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House-dust mite sublingual-swallow immunotherapy in perennial conjunctivitis: a double-blind, placebo-controlled study

B. Mortemousque, F. Bertel, J. De Casamayor, P. Verin and J. Colin

Department of Ophthalmology, University Hospital of Bordeaux (Hôpital Pellegrin), Bordeaux, France

Summary

Background The safety and the efficacy of sublingual-swallow immunotherapy (SLIT) in perennial conjunctivitis caused by house dust mite were evaluated in a double-blind, placebo-controlled study including 60 patients for 24 months.

Methods Patients received either placebo or SLIT with standardized *Dermatophagoides pteronyssinus* (*D.pt.*) and *D. farinae* (*D.f.*) 50/50 extract. The evaluation of the efficacy of the SLIT was obtained by using standardized *D. pteronyssinus* (*D.pt.*) extracts on the antigen-specific conjunctival provocation test (CPT). Specific CPT, skin sensitivity and serum-specific IgE were performed before starting treatment and 6, 12, 18 and 24 months.

Results Of the 60 patients included, only 45 completed the study (26 in the active group and 19 in the placebo group, $P < 0.05$). Two out of 30 (6.6%) patients dropped out because of insufficient efficacy in the active group compared to eight out of 30 (26.6%) in the placebo group ($P < 0.05$). There was a significant increase in the antigen threshold required to obtain a positive CPT from 8.2 IR [95% confidence interval (CI) 5.9–11.4] at baseline to 21.7 IR (95% CI 15.4–30.4) at 2 years (M24) in the active group, compared to 8.1 IR (95% CI 5.4–12.1) at baseline to 8.1 IR (95% CI 5.1–12.4) at M24 in the placebo group ($P < 0.04$ for overall treatment difference, $P < 10^{-7}$ for the time–treatment interaction; repeated measures ANOVA). Differences between groups at individual time points were significant from 18 months on. Despite increased antigen concentrations, CPT scores were lower overall in the active than in the placebo group ($P < 0.001$). No serious adverse effects were reported.

Conclusion SLIT effectively increased the antigenic threshold required to obtain a positive CPT to house-dust mite antigen. Tolerance to and innocuity of SLIT were comparable to previous studies. This could justify recommending SLIT for the preventive treatment of perennial conjunctivitis caused by house dust mites.

Keywords house dust mite, sublingual immunotherapy

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Introduction

Subcutaneous injections of increasing doses of certain allergens is a clinically effective therapy for allergic disorders like rhino-conjunctivitis or asthma. As this route of application is inconvenient and is occasionally associated with severe systemic side-effects, local, oral and sublingual immunotherapy (IT) have been tested in a number of controlled studies over the last 15 years [1–3] with contradictory results. In 1993, the European Academy of Allergology and Clinical Immunology stated that more conclusive data were required before these application routes can be recommended for treatment [4]. Nine well-designed studies demonstrated the clinical effectiveness of sublingual-swallow immunotherapy (SLIT) with grass, Parietaria, olive pollen and mite allergens [1, 5–12]. Bousquet, in a WHO position paper [1] stated that SLIT may be a viable alternative to parenteral injection therapy for treating allergic arways diseases, but further studies were still needed to define the indications and therapeutic target dose of this form of immunotherapy.

In line with this reccomendation, the present study was designed to evaluate the safety and efficacy of SLIT using standardized *Dermatophagoides pteronyssinus* (*D.pt.*) extracts on the antigen-specific conjunctival provocation test (CPT) in patients with isolated perennial conjunctivitis caused by house-dust mites.

Correspondence: Mortemousque Bruno, Department of Ophthalmology, University Hospital of Bordeaux (Hôpital Pellegrin), Place Amélie Raba-Leon, 33076 Bordeaux Cedex, France.
E-mail: bruno.mortemousque@chu-bordeaux.fr

Material and methods

Patients

Sixty patients (mean age 24.3 $\pm$ 12 years; range 6–60 years) with a history of perennial conjunctivitis agreed to take part in this clinical trial. Careful evaluation of their clinical history clearly

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indicated the role of exposure to domestic house-dust mites as the major agent of the symptoms. Patients also experienced symptoms not sufficiently controlled by topical medications and/or oral antihistamines. IgE-mediated conjunctivitis was demonstrated in all patients selected by positive skin prick tests to *D. pteronyssinus* (*D.pt.*) *and D. farinae* (*D.f.*) standardized extract (Stallergènes® SA, Antony, France), specific IgE (*D.pt.* and *D.f*) positivity (RAST ≥ class 2) and CPT positivity to *D.pt.* [cumulative clinical score of Abelson, Chambers and Smith [12] ≥ 5; Stallergènes SA]. Patients sensitized to cat or dog allergens and living with pets at home were excluded. No patient had previously received immunotherapy with *D.pt.*, was using oral steroids or had any other significant illness. The study protocol was submitted to the approval of the ethics committee of the University Hospital of Bordeaux, France, and patients, or both parents of minors, signed informed consent forms before starting the study. During the trial, all patients were asked to continue their usual antiallergic eye drops (antihistamines and cromones) without change, apart from oral corticosteroids which were not allowed, though topical ones were. This was verified with a diary. Before starting the treatment, all patients were asked not to take any specific measure to avoid house-dust mites during the study, in order to maintain the same level of exposure throughout.

Conjunctival provocation test

CPTs were not performed in periods of acute conjunctivitis or ocular symptoms. Contact lens wearers were requested to remove their lenses on the evening before the CPT. Any antihistamine, corticosteroid or anti-inflammatory treatment was stopped at least 7 days before the test. Treatments with eye drops were curtailed 5 days before the test. CPTs were performed with lyophilized allergenic extracts of *D.pt.* (Stallergènes SA) diluted in a nonphenolic solution in five incremental concentrations: 1.2, 3.7, 11, 33 and 100 IR (index of reactivity).

For each CPT we arbitrarily decided that the left eye would receive a drop (40 mL) of allergenic extract while the right eye would receive a drop of isotonic solution. The asymmetry of ocular response therefore gave an assessment of allergic response.

Conjunctival response was evaluated 10 min after administration of the allergen according to the cumulative clinical score of Abelson, Chambers and Smith [12], which includes four criteria (Table 1): redness, watering, oedema and pruritis. If the score was equal to or exceeded 5, the test was considered positive. If this score was not obtained, the next higher allergenic concentration was administered until the final concentration. At the end of the CPT, the allergic reaction was controlled by administering a drop of levocabastine (Levophta®). This CPT was previously found to have 93% sensitivity and 100% specificity [13]. A CPT was performed for each patient before treatment, after 6, 12, 18 months, and at end of the trial (24 months).

Allergen preparation and immunotherapy

The standardized extract used throughout the study was the same batch of equal proportions of a mixture of *D.pt and D.f.* (Stallergènes SA). The extract was graded into four concentrations: 1, 10, 100 and 300 IR/mL. The inhouse reference extract (labelled 100 IR) is defined as the concentration eliciting by skin prick test a geometric mean wheal size of 7 mm diameter in 30 subjects sensitive to the corresponding allergen. The Der p 1 and Der f 1 contents per 1 mL of the 100 IR allergen extract used in this study were 4.8 and 3.7 μg, respectively.

After selection, patients were randomized to receive either a *D.pt./D.f.* preparation in 50% aqueous glycerol or a placebo. The placebo preparation was identical to active treatment in terms of composition, appearance, presentation, taste and colour, but obviously did not contain allergens. The duration of treatment was 24 months. Treatment was initiated between January 1997 and March 1997 and the study was completed in March 1999. During a 4-week incremental dose period, patients took daily increasing doses from 1 to 10 drops of the 1-IR/mL vial from day 1 to day 4, then 1–10 drops of the 10-IR/mL vial from day 5 to day 8, then 1–20 drops of the 100-IR/mL from day

Table 1. Abelson, Chambers and Smith score

Symptoms	Score
Redness of conjunctivitis	0 = none
	1 = slight
	2 = moderate
	3 = severe
Chemosis	0 = none
	1 = discreet (detectable with slit lamp, conjunctiva separate from sclera)
	2 = moderate (clearly visible, conjunctiva raised in inferior part)
	3 = severe (swollen conjunctiva)
Watering	0 = none
	1 = slight
	2 = moderate (occasional nasal flow)
	3 = severe (tears running down cheeks)
Pruritis (self-evaluated)	0 = none
	1 = slight (occasional tingling sensation)
	2 = moderate (permanent hindrance but no need to rub eye)
	3 = severe (permanent hindrance with need to rub eye)
	4 = unbearable pain (compulsive desire to rub eye)

9 to day 15, and finally 5–20 drops of the 300-IR/mL vial during the next 2 weeks. The highest dose (20 drops from the 300-IR/mL vial) was administered daily for 4 weeks and then 3 days per week throughout the maintenance phase. Each dose had to be taken in the morning before breakfast, and drops of allergen had to be kept under the tongue for at least 2 min before swallowing. With this schedule, an average cumulative dose of 90 000 IR (equivalent to 2.2 mg of Der p 1 and 1.7 mg of Der f 1) of allergens had been administered to each patient undergoing active treatment by the end of the trial.

Skin reactivity

Before treatment, at 12 months and at the end of the study, a quantitative skin prick test was performed with a Stallerpoint device with five serial threefold dilutions ot *D.pt.* and *D.f.* extracts (300, 100, 30, 10 and 1 IR/mL; Stallergènes SA). The wheals were marked after 15 min with a thin pen and then transferred onto a transparent adhesive tape.

Clinical scores

Symptoms were recorded at each visit, and scored on a scale from 0 to 3: 0 = absence of signs or symptoms; 1 = signs or symptoms present but not disturbing; 2 = signs or symptoms disturbing; and 3 = very disturbing with impaired social or professional life.

Statistical analysis

The main study variable was the threshold concentration needed to elicit an allergic reaction scored above 5 in the CPT. Differences between these threshold concentrations in the active and placebo groups were compared using repeated measures ANOVA on log-transformed data. Individual time points were also compared using Mann–Whitney's non-parametric test. Threshold concentration results are shown as mean and upper and lower bounds of the 95% CI of the mean, calculated on the log-transformed data and back-transformed.

Scores obtained in the CPT were compared using repeated measures ANOVA, completed by Mann–Whitney's non-parametric test for individual time points. Results are shown as mean $\pm$ SEM. Other variables were compared using non-parametric tests as needed.

All tests were two-tailed and the level of significance was set at 0.05.

Results

Sixty patients were included in the study, 45 of whom completed the study (26 in the active group and 19 in the placebo group). Nine patients dropped out during the first year (three in the active group and six in the placebo group) and another six patients dropped out during the second year (one in the active group and five in the placebo group). The number of dropouts was significantly greater in the placebo group than in the active group (11 patients and four patients, respectively; $P < 0.05$). Two out of 30 (6.6%) dropped out because of insufficient efficacy in the active group compared to eight out of 30 (26.6%) in the placebo group ($P < 0.05$). Other reasons for discontinuing immunotherapy were poor compliance and loss to follow-up for five patients (two in the active group and three in the placebo group). There was no significant difference between the initial threshold concentrations and CPT scores between dropouts and study completers.

There was no difference in age distribution between the two treatment groups either at inclusion or for study completers [mean age for the final active group 25.15 $\pm$ 11.57 years (range 6–60 years) and for the placebo group 24.25 $\pm$ 9.3 years (range 7–47 years)].

There was no difference in baseline clinical data, threshold concentrations and CPT scores for the 45 patients eligible for analysis between the active treatment and placebo groups. Individual patient data are available upon request.

Tolerance of therapy

Two patients in the active group reported more marked episodes of ocular pruritis in the first month of treatment, which then waned. One of these complained of local swelling, reddening and tingling of the tongue and the gingiva within less than 30 min of application during the first month. Neither patient dropped from the study. No general reaction was observed during the study.

Skin reactivity

No significant decrease in wheal areas obtained by the prick test with *D.pt.* was observed for either group.

Specific IgE

There was no significant change in specific *D.pt.* serum IgE concentrations in the either treatment group.

Conjunctival provocation test

There was no significant difference at baseline between the groups for threshold concentration or CPT score. There was a significant increase in the antigen threshold required to obtain a provocation test, score above 5 (Fig. 1), from 8.2 IR (95% CI 5.9–11.4) at baseline to 21.7 IR (95% CI 15.4–30.4) after 2 years. In the placebo group the thresholds remained stable, from 8.1 IR (95% CI 5.4–12.1) at baseline to 8.1 IR (95% CI 5.3–12.4) at 2 years. Repeated-measures ANOVA on log-transformed data

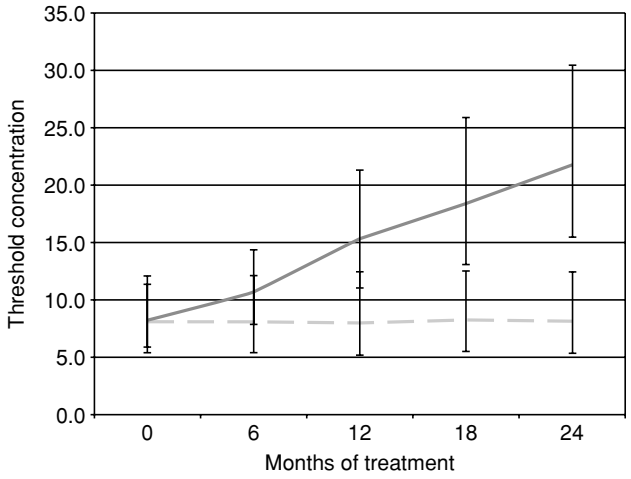

Fig. 1. Evolution over time of the threshold concentration needed to elicit an allergic reaction with a score ≥ 5. Results are shown as mean and 95% confidence interval of mean computed on log-transformed data. Full line, active treatment; dashed line, placebo.

found a significant difference between treatment arms overall [F $(1, 43) = 4.48$, $P = 0.04$], with a highly significant time–treatment interaction [F $(3, 129) = 17.02$, IP $= 0.1\ 10^{-7}$], with a linear trend ($P < 10^{-5}$). The comparison of individual datapoints showed that the difference between the groups was significant at 18 ($P < 0.01$) and 24 ($P < 0.0007$) months.

The mean scores obtained during these provocation tests are shown in Fig. 2. Repeated measures ANOVA showed a significant overall between-treatment difference [F $(1, 43) = 9.38$, $P = 0.004$], but a non-significant time–treatment interaction [F $(3, 129) = 2.47$, $P = 0.06$]. At individual time points, the CPT score was lower in the active treatment group than in the placebo group at 18 ($P < 0.003$) and 24 months ($P < 0.0006$).

Clinical scores

There were significantly more dropouts in the placebo group (11 subjects) than in the active treatment group (four subjects, $P < 0.05$). Most dropped out because of perceived lack of efficacy. The evolution of the number of patients in each score group was different between the treatment groups, over time (X^2 $P < 0.01$ including dropouts): in the active group, a decrease in the number of patients scored 2 (moderate symptoms) was accompanied by an increase of the number of patients scored 1 (mild symptoms; Fig. 3). In contrast, in the placebo group, the number of patients in score group 1 remained constant, while the number of patients scored 2 decreased, in parallel with the number of dropouts. The only patient in whom all symptoms disappeared was in the active treatment group (Fig. 3).

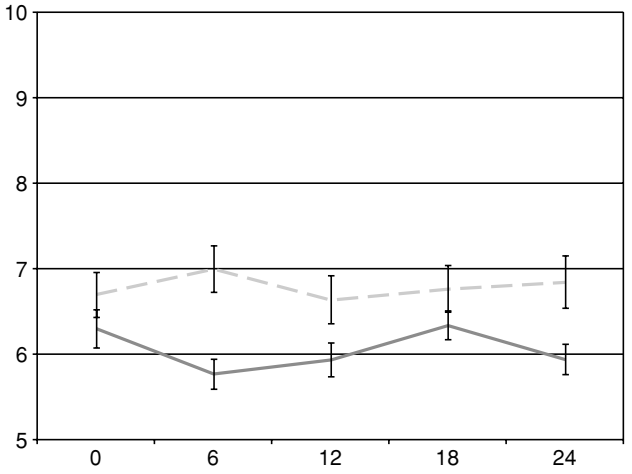

Fig. 2. Mean ± SEM of conjuctival reaction scores in CPT, elicited by the threshold antigen concentrations (see Fig. 1). Full line, active treatments; dashed line, placebo.

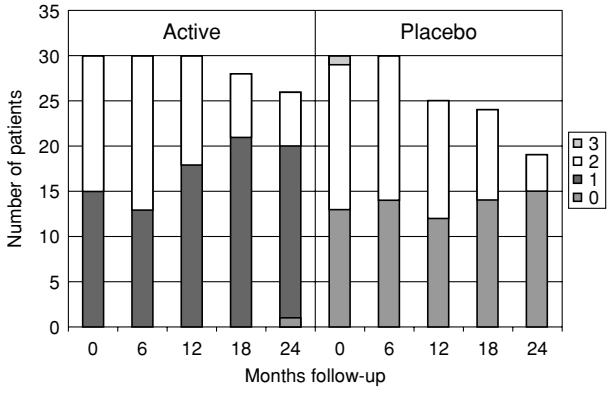

Fig. 3. Clinical scores at each study time/number of patients. Clinical score: 0, no symptoms since last consultation; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms.

Discussion

This study compares house-dust mite SLIT and placebo administered for 24 months for perennial allergic conjunctivitis. It showed that significantly more patients dropped out, for lack of efficacy, in the control group. In those remaining in the study, the threshold value to trigger a conjunctival reaction was higher in the active treatment group, and the conjunctival symptom scores were better. This was observed despite the small number of subjects in our study, a consequence of the small number of patients with perennial conjunctivitis not responsive to local and/or general medical treatment. There are to our knowledge no previous study of the use of SLIT in perennial conjunctivitis due to house-dust mites. Only Nunez et al. [3] have assessed the efficacy of local conjunctival immunotherapy (LCI) in the same indications, using CPT.

There is a possible hypothetic mechanism that might explain the efficacy of SLIT. The mucosa of the mouth is particularly rich in Langerhans' cells. These cells pick up antigens and allergens and subsequently migrate to the draining lymph nodes, where they become dendritic cells. In animal studies, tolerance induction via the oral mucosa showed that dendritic cells derived from the oral mucosa acted as antigen-presenting cells [14]. The basic mechanism for oral mucosa-induced tolerance is subsequent clonal anergy of specific T cells. Dendritic cells are also known to produce IL-12, a cytokine that could preferentrially drive the development of T helper type-1 (Th1) cells [15].

We found clear differences between the placebo and active treatment groups in three outcome criteria: patient dropout rate, antigen threshold for the CPT and CPT symptoms score.

High patient dropout rates are not a desired feature in a clinical trial, because it decreases the power of the analysis, and makes the ensuing results less persuasive or applicable. However, in our case, the high dropout rate could be anticipated because of the duration of the study and the treatment complexity and constraints. Staying in the study would in fact be a marker of patient compliance or satisfaction. In this case, that 11 of the placebo patients and only four of the active patients dropped out is revealing. Additionally, eight of the 11 placebo dropouts did so because of explicitly stated lack of efficacy, versus two of the four active dropouts. This could also explain the very small difference in the clinical scores between the groups: patients with poor clinical scores would tend to drop out, and this is illustrated by the fact that the percentage of patients who rated their symptoms as moderately severe decreased from about 50% to around 20% between the onset and the end of the study in both groups. This was, however, related to a shift towards a lower symptoms score in active treatment patients, and to a dropout of the more severe patients in the placebo group (Fig. 3). The same phenomenom of more patient attrition in the placebo group than in the SLIT group has already been noted by Guez et al. [5] in perennial rhinitis and by Hirsch et al. [16] in allergic childhood asthma.

The CPT we used has proved to be an ideal tool to assess clinical efficacy of antiallergic treatments and to be more precise than clinical symptoms, skin test or specific IgE [3, 13, 16–18]. As in previous studies [5, 16, 19, 20], we found no difference between skin test findings and specific IgE levels between baseline and final analyses. Different results were obtained for specific IgE [20, 21]. Whatever the mode of administration, the aim of immunotherapy is to raise the antigenic threshold above which mastocyte degranulation is triggered. In our opinion, CPT is the best technique to assess this threshold. Unlike previous studies which relied on ocular [3, 19] or nasal [20] provocation tests, we only collated overall ocular scores (pruritis + redness + watering + oedema). In addition, our main outcome criterion was not the score at a fixed antigen concentration, but the concentration needed to elicit a reaction with a score of at least 5. The result of this choice was that we could demonstrate an increasing threshold value over time. Eighteen patients (60%) increased their threshold by one concentration and five (16.6%) by two concentrations, over the 2-year timespan of the study. The increased in threshold concentration was linear over time. This increasing antigen concentration actually resulted in slightly lower ophthalmic reaction scores. In the same condition, there was no change at all in the threshold concentration for placebo or in the resulting CPT scores.

On a cautious note within these very positive results, in seven (23.3%) of the active treatment subjects there was no change in the threshold. No significant difference was observed for any parameter (age, sex, specific IgE, threshold and initial CPT score) in these seven non-responders compared with the responders. Though we did not document compliance, non-response could be a result of poor treatment compliance, a problem already described by other authors [5, 16]. It could also be related to individual patient resistance to immunotherapy or insufficient dosage in these patients. There is no other trial of the clinical efficacy of immunotherapy in the treatment of isolated conjunctivitis due to house-dust mites. We can only therefore compare our results with those of immunotherapeutic protocols used for treating rhinitis and rhino-conjunctivitis, which have hardly been more studied [5, 22–25]. Allergic rhinitis may respond better to SLIT than allergic conjunctivitis [26], but these studies focused primarily on nasal symptoms and rarely used ocular provocation as a primary end point. The total cumulative amount of antigen administered seems to have a considerable influence on the results of SLIT. For example, in studies using doses similar to ours [5, 16], the results were only moderate or disappointing. On the other hand, higher doses [27] provide more convincing arguments for SLIT, including when used in conjunctivitis [28, 29]. Therefore, it seems essential to define the optimal dose of antigen required to obtain homogeneous results. There is still much work to do in that direction.

In conclusion, our findings are comparable to those in previous studies in terms of tolerance to SLIT and its innocuity. With regard to efficacy, our data are clearly in favour of SLIT to treating perennial conjunctivitis caused by house-dust mites, with a significant increase in the antigenic threshold required to obtain a positive CPT, and indications of clinical benefit. However, one-quarter of the patients may not respond. Further research is needed to better understand the value of SLIT and to define the minimum total allergen dose required.

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