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The effect of ketotifen on inflammatory markers in allergic conjunctivitis: an open, uncontrolled study

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Abstract

Background: The efficacy and safety of ketotifen eye drop treatment in allergic conjunctivitis (AC) management is perfectly known by several studies, but the mechanism of action at the biochemical levels is poorly understood so we decided to perform an open, uncontrolled study in order to investigate the effect of the topical administration of ketotifen fumarate 0.05% on biochemical markers of inflammation on conjunctival cells in patients with AC.

Methods: Nineteen patients with symptoms and signs of AC (itching, discharge, burning, redness, increase in the watery discharge, swelling and follicles) and with a history of allergy were prescribed with two daily instillation of one drop of eyewash ketotifen fumarate 0,05% in both eyes during thirty days. They were studied by measuring clinical and immunologic parameters.

Results: Ketotifen fumarate treatment significantly reduced the total symptoms and signs score for each patient as well as each symptoms and signs at all time points compared with day 0 ($p < 0.0001$ and $p < 0.016$, respectively). Although the percentage of HLA-DR+ epithelial cells diminished only in 58% of patients, the numbers of CD29+ and eotaxin+ epithelial cells dropped significantly in 68% and 73 % of them ($p < 0.0062$ and <0.0082, respectively) as a consequence of the treatment. In 9 out of 19 patients a simultaneous decrease in the percentage of epithelial cells positive for CD29 and eotaxin was observed.

Conclusion: Ketotifen besides the well-known effect in reducing signs and symptoms of AC significantly diminished production of eotaxin and expression of CD29 by epithelial cells in patients with seasonal AC.

Background

Allergic conjunctivitis (AC) is an ocular surface inflammatory disease that affects approximately 25% of the general population [1–3] and has a significant impact on the social and economic aspects of life. It can appear alone or associated with other allergic diseases, especially allergic rhinitis. AC is an immunopathological disease which the number of mast cells in the substantia propria increase, and also the epithelium becomes densely infiltrated [4,5]. Activation of mast cells by IgE bound-receptor crosslinking by allergens promotes the release of several mediators such as histamine, prostaglandins, leukotrienes, tryptase and cytokines, all of them responsible for the symptoms of AC and the inflammatory changes in conjunctival cells [6–8].

Conjunctival epithelial cells (EC) play an important proinflammatory role in chronic ocular allergic diseases, the expression profile levels of different cellular adhesion molecules and surface inflammatory antigens have been reported in normal and altered human conjunctival epithelium [9–11]. Recent results have demonstrated that expression of ICAM-1 may allow EC to recruit, retain and locally concentrate leukocytes and the presence of HLA-DR raises the question of conjunctival EC antigen presentation [12].

Although the level of expression of CD29 (the common chain of the beta 1 integrins) on normal conjunctival ECs is already known [10], there is not information about changes in its expression during AC. In addition, different epithelial cytokines/chemokines, which are upregulated actively, participate in allergic inflammation [13]. Among those chemokines eotaxin not only play an important role in eosinophilic recruiting and in damaging the tissue, but also maintain this type of immune response [14].

AC can be treated with local anti-allergic agents such as antihistamines, either alone or in combination with alpha-adrenergic agents and mast cell stabilizers [15–18]. Ketotifen fumarate is derived from cyproheptadine, a serotonin and histamine antagonist [19]. The efficacy and safety of this drug treatment in AC management is well known [20,21] and its clinical use has also been widely studied in relation to bronchial asthma where have been demonstrated that ketotifen treatment significantly decreased EG2+ activated eosinophils, CD3+ and CD4+ T cells in the bronchial mucosa and inhibit the expression of E-selectin and ICAM-1 on vascular endothelial cells [22–24].

The effects of different H1 antihistamines on ICAM-1 expression have been extensively studied [24–29], but since there is not too much information about the effects of ketotifen on expression of CD29, HLA-DR and eotaxin on conjunctival EC during AC, we performed an open, uncontrolled study on patients with AC to investigat the effect of this drug on clinical features and those markers of inflammation.

Methods

Human subjects

Nineteen subjects (8 males and 11 females, between 6 and 63 years) with seasonal AC [30] and with a history of allergy (allergic conjunctivitis, hay fever, asthmatic bronchitis and dermatitis) who visited the Department of Ophthalmology, Hospital Privado, Córdoba, Argentina, during the spring season were evaluated. Those patients were diagnosed as previously described [31] based on positive allergen-skin prick test, IgE in tears, conjunctival eosinophils, and symptoms and signs such as itching, discharge, burning, redness, increase in the watery discharge, swelling and presence of follicles. At day 0 they had normal levels of secretory IgA and lisozime and did neither suffer from other eye disorder, nor had they experienced any ophthalmologic treatment in the two previous weeks to their enrollment. The use of contact lenses was suspended for seventy two hours before and during the whole study. All subjects gave informed consent and the study was approved by the ethics committee of the Hospital Privado.

Each patient was prescribed with two daily instillations of one drop of eyewash ketotifen fumarate 0,05% in both eyes, during thirty days.

Ocular status assessment

Different symptoms (itching, tearing, burning, redness) and signs (watery discharge increase, swelling, presence of follicles) of allergic conjunctivitis were evaluated at their enrollment (day zero) and at different times after starting treatment (7, 15 and 30 days). Symptoms and signs were classified in four stages: 0-Absent; 1-Mild; 2-Moderate and 3-Severe. The total symptoms and signs score (TSSS) for each subject were obtained adding the values of each symptoms and signs divided by the total number of them.

Immunological studies

Samples of conjunctival scrapings were collected from both eyes of all patients treated on days 0 and 30 using a disposable plastic scoop (Rhino probe™). The EC obtained were suspended in 20% AB human serum – PBS and incubated with anti-CD29 (beta chain of beta 1 integrin) RD1 (COULTER), anti-HLA-DR FITC (Becton Dickinson), and anti-CD45 (Leukocyte common antigen) PerCP (Becton Dickinson) monoclonal antibodies (mAb), or isotopic antibody controls (Becton Dickinson) for 45 min on ice. Another fraction of the EC was incubated with anti-eotaxin (CCL11) mAb (6H9, kindly provided by LeukoSite Inc., Cambridge, MA) diluted with Triton

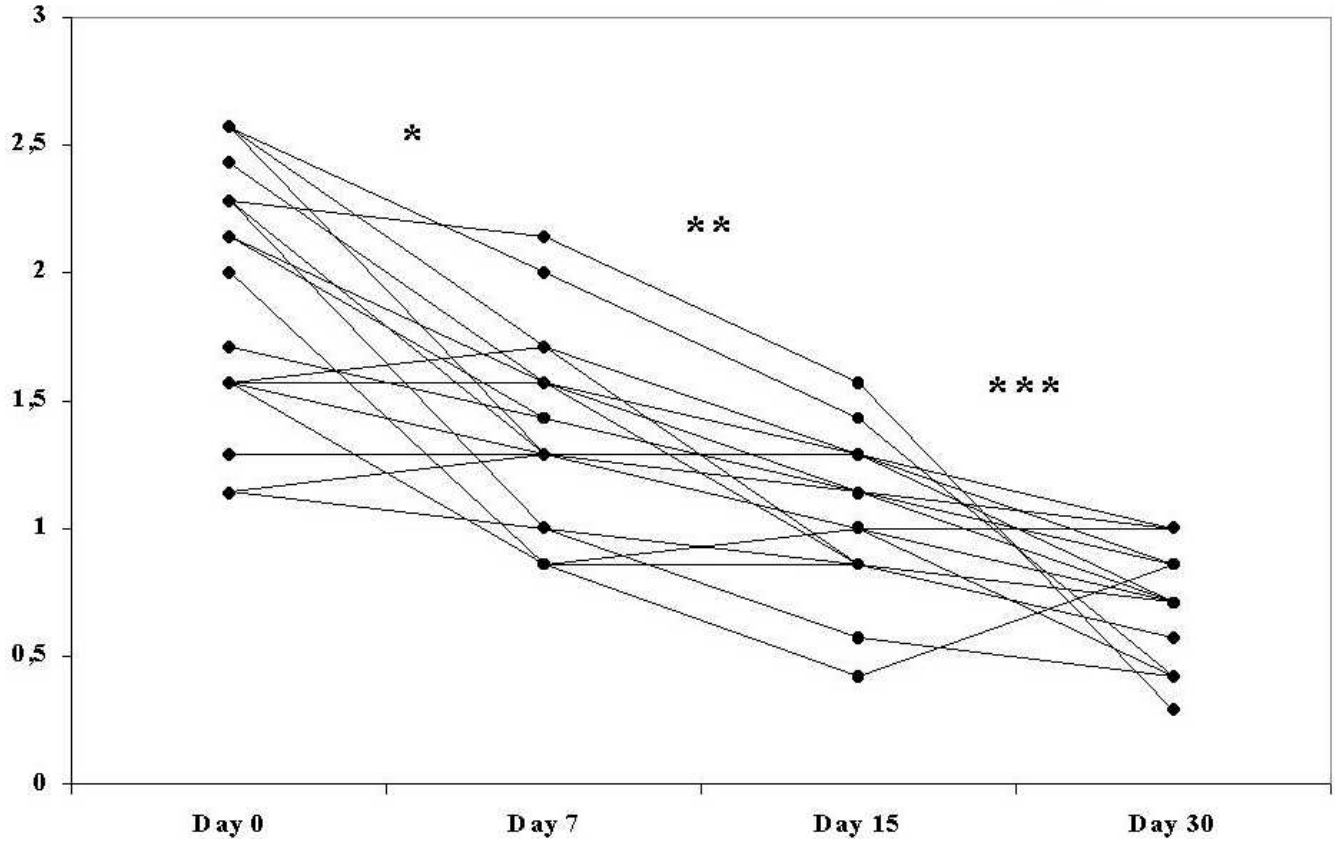

Figure 1
Variation of total score of symptoms and signs for each patient with ketotifen treatment. The differences between time points were analyzed by Wilcoxon signed-rank test (*, $p < 0.0001$, day 0 vs 7, 15 and 30; and 7 vs 30; **, $p < 0.0002$, day 7 vs 15; ***, $p < 0.0008$ day 15 vs 30).

2%-PBS (Sigma) for 1 hour at 37°C, then it was incubated with goat anti-mouse IgG FITC (SIGMA) antibody for 30 min on ice and finally with anti-CD45 PerCP (Becton Dickinson) mAb, or isotopic antibody controls (Becton Dickinson) for 45 min on ice. The samples were properly washed and fixed with paraformaldehyde 1% and analyzed on a flow cytometer (Cytoron Absolute), equipped with an argon laser emitting at 488 nm. Analytical gates were set to discard cell debris and CD45+ cells. The number of antigen-positive EC was then obtained from a quadrant graphic paper representing mean fluorescence intensities (MFI) on a 3-decade logarithmic amplifier. The upper limit of intensity of fluorescence from the control antibody was regarded as positivity threshold for the tested antibodies. For each sample, 1.000–5.000 EC were analyzed.

Statistical analysis

Variability of the parameters studied was analyzed with Wilcoxon matched-pairs signed-rank test. For all tests, $p \leq 0,05$ was considered significant.

Results

After 7 days of treatment, 53% of patients showed improvements of their symptoms and signs ($p < 0.0001$). With continued treatment through day 14, symptoms control was achieved in 76% of patients ($p < 0.0001$). Moreover, administration of 0,05% ketotifen eye drops for thirty days significantly ($p < 0.0001$) reduced the TSSS for each patient (Figure 1) between days 0 and 30.

Treatment with this drug has clinical effect on burning, watery discharge, and swelling, particularly, at the end of the treatment. Every other symptom were significantly reduced ($p < 0.016$) at any time of clinical evaluation (Figure 2).

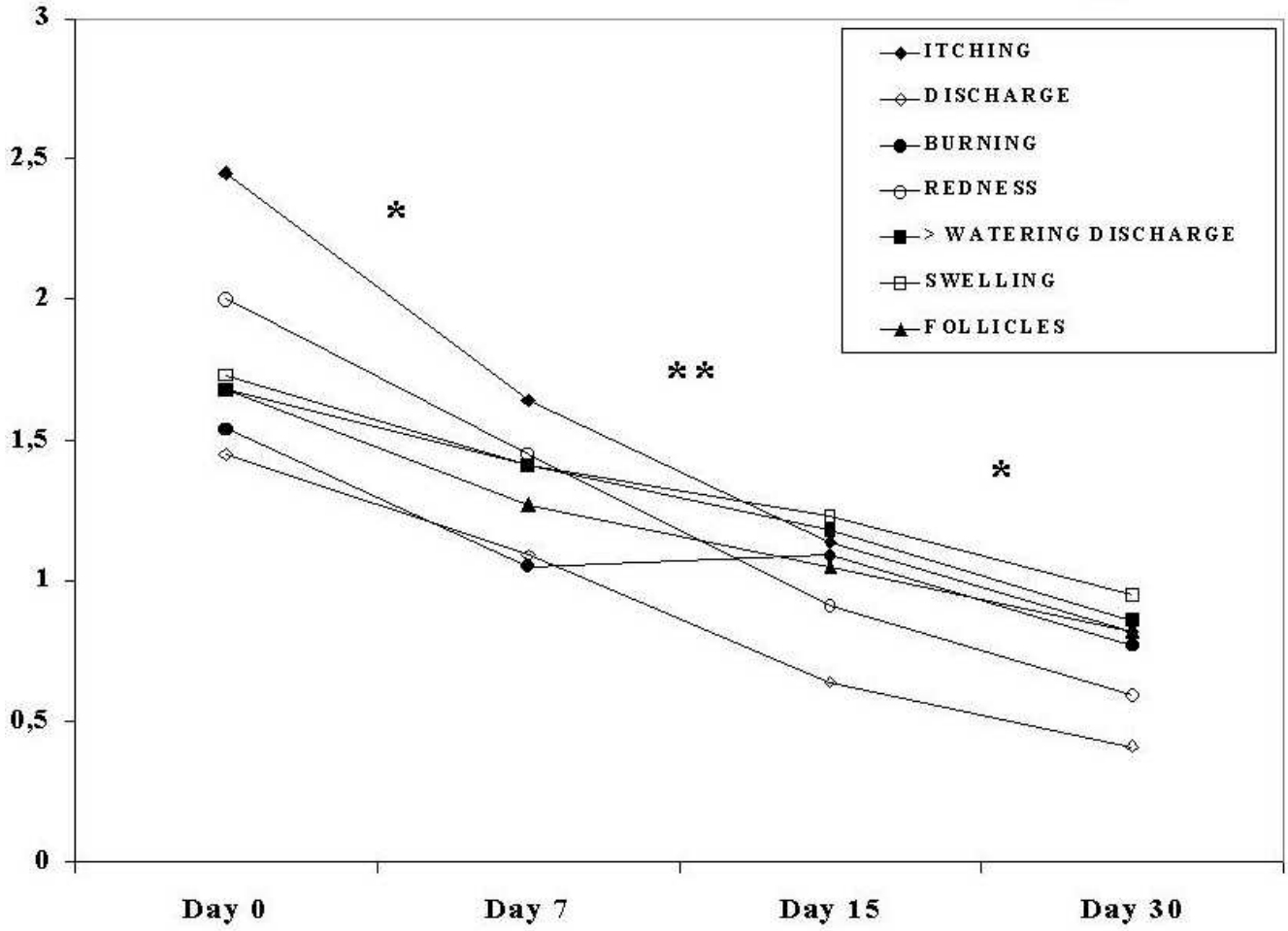

Figure 2
Variation of total score of symptoms and signs with ketotifen treatment. The differences between time points were analyzed by Wilcoxon signed-rank test (*, $p < 0.016$, day 0 vs 7, 15 and 30; day 7 vs 30, and day 15 vs 30; **, $p < 0.031$, day 7 vs 15).

The effect of the treatment was also studied on the expression of different molecules on CD45 negative cells (EC) by FACS. It is worth to note that cells studied which were obtained by conjunctival scraping never contain more than 3 % of CD45 + cells (data not shown).

When the expression of HLA-DR, CD29 and eotaxin on EC was evaluated after the treatment we found a drop in the percentage of those positive EC in 58%, 68% and 73% of patients, respectively. Although the variation in percentage of HLA-DR+ EC was not significant, the percentage of CD29+ and eotaxin + EC significantly decreased ($p < 0.0062$ and <0.0082, respectively) at the end of the treatment (Table 1). In 9/19 patients a simultaneous drop in EC positive for CD29 and eotaxin was observed. Figure 3 shows a representative case from this group of patients.

We did not find any correlation between variations in mean fluorescent intensity and percentage of positive EC for these markers before and after the treatment.

Discussion

AC is a common, prevalent, and clinically significant IgE mediate hypersensitivity response in which signs and symptoms associated with the progression from early to late phase reaction start four to eight hours after challenge and persist up to 24 hours. The clinical reaction is accompanied by a significant recruitment of inflammatory cells (mainly neutrophils) in tears 20 minutes after challenge, and eosinophils and lymphocytes 6 to 24 hours after challenge [32]. In the chronic and more severe forms of AC, other mechanisms and cells contribute to a more complex clinical profile [33]. In addition, the mast cell is consid-

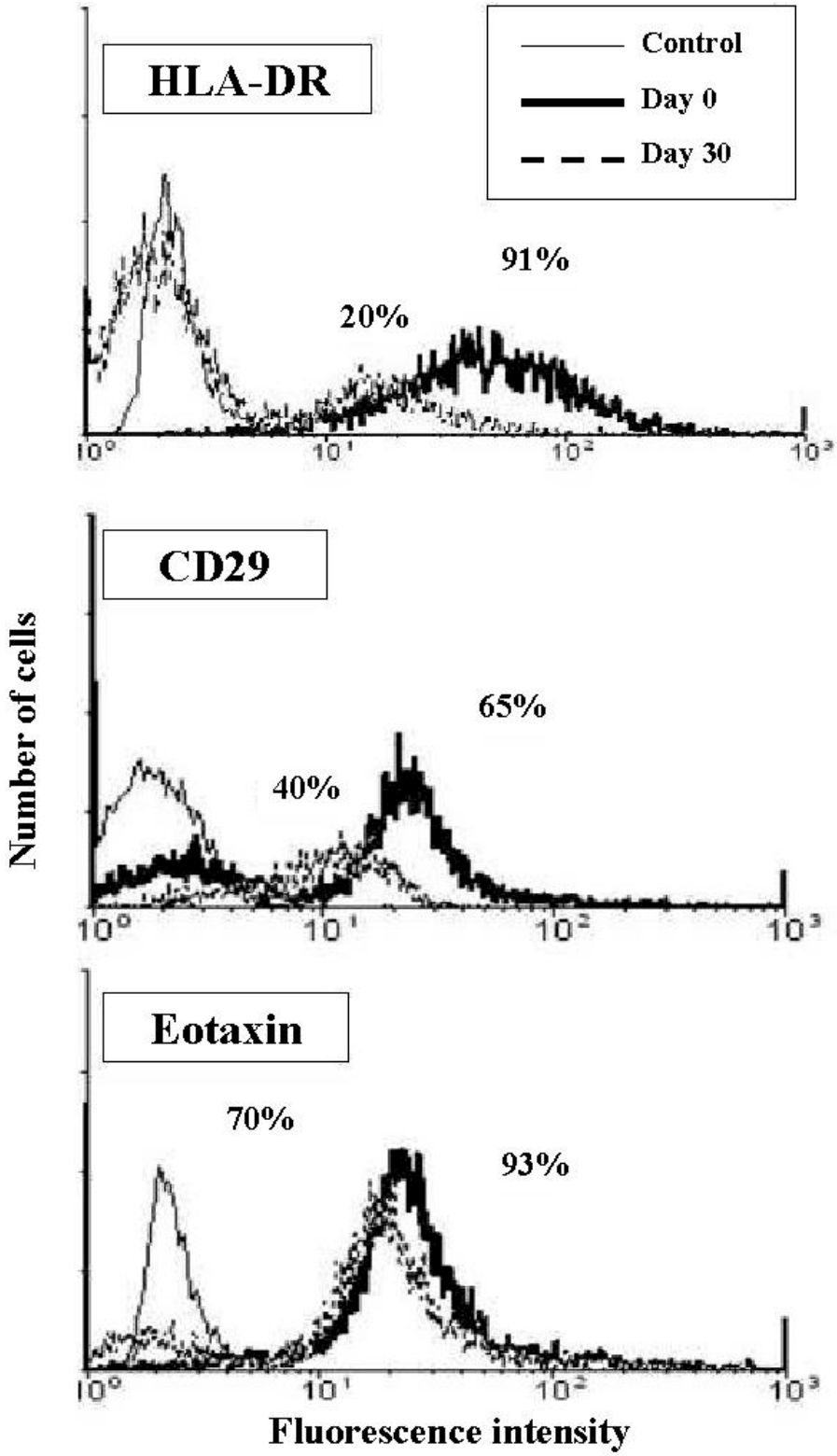

Figure 3
HLA-DR, CD29 and eotaxin expression by EC from patients with AC before and after the treatment with ketotifen fumarate 0.05%. Data show one representative case from nine patients who had a simultaneous drop in CD29+ and eotaxin+ EC.

Table 1: Percentage of positive EC before and after the treatment with ketotifen fumarate 0.05%.

Patients	HLA-DR positive cells*		CD29 positive cells*		Eotaxin positive cells*	
	Day 0	Day 30	Day 0	Day 30	Day 0	Day 30
1	60	30	82	38	69	52
2	18	31	67	34	81	66
3	50	34	86	70	75	64
4	46	30	59	27	83	69
5	10	65	61	50	86	64
6	45	80	81	73	85	84
7	12	55	78	45	98	72
8	75	32	85	25	94	83
9	91	19	65	51	93	70
10	32	70	36	54	51	66
11	21	11	64	13	68	82
12	24	27	22	23	74	60
13	20	12	17	24	76	65
14	62	31	81	37	63	72
15	55	72	54	83	77	57
16	82	41	91	41	81	89
17	80	3	75	80	84	72
18	67	11	81	48	79	84
19	17	63	42	45	76	56
Media	**46**	**38**	**64**	**45**	**79**	**70**
SD	**26**	**23**	**22**	**19**	**11**	**10**

* See Methods.

ered to play a pivotal role in causing signs and symptoms since Bacon AS et al found increased levels of histamine, tryptase, PGD2, and leukotriene C4 in the tears of patients with Seasonal Allergic Conjunctivitis after conjunctival allergen challenge [34].

It is now becoming clear that EC may be viewed as active participants in the regulation of inflammation and leukocyte behavior. EC from a number of mucosal sites have been shown to be capable of: 1) expressing adhesion molecules like ICAM-1 (CD54) or beta 1 integrin (CD29), which are important for leukocyte emigration into tissue; 2) expressing MHC class II molecules and 3) synthesizing and releasing prostaglandins and leukotrienes which attract and activate leukocytes and 4) producing a variety of pro-inflammatory cytokines and chemokines. The adhesion molecules expressed on EC not only allow interactions between cells and cells with matrix but also they might be involved in intracellular signals [12,13,35–37].

Although we did not found significant variations in the percentage of HLA-DR+ EC before and after treatment, the majority of patients exhibited a significant decrease in the percentage of CD29+ cells suggesting that the expression of these molecules are controlled by different mechanisms.

Eotaxin, a CC chemokine, is a potent and selective eosinophil chemoattractant to the site of inflammation but also plays an important role in damaging tissue by its capacity to induce the release of reactive oxygen species [38,39]. It has been previously demonstrated that eotaxin mRNA and protein are constitutively expressed by bronchial and nasal epithelium from normal individuals and increased within the airways of asthmatic individuals, and in nasal biopsy specimens from individuals with allergic rhinitis. In addition eotaxin immunoreactivity was found in macrophages, eosinophils, T cells, mast cells, and neutrophils, suggesting that this chemokine is an important mediator in the physiological and pathological trafficking of eosinophils [40,41].

It has been reported that in EC from normal conjunctival biopsies there is a weak cytoplasmic expression of eotaxin [13]. In our study we found a strong expression of eotaxin on EC from patients with AC which decrease significantly after treatment with ketotifen.

Current therapy of ocular allergic disease focuses on allergen elimination, modulation of the immune system and pharmacological inhibition of the chemical mediators involved in the immune response. The most commonly used therapeutic option is the pharmacological inhibition of chemical mediators. Mast cells stabilizers and antihista-

mines are two of the most commonly used group of therapeutic agents; they stabilize the mast cell membranes by preventing calcium influx across the mast cell membranes, thereby preventing mast cell degranulation and mediator release and the new antihistamines have been demonstrated to be capable of affecting several phenomena of the allergic inflammation, including mediator release, cellular activation and adhesion molecule expression [42,43]. Among these drugs, ketotifen fumarate, attenuated the local allergic reaction in patients with AC [20,21] and the efficacy and safety of this drug in AC management has also been shown in human conjunctival allergen challenge model [44,45].

Here, we corroborated the effectiveness of ketotifen fumarate in decreasing the symptoms and signs of AC in the majority of patients, but more importantly we showed that this drug even though was not effected in reducing the expression of HLA-DR on EC, it significantly decreased the percentage of CD29+ and eotaxin+ EC.

The mechanism of action by which this drug produce this effects are probably related to the decrease amounts of inflammatory cytokines/chemokines released by effector cells at the conjunctival level. Clearly, additional studies are needed to fully understand the complex mechanisms of the anti-inflammatory effect of Ketotifen fumarate in patients with AC.

List of abbreviations

AC = Allergic Conjunctivitis.

EC = Epithelial cells.

TSSS = Total symptoms and signs score.

mAb = Monoclonal antibody.

Competing interests

None declared.

Authors' contributions

Martín AP: collected conjunctival scrapings and carried out immunofluorescence studies, performed the statistical analysis and drafted the manuscript.

Urrets-Zavalia J: performed the ophthalmological evaluation of the patients with AC.

Berra A: performed conjunctival cytology studies and quantification of tear IgE.

Mariani AL: carried out the quantification of serum IgE, secretory IgA and lisozime.

Gallino N: carried out the prick tets and clinical evaluation of the patients with AC.

Gomez Demel E: performed the ophthalmological evaluation of the patients with AC.

Gagliardi J: carried out the prick tets and clinical evaluation of the patientswith AC.

Baena-Cagnani CE: carried out the prick tets and clinical evaluation of the patientswith AC.

Urrets-Zavalia E: conceived of the study, and participated in its design and coordination.

Serra, HM: conceived of the study, and participated in its design and coordination.

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