ORIGINAL ARTICLES

Local conjunctival immunotherapy: the effect of dermatophagoides pteronyssinus local conjunctival immunotherapy on conjunctival provocation test in patients with allergic conjunctivitis

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SUMMARY

Background: we evaluated the effect of local conjunctival immunotherapy (LCIT) with standardized dermatophagoides pteronyssinus (Dp) extracts on antigen-specific conjunctival provocation test (CPT) in patients with allergic conjunctivitis in a double blind, placebo-controlled study. We use the CPT because in our experience is the more objective parameter to evaluate the sensitivity to allergens in this patients.

Methods: the patients were selected on the basis of symptoms, positive prick test, positive CPT and elevated serum and tears total and specific IgE. The CPT was assessed with increased dilution of Dp extracts instilled into the lower fornix. Conjunctival hyperemia, tearing, itching, burning and swelling of eyelids were scored according a 4-point rating scale. Patients were randomly assigned to 2 groups of 12. The first group was treated with Dp extracts and the second group with placebo during 6 months. A drop of diluted antigen was instilled in both eyes daily, in 2-fold increased concentrations, the first 10 AU/ml. The maintenance dose was 1,000 AU/ml or the maximal dose which did not provoke symptoms. The symptoms were controlled with oral and/or local antihistamines. We evaluated the CPT before and after the treatment. The patients did not receive antihistamines during the 15 previous days to carrying-out the CPT.

Results: ten of the twelve patients of the active group complete the treatment. One of the patients dropped out of the study because experienced local reaction with a dose of 1,000 AU/ml and refused to continue with the treatment. Other patient was disqualified for failure to comply with the protocol.

One patient, which experienced itching and tearing with a dose of 1,000 AU/ml, tolerate 100 AU/ml. We continue with this dose until the end of treatment. The remaining patients tolerate 1,000 AU/ml as maintenance dose.

A significant difference was observed in the score of CPT between LCIT treated patients and placebo group after 6 month of LCIT.

Conclusions: we propose LCIT as a useful alternative to traditional subcutaneous immunotherapy in patients with allergic conjunctivitis.

Key words: Allergic conjunctivitis. Conjunctival immunotherapy. Conjunctival provocation test. Dermatophagoides pteronyssinus.

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INTRODUCTION

Allergic conjunctivitis is often associated with allergic rhinitis and/or asthma, its symptoms are usually mild, and from the patient's point of view are less annoying than the symptoms of the associated pathology. But sometimes conjunctivitis is the principal manifestation of the allergic diseases and disturb the normal life of the patient.

Allergy to house dust mites is the most frequent cause of perennial allergic rhinoconjunctivitis (1, 2).

Parenteral immunotherapy with mite extracts for

allergic rhinoconjunctivitis has been proven effective in many controlled trials (3, 4), however, it has some disadvantages, including compliance, cost, and, in few cases, severe anaphylactic reactions (5).

Alternative routes for immunotherapy have been reported. Oral and sublingual immunotherapy have been used with conflicting results (6, 7).

Many previous studies have suggested that local nasal immunotherapy is effective in the treatment of pollen (6) and mite (8, 9) induced rhinitis.

We did not find reports of local conjunctival immunotherapy for allergic conjunctivitis in the literature.

In our study, we developed a method for local conjunctival immunotherapy (LCIT), similar to local nasal immunotherapy proved in patients with allergic rhinitis.

Conjunctival provocation test (CPT) with specific allergens has been shown to be a safe, consistent method of reproducing the conjunctival allergen response by triggering IgE mediated mast cell degranulation (10-12). A strong correlation exists between positive CPT and other test as skin test and serum RAST values (13), Furthermore, few patients exhibit a positive CPT in the absence of systemic sensitivity implying that IgE production in the conjunctiva may be locally regulated. The validity of CPT as a model for conjunctival allergen response has formed the basis for numerous studies, particularly those involving therapeutic intervention (12, 14).

We use CPT as a method for evaluate the efficacy of LCIT.

MATERIAL AND METHODS

Patients

Twenty-four patients (14 females and 10 males) aged between 18 and 43 years, suffering from allergic conjunctivitis as main symptom of allergy for at least 3 years were selected.

Criteria for inclusion were:

1. Typical history of perennial allergic conjunctivitis.
2. Elevated levels of serum and tears IgE.
3. Positive prick test (2+ to 3+) to *Dermatophagoides pteronyssinus* (Dp) extracts in glycerinated solution.
4. RAST positive (al least class 3) to Dp.
5. Positive response to CPT.

Criteria for exclusion were:

1. Long-term use of conjunctival corticosteroids or decongestants.
2. Prior specific immunotherapy.
3. Other allergen sensitivity.
4. State of pregnancy or lactation.

Antigens

Highly purified preparation of Dp standardized in Allergy Units (AU), purchased from Greer Labs, was used for skin test, conjunctival provocation test and LCIT.

Study design

The design of the study was a double blind placebo-controlled trial.

Patients were randomly assigned to two groups. Group 1 (12 patients) received LCIT with dermatophagoides pteronyssinus extracts during 6 months. Group 2 (12 patients) received placebo.

Before therapy the following baseline data were collected: skin test, serum and tears total and specific IgE and conjunctival provocation test with Dp.

Placebo and active LCIT were self-administered during six month according to the following schedule: A drop of diluted allergen was instilled in both eyes daily, in 2-fold increased concentrations, the first 10 AU/ml. The maintenance dose was 1,000 AU/ml or the maximal dose which did not provoke symptoms. The placebo group received drops of saline solution.

The patients could use oral and/or topical antihistamines when recorded symptoms.

After 6 month of treatment CPT with Dp was performed. The patients did not receive antihistamines during the 15 previous days to carrying-out the CPT.

Skin Prick Tests

Skin prick tests were performed with Dp extracts in glycerinated solution with a concentration of 10,000 AU/ml. Histamine and saline solutions were used as controls. The results were scored according the size of the wheal in a 4-point rating scale (0 to 3).

SPECIFIC Der p IgE

Serum and tear samples were collected and Dp IgE by RAST and total IgE were assayed.

Conjunctival Provocation Test

Conjunctival provocation test was performed by applying inside the conjunctival cul-de-sac of the right eye 20 microliters of increased concentrations of allergen, while saline solution, used to dilute the allergen, was administered into the contralateral eye to exclude possible non-specific reactions. The initial

Table I

Score of symptoms in the conjunctival provocation test

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-burning
0: none
1: mild (intermittent tickling sensation)
2: moderate (continual awareness with the desire to rub the eyes)
3: severe (subject insist on rubbing eyes)

Adapted from Abelson, et al (10).

allergen concentration was 100 AU/ml and increased with 10-log steps until a positive reaction occurred. Conjunctival redness, tearing, itching-burning, chemosis and eyelid swelling was scored according to an arbitrary 4-point rating scaled, similar to the score proposed by Abelson, et al (10) (table I). Total symptom score > 6 was considered positive.

The allergen dose used in the CPT after treatment was the same as those used before treatment.

Statistical Analysis

The Wilcoxon Signed-Rank test was used to assess differences between groups for two continuous variables (before-after), and for two different samples (treatment-placebo) was used the Mann-Whitney Rank-Sum test. Statistical significance was taken as $p < 0.05$. The software used was CSS/Statistica, 5.1 (Statsoft Corp. Tulsa, Okla, USA).

RESULTS

Four patients dropped out of the study, two from the active group and two from the placebo group. One of the patients of the active group withdraw because experienced local reaction with a dose of 1,000 AU/ml and refused to continue with the treatment. The other three patients were disqualified for failure to comply with the protocol.

Table II

Score of CPT (Conjunctival provocation test) before treatment

Patients	Redness	Chemosis	Tearing	Itching	Total Score
1	3	1	3	2	9
2	3	1	2	2	8
3	2	1	2	2	7
4	2	2	2	1	7
5	2	2	2	2	8
6	3	2	2	2	9
7	3	2	2	2	9
8	2	1	2	2	7
9	2	2	2	3	9
10	2	2	2	3	9
11	2	3	2	2	9
12	3	1	2	2	8
13	2	1	2	2	7
14	1	1	2	3	7
15	3	2	2	3	10
16	2	2	2	2	8
17	1	2	2	2	7
18	3	2	3	3	11
19	1	1	2	2	6
20	2	2	2	3	9
21	2	2	2	2	8
22	3	2	2	2	9
23	1	2	3	2	8
24	2	2	2	2	8

Patients 1-12: LCIT (Local conjunctival Immunotherapy).
Patients 13-24: Placebo.

Other patient, which experienced itching and tearing with a dose of 1,000 AU/ml, tolerate 100 AU/ml. We continue with this dose until the end of treatment. The remaining patients tolerate 1,000 AU/ml as maintenance dose.

The individual symptoms scores of CPT before and after treatment are shown in table II and table III.

The average symptoms score before and after treatment are shown in table IV.

The average symptoms score of the treated group versus placebo group are shown in table V.

Then, after six months of LCIT the scores of symptoms after CPT significantly decreased in the actively treated group, whereas no difference was observed in the control group (Fig. 1).

DISCUSSION

In our study, we developed a method for LCIT in a group of patients with conjunctivitis as the main

Table III

Score of CPT after treatment

Patients	Redness	Chemosis	Tearing	Itching	Total Score
1	1	1	1	1	4
2	2	0	1	1	4
3	2	1	1	1	5
4	1	0	1	1	3
5					
6	1	0	0	1	2
7	2	1	1	2	6
8	1	1	1	2	5
9	1	1	1	1	4
10					
11	1	1	0	1	3
12	1	0	0	1	2
13	2	2	2	2	8
14	2	1	2	2	7
15	2	2	2	3	9
16					
17	2	2	3	2	9
18					
19	2	1	2	2	7
20	2	2	2	2	8
21	2	1	1	1	5
22	1	1	1	2	5
23	1	1	2	2	6
24	1	2	2	2	7

Patients 1-12: LCIT.
Patients 13-24: Placebo.

Table IV

Score of Conjunctival Provocation Test before and after treatment

1. Treated Group (n: 10) Symptom	Score: Mean ± STD (median) Before	After	*p value**
Redness	2.5 ± 0.53 (2.5)	1.3 ± 0.48 (1.0)	< 0.00062
Chemosis	1.6 ± 0.7 (1.5)	0.6 ± 0.52 (1.0)	< 0.0035
Tearing	2.1 ± 0.32 (2.0)	0.7 ± 0.48 (1.0)	< 0.00005
Itching	2.0 ± 0.47 (2.0)	1.2 ± 0.42 (1.0)	< 0.0021
Total score	8.2 ± 0.92 (8.5)	3.8 ± 1.32 (4.0)	< 0.00015
2. Placebo Group (n: 10) Symptom	**Score: Mean ± STD (median) Before**	**After**	***p value****
Redness	1.8 ± 0.79 (2.0)	1.7 ± 0.48 (2.0)	< 0.865
Chemosis	1.7 ± 0.48 (2.0)	1.5 ± 0.52 (1.5)	< 0.373
Tearing	2.1 ± 0.32 (2.0)	1.9 ± 0.57 (2.0)	< 0.330
Itching	2.3 ± 0.48 (2.0)	2.0 ± 0.47 (2.0)	< 0.177
Total score	7.9 ± 1.2 (8.0)	7.1 ± 1.45 (7.0)	< 0.244

* Wilcoxon Signed-Rank Test.

Table V

Score of Conjunctival Provocation Test. LCIT versus placebo

1. Before Treatment Symptom	Score: Mean ± STD (median) LCIT (n: 10)	Placebo (n: 10)	*p value**
Redness	2.5 ± 0.53 (2.5)	1.8 ± 0.79 (2.0)	< 0.042
Chemosis	1.6 ± 0.7 (1.5)	1.7 ± 0.48 (1.0)	< 0.575
Tearing	2.1 ± 0.32 (2.0)	2.1 ± 0.32 (1.0)	< 0.999
Itching	2.0 ± 0.47 (2.0)	2.3 ± 0.48 (1.0)	< 0.177
Total score	8.2 ± 0.92 (8.5)	7.9 ± 1.2 (8.0)	< 0.502
2. After treatment Symptom	**Score: Mean ± STD (median) LCIT (n: 10)**	**Placebo (n: 10)**	***p value****
Redness	1.3 ± 0.48 (1.0)	1.7 ± 0.48 (2.0)	< 0.081
Chemosis	0.6 ± 0.52 (1.0)	1.5 ± 0.52 (1.5)	< 0.0033
Tearing	0.7 ± 0.48 (1.0)	1.9 ± 0.57 (2.0)	< 0.00047
Itching	1.2 ± 0.42 (1.0)	2.0 ± 0.47 (2.0)	< 0.0021
Total score	3.8 ± 1.32 (4.0)	7.1 ± 1.45 (7.0)	< 0.00052

* Mann-Whitney Rank-Sum Test.

symptom of their allergic diseases. We did not find other reports of this route of immunotherapy in the literature.

The evaluation of the results was done with CPT because it was proved to be an ideal tool to evaluate clinical efficacy of an anti-allergic treatment, more exact than clinical symptoms, skin test or specific IgE (12, 14).

We used a symptom score to evaluate CPT because, according with Melillo (12), we consider that is better than level of mediators in tears, conjunctival cytology or measure of conjunctival temperature for de evaluation of conjunctival response.

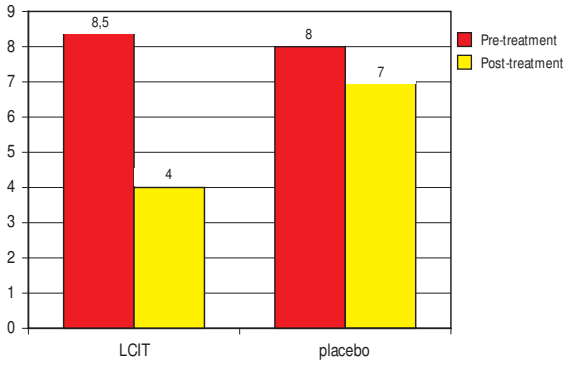

Figure 1.—Average symptom score of CPT.

Ten of the twelve patients tolerated the treatment. Occasionally they experienced conjunctival symptoms controlled with topical and/or oral antihistamines. In the placebo group we used similar medication, but with significantly more consumption. Two patients experienced local reaction with a dose of 1,000 AU/ml and one of them refused to continue with the treatment.

A significant difference was observed in the scores between the patients treated with LCIT and the patients of the placebo group. This results demonstrate the efficacy and compliance of LCIT in the treatment of allergic conjunctivitis and agree with the results of the studies with local nasal immunotherapy in patients with allergic rhinitis.

In conclusion, we propose LCIT as an useful alternative route of immunotherapy in monosensitized patients with allergic conjunctivitis, without the disadvantages of the traditional subcutaneous immunotherapy and the conflicting results of other alternative routes. However, further investigations on a wider population are needed to confirm its clinical efficacy and clarify its mechanisms.

RESUMEN

Antecedentes: efectuamos inmunoterapia local por vía conjuntival con extractos alergénicos estandarizados de dermatophagoides pteronyssinus en pacientes con conjuntivitis alérgica sensibles a dicho alergeno. Evaluamos la efectividad del tratamiento determinando las modificaciones producidas en el test de provocación conjuntival específico con el alergeno, ya que consideramos a dicho test como el más confiable para evaluar la sensibilidad a un alergeno en este tipo de pacientes. El estudio se efectuó a doble ciego controlado con placebo.

Métodos: los pacientes fueron seleccionados en base a presentar síntomas típicos de conjuntivitis alérgica, valores elevados de IgE específica en suero y en lágrima, y prick test y test de provocación conjuntival positivos con dermatophagoides pteronyssinus. El test de provocación conjuntival se efectuó instilando en la conjuntiva diluciones crecientes de extractos de dermatophagoides pteronyssinus. Se evaluaron la hiperemia, lagrimeo, prurito y quemosis en base a una escala arbitraria de cuatro puntos. Los pacientes fueron separados aleatoriamente en dos grupos de 12 y se los sometió a tratamiento durante seis meses. El primer grupo recibió inmunoterapia local conjuntival con dermatophagoides pteronyssinus y el segundo placebo. Se instiló diariamente una gota de diluciones crecientes del antígeno, comenzando con 10 AU/ml. La dosis de mantenimiento fue de 1.000 AU/ml o la máxima dosis que no provocara síntomas. Los síntomas fueron controlados mediante la administración de antihistamínicos locales u orales. Luego de seis meses efectuamos nuevamente el test de provocación conjuntival. No se administró ninguna medicación en los 15 días previos al test de provocación.

Resultados: 10 de los 12 pacientes del grupo activo completaron el tratamiento. Uno de los pacientes abandonó al experimentar reacción con la dosis de 1.000 AU/ml. El otro fue descalificado por no cumplir con el protocolo establecido. Uno de los pacientes, que experimentó prurito y lagrimeo con la dosis de 1.000 AU/ml, continuó el tratamiento con 100 AU/ml.

Se observó una diferencia estadísticamente significativa entre el score del test de provocación conjuntival del grupo tratado y del grupo placebo.

Conclusiones: consideramos que la inmunoterapia local por vía conjuntival es una buena alternativa a la inmunoterapia subcutánea tradicional en pacientes con conjuntivitis alérgica.

Palabras clave: Conjuntivitis alérgica. Inmunoterapia conjuntival. Test de provocación conjuntival. Dermatophagoides pteronyssinus.

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PRINTER'S ERROR

The correct name of the first author of the article titled "Hereditary angioedema. Long-term follow-up of 88 patients. Experience of the Argentine Allergy and Immunology Institute", published in Allergologia et Immunopathologia (2000;28/5:267-71), is the doctor **J. E. Fabiani,** not Fabiana, as appeared by error.