Precision of conjunctival provocation tests in right and left eyes

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Background: *Conjunctival provocation tests (CPTs) are used for assessing the efficacy of antiallergic treatments, but their reproducibility is not well characterized. A study was carried out to assess the reproducibility of CPTs and the release of mediators during CPTs.*
Methods: *Both eyes of 30 grass-pollen–allergic patients were challenged with threefold increasing concentrations of a standardized orchard grass pollen extract. The positivity of the CPT was assessed by a cumulative symptom score. The release of mediators was examined by means of histamine (radioimmunoassay), prostaglandin D_2 and leukotrienes C_4 and D_4 (enzyme immunoassay).*
Results: *There was a significant correlation between the concentrations of allergen inducing a positive CPT in both eyes ($p < 0.0001$, Spearman). All but one patient had a significant release of at least one mediator. After allergen CPT there was a significant release in both eyes in 13 of 20 patients for prostaglandin D_2, 11 of 19 for leukotrienes C_4 and D_4 and 15 of 18 for histamine. The correlations between the levels of mediators released during diluent and allergen challenges in both eyes were significant for prostaglandin D_2 (diluent and allergen challenges) and leukotrienes C_4 and D_4 (allergen challenge).*
Conclusion: *Considering the whole group of patients, CPT is reproducible in both eyes, but the results are less satisfactory when patients are examined individually. (J ALLERGY CLIN IMMUNOL 1993;92:49-55.)*

Key words: *Conjunctival challenge, allergen, histamine, PGD_2, LTC_4*

Conjunctival provocation tests (CPTs) are being used to assess the efficacy of various antiallergic treatments including H_1-blockers and specific immunotherapy.[1-8] The positivity of the challenge may be assessed by symptom and/or medication scores,[9, 10] and more recently, by the measurement of inflammatory mediators released in tears[11, 12]; the enumeration of cells should be usually obtained by scraping.[13, 14] It has been shown that CPTs are highly reproducible when symptom scores are examined,[9] but the reproducibility of the doses inducing a positive CPT result or of the release of mediators during CPT has never been published.

Abbreviations used
CPT: Conjunctival provocation test
IR: Index of reactivity
LT: Leukotriene
PGD_2: Prostaglandin D_2

A study was carried out in 20 patients allergic to grass pollens to assess the reproducibility of symptoms and mediators released during CPTs with grass pollen extracts.

METHODS

Patients

Twenty patients allergic to grass pollens who ranged in age from 21 to 31 years (13 men) were studied after informed consent and approval by the ethical committee of the hospital were obtained. Subjects were selected on the following criteria. All had symptoms of rhinoconjunctivitis between April and July during the grass pollen season. The duration of symptoms ranged from 2 to 14 years. All patients had a positive prick test result to a 100 index of reactivity (IR) per milliliter of

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TABLE I. Scoring system to measure the signs and symptoms of allergic conjunctivitis

Redness, eyelid swelling
0: none
1: mild
2: moderate
3: severe
Chemosis
0: none
1: mild, detectable with slit lamp, conjunctiva separated from sclera
2: moderate (visually evident, raised conjunctiva, especially at the limbal area)
3: severe (ballooning of conjunctiva)
Tearing
0: none
1: mild (eyes feel slightly watery)
2: moderate (blows nose occasionally)
3: severe (tears rolling down cheeks)
Itching (to be graded by subject)
0: none
1: mild (intermittent tickling sensation)
2: moderate (continual awareness but without the desire to rub)
3: severe (continual awareness with the desire to rub the eyes)
4: incapacitating itching (subject insists on rubbing eyes)

Data from Abelson et al. Arch Ophthamol 1990;18:84-8.

standardized orchard grass pollen extract (Stallergènes Laboratories, Fresnes, France) and the presence of orchard grass-pollen–specific IgE (Phadebas RAST or CAP System, Pharmacia Diagnostics, Uppsala, Sweden).

No subject was undergoing treatment that would effect the performance of a CPT.[15, 16] More specifically, the patients had not taken aspirin or nonsteroidal antiinflammatory drugs or antihistamines for 1 week before the test and had not taken astemizole for 8 weeks before the study.

Procedure

Orchard grass pollen extract. Freeze-dried standardized extracts from orchard grass *(Dactylis glomerata)* were prepared according to the proposals of the Allergen Subcommittee of the International Unions of Immunological Societies by the Laboratories des Stallergènes (Fresnes, France). A complete description of the preparation of the extracts was previously published.[17] A control of the potency of an aliquot was done by RAST inhibition, isoelectric focusing, IgE-immunoblotting, and cross-immunoelectrophoresis and was compared with the internal standards, which were controlled by the same in vitro assays and skin test titration. Extracts were labeled in biological units (IR: index of reactivity) with a method derived from the proposals of the "Nordic Council of Medicines Guidelines on Allergen Standardization"[18] with codeine phosphate as a positive control. The freeze-dried extracts were stored at +4° C. The same batch of allergen extract was used throughout the study. Each test day, a new vial was diluted in sterile isotonic saline solution, which was prepared without preservatives.

Conjunctival provocation test. None of the patients had experienced any form of allergic conjunctivitis for at least 2 months before the study. On entering the clinic, all subjects were examined to establish that no visible ocular symptoms were present at the time of the challenge. CPTs were performed by applying inside the conjunctival cul-de-sac 20 μl of the diluent and then 20 μl of 10-fold increasing allergen solutions ranging in concentration from 0.14 to 100 IR/ml to the eyelid every 10 minutes until a composite symptom score of 5 was reached. This score was proposed by Abelson et al.[10] and includes redness, tearing, chemosis, and itching (Table I). The patients were examined by an ophthalmologist using a slit lamp.

Collection of tears. Conjunctival secretions were collected with the method of Proud et al.[11] On the challenge day, tears were collected by placing three preweighted strips of filter paper (Schirmer strips into the inferior fornix just after the challenge was considered to be positive by symptom score. The strips were left in place for 5 minutes and then removed. After the strips were weighed, one strip was placed in 1 ml of isotonic saline solution for the later measurement of histamine, and the other two were placed in 1 ml of 95% ethanol for the titration of prostaglandin D_2 (PGD_2) and leukotrienes (LTs) C_4/D_4. Each collection tube was then stored at −20° C until assay. On the baseline day, a diluent was instilled inside the conjunctival cul-de-sac, and tears were collected as they were on the challenge day.

Measurement of mediators. Histamine was measured by radioimmunoassay with a monoclonal antibody against acylated histamine (Immunotech, Luminy, France.)[19] PGD_2 and LTC_4/D_4 were assayed by enzyme immunoassay[20, 21] (Stallergènes)). The specificity and sensitivity of these assays have been previously published in detail for histamine and PGD_2.[22] For LTC_4/D_4, the antibody used in this assay shows a cross-reactivity at 50% binding/zero binding of 46% with LTD_4 and 2% with LTE_4 at +22° C. The limit of detectability is 15 pg/ml. Mediators were considered to be released after allergen challenge when their level after allergen challenge was at least 200% greater than that of baseline (diluent challenge).

Design of the study

Patients were challenged at a 1-week interval with diluent or allergen administered in random order. The allergen CPT was performed with three-fold increasing concentrations of the orchard grass pollen extract until a clinically apparent reaction was observed.

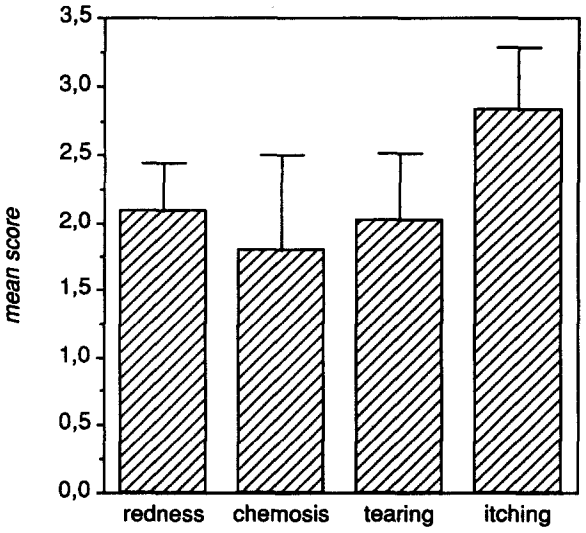

FIG. 1. Mean symptom scores during positive conjunctival provocation test with allergen.

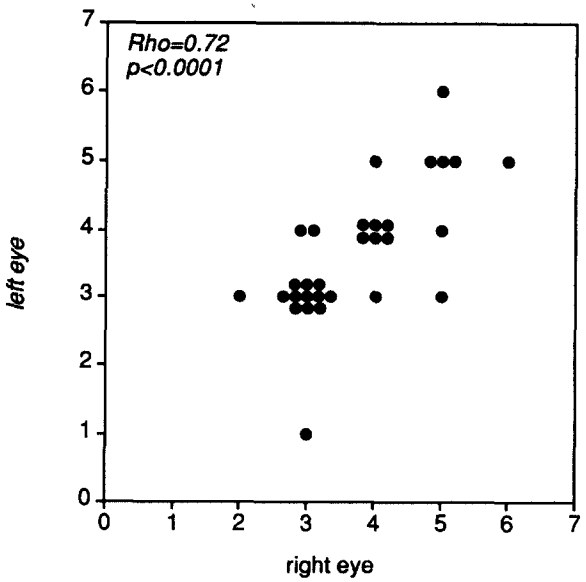

FIG. 2. Correlation between the dose of allergen inducing a positive conjunctival challenge in both eyes. Allergen doses: log-3 concentrations of orchard grass pollen extract. Statistical analysis was done by Spearman's rank correlation coefficient.

Results were expressed as means ± SD. Statistical analyses of the data were performed by means of nonparametric tests.

RESULTS

Clinical evaluation of the CPT

The diluent did not induce any symptoms. All patients had positive CPT results to allergen in both eyes. Mean symptom scores are presented in Fig. 1. All CPTs induced redness, tearing, itching; 90% of the CPTs induced chemosis. The mean cumulative score was 8.8 ± 1.2. When chemosis was deleted from this cumulative score all patients had a subtotal score of over 5.

The doses of allergen that induced a positive CPT result ranged from 0.41 IR/ml to 100 IR/ml. In 29 of 30 patients both eyes reacted for the same dose of allergen or the next dose (Fig. 2). Mean doses were 7.2 ± 605 IR/ml for the right eye and 9.8 ± 17.6 IR/ml for the left eye. As determined by Spearman's rank correlation coefficient, it is shown that there was a highly significant ($p < 0.0001$) correlation between allergen doses that elicit a positive CPT result in both eyes.

Release of mediators during CPT

All three mediators were released after diluent or after allergen challenges (Table II and Fig. 3). All but one patient had a significant release of at least one mediator. When right and left eyes were studied, PGD_2 was released in 11 and 12 allergen challenges, LTC_4/D_4 in 14 and 11 allergen challenges, and histamine in 13 and 16 allergen challenges. There was a significant increase in the release of all three mediators after provocation with allergen, ranging from 9.2 to 12.5 times for PGD_2, 6 to 7 times for LTC_4/D_4, and 5.2 to 6.5 times for histamine (Table III). There was no significant correlation between the symptom scores and the expression of each mediator in each patient.

After allergen challenge there was a concordant release in both eyes (positive-positive release or negative-negative release) in 13 of 20 patients for PGD_2, 11 of 19 patients for LTC_4/D_4, and 15 of 18 patients for histamine. The correlations between the levels of mediators released during diluent and allergen challenges in both eyes were significant for PGD_2 (diluent and allergen challenge) and LTC_4/D_4 (allergen challenge) (Fig. 4).

DISCUSSION

The results presented herein confirm that mediators are released during CPTs and can be recovered by means of a simple method. Considering the whole group of patients, the reproducibility of CPTs was demonstrated for the dose of allergen inducing a positive challenge and the release of mediators. However, as expected, the results are less satisfactory when patients are examined individually.

Conjunctival challenge is a useful technique that can be used to model some of the symptoms present in allergic conjunctivitis, and its positivity

TABLE II. Mediators released after challenge with diluent or allergen

	Eye	PGD_2 (ng/ml)	LTC_4/D_4 (ng/ml)	Histamine (ng/ml)
Diluent	Right	0.041 ± 0.030	0.016 ± 0.017	7.67 ± 7.45
	Left	0.040 ± 0.027	0.014 ± 0.012	7.81 ± 10.32
Allergen	Right	0.277 ± 0.314	0.057 ± 0.036	18.72 ± 8.39
	Left	0.256 ± 0.312	0.053 ± 0.045	17.22 ± 16.73
Increase in mediator release after allergen*	Right	9.2 ± 11.5	7.0 ± 8.8	6.5 ± 8.0
	Left	12.5 ± 15.4	6.0 ± 9.2	5.2 ± 5.2

*The increase in mediator release was calculated for each patient and then averaged for the group. Results are expressed as means ± SD.

TABLE III. Increase in mediator release after allergen challenge

PGD_2		LTC_4/D_4		Histamine	
Right	Left	Right	Left	Right	Left
11.2	10	39	1.4	6	6.2
1.3	0.9	10.6	7.1	5.8	10.9
0.3	2.3	17	–	10	4.7
1	0.3	1	1	1.5	2.1
2.4	0.4	8.8	1.9	–	–
0.5	3.2	3.1	4	1	0.5
8.8	1	13.2	31.8	2.7	3.4
1	0.5	8.7	3.5	1.3	10
1.7	42	1	1	2	7.9
2.5	1	4.1	1	14.5	2
6.2	16.7	9.2	1.7	5.7	2.7
1.5	1.3	1.5	30	35	3.7
23.7	25.2	4.8	2.4	3.4	4.9
11.7	17.7	2	3	1.4	22.5
27.8	26.5	6	1.1	10	2
42.5	17.5	3.2	12.4	5.5	2.6
1	1	1	1	6	2
21.8	59.5	1	1	3.8	6
3.1	1.2	4.2	5.5	1.4	1
13.9	6.6	1	3.5	–	–

Results present the magnitude of increase of a mediator comparing the levels after allergen and diluent challenge.

can be assessed by symptom score or by the release of mast cell–derived mediators.[11, 12, 14] In the present study we used a composite symptom score, first proposed by and found to be reproducible by Abelson et al.,[10] as well as by our research group (manuscript in preparation). The assessment of redness is relatively easy, although a precise scoring system may require more sophisticated equipment.[23] The assessment of chemosis requires a slit lamp so that we could determine whether this symptom is absolutely required for a positive CPT result. It was observed that the symptom score of each CPT was over 5 when chemosis was not considered in the cumulative score. This finding suggests that a slit lamp is not absolutely required to assess the positivity of a CPT with the cumulative symptom score used in this study. The study was not double-blind placebo-controlled because in its design we proposed to increase allergen dose up to a positive challenge. Using the diluent to measure mediators at baseline, we only instilled the diluent once. It is clear from this design that there was no blinded challenge per se.

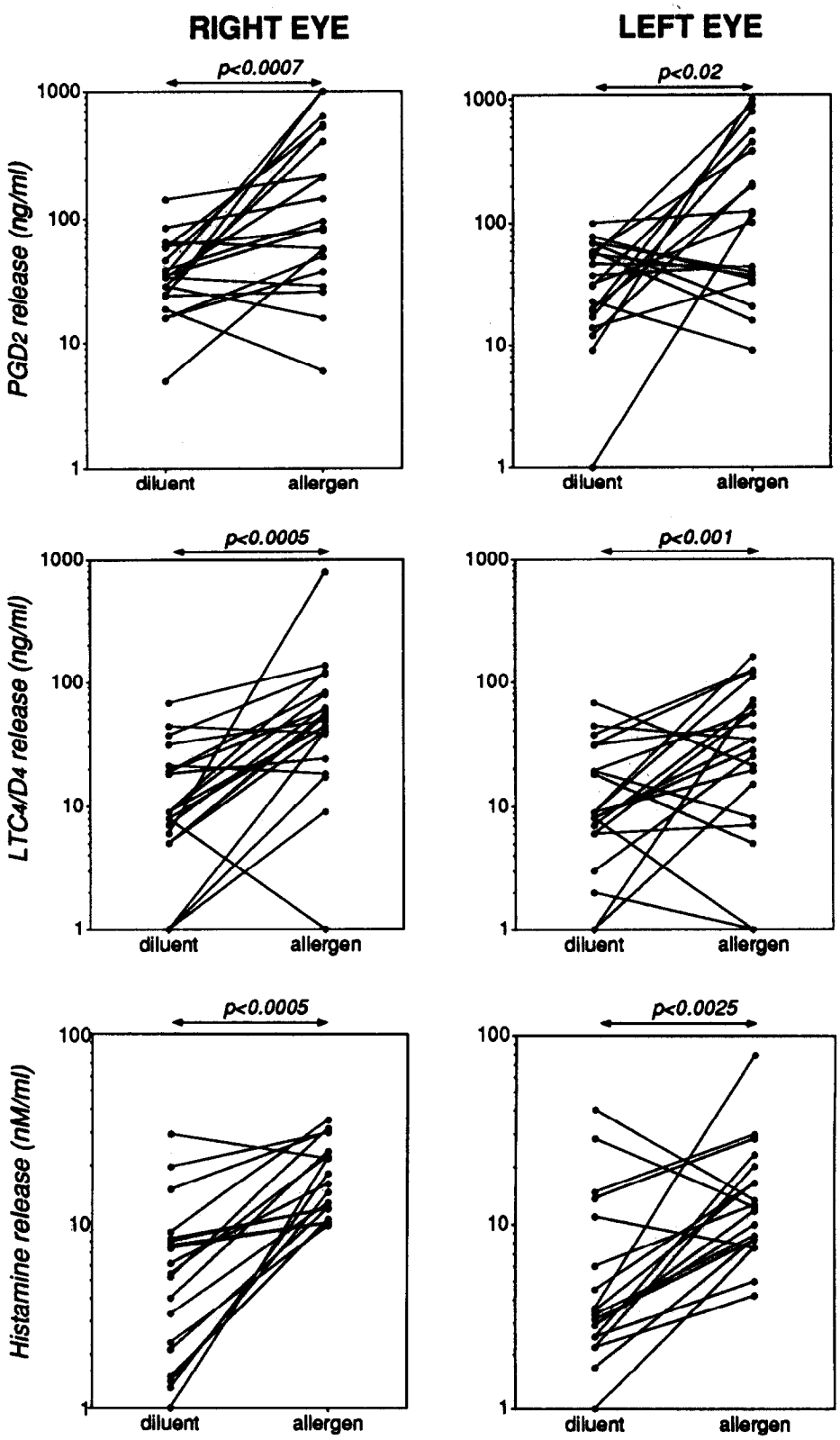

FIG. 3. Release of mediators in both eyes after diluent and allergen challenge. Statistical analysis was done by Wilcoxon W test.

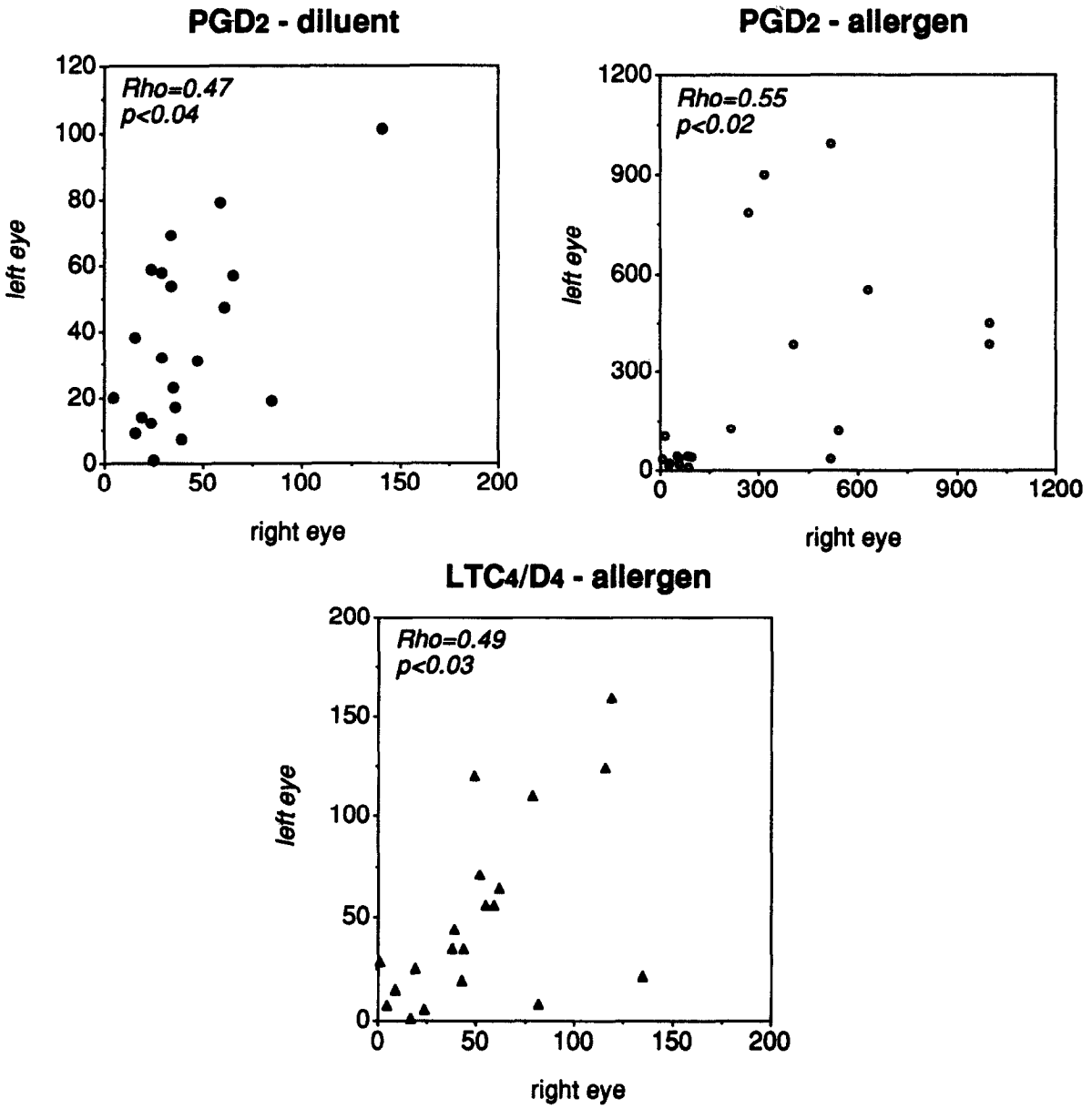

FIG. 4. Correlation between the release of mediators in both eyes after diluent or allergen challenge. Correlation was determined by Spearman's rank test.

The measurement of mediators is more difficult in tears than in nasal secretions because of the methods of tear collection. Among the methods used, collection with a capillary tube, the Schirmer strips, and more recently, the cellulose sponge[24] are the most appropriate. However, they have some defects. With the capillary tube, the amount collected is very small and does not allow the titration of several mediators. The Schirmer strips make it possible to recover a greater amount of secretions, but use of these strips induces some irritation of the eye, and a challenge cannot be performed later on the same eye. We used the Schirmer strips because we wanted to analyze three mediators, and the method was reported to be efficient.[11] The release of the three mediators was observed and the results confirm those of the study by Bisgaard et al.,[12] although in the study of Proud ed et.[11] sulfidopeptide leukotrienes were not released in a comparable amount. The difference among the results of the three studies are difficult to understand because, like Proud et al.[11] we used an assay that detected LTC_4 and LTD_4. This study therefore confirms that the release of mediators can be used to assess the positivity of a CPT.

The reproducibility of CPTs was examined with two parameters: doses of allergen inducing a positive symptom score and release of mediators. The doses of allergen inducing a positive CPT were found to be highly precise because both eyes reacted for the same dose or the next dose in 28 of 30 patients. We therefore confirmed the results of the study by Møller et al.,[9] showing the high reproducibility of CPT with the use of symptom scores, and CPT can be considered for use in the study of antiallergic treatments.

The reproducibility of the release of mediators is more difficult to appreciate than that of the dose inducing a positive CPT result. At baseline, the release of PGD_2 between right and left eyes was significantly correlated but there was no significant correlation for histamine and LTC_4/D_4. The lack of correlation after diluent CPT for LTC_4/D_4 may be due to the low levels present in the fluid, which were close to the detectability limit. After CPT with allergen, the concordance of release was higher for histamine than for the arachidonic acid metabolites, but the amount of histamine released in both eyes was not significantly correlated. On the other hand, the concordance of release was lower for PGD_2 and LTC_4/D_4, the levels released in both eyes were significantly correlated. These results indicate that the use of mediators during pharmacologic studies may lead to some difficulty in interpretation unless the treatment used is very potent in its ability to block the release of a given mediator.

This study shows that CPTs are very precise when the threshold concentration of allergen inducing a positive test is considered, and as such, can be used for the assessment of antiallergic treatments. However, the release of mediators during CPT is less reproducible.

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