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Tear and serum eosinophil cationic protein levels in seasonal allergic conjunctivitis

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BACKGROUND/PURPOSE. *Eosinophil cationic protein (ECP) levels in tear fluid and sera of patients with seasonal allergic conjunctivitis (SAC) were measured to assess local and systemic eosinophilic activity in SAC. The correlation between ECP levels and disease activity was evaluated.*

METHODS. *Tears and sera were collected from 21 patients with SAC and 13 healthy control subjects. ECP levels in tears and sera were measured before and 4 weeks after treatment with 0.1% lodoxamide eyedrops. Clinical signs and symptoms of SAC were scored and the correlation of ECP levels with the clinical scores was evaluated.*

RESULTS. *Tear and serum levels of ECP were significantly increased (p=0.01, p=0.02, respectively) in patients with SAC compared with the control subjects, but ECP levels were not correlated with the severity of the disease. Following treatment with topical 0.1% lodoxamide eyedrops, the mean level of ECP in tears decreased significantly (p=0.02), whereas no significant change was observed in serum ECP levels. Furthermore, a significant decrease in clinical signs and symptoms scores was found after treatment (both p<0.0001).*

CONCLUSIONS. *Increased serum and tear ECP levels in patients with SAC confirms that both local and systemic eosinophil activation occurs in SAC. However, clinical signs and symptoms of SAC were not found to be correlated with the degree of eosinophilic activity. Thus ECP does not seem to have an important role in clinical manifestations of SAC. (Eur J Ophthalmol 2003; 13: 671-5)*

KEY WORDS. *Seasonal allergic conjunctivitis, Eosinophil cationic protein, Lodoxamide*

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INTRODUCTION

Seasonal allergic conjunctivitis (SAC), the most common ocular allergy, occurs after single or repeated natural challenges with environmental allergens that induce IgE-specific mast cell activation and the subsequent cascade of inflammatory mediators and cellular infiltration. This immediate hypersensitivity reaction is biphasic (early and late phase reaction) (1). Eosinophils are the predominating cell type in tissues infiltrated in the late phase (2). Because the conjunctiva is normally void of any eosinophils (3), the presence of these cells is significant in the diagnosis of allergic conjunctivitis. However, as eosinophils are frequently located in deeper layers of the conjunctiva, the sensitivity of eosinophil detection in conjunctival scrap-

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ings is low (4, 5). Eosinophils also appear to be involved in disease conditions unrelated to atopy. The presence of eosinophils and their toxic granule proteins has been reported in various forms of nonatopic conjunctivitis or blepharoconjunctivitis (6, 7), and in ocular cicatricial pemphigoid (8). Activated eosinophils release granule stored, pharmacologically specific proteins, enzymes, and newly formed mediators, which contribute to the allergic manifestations by inducing further histamine release from mast cells and by exerting cytotoxic effects on corneal epithelium (9, 10). Eosinophil cationic protein (ECP) is a highly cationic protein that comprises 30% of the eosinophil granule matrix (11). The degree of local involvement of eosinophils was evaluated by ECP measurements in various biologic fluids such as tears and nasal and bronchoalveolar lavage fluids and was reported to be increased in respective allergic diseases (12-15). The presence of ECP in biological fluids and tissues is now considered as a specific marker for eosinophil activation (16-19).

In the current study, we measured ECP in tears and sera of patients with SAC before and after treatment with a potent (20) topical mast cell stabilizer (lodoxamide 0.1%) that has inhibitory effects on eosinophil activation (21) to assess local and systemic eosinophil activity in SAC. We also attempted to determine whether ECP levels were related with the severity of clinical manifestations of the disease.

PATIENTS AND METHODS

Patients

A total of 21 patients with active SAC (mean age 23.0 ± 3.4 years; 9 men, 12 women) and 13 normal volunteers (mean age 23.0 ± 3.6 years; 7 men, 6 women) with no history of atopic disorders or allergic conjunctivitis were included in this study. The study protocol was approved by the institutional research council and informed consent was obtained from all patients. None of the subjects was using topical/oral medications or contact lenses for at least a month. Ocular allergic complaints were recorded and full ophthalmologic examination of each subject (best uncorrected/corrected visual acuity, slit-lamp examination, applanation tonometry, funduscopy) was performed. No other accompanying ocular disease was detected in the subjects.

All of the subjects with SAC were found to be sensitive for pollen/grass with skin prick tests and not to be sensitive for other allergens. Active allergic rhinitis was present in 8 of 21 patients with SAC, but none of them had atopic eczema or asthma.

Tear and serum samples were collected from 21 patients with SAC and 13 control subjects for determination of ECP levels; clinical signs and symptoms of ocular allergy were assessed and recorded before and 4 weeks after treatment with 0.1% lodoxamide eyedrops four times daily.

Assessment of clinical scores of SAC

A clinical score was given considering the severity of subjective symptoms (itching, tearing, photophobia, foreign body sensation) and signs (conjunctival hyperemia, chemosis, secretion, papillary reaction). All were assessed using a four-point scale – 0 (absent), 1 (mild), 2 (moderate), and 3 (severe) – with the exception of itching. Itching was assessed on a scale of 0 to 4; an additional category of very mild was added.

Laboratory diagnosis and determination of ECP in sera and tears

Peripheral blood and tear samples were collected. Blood samples were allowed to clot at room temperature for 60 ± 10 minutes followed by centrifugation at 2000 rpm for 10 minutes. With the use of a glass microcapillary tube, tears were gently collected from the external canthus of the more severely involved eye, taking precautions to avoid the tearing reflex. The samples were immediately transferred to Eppendorf tubes and stored at –70 °C for subsequent determination of ECP.

The level of ECP in serum and tear samples was measured in duplicate by the commercially available Unicap fluoroenzymeimmunoassay and Unicap 100 device (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Tear samples (minimum 70 μL) were first diluted $1^1/_2$ times by adding assay buffer. The ECP values in tears were adjusted to the dilution coefficient. The lower detection limit of the test with 100 μL of sample was 2 μg/L.

Statistical analysis

Mann-Whitney *U* test was used to compare serum and tear levels of ECP in SAC patients and controls. Correlations between ECP levels and clinical scores in SAC subjects were analyzed by Spearman rank correlation test. The differences between pretreatment and posttreatment levels of serum and tear ECP and clinical scores were assessed by Wilcoxon signed-rank test. A p value less than 0.05 was considered significant.

RESULTS

ECP levels in tears and sera of patients with SAC were found to be significantly higher than those of the control group (p=0.01 and p=0.02, respectively) (Tab. I). In allergic patients, no significant difference was noted between the median values of ECP in tears and sera (p=0.9). There was no correlation between serum and tear levels of ECP (r=0.02, p=0.7). Serum ECP levels did not differ significantly between SAC patients with (n=8; median, 11.6; range, 2.4 to 31.3 μg/L) or without (n=13; median, 15.2; range, 2 to 71.1 μg/L) active allergic rhinitis (p=0.3). No significant correlations were noted between the clinical activity (symptoms and signs) scores of SAC and ECP levels in tears or sera of patients.

Table II gives the ECP levels in tears and sera and clinical symptoms and signs scores of SAC patients before and 4 weeks after treatment with 0.1% lodoxamide. The tear level of ECP decreased significantly after treatment (p=0.02), whereas serum level of ECP remained unchanged. The total symptoms and signs scores decreased significantly after treatment (both p<0.0001). In the control group, there was no statistically significant change in tear and serum levels of ECP after treatment.

DISCUSSION

In the present study, we found that ECP levels are increased in serum and tears of patients with SAC, which provides evidence of local and systemic eosinophil activation in the pathogenesis of the disease. A number of studies (7, 12, 13) have shown high-

TABLE I - COMPARISON OF LEVELS OF EOSINOPHIL CATIONIC PROTEIN (ECP) IN TEARS AND SERA OF PATIENTS WITH SEASONAL ALLERGIC CONJUNCTIVITIS AND HEALTHY CONTROLS

ECP	Seasonal allergic conjunctivitis (n=21)	Control (n=13)	p value
Tear, μg/L			
Mean ± SD	30.5 ± 51.5	3.5 ± 1.8	0.01
Median	9.6	3	
Range	3.7–251	3–7.9	
Serum, μg/L			
Mean ± SD	19.8 ± 18.4	7.2 ± 5.4	0.02
Median	15.2	5.6	
Range	2–71.1	2–27.9	

TABLE II - TEAR AND SERUM EOSINOPHIL CATIONIC PROTEIN (ECP) LEVELS AND TOTAL SYMPTOMS AND SIGNS SCORES BEFORE AND AFTER TREATMENT AMONG PATIENTS WITH SEASONAL ALLERGIC CONJUNCTIVITIS (N=21)

ECP	Before	After	p value
Tear, μg/L			
Mean ± SD	30.5 ± 51.5	13.3 ± 23.9	0.01
Median	9.6	6.8	
Range	3.7–251	3–98.7	
Serum, μg/L			
Mean ± SD	19.8 ± 18.4	19.5 ± 15.3	0.6
Median	15.2	11.6	
Range	2–71.1	2–47.9	
Total symptoms score			
Mean ± SD	8.2 ± 0.3	3.2 ± 0.3	<0.0001
Median	8	3	
Range	2–11	1–6	
Total signs score			
Mean ± SD	7.5 ± 0.9	2.8 ± 1.3	<0.0001
Median	8	2	
Range	6–10	1–5	

er tear levels of ECP in subjects with allergic conjunctivitis, the highest levels (470 μg/ml and 215 μg/ml, respectively) being present in the most serious and chronic forms of allergic disease like vernal and atopic keratoconjunctivitis (13). In our study, we observed a less pronounced (30.5 μg/ml) increase in tear levels of ECP in patients with SAC. We also noticed considerable individual variability in tear ECP levels. This could be explained by exposure to varying allergen concentrations to elicit eosinophil migration and activation. Interestingly, increased levels of tear ECP have been reported in patients with blepharoconjunctivitis (7, 13) and bacterial conjunctivitis (7). Similarly, Rasp et al (14) reported high nasal ECP concentrations in patients with nonallergic nasal polyposis and chronic purulent nonallergic sinusitis. Thus local presence of ECP may not be considered specific for allergic conditions.

In our study, subjects with SAC had significantly increased serum levels of ECP. In accordance with this finding, increased serum levels of ECP have previously been reported in subjects with allergic rhinitis (14, 22) and allergic conjunctivitis (7, 13, 23). Although the diffusion of locally released ECP from conjunctiva into the systemic circulation can be thought to have a role in increased serum ECP levels, this possibility seems unlikely as this highly cationic protein binds tightly to negatively charged tissue intercellular proteins after being released from eosinophils (24). Our findings show that no correlation exists between serum and tear levels of ECP, which is in accordance with the results of previous studies (7, 13). Also, the increase of ECP in serum was not related to the severity of allergic conjunctivitis, which confirms the previous reports on allergic rhinitis (14) and asthma (25). Following lodoxamide treatment, we observed a significant improvement in clinical signs and symptoms of SAC together with a significant decrease in tear ECP levels; however, serum levels of ECP remained unchanged. Thus, systemic eosinophil activation in SAC seems to be independent of local disease activity. It has been shown that allergen-specific CD4+ T cell clones that exhibit type 2 helper (Th2) phenotype play a crucial role in allergic diseases (26). Th2 cells from atopic individuals are characterized by a distinct cytokine profile including IL-3, IL-4, IL-5, and GM-CSF. These cells induce IgE production via IL-4, favor the differentiation and activation of mast cells via IL-3 and IL-4, and regulate proliferation, activation, and degranulation of eosinophils via IL-5 (26, 27). Aberrant IL-5 production by Th2 cells may represent one of the immune alterations responsible for enhanced eosinophil activation in atopic people. Taken together, these data may support the hypothesis that SAC, like vernal and atopic keratoconjunctivitis, may be considered a systemic disorder with an increased activation of peripheral eosinophils (7, 23).

Granules released from activated eosinophils are responsible for pathologic changes in target tissues in allergic diseases. *In vitro* studies have shown the cytotoxic effect of ECP on human corneal epithelial cells (9). Moreover, ECP and eosinophil major basic protein have been shown to play a role in pathogenesis of epithelial erosions in vernal conjunctivitis (28, 29). Bacon et al (30) reported that patients with chronic allergic conjunctivitis with keratopathy had higher cell numbers in the tarsal conjunctiva staining for ECP than patients without keratopathy. Leonardi et al (31, 32) suggested the usefulness of ECP measurement in the monitoring of vernal conjunctivitis as well as in the evaluation of topical therapies. The relation of tear ECP levels with the severity of different types of ocular allergies has been previously described and it is clearly recognized that this correlation is highest in subjects with vernal and atopic keratoconjunctivitis, and weaker in subjects with SAC (13). In this study, possible correlations between the concentrations of ECP in tears and sera with the severity of SAC were evaluated, but no significant correlation could be demonstrated. Thus our data suggest that this relatively limited eosinophil activation does not appear to be directly involved in the clinical manifestations of this milder form of allergic conjunctivitis without corneal involvement.

In conclusion, both local and systemic eosinophil activation occurs in SAC; however, as the degree of eosinophilic activity was not found to be related to clinical signs and symptoms, ECP does not seem to have as important a role as other inflammatory mediators in clinical manifestations of SAC.

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