

Research article

Open Access

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

Andrea P Martín¹, Julio Urrets-Zavalía², Alejandro Berra³, Ana Lía Mariani⁴, Norberto Gallino⁵, Eduardo Gomez Demel⁶, Julio Gagliardi⁵, Carlos E Baena-Cagnani⁷, Enrique Urrets-Zavalía^{2,8} and Horacio M Serra*^{1,8}

Address: ¹Inmunología, Dpto. Bioquímica Clínica, Facultad Ciencias Químicas, Universidad Nacional de Córdoba, Argentina, ²Servicio de Oftalmología, Clínica Reina Fabiola. Córdoba, Argentina, ³Dpto. de Patología, Facultad Medicina, Universidad de Buenos Aires, Argentina, ⁴Inmunología, Universidad Nacional de Misiones, Argentina, ⁵Servicio de Alergia e Inmunología, Hospital Privado. Córdoba, Argentina, ⁶Servicio de Oftalmología, Hospital Privado. Córdoba, Argentina, ⁷Servicio de Pediatría, Hospital Infantil Municipal. Córdoba, Argentina and ⁸These authors contributed equally to this work

Email: Andrea P Martín - amartin@bioclin.fcq.unc.edu.ar; Julio Urrets-Zavalía - jauz@arnet.com.ar; Alejandro Berra - hserra@bioclin.fcq.unc.edu.ar; Ana Lía Mariani - almariani@arnet.com.ar; Norberto Gallino - hprivadoalergia@arnet.com.ar; Eduardo Gomez Demel - oftalmologia_h_priv@cordoba.com.ar; Julio Gagliardi - hprivadoalergia@arnet.com.ar; Carlos E Baena-Cagnani - baena@powernet.net.ar; Enrique Urrets-Zavalía - jauz@arnet.com.ar; Horacio M Serra* - hserra@bioclin.fcq.unc.edu.ar

* Corresponding author

Published: 6 January 2003

Received: 26 September 2002

BMC Ophthalmology 2003, 3:2

Accepted: 6 January 2003

This article is available from: <http://www.biomedcentral.com/1471-2415/3/2>

© 2003 Martín et al; licensee BioMed Central Ltd. This is an Open Access article: verbatim copying and redistribution of this article are permitted in all media for any purpose, provided this notice is preserved along with the article's original URL.

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 investigate 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 lysozyme 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

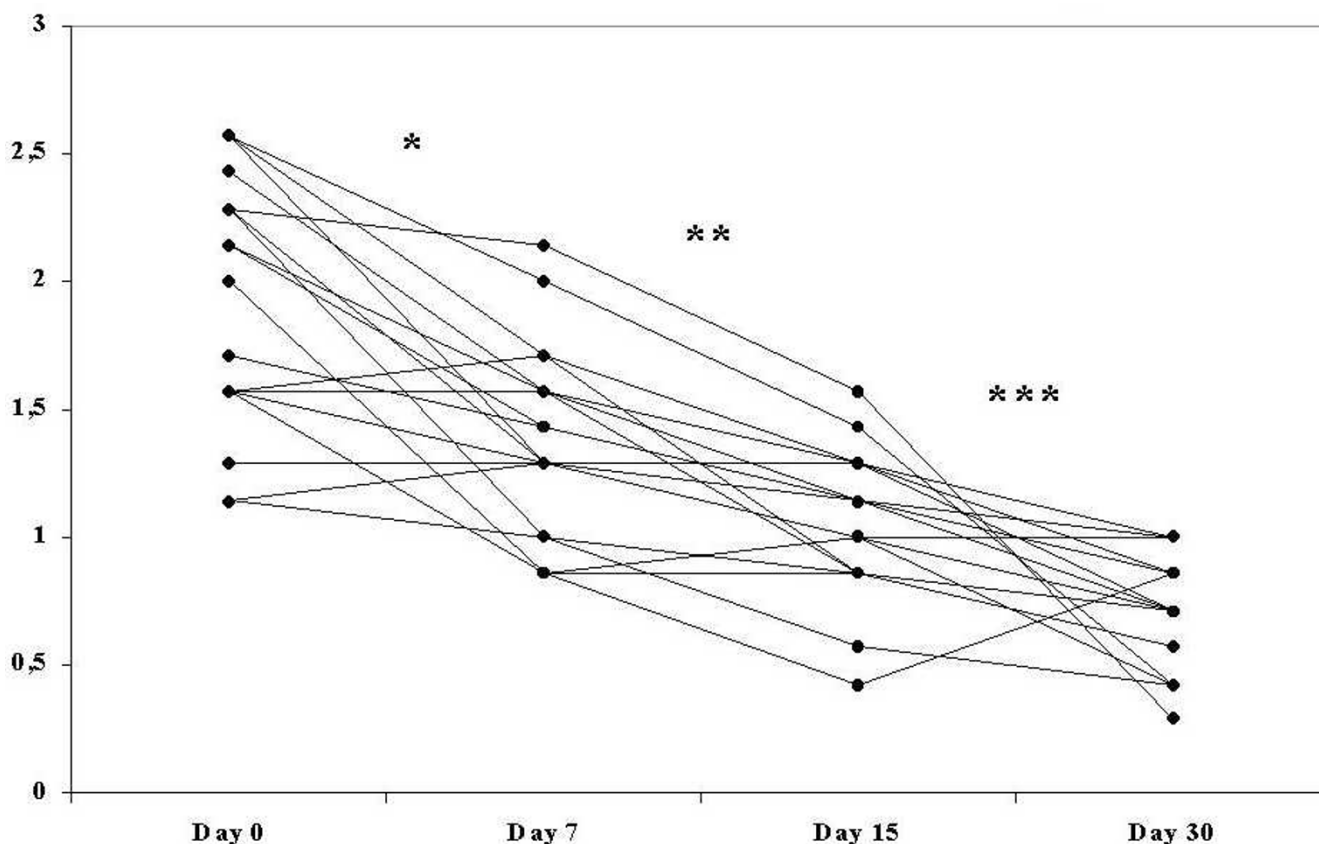


Figure 1
Variation of total score of symptoms and signs for each patient with ketotifen treatment. The differences between time points were analyzed by Wilcoxon signed-rank test (*, $p < 0.0001$, day 0 vs 7, 15 and 30; and 7 vs 30; **, $p < 0.0002$, day 7 vs 15; ***, $p < 0.0008$ day 15 vs 30).

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).

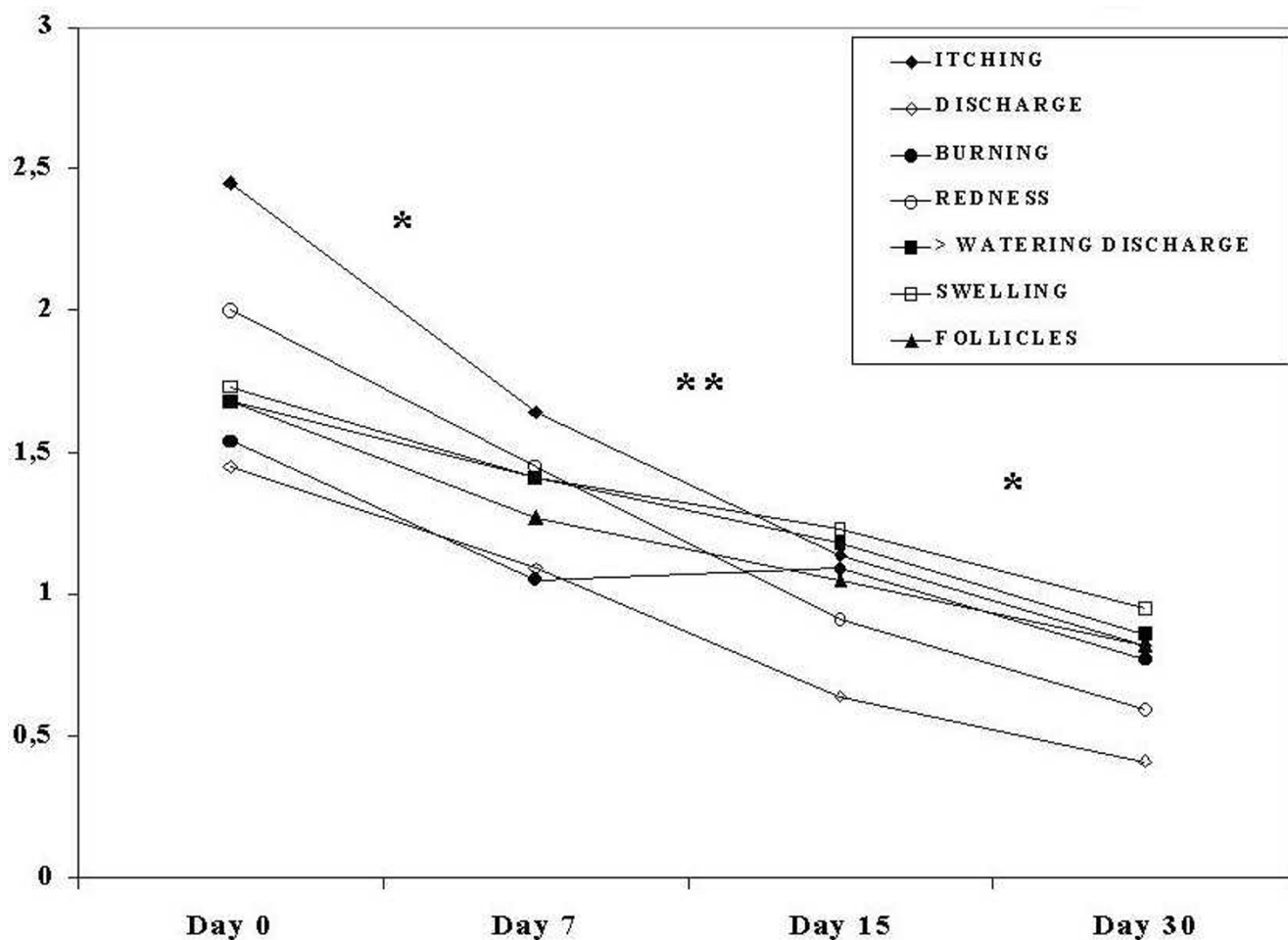


Figure 2
Variation of total score of symptoms and signs with ketotifen treatment. The differences between time points were analyzed by Wilcoxon signed-rank test (*, $p < 0.016$, day 0 vs 7, 15 and 30; day 7 vs 30, and day 15 vs 30; **, $p < 0.031$, day 7 vs 15).

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-

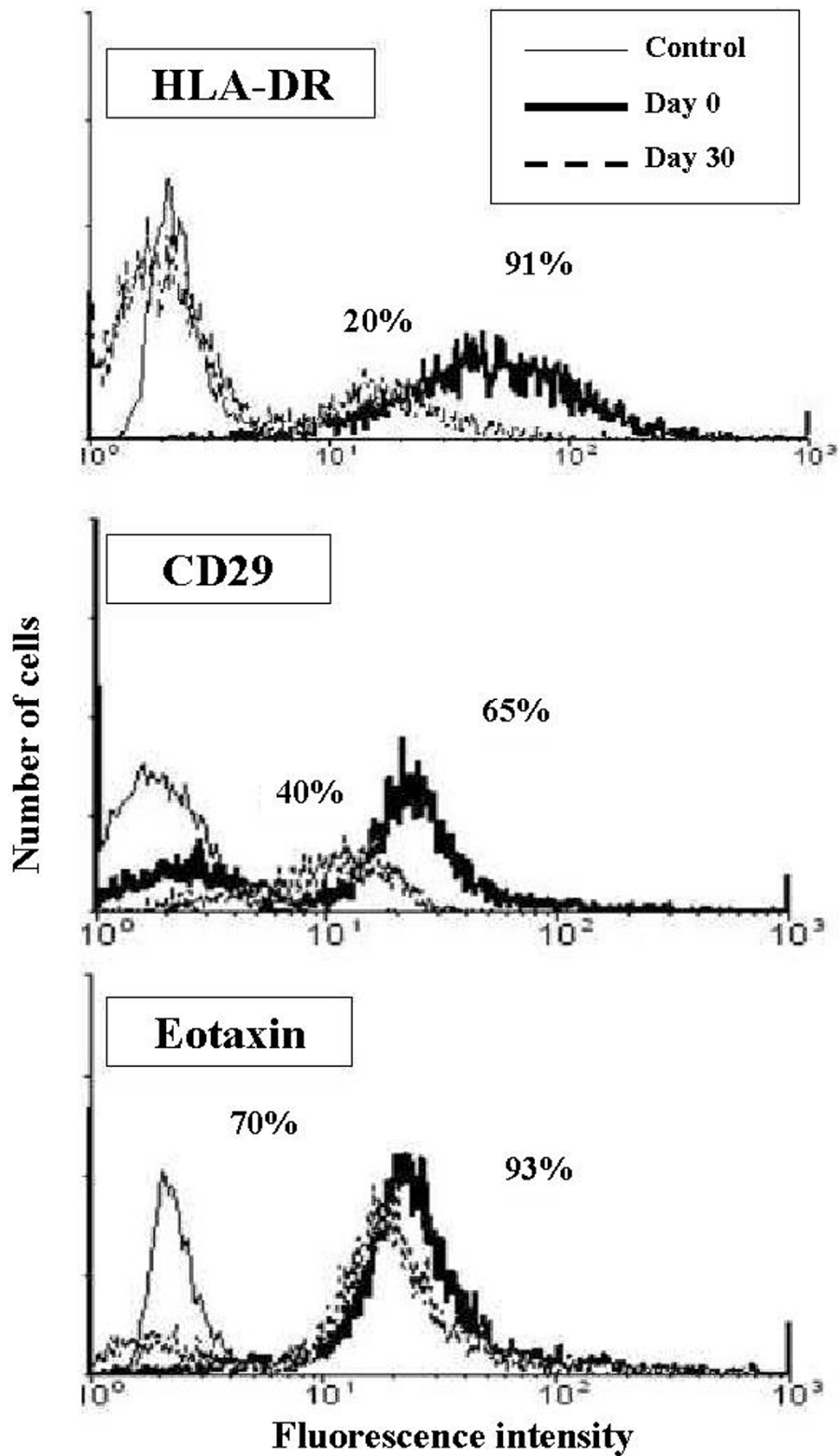


Figure 3
HLA-DR, CD29 and eotaxin expression by EC from patients with AC before and after the treatment with ketotifen fumarate 0.05%. Data show one representative case from nine patients who had a simultaneous drop in CD29+ and eotaxin+ EC.

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-Zavalía 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 lisozyme.

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

Baena-Cagnani CE: carried out the prick tests and clinical evaluation of the patients with AC.

Urrets-Zavalía 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.

Acknowledgements

We wish to thank the excellent technical assistance of Yanina Eberhard and Pablo Romagnoli.

References

1. A Solomon, Pe'er J and Levi-Schaffer F **Advances in ocular allergy: basic mechanisms, clinical patterns and new therapies.** *Curr Opin Allergy Clin Immunol* 2001, **1**:477-482
2. Bielory L **Update on Ocular Allergy Treatment.** *Expert Opin Pharmacother* 2002, **3**:541-553
3. Abelson MB, George MA and Garofalo C **Differential diagnosis of ocular disorders.** *Ann Allergy* 1993, **70**:95-109
4. MacLeod JD, Anderson DF, Baddeley SM, Holgate ST, McGill JF and Roche WR **Immunolocalization of cytokines to mast cells in normal and allergic conjunctiva.** *Clin Exp Allergy* 1997, **27**:1328-1334
5. Irani AM **Ocular mast cells and mediators in ocular allergy.** In: *Immunology and allergy clinics of North America (Edited by: Bielory)* Philadelphia, WB Saunders 1997, 1-13
6. MacDonald SM **Histamine-releasing factor.** *Curr Opin Immunol* 1996, **8**:778-783
7. Desreumaux P and Capron M **Eosinophils in allergic reactions.** *Curr Opin Immunol* 1996, **8**:790-795
8. Anderson DF, MacLeod JD, Baddeley SM, Bacon AS, McGill JI, Holgate ST and WR Roche **Seasonal allergic conjunctivitis is accompanied by increase mast cell numbers in the absence of leukocyte infiltration.** *Clin Exp Allergy* 1997, **27**:1060-1066
9. Fujihara T, Takeuchi T, Saito K and Tsuboda K **Flow cytometric analysis of surface antigens on human conjunctival epithelial cells.** *Ophthalmic Res* 1997, **29**:103-109
10. Avunduk AM, Avunduk MC, Dayioglu YS and Centinkaya K **Flow cytometric tear analysis in patients with chronic allergic conjunctivitis.** *Jpn J Ophthalmol* 1997, **41**:67-70
11. Vorkauf M, Duncker G, Nolle B and Sterry W **Adhesion molecules in normal human conjunctiva. An immunohistological study using monoclonal antibodies.** *Graefes Arch Clin Exp Ophthalmol* 1993, **231**:323-330
12. Hingorani M, Calder V, Buckley RJ and Lightman S **The Role of Conjunctival Epithelial Cells in Chronic Ocular Allergic Disease.** *Exp. Eye Res* 1998, **67**:491-500
13. Abu-El-Asrar AM, Struyf S, Al-Kharashi SA, Missotten L, Van Damme J and Geboes K **Chemokines in the limbal form of vernal keratoconjunctivitis** *Br J Ophthalmol* 2000, **84**:1360-1366
14. Garcia-Zepeda EA, Rothenberg ME, Ownbey RT, Celestin J, Leder P and Luster AD **Human eotaxin is a specific chemoattractant for eosinophil cells and provides a new mechanism to explain tissue eosinophilia.** *Nat Med* 1996, **2**:449-456
15. Abelson MB, Allansmith MR and Friedlaender MH **Effect of topical applied ocular decongestant and antihistamine.** *Am J Ophthalmol* 1980, **90**:254-257

16. Naclerio RM **Optimizing treatment options.** *Clin Exp Allergy* 1998, **28**:54-59
17. Joss JD and Craig TJ **Seasonal allergic conjunctivitis: overview and treatment update.** *J Am Osteopath Assoc* 1999, **99**:S13-18
18. Friedlaender MH **The current and future therapy of allergic conjunctivitis.** *Curr Opin Ophthalmol* 1998, **9**:54-58
19. Grant SM, Goa KL, Fitton A and Sorkin Ketotifen EM **A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in asthma and allergic disorders.** *Drugs* 1990, **40**:412-448
20. Mimura Y **Clinical efficacy and optimal concentration of ketotifen ophthalmic solution on allergic conjunctivitis and vernal conjunctivitis.** *J Clinical Ther Med* 1989, **5**:709-721
21. Aguilar AJ **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 Suppl* 2000, **230**:52-55
22. Kabra SK, Pandey RM, Singh R and Seth V **Ketotifen for asthma in children aged 5 to 15 years: a randomised placebo-controlled trial.** *Ann Allergy Asthma Immunol* 2000, **85**:46-52
23. Hoshino M, Nakamura Y, Sim JJ and Tomioka H **A comparative study of the effects of ketotifen, disodium cromoglycate, and beclomethasone dipropionate on bronchial mucosa and asthma symptoms in patients with atopic asthma.** *Respir Med* 1998, **92**:942-950
24. Miki I, Kusano A, Ohta S, Hanai N, Otoshi M, Masaki S, Sato S and Ohmori K **Histamine enhanced the TNF-alpha-induced expression of E-selectin and ICAM-1 on vascular endothelial cells.** *Cell Immunol* 1996, **171**:285-288
25. Ciprandi G, Buscaglia S, Pesce G, Passalacqua G, Rihoux JP, Bagnasco M and Canonica GW **Cetirizine reduces inflammatory cell recruitment and ICAM-1 (or CD54) expression on conjunctival epithelium in both early- and late-phase reactions after allergen-specific challenge.** *J Allergy Clin Immunol* 1995, **95**:612-621
26. Bagnasco M and Canonica GW **Influence of H1-receptor antagonists on adhesion molecules and cellular traffic.** *Allergy* 1995, **50**(24 Suppl):17-23
27. Ciprandi G, Buscaglia S, Pronzato C, Benvenuti C, Cavalli E, Bruzzone F and Canonica GW **Oxatomide reduces inflammatory events induced by allergen-specific conjunctival challenge.** *Ann Allergy Asthma Immunol* 1995, **75**:446-452
28. Ciprandi G, Pronzato C, Ricca V, Varese P, Del Giacco GS and Canonica GW **Terfenadine exerts antiallergic activity reducing ICAM-1 expression on nasal epithelial cells in patients with pollen allergy.** *Clin Exp Allergy* 1995, **25**:871-878
29. Vignola AM, Crampette L, Mondain M, Sauvere G, Czarlewski W, Bousquet J and Campbell AM **Inhibitory activity of loratadine and descarboethoxyloratadine on expression of ICAM-1 and HLA-DR by nasal epithelial cells.** *Allergy* 1995, **50**:200-203
30. Friedlaender MH **Conjunctivitis of Allergic origin: Clinical presentation and differential diagnosis.** *Surv Ophthalmol* 1993, **38**:105-114
31. Martín A, Gagliardi J, Gomez Demel E, Berra A, Gallino N, Daraio MC, Copello A, Urrets-Zavalía J, Mariani AL, Urrets-Zavalía E and Serra HM **Toward the best diagnosis of allergic conjunctivitis.** *Archivos de Alergia e Inmunología Clínica* 2001, **32**:16-25
32. Bonini S and Ghinelli E **The Early and Late Phase of the Ocular Allergic Reaction.** *Acta Ophthalmol Scand* 2000, **78**:41
33. Abelson MB and Udell IJ **Allergic and toxic reactions.** In: *Principles and Practice of Ophthalmology: Clinical Practice Philadelphia: WB Saunders Company* 1994, 77-100
34. Bacon AS, Ahluwalia P, Irani AM, Schwartz LB, Holgate ST, Church MK and McGill JI **Tear and conjunctival changes during the allergen-induced early and late phase responses.** *J Allergy Clin Immunol* 2000, **106**:948-954
35. Abu El-Asrar AM, Geboes K, Al-Kharashi S, Al-Mansouri S, Missotten L and Geboes K **Adhesion molecules in vernal keratoconjunctivitis.** *Br J Ophthalmol* 1997, **81**:1099-1106
36. Bacon AS, McGill JI, Anderson DF, Baddeley S, Lightman SL and Holgate ST **Adhesion molecules and relationship to leukocyte levels in allergic eye disease.** *Invest Ophthalmol Vis Sci* 1998, **39**:322-330
37. Ciprandi G, Buscaglia S, Pesce G, Bagnasco M and Canonica GW **Allergic subjects express intercellular adhesion molecule-1 (ICAM-1 or CD54) on epithelial cells of conjunctiva after allergen challenge.** *J Allergy Clin Immunol* 1993, **91**:783-792
38. Jose PJ, Griffiths-Johnson DA, Collins PD, Walsh DT, Moqbel R, Totty NF, Truong O, Hsuan JJ and Williams TJ **Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation.** *J Exp Med* 1994, **179**:881-887
39. Elsner J, Hochstetter R, Kimmig D and Kapp A **Human eotaxin represents a potent activator of the respiratory burst of human eosinophils.** *Eur J Immunol* 1996, **26**:1919-1915
40. Taha RA, Minshall EM, Miotto D, Shimbara A, Luster A, Hogg JC and Hamid QA **Eotaxin and monocyte chemotactic protein-4 mRNA expression in small airways of asthmatic and nonasthmatic individuals.** *J Allergy Clin Immunol* 1999, **103**:476-483
41. Minshall EM, Cameron L, Lavigne F, Leung DY, Hamilos D, Garcia-Zepeda EA, Rothenberg M, Luster AD and Hamid QA **Eotaxin mRNA and protein expression in chronic sinusitis and allergen-induced nasal responses in seasonal allergic rhinitis.** *Am J Respir Cell Mol Biol* 1997, **17**:683-690
42. Abelson MB and Schaefer K **Conjunctivitis of allergic origin: Immunologic mechanisms and current approaches to therapy.** *Surv Ophthalmol* 1993, **38**(suppl):115-127
43. Ciprandi G, Passalacqua G and Canonica GW **Effects of H1 antihistamines on adhesion molecules: a possible rationale for long-term treatment.** *Clin Exp Allergy* 1999, **29**(Suppl 3):49-53
44. Berdy GJ, Spangler DL, Bensch G, Berdy SS and Brusatti RC **A comparison of the relative efficacy and clinical performance of olopatadine hydrochloride 0.1% ophthalmic solution and ketotifen fumarate 0.025% ophthalmic solution in the conjunctival antigen challenge model.** *Clin Ther* 2000, **22**:826-833
45. D'Arienzo PA, Leonardi A and Bensch G **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* 2002, **24**:409-416

Pre-publication history

The pre-publication history for this paper can be accessed here:

<http://www.biomedcentral.com/1471-2415/3/2/prepub>

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

