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Correlation Between Conjunctival Provocation Test (CPT) and Systemic Allergometric Tests in Allergic Conjunctivitis

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Summary

In order to assess the potential usefulness of CPT as a diagnostic tool for ocular allergy, the correlation between skin/RAST tests and CPT was determined in 144 patients affected by allergic 'hay fever' type conjunctivitis. The results showed that an agreement between skin/RAST tests and CPT occurred in 71% of the cases (130/183). Of the 29% uncorrelated cases, 23% (43/183) were positive for at least one specific antigen by skin/RAST tests but not by CPT, while 6% (10/183) were positive for at least one specific antigen by CPT, but not by skin/RAST tests. CPT dramatically increased the histamine levels in tears ($p<0.001$). These findings show that (1) systemic tests can be misleading in that they may suggest a specific sensitisation which, in fact, does not involve the conjunctiva (systemic test positive/CPT negative); (2) CPT can identify local conjunctival sensitisation in the absence of a systemic sensitisation (systemic test negative/CPT positive); (3) CPT can demonstrate that allergic 'hay fever' type conjunctivitis may be related to allergens different from those responsible for a systemic sensitisation.

A diagnosis of true allergic conjunctivitis can be elusive when the signs and symptoms presented by the patients are not related to other allergic manifestations, such as rhinitis, asthma, or urticaria. A clinical history suggestive of allergic conjunctivitis needs the objective support of reliable tests. Total and specific IgE antibodies in serum, and the results of skin tests often confirm the clinical diagnosis of an allergic disease. However, some patients show negative results to the common systemic tests and require a more intensive study. The detection of IgE immunoglobulins and of mediators of inflammation in tears should be supported by a positive response to the conjunctival provocation test (CPT). This procedure has been increasingly reported as a simple method for identifying the specificity of the ocular response to allergen.[1–3] Theoretically, in fact, the most appropriate method for identifying the aetiology of an allergic conjunctivits should test the local specific sensitivity to various allergens.[4]

CPT can be used not only for the diagnosis of allergic conjunctivitis, but also for demonstrating the efficacy of the different kinds of treatment. CPT also represents a human model for studying allergic inflammation,[5] and is an excellent tool for the evaluation of anti-allergic drugs.[6,7]

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In this study, we have subjected 144 patients with a history of allergic disease, who were in a quiescent state for both ocular signs and symptoms to CPT. The results of the CPTs were correlated to the results of systemic tests (skin test/RAST). Total IgE and histamine levels in tears before and after antigen challenge were also measured.

Materials and Methods

Patients

A total of 144 patients, 81 females and 63 males, aged 7 to 48 years, were tested. While the majority of the patients were diagnosed as having multiple allergic disorders, 46 subjects were clinically diagnosed as affected by allergic conjunctivitis only. All patients were tested for skin reactivity and/or level of specific serum IgE (RAST) in the Allergy Unit of the Padova General Hospital. All patients were free of ocular signs or symptoms for at least two weeks prior to the CPT. Exclusion criteria in selecting patients included the presence of other ocular inflammatory disorders, and the use of any systemic or topical ocular medications which might have interfered with the test.

Conjunctival provocation test

Allergens for the CPT were chosen according to the results of the skin tests or the clinical history. Three dilutions of each allergen (10, 100, 1000 A.U./ml) were made at the time of testing, starting from the basic solution (Lofarma, Milano). A saline buffer was used as diluent. One drop of saline was administered to the conjunctival sac of both eyes to test individual non-specific reactivity. One drop of the 10 A.U./ml allergen solution was then administered to both eyes. If no reaction was noted within 20 minutes, one drop of the next more concentrated dilution was used. The criteria for a positive CPT was the appearance of itching and/or burning and/or tearing with conjunctival redness or oedema. A quantitative score was not used. Additional CPTs were eventually performed in the same patients with different antigens at weekly intervals.

Before the CPT and within five minutes of the appearance of signs and symptoms, 10–100 µl of tears were collected using a capillary tube positioned in the inferior conjunctival sac, being careful to avoid any irritation. Tear samples were analysed for histamine and total IgE content.

Total tear IgE assay

A fluorescent enzyme immunoassay technique (3M total IgE FAST, 3M Diagnostic Systems, Santa Clara, CA distributed by Eurospital Phaerus, TS) was used.

Tear histamine assay

Tear histamine levels were determined using the enzymatic isotopic assay described by Beaven *et al*[8] and modified by Allansmith *et al*.[9]

Statistics

The correlation between two different tests was evaluated by the agreement coefficient (Cohen's K). Values greater than 0.75 may be taken to represent excellent agreement beyond chance, values below 0.40 may be taken to represent poor agreement beyond chance, and values between 0.40 and 0.75 may be taken to represent fair to good agreement beyond chance.[10] The difference in the tear levels of histamine and IgE before and after the CPT, was analysed by the Wilcoxon tests.

Results

All 144 patients were skin and/or serum RAST tested. Results showed that 68 subjects were positive to only one allergen; 59 were plurisensitised; and 17 were negative to the allergens tested. Thirty-three patients were challenged with more than one allergen, and a total of 183 CPT were thus performed on these 144 patients. On the basis of the clinical history and skin/RAST results, 80 CPT were performed with graminaceae; 55 with dermatophagoides; 22 with parietariae; 14 with compositae; six with alternariae; three with plantago, and one for each of the following antigens: house dust, cat hair and dander, and betullae. No side effects were noted in any of the subjects.

The correlation between the results of the skin/RAST tests and the results of CPT gave the following results: in 71% of the cases, there was agreement between skin/RAST test

Table I *Correlation between skin/RAST tests and CPT in 183 challenges*

	CPT positive	*CPT negative*
skin/RAST positive	(104) 57%	(43) 23%
skin/RAST negative	(10) 6%	(26) 14%

The agreement between skin and/or RAST and CPT was found in 71% of the challenges: the Cohen's coefficient proved a poor relationship between these tests (k=0.31)

results and CPT results. The remaining 29% were discordant: 23% of the cases, which were skin test positive, tested negative by CPT performed with the same antigen. Conversely, the remaining 6% of the cases were skin test negative, but positive for CPT chosen on the basis of the patient's clinical history. Statistical analysis did not show a good agreement between CPTs and skin/RAST tests (Cohen's K=0.31) (Table I).

The correlation between skin/RAST and CPT was then evaluated for each specific allergen subgroup (Table II). In the graminaceae group, a positive skin test was correlated with a positive CPT in 62% of the cases, and a negative skin test with a negative CPT in 18%. Five per cent of the cases were positive only to ocular challenge, and 15% only to skin/RAST test. The agreement coefficient (K=0.5) demonstrated a fair correlation between the two tests. We found a worse correlation in patients challenged with parietariae or compositae (K=0.30 and K=0.35, respectively) and in the dermatophagoides group the correlation between systemic and ocular tests was even lower (K=0.11).

Table II *Correlation between skin/RAST and CPT for the four major allergens*

Graminaceae (N=80)		
	CPT positive	CPT negative
skin/RAST positive	(50) 62%	(12) 15%
skin/RAST negative	(4) 5%	(14) 18%
Dermatophagoides (N=55)		
	CPT positive	CPT negative
skin/RAST positive	(34) 62%	(14) 25%
skin/RAST negative	(4) 7%	(3) 17%
Parietariae (N=22)		
	CPT positive	CPT negative
skin/RAST positive	(10) 45%	(6) 27%
skin/RAST negative	(1) 5%	(5) 23%
Compositae (N=14)		
	CPT positive	CPT negative
skin/RAST positive	(5) 36%	(4) 28.5%
skin/RAST negative	(1) 7%	(4) 28.5%

The correlation between skin/RAST and CPT proved a fair agreement between tests performed for graminaceae (k=0.50), a poor agreement for paritariae (k=0.35), compositae (0.30), and dermatophagoides (k=0.11).

In 20 patients who skin tested positive to more than one antigen, we performed the CPT at weekly intervals, with each antigen to which they were sensitive. In this group, a positive skin tests correlated with positive CPTs in 10% of the patients; positive skin tests with negative CPTs in 5%; 15% of the patients were positive only to ocular challenge. In 70% of the patients the CPTs were positive to some of the skin tested positive allergens.

Of the 46 patients with a clinical history of allergic conjunctivitis only, 24 were skin/RAST positive to only one antigen (monosensitised); 11 were positive to one or more antigens (plurisensitised); and 11 were skin test negative. In the monosensitised group, 50% of the patients were also positive to CPTs performed with the same antigen. The correlation between skin/RAST and CPT was very low (K=0.11) in this group (Table III).

In the group of plurisensitised patients (N=11) with only allergic conjunctivitis, 46% were positive CPTs to the same antigens skin tested positively; 27% were CPT positive to some of the antigens positive by skin tests; and the remaining 27% were CPT negative to

Table III *Correlation between skin/RAST and CPT in monosensitised patients*

	CPT positive	*CPT negative*
skin/RAST positive	(12) 44%	(11) 41%
skin/RAST negative	(1) 4%	(3) 11%

The agreement between skin/RAST and CPT was found in 55% of challenges; the tests were poorly related (k=0,117) in this group of patients.

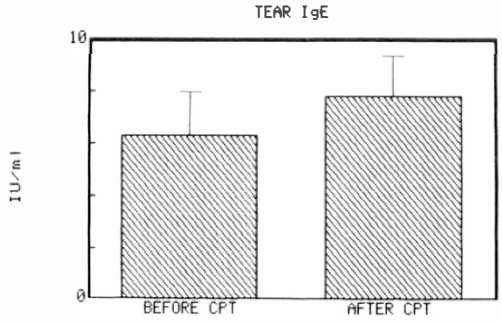

Fig. 1. *Total IgE levels before and after antigen challenge. The difference was not statistically significant.*

all of the skin test positive antigens available for ocular challenge. Of the 11 skin/RAST negative patients, with a positive clinical history of ocular allergic disease, only one showed a positive CPT.

Total tear IgE (N=22 patients) and histamine (N=43 patients) levels were measured before and after CPT. IgE did not change significantly when considered before (6.3±2 IU/ml) and after the test (7.8±1.8 IU/ml) Fig. 1. Histamine levels, on the contrary, after CPT (11.0±1.5 ng/ml) were significantly increased ($p<0.025$) when compared to basal levels (6.8±1 ng/ml) (Fig. 2).

Discussion

The diagnosis of allergic conjunctivitis is often based on the clinical history, and the presence of other allergic disorders such as rhinitis or asthma. In these cases, skin tests can be supported by the finding of increased systemic total and specific IgE to confirm the diagnosis and indicate whether immunotherapy should be considered. A high percentage of allergic subjects (32% in our group), however, have only eye signs and symptoms. The conjunctiva may be the only affected tissue in a subgroup of patients with systemic sensitisation or, conversely, it may represent the only sensitised tissue. Specific IgE antibodies can be produced, in fact, in the conjunctival mucosa and detected only in the tears, being substantially absent in the serum.[11–13] In this study, we evaluated the correlation between skin/RAST tests and conjunctival provocation tests. In our group of patients the two tests were in agreement in 71% of the cases; however, 29%, on the contrary, did not correlate. The lack of correlation was more evident in the group of patients with only allergic conjunctivitis. The possibility of identifying a conjunctival sensitisation to specific antigen(s) in the absence of a positive skin test, enables the ophthalmologist, or the allergist to make a diagnosis of allergic conjunctivitis when systemic tests fail. Similarly, if a plurisensitised patient is skin test positive for a variety of allergens, but CPT positive to only one, the aetiology of the allergic conjunctivitis may be better identified, and the potential for a successful specific immunotherapy greatly increased. In either case, therefore, the CPT represents a useful tool in the diagnosis of allergic conjunctivitis, to be used in conjunction with other systemic and local tests such as skin/RAST tests, and the search for specific tear IgE.

A negative CPT response to specific antigens, in skin and serum RAST positive patients, probably reflects a lack of conjunctival reactivity to that specific antigen(s) sensitisation. The potential for multiple ocular challenges to elicit a down-regulation, or a diminution of the allergic response in the conjunctival mucosal tissue,[14,15] was prevented by waiting at least one week between ocular challenges. The possibility for mucosal tissues to modulate locally the allergic response, by inducing cellular and humoral mediated repressor pathways after multiple antigen challenges,[16,17] may hypothesise a new treatment for ocular allergy by means of a local specific immunotherapy.

A significant increase of tear histamine was found, as expected, within ten minutes of antigen challenge, coinciding with the

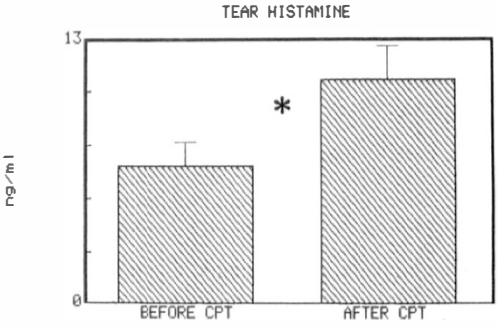

Fig. 2. *Tear histamine levels after antigen challenge were statistically increased compared to basal levels (*$p<0.025$).*

patient's appearance of symptoms and signs. The IgE antibody levels did not change significantly in the course of the allergic reaction. These results confirm the major role of histamine as mediator of immediate conjunctival anaphylactic reactions.[18]

In conclusion, the ocular response to antigen challenge represents the most clinically relevant of all the ocular models of experimental allergy, including topical instillation of histamine, compound 48/80, arachidonic acid or platelet activating factor. It can be used for studying the pattern of clinical early and late phase reactions,[19] the release of mediators,[20] the cytology of tear film,[21] and the drug modulation of ocular allergic responses.[6,7]

In addition, the conjunctival provocation test may be considered as a valuable tool for the diagnosis of true allergic conjunctivitis, to be used in conjunction with other more traditional methods, and also with newer tests such as tear cytology, tear specific and total IgE, and tear mediators analysis.[22] Finally, repetitive ocular challenge with specific antigens may represent a new hypothesis for the treatment of allergic ocular disorders, taking advantage of the mucosal tissue's natural capacity for a down-regulation of the allergic response.

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