderson, H.R., Burr, M., Harkins, L.S., Strachan, D.P., Warner, J.O., 1999. Hay fever, eczema, and wheeze: a nationwide UK study (ISAAC, international study of asthma and allergies in childhood). *Arch. Dis. Child.* 81, 225–230.
- Avunduk, A.M., Tekelioglu, Y., Turk, A., Akyol, N., 2005. Comparison of the effects of ketotifen fumarate 0.025% and olopatadine HCl 0.1% ophthalmic solutions in seasonal allergic conjunctivitis: a 30-day, randomized, double-masked, artificial tear substitute-controlled trial. *Clin. Ther.* 27, 1392–1402.
- Bayer, A., Uludag, H.A., Sobaci, G., Mutlu, F.M., 2003. Comparison of antiallergic drugs in an experimental model of ocular anaphylaxis. *Ophthalmologica* 217, 119–123.
- Bielory, L., 2007. Differential diagnoses of conjunctivitis for clinical allergist-immunologists. *Ann. Allergy Asthma Immunol.* 98, 105–114. quiz 114–117, 152.
- Bonini, S., Rasi, G., Brusasco, V., Carlsen, K.H., Crimi, E., Popov, T., Schulze-Werninghaus, G., Gramiccioni, C., Bonini, M., Passali, D., Bachert, C., van Cauwenberge, P.B., Bresciani, M., Bonini, S., Calonge, M., Montan, P.G., Serapiao Dos Santos, M., Belfort Jr., R., Lambiase, A., Sacchetti, M., 2007. Nonspecific provocation of target organs in allergic diseases: EAACI-GA(2)LEN consensus report. *Allergy* 62, 683–694.
- Calonge, M.C., Pastor, J.C., Herreras, J.M., Gonzalez, J.L., 1990. Pharmacologic modulation of vascular permeability in ocular allergy in the rat. *Invest. Ophthalmol. Vis. Sci.* 31, 176–180.

- Calonge, M., Montero, J.A., Herreras, J.M., Ramon, J.J., Pastor, J.C., 1996. Efficacy of nedocromil sodium and cromolyn sodium in an experimental model of ocular allergy. *Ann. Allergy Asthma Immunol.* 77, 124–130.
- Calonge, M., Siemasko, K.F., Stern, M.E., 2003. Animal models of ocular allergy and their clinical correlations. *Curr. Allergy Asthma Rep.* 3, 345–351.
- Costagliola, C., Prete, A.D., Incorvaia, C., Fusco, R., Parmeggiani, F., Di Giovanni, A., 2001. Ocular surface changes induced by topical application of latanoprost and timolol: a short-term study in glaucomatous patients with and without allergic conjunctivitis. *Graefes Arch. Clin. Exp. Ophthalmol.* 239, 809–814.
- D'Arienzo, P.A., Leonardi, A., Bensch, G., 2002. Randomized, double-masked, placebo-controlled comparison of the efficacy of emedastine difumarate 0.05% ophthalmic solution and ketotifen fumarate 0.025% ophthalmic solution in the human conjunctival allergen challenge model. *Clin. Ther.* 24, 409–416.
- EMA, 2007. EPARs for authorised medicinal products for human use. <http://www.ema.europa.eu/htms/human/epar/a.htm>.
- Fuchs, T., Meinert, A., Aberer, W., Bahmer, F.A., Peters, K.P., Lischka, G.G., Schulze-Dirks, A., Enders, F., Frosch, P.J., 1993. Benzalkonium chloride—a relevant contact allergen or irritant? Results of a multicenter study of the German Contact Allergy Group]. *Hautarzt* 44, 699–702.
- Ganz, M., Koll, E., Gausche, J., Detjen, P., Orfan, N., 2003. Ketotifen fumarate and olopatadine hydrochloride in the treatment of allergic conjunctivitis: a real-world comparison of efficacy and ocular comfort. *Adv. Ther.* 20, 79–91.
- Goldschmidt, P., Luyckx, J., 1996. Effects of lodoxamide (LOD), disodium cromoglycate (DSCG) and N-acetyl-aspartyl-glutamate sodium salt (NAAGA) on ocular active anaphylaxis. *Allerg. Immunol. (Paris)* 28, 124–126.
- Greiner, J.V., Udell, I.J., 2005. A comparison of the clinical efficacy of pheniramine maleate/naphazoline hydrochloride ophthalmic solution and olopatadine hydrochloride ophthalmic solution in the conjunctival allergen challenge model. *Clin. Ther.* 27, 568–577.
- Groneberg, D.A., Bielory, L., Fischer, A., Bonini, S., Wahn, U., 2003. Animal models of allergic and inflammatory conjunctivitis. *Allergy* 58, 1101–1113.
- Herreras, J.M., Pastor, J.C., Calonge, M., Asensio, V.M., 1992. Ocular surface alteration after long-term treatment with an antiglaucomatous drug. *Ophthalmology* 99, 1082–1088.
- Hesselmar, B., Aberg, B., Eriksson, B., Aberg, N., 2001. Allergic rhinoconjunctivitis, eczema, and sensitization in two areas with differing climates. *Pediatr. Allergy Immunol.* 12, 208–215.
- Horak, F., Stubner, P., Ziegelmayer, R., Kawina, A., Moser, M., Lanz, R., 2003. Onset and duration of action of ketotifen 0.025% and emedastine 0.05% in seasonal allergic conjunctivitis: efficacy after repeated pollen challenges in the Vienna Challenge Chamber. *Clin. Drug Investig.* 23, 329–337.
- Joseph Titi, M., 1996. A critical look at ocular allergy drugs. *Am. Fam. Physician* 53, 2637–2642. 2645–2646.
- Kamei, C., Izushi, K., Tasaka, K., 1991. Inhibitory effect of levocabastine on experimental allergic conjunctivitis in guinea pigs. *J. Pharmacobiodyn.* 14, 467–473.
- Khosravi, E., Elena, P.P., Hariton, C., 1995. Allergic conjunctivitis and uveitis models: reappraisal with some marketed drugs. *Inflamm. Res.* 44, 47–54.
- Leonardi, A., Abelson, M.B., 2003. 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. *Clin. Ther.* 25, 2539–2552.
- Merayo-Lloves, J., Zhao, T.Z., Dutt, J.E., Foster, C.S., 1996. A new murine model of allergic conjunctivitis and effectiveness of nedocromil sodium. *J. Allergy Clin. Immunol.* 97, 1129–1140.
- Netland, P.A., Leahy, C., Krenzer, K.L., 2000. Emedastine ophthalmic solution 0.05% versus levocabastine ophthalmic suspension 0.05% in the treatment of allergic conjunctivitis using the conjunctival allergen challenge model. *Am. J. Ophthalmol.* 130, 717–723.
- Purello D'Ambrosio, F., Gangemi, S., Ricciardi, L., Cuzzocrea, S., Di Lorenzo, G., 1997. Lodoxamide versus spaglumic acid: a comparative double-blind trial on patients suffering from seasonal allergic conjunctivitis induced by Parietaria pollen. *Allergol. Immunopathol. (Madr.)* 25, 233–237.
- Schoch, C., 2003. Effects of ketotifen 0.025% and lodoxamide 0.1% on eosinophil infiltration into the guinea pig conjunctiva in a model of allergic conjunctivitis. *J. Ocul. Pharmacol. Ther.* 19, 153–159.
- Schoch, C., 2005. Effect of ketotifen fumarate, olopatadine, and levocabastine on ocular active anaphylaxis in the guinea pig and ocular immediate hypersensitivity in the albino rat. *Ocul. Immunol. Inflamm.* 13, 39–44.
- Secchi, A., Ciprandi, G., Leonardi, A., Deschenes, J., Abelson, M.B., 2000. Safety and efficacy comparison of emedastine 0.05% ophthalmic solution compared to levocabastine 0.05% ophthalmic suspension in pediatric subjects with allergic conjunctivitis. Emedastine Study Group. *Acta Ophthalmol. Scand.* 78 (Suppl. 230), 42–47.
- Shoji, J., Sakimoto, T., Muromoto, K., Inada, N., Sawa, M., Ra, C., 2005. Comparison of topical dexamethasone and topical FK506 treatment for the experimental allergic conjunctivitis model in BALB/c mice. *Jpn. J. Ophthalmol.* 49, 205–210.
- Spangler, D.L., Bensch, G., Berdy, G.J., 2001. Evaluation of the efficacy of olopatadine hydrochloride 0.1% ophthalmic solution and azelastine hydrochloride 0.05% ophthalmic solution in the conjunctival allergen challenge model. *Clin. Ther.* 23, 1272–1280.
- Vazquez Nava, F., Salas Ramirez, E., Mogica Mogica Jde, D., Beltran Guzman, F.J., 2002. Prevalence and risk factors for allergic conjunctivitis in children. Comparative study in two communities]. *Rev. Alerg. Mex.* 49, 33–41.
- Yanni, J.M., Stephens, D.J., Parnell, D.W., Spellman, J.M., 1994. Preclinical efficacy of emedastine, a potent, selective histamine H1 antagonist for topical ocular use. *J. Ocul. Pharmacol.* 10, 665–675.
- Yanni, J.M., Stephens, D.J., Miller, S.T., Weimer, L.K., Graff, G., Parnell, D., Lang, L.S., Spellman, J.M., Brady, M.T., Gamache, D.A., 1996. The in vitro and in vivo ocular pharmacology of olopatadine (AL-4943A), an effective anti-allergic/antihistaminic agent. *J. Ocul. Pharmacol. Ther.* 12, 389–400.