

NASAL ECP PATTERNS AND SPECIFIC IMMUNOTHERAPY IN MITE-ALLERGIC RHINITIS PATIENTS

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SUMMARY:

Background: Specific immunotherapy (SIT) is an effective treatment in allergic rhinitis and it has been shown to decrease nasal ECP rise after allergen challenge.

Aims: To evaluate if the kinetics of nasal ECP release after specific nasal challenge could be changed by SIT and if these changes were related to the dose of allergen administered.

Material and Methods: 75 allergic rhinitis patients, monosensitized to house dust mites were included: 25 controls and 50 SIT-treated patients. These patients were divided in two groups: one receiving a high dose of the allergenic extract (Group 2) and other receiving a lower dose (Group 1). Nasal challenges were performed at the beginning of the study (T0), after 6 (T1) and after 12 months (T2). Nasal ECP values were measured in nasal lavages before challenge and one and four hours after obtaining a positive reaction. Patients were also asked in every visit to evaluate on a visual analog scale the intensity of their disease in the previous month.

Results: SIT was effective in improving subjective (visual analog scale) and objective parameters (nasal allergenic reactivity). Both SIT groups had significant differences between T0 and T2 values and comparing with control patients. SIT reduced significantly mean post-provocation ECP values in both groups and reduced the number of patients that showed increase in nasal ECP >100% of basal values in each of the SIT-treated groups. The changes in nasal ECP values and particularly in nasal ECP patterns were more pronounced in Group 2 than in Group 1 but the differences did not reach statistical significance.

Conclusions: SIT can inhibit nasal ECP increase after allergen challenge, frequently present in non SIT-treated patients. This effect is more pronounced and reaches statistical significance earlier in patients receiving higher doses of the allergenic extract used in SIT.

Key-Words: Allergic rhinitis - Nasal ECP - Nasal provocation - Specific immunotherapy.

INTRODUCTION

Several papers have already unequivocally demonstrated efficacy of SIT with mite extracts in mite-allergic rhinitis, in double-blind placebo-controlled studies (1, 2) or in case-control studies (3) and clinical and/or immunological efficacy could already be demonstrated after 12 months of SIT (1, 3). SIT has also been recognized as an effective treatment in recent international consensus on allergic rhinitis (4). Nevertheless, despite its efficacy SIT is not equally effective in all patients, even with adequate selection criteria.

It is well known that in the long term SIT can induce changes in cytokine/chemokine profiles that result, among other effects, in an isotype switch with a decrease in specific IgE levels. However, in the short term it is also possible to document beneficial immunologic effects of SIT. It has been demonstrated that SIT can decrease cellular releasability, namely baso-

phil histamine release, in response to IgE-mediated stimuli (allergen or anti-IgE antibodies) in mite-allergic asthmatic children, immediately at 4 and 9 months (5). A similar decrease in histamine release and leukotriene production from peripheral blood leukocytes has also been reported immediately after induction phase of rush venom SIT (6). The rapid desensitization observed in rush venom SIT is not due to IgE or IgG changes but could therefore be explained by early changes in cytokine or chemokine production that induce a rapid decrease in basophil or mast cell releasability (6, 7). In fact, some allergic models also show that decrease in histamine release following SIT was partially independent of reduction of specific IgE (8). The existence of intrinsic factors, IgE-independent, was already suggested in older studies (9-10) and the presence of inhibitory signaling receptors on mast cells (11) and on eosinophils (12) may represent one of the targets for the early effects of SIT on cell activation. Therefore, one of the aims of our work was to evaluate if SIT could also decrease eosinophil releasability, measured by kinetics of nasal ECP release after specific nasal challenge. Additionally we wanted

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to determine if that decrease could also be demonstrated immediately at 6 or 12 months of SIT and if it had relation with the allergen dose used in SIT.

MATERIAL AND METHODS

Patients

We included 75 moderate-severe persistent allergic rhinitis patients, monosensitized to house-dust mites (*Dermatophagoides*), as suggested by clinical history, skin prick tests and serum specific IgE. There were 37 females and 38 males, mean age 22.3 ± 7.7 years (range: 15-46 years), which were randomly assigned either to a control group which did not receive SIT, to a low-dose SIT group which received injections of a modified *Dermatophagoides pteronyssinus* extract with a monthly dose of 5.5 mg of the extract (annual dose = 75 µg) or to a high-dose SIT group which received injections of a modified *Dermatophagoides pteronyssinus* extract with a monthly dose of 55 mg of the extract (annual dose = 710 mg). All patients were allowed to take oral antihistamines and/or nasal steroids when needed. This study was approved by the Ethical Committee of the Faculty of Medicine of Lisbon and the patients gave their informed consent. Patients were assessed before starting immunotherapy (T0), after 6 months (T1) and after 12 months (T2).

■ **Specific immunotherapy (SIT):** We used a *Dermatophagoides pteronyssinus* extract, depigmented and polymerised with glutaraldehyde (DEPIGROID®, CBF LETI Laboratories, Spain), described in detail elsewhere (13).

All SIT-treated patients received a build-up phase with 5 weekly injections of increasing allergen doses (0.1, 1, 2, 4 and 5.5 µg in Group 1; 1, 5, 11, 33 and 55 µg in Group 2) and a maintenance phase with 11 monthly doses of 5.5 µg in Group 1 or 55 mg in Group 2. SIT was administered according to European Academy of Allergy and Clinical Immunology rules (14).

■ **Skin Prick Tests (SPT):** SPT were performed according to standard procedure (15) in the volar side of the forearm by means of puncture with the Prick Lancettes Dome-Hollister-Stier; one sterile device was used for each test. Common inhalant extracts tested were mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Blomia kulagini*, *Euroglyphus maynei*, *Tyrophagus putrescentia*, *Lepidoglyphus destructor*, *Acarus siro*), cockroach, moulds (*Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Mucor*, *Neurospora crassa*,

Penicillium notatum), pollens from grass (*Lolium perenne*, *Dactylis glomerata*, *Secale cereale*), trees (*Betula verrucosa*, *Olea europaea*, *Platanus acerifolia*) and weeds (*Artemisia vulgaris*, *Plantago lanceolata*, *Parietaria judaica*, *Salsola kali*) and dander from cat and dog. Histamine phosphate (10 mg/ml) and sterile 0.9% saline were used as positive and negative controls, respectively. We measured the area of the wheals induced by SPT, considering a positive response a mean wheal area of 7 mm² or greater compared with the negative control, 15 minutes after puncture.

■ **Visual Analog Scale (VAS):** In each visit patients were asked to mark on a VAS with 10 cm long the point which would best correspond to the intensity of their rhinitis symptoms during the previous month (10 cm = best possible; 0 cm = worst possible).

■ **Specific Nasal Provocation:** It was performed in each visit with a lyophilised *Dermatophagoides pteronyssinus* allergenic solution (CBF LETI Laboratories, Spain), which was reconstituted 30 minutes before provocation in order to obtain a solution with an allergenic potency of 100 HEP/ml (*Histamine Equivalent Potency*). This solution was serially tenfold diluted in order to obtain four other allergenic solutions with 10 HEP/ml, 1 HEP, 0.1 HEP and 0.01 HEP, respectively. We did not use any vasoconstrictor medication before nasal challenge and patients were asked not to use any nasal steroids during the two weeks and antihistamines during the three days that preceded nasal challenge.

Nasal provocation was always performed in the same room and by the same investigator. It started with nebulization of 0.05 ml of negative control, followed by 0.05 ml nebulizations of the allergenic solutions (20 minutes interval) with increasing potency. Nasal challenge was stopped when the sum of a nasal score (sneezes, itching, rhinorrhea, nasal obstruction; 0-no symptoms, 1-mild, 2-moderate, 3-severe) was superior or equal to 6 (maximum 12). Allergic threshold was therefore the potency of the last allergenic solution administered.

■ **Nasal Lavage Fluid (NLF) and ECP measurements in NLF:** Nasal lavage was performed before provocation and one and four hours after nasal challenge. We used the technique with warm saline described by Naclerio *and al.* (16), slightly modified in order to obtain a final volume of NLF of 2 ml per each nostril, to avoid excessive dilution. NLF was centrifuged at 1500 g for 15 minutes. We removed the upper 3 ml and resuspended the centrifuge in the remaining 1 ml. ECP was measured in this 1 ml suspension by immuno-fluoroenzymatic method ECP UNICAP FEIA (Pharmacia Diagnostics, Sweden).

Besides measuring individual ECP values in each NLF, we considered the existence of two main nasal ECP patterns: an "activation" pattern if nasal ECP values at 1 or 4 hours increased at least 100% comparing to pre-provocation values and a "non-activation" pattern if there were no such increases.

RESULTS

Regarding subjective evaluation in the VAS (diagram 1) we have observed in control patients maintenance of mean VAS scores, while in the two SIT-treated groups mean VAS scores were significantly ($p < 0.05$) improved, comparing initial and final values (from 5.71 to 6.74 cm in Group 1 and from 5.61 to 6.99 cm in Group 2 patients).

Regarding objective evaluation in nasal challenges we have observed an increase in the allergenic concentration that SIT-treated patients tolerated, while in the control group there were no significant differences. In the control group, the mean concentration that induced a positive nasal challenge was 5.24 ± 4.66 HEP at the beginning of the study, 5.64 ± 4.63 HEP at 6 months and 4.88 ± 4.63 HEP at the end. On the contrary, in Group 1 there was an increase from 4.49 ± 4.59 HEP observed at the beginning of the study to 5.53 ± 4.75 HEP at 6 months and to 11.40 ± 18.87 HEP at the end of the study. In Group 2 there was an even more pronounced increase in allergenic threshold for a positive provocation from 4.34 ± 4.72 HEP at the beginning of the study to 6.68 ± 4.51 HEP at 6 months and to 23.32 ± 34.28 HEP at the end of the study.

In the distribution of individual allergenic thresholds (diagram 2) we could clearly see this improvement in allergenic tolerance in SIT-treated

patients, with reduction (in Group 1) or even disappearance (in Group 2) of positive challenges with lower allergenic concentrations (0.1 HEP) and appearance of some patients (one in Group 1 and four patients in Group 2) who had positive challenges only with the highest concentrations (100 HEP). Both SIT-treated groups had 80% increase in the number of patients who had positive challenges with a relatively high allergen concentration (10 HEP).

These differences were statistically significant whether comparing evolution within each one of the SIT-treated groups before and after 12 months treatment ($p < 0.01$) or comparing each one of SIT-treated and control groups ($p < 0.05$).

In diagram 3 we have represented mean nasal ECP values of the three groups in T0, T1 and T2. In the beginning of the study, all three groups showed similar behaviour after nasal provocation, with mean increases superior to 70% in all groups, without any significant differences between them. However, not all patients displayed similar magnitude of nasal ECP increases and some patients had maximum values at one hour while other had maximum ECP values at four hours after nasal challenge. In view of this heterogeneity we decided to categorize variations in nasal ECP values into two patterns of nasal ECP kinetics. These patterns were evaluated during each nasal provocation and classified in "activation" or "non-activation" patterns, as defined above. The relative percentage of "activation" and "non-activation" patterns in each group in different timings is indicated in diagram 4. In T0 we could see that there was a similar number of patients in each group with these two patterns and therefore we believe that the introduction of these patterns did not lead to any distortion of nasal ECP results.

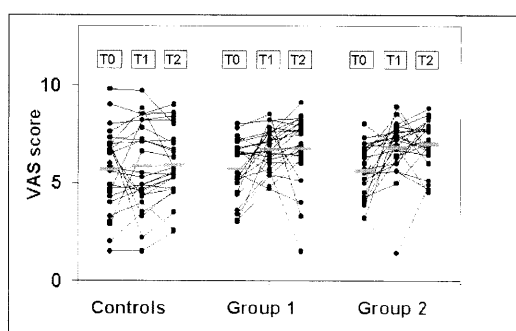


Diagram 1: Evolution of VAS scores in the three groups.
- mean values
T0 – beginning of the study; T1 – 6 months; T2 – 12 months.

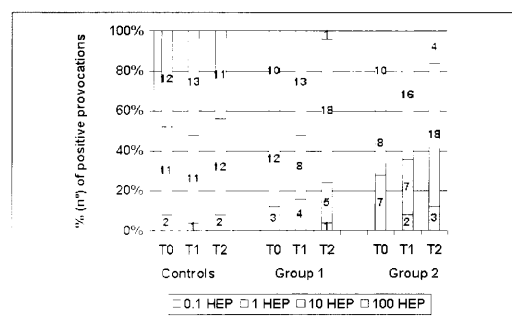


Diagram 2: Evolution of allergenic thresholds in nasal challenges.

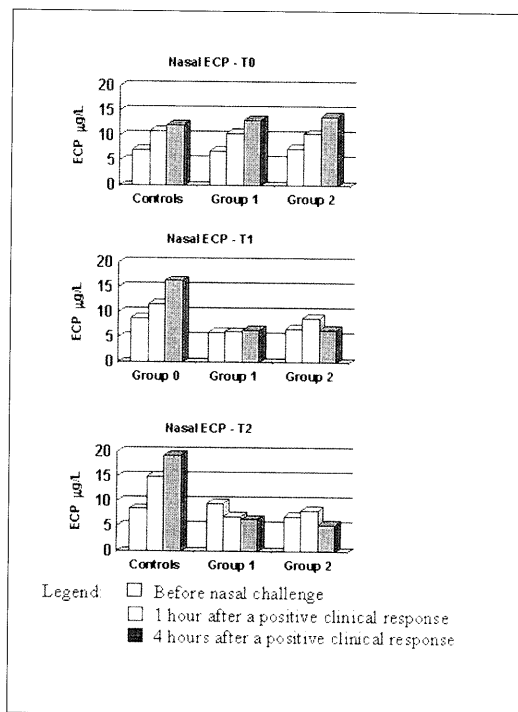


Diagram 3: Evolution of mean ECP nasal values in the three groups.

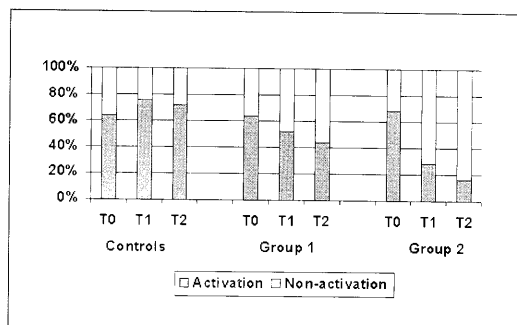


Diagram 4: Evolution of relative percentage of ECP patterns in the three groups.

In T0 the three groups are absolutely comparable but it is clear that evolution in T1 and T2 of the two SIT-groups is quite different from evolution of the control group. In the latter, we registered a small increase of mean nasal ECP levels after nasal provocation as well as small increase in the number of patients displaying "activation" patterns. These changes were not statistically significant. However, in both SIT-treated groups we could observe, immediately at T1 and also at T2, an absence of any increase in mean nasal ECP levels after nasal provocation, a fact that was similar in both SIT-groups, without any statistically significant differences between the two SIT groups but with significant differences comparing at T2 each SIT-treated and control groups. In nasal ECP patterns we observed a

progressive reduction in the number of patients with activation patterns from T0 to T1 but also from T1 to T2, in both SIT groups. Chi-square statistical analysis at T1 showed a significant difference between control group and Group 2 ($p=0.002$) but not between control group and Group 1 ($p=0.141$). At T2 a difference between Groups 1 and 2 was visible, the latter having a clearly more pronounced decrease in the number of patients with "activation" patterns. Despite visible differences, statistical analysis by chi-square test did not show significant differences between the two SIT groups at T2 ($p=0.115$). However, comparing SIT-groups with control group at T2 we found only a slightly significant difference between control group and Group 1 ($p=0.046$) but a highly significant difference between control and Group 2 ($p<0.001$).

DISCUSSION

Visual analog scales are a frequently used method to evaluate allergic rhinitis. They are considered reliable (17), with correlation with clinical improvements (18) and with other parameters of assessment of SIT efficacy (19). Our results showed that in groups 1 and 2 there are mean improvements at T1 of 17.5% and 21.25%, respectively, with a small additional improvement at T2 in Group 2. Other studies have also demonstrated such early improvements in SIT-treated patients (20, 21). This early response is clearly an important aspect to patients and should be taken into account when deciding the prescription of SIT.

Reduction in nasal allergenic reactivity after specific challenge is an objective parameter, frequently used in studies evaluating SIT efficacy. In some studies significant differences were documented between pre and post-treatment values and also between control and SIT-treated patients (22-24).

In a similar way our study also clearly demonstrates a statistically significant reduction in nasal specific reactivity at T2, more intense in Group 2. Both improvements in VAS and allergenic thresholds show that SIT is effective, this efficacy being higher in patients receiving higher doses of the allergenic extract.

Nasal ECP values have been shown to increase after allergen challenge (25), presenting a discriminative value between allergic and non-allergic rhinitis (26) and between symptomatic and non-symptomatic allergic rhinitis patients (27, 28). In some studies, nasal ECP values also correlated with clinical scores (29). However, not all patients display significant ECP increase after nasal allergen challenges. This eosinophil "non-reactivity" that we have observed at T0 in

about one-third of our mite-allergic patients has also been reported in nasal challenges of other mite-allergic patients (30), reflecting heterogeneity in individual responses of allergic patients. In our study SIT practically abolished the increase in nasal ECP after allergen challenge in several SIT-treated patients. This fact was shown not only by reductions in mean nasal ECP levels after challenge but also by the reductions in the number of patients with "activation" patterns, both changes were statistically significant in the two SIT groups.

It is interesting that some studies showed that nasal ECP values could be decreased more effectively by SIT than by nasal steroids (31,32), even after short-term (one-year) SIT. In our opinion, all these data imply that in many allergic rhinitis patients, local eosinophil activation has clinical relevance and can be effectively modulated by SIT.

Although we did not find any good *in vitro* studies that analyzed the influence of SIT on eosinophil releasability after incubation with specific allergens, it is probable that the same mechanisms that induce a decrease in mast-cell and basophil reactivity may also act in a similar way in the eosinophil population. Besides, there are some *in vivo* studies showing that SIT can decrease allergen-induced eosinophil migration to target-organs (33, 34), possibly through reduction in the production of eosinophil chemotactic factors by T cells (35) or by inhibiting increase in adhesion to VCAM-1 and ICAM-1 induced by allergen exposure (36). This latter aspect was also focused in a very interesting study using an eosinophil locomotion assay where the authors found that blood eosinophils from allergic asthmatic patients migrated more than eosinophils from healthy non-allergic donors (37) and that eosinophil activation was accompanied by increase in cellular expression of VLA-4 (38), the ligand for VCAM-1, suggesting this finding as the explanation for the increased eosinophil migration in allergic patients. Cell migration and cell activation frequently coexist and therefore it is logical to assume from these studies that many eosinophils from allergic individuals are more activated than eosinophils from non-allergic individuals and that SIT can reduce this activation.

Since SIT can rapidly induce decreases in cell releasability in mast cell and basophils, our hypothesis was that effective SIT would also reduce local eosinophil releasability, measured by nasal ECP kinetics after allergen challenge. In our study we measured nasal ECP levels but we were more interested in documenting the presence of clear-cut allergen-induced early ECP release that would indicate the presence in nasal mucosa of eosinophils that rapidly and significantly degranulate after allergen exposure, showing that

these type of patients had a population of nasal eosinophils with a heightened releasability. Our results showed statistically significant reductions in ECP release after nasal challenge, reflected in mean nasal ECP levels and in the number of patients with eosinophil "activation" patterns. In mean nasal ECP levels there is no evidence of differences between Group 1 and Group 2 patients. However, considering "activation" pattern Group 2 had from T0 to T2 a 76% reduction in the number of patients with "activation", while Group 1 had only a 38% reduction, a difference that although clear did not reach statistical significance due to the relatively small number of patients involved in this study. These nasal ECP changes reflect, in our opinion, a decrease in activation and/or releasability of eosinophils already present in the nasal mucosa. This decrease is clearly induced by SIT (it does not occur in control patients) and is more pronounced (although as mentioned the difference at T2 was not statistically significant) and more early documented in patients receiving higher doses of the allergenic extract, since at T1 there were only significant differences between control and Group 2 patients ($p=0.002$).

One possible criticism to our work is the absence of measurements of eosinophil numbers in nasal mucosa. However, in our opinion the number of eosinophils in nasal mucosa is not very relevant to our findings for two main reasons. The first is that mean pre-provocation nasal ECP levels were not reduced in any group and therefore it is not probable that there would be very significant variations in the number of eosinophils. The second is that we measured the magnitude of individual increases in nasal ECP values after allergen challenge and the magnitude of those increases is influenced not by the number of eosinophils but by the degree of eosinophil responsiveness to allergen challenge. Furthermore, counting the number of eosinophils does not give any information on the activation state of that population, which we believe is a much more relevant fact.

Although more studies with more patients are needed, we think that our data point to an interesting area of research that should be looked into and we strongly believe that, if our findings are corroborated by other groups, the interference with local eosinophil activation may well become recognized as one of the mechanisms of action of SIT.

CONCLUSIONS

Mite-allergic rhinitis patients may show an early rise of nasal ECP levels after specific nasal provocation. In approximately two-thirds of our patients, these early

increases were quite marked (>100%), in comparison to pre-provocation levels. Mechanisms by which SIT acts and induces clinical benefits are multiple and in this study we have shown that 6 and 12 months of SIT can effectively inhibit the increase in nasal ECP after allergen challenge that remained present in control, non SIT-treated patients. This inhibition was demonstrated not only by evaluating mean nasal ECP levels in each group but also by evaluating the number of patients with nasal ECP "activation" patterns. In this latter evaluation, SIT had a more pronounced effect and reached statistical significance earlier in patients receiving higher doses of the allergenic extract (Group 2), a fact which found correspondence in the clearly more marked improvement in nasal challenges observed in Group 2 than in Group 1 patients.

Although more studies with more patients are needed, our study provides evidence that SIT can decrease, either directly or indirectly, local target-organ eosinophil activation. This mechanism of action of SIT is more intense and more clinically meaningful if high doses of the allergenic extract are used.

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PREMIES DELIVERED CESAREAN FACE HIGHER ASTHMA RISK

ARLINGTON HEIGHTS, IL – Cesarean delivery is associated with an increased risk of childhood asthma hospitalization in premature infants, but not in full-term infants, according to a report published in this month's *Annals of Allergy, Asthma & Immunology*, the scientific journal of the American College of Allergy, Asthma and Immunology (ACAAI).

The investigation by Jason S. Debley, M.D., M.P.H., and colleagues at the University of Washington, Seattle, provides new insight into why cesarean delivery may predispose an infant to having asthma. Previous study results on the association between mode of birth and asthma are conflicting, and do not take into account the effects of prematurity and maternal asthma according to the investigators.

Cesarean delivery alters the bacterial colonization of the gut, which normally occurs shortly after birth and may have a protective effect against the predisposition to asthma and allergies. Some investigators suggest that modification of the bacterial colonization by cesarean delivery may hinder the infant's tolerance to allergenic substances and increases the risk of asthma, in agreement with the hygiene hypothesis.

When analyzed separately, Dr. Debley and colleagues found a significant association between cesarean delivery and asthma hospitalization in premature infants, but not in full-term infants. They also reported that, since women with asthma have higher rates of both premature infants and Cesarean delivery, premature infants are more likely than full-term infants to have a maternal genetic predisposition for asthma.

The study evaluates more than 10,000 children aged 6 to 12 years using the Washington State Birth Events Record Database linked to state-wide hospitalization data.

The study was supported by a grant from Maternal and Child Health Bureau, Health Resources and Services Administration, Department of Health and Human Services, and a National Research Service Award grant.

Patient information on allergic diseases including asthma is available by calling the ACAAI toll free number at (800) 842-7777 or visiting its Web site at www.acaa.org.

The ACAAI is a professional medical organization comprising nearly 5,000 qualified allergists-immunologists and related health care professionals. The College is dedicated to the clinical practice of allergy, asthma and immunology through education and research to promote the highest quality of patient care.

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