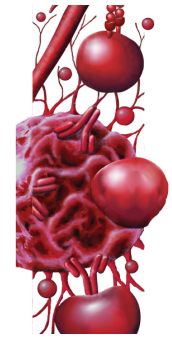
Research Article

Immunotherapy



Randomized dose–response study of subcutaneous immunotherapy with a *Dermatophagoides pteronyssinus* extract in patients with respiratory allergy

Aim: To evaluate the efficacy of *Dermatophagoides pteronyssinus* (DPT) subcutaneous immunotherapy in allergic rhinoconjunctivitis patients. **Patients & methods:** This 17-week double-blind study randomized 136 patients (95 evaluable) to five dose groups of DPT depot extract (0.0625–0.75 skin prick test [SPT] units) or placebo, administered in a six updosing schedule. **Results:** A dose–response was observed for clinical efficacy (allergen concentration needed to induce a positive nasal provocation test response from baseline to final visit) and safety (adverse reactions). Local and systemic reactions occurred with 14.8 and 6.4% of administered doses, respectively; a single anaphylactic reaction occurred in each of Groups 3, 4 and 5 (0.3% of doses). **Conclusion:** The risk–benefit profile appeared most favorable with a DPT dose of 0.125 SPT units.

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Keywords: *Dermatophagoides pteronyssinus* • house dust mite • rhinoconjunctivitis • subcutaneous immunotherapy

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Allergic rhinitis is a common disease, particularly in western countries where it is estimated to affect at least 10–25% of the population [1–3]. Its prevalence is increasing globally as more societies adopt western lifestyles [3,4]. Allergic rhinitis is often associated with conjunctivitis [5], and comorbidity between allergic rhinitis/rhinoconjunctivitis and asthma is common [3,5–7]. Moreover, sensitization to house dust mite airborne allergens is recognized as a causative factor for both allergic rhinitis/rhinoconjunctivitis and allergic asthma [8–10]. In western countries, approximately 20% of the population are sensitized to the most common house dust mite species, *Dermatophagoides pteronyssinus* (DPT), and this figure increases to 37% among people with asthma [11].

General options for the management of allergic rhinitis/rhinoconjunctivitis include allergen avoidance, pharmacotherapy and specific immunotherapy [12,13]. Avoidance of house dust mites is not always feasible however and, while drug therapy can be effective at relieving symptoms, it does not alter the course of the disease. Allergen-specific immunotherapy involves repeated administration of extracts of the specific offending allergen in order to modulate the immune system and induce tolerance to the antigen. In contrast to pharmacotherapy, allergen-specific immunotherapy has the potential to provide long-term benefits after treatment has ended [14,15]. Meta-analyses and evidence-based reviews focusing specifically on immunotherapy directed against house dust mites support the use of immunotherapy for allergic rhinitis/rhinoconjunctivitis [16–18], although it is apparent that additional well-designed studies are needed to confirm the benefits of such therapies [18].

A new DPT extract for subcutaneous immunotherapy is under evaluation in accordance with the European Medicines Agency (EMA) guideline on clinical development of immunotherapy products [19]. A randomized

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placebo-controlled Phase 1 study conducted in adults with allergic rhinoconjunctivitis (with or without asthma) found that all three dose-escalation schedules of DPT extract under investigation had good short-term safety and tolerability profiles, and that all active treatment groups demonstrated decreased cutaneous reactivity and increased specific immunoglobulin G (IgG) levels [20]. The study also established the optimal dose-escalation scheme to use for future studies.

Although a dose–response relationship has been demonstrated in some allergen immunotherapy studies, few dose–response studies have been performed with subcutaneous house dust mite immunotherapy [21]. The aim of the current randomized controlled trial was to compare the efficacy of five different doses of the new depot DPT extract subcutaneous immunotherapy in patients with allergic rhinoconjunctivitis who were sensitized to DTP.

Patients & methods

Study design & ethical considerations

This double-blind, randomized, placebo-controlled, dose-ranging study was performed at 12 hospitals in Spain, in order to establish the dose–response relationship of different doses of depot DPT subcutaneous immunotherapy in patients with allergic rhinoconjunctivitis (with or without asthma). Patients were randomized to one of five active-treatment groups or placebo for a 17-week treatment period, which comprised a 5-week initiation phase, during which the concentration of DPT extract was increased gradually to reach the target maintenance dose, and a 12-week maintenance phase. Efficacy was evaluated using the nasal provocation test (primary variable), and by immunological assessments and skin prick tests (surrogate efficacy endpoints). Safety was assessed through evaluation of the number and severity of adverse events. The data collection period was from May 2012 to May 2013.

The study was conducted in accordance with the principles of the Declaration of Helsinki and the ICH guideline on good clinical practice and was approved by relevant ethics committees and the Spanish regulatory authorities. All patients provided written informed consent to participate.

Patients

To be eligible for the study, patients had to be adults aged 18–60 years with perennial allergic rhinoconjunctivitis due to DPT sensitization for at least 2 years prior to the study. Patients with concurrent mild or moderate asthma were allowed to participate. Patients had to have a skin prick test result of ≥3 mm diameter against DPT, and specific immunoglobulin E (sIgE) levels of ≥0.7 kUA/L against DPT determined by ImmunoCAP® (Thermo Scientific, Uppsala, Sweden). Patients were preferably sensitized only to DPT, although patients sensitized to other mites with cross reactivity to DPT were also accepted. Patients were tested toward the more prevalent allergenic sources in the geographic area in which they were recruited. Patients who were polysensitized to other allergens could be included only in the following circumstances: sensitizations to pollens whose season did not overlap with the data collection period (or, if there was overlap, were associated with sIgE levels of <0.7 kUA/l); sensitizations to perennial allergens where sIgE levels were <0.7 kUA/l; sensitizations to allergens where environmental levels were not high enough to produce symptoms during the study period.

Patients were excluded from participation if they were sensitized to other perennial or seasonal allergens and had sIgE levels of ≥0.7 KUA/l (including other mites unless they were cross-reactive to DPT). They were also excluded if they had used continuous medication to treat their allergic condition during the 2 weeks prior to the study, were receiving immunotherapy for any other allergen or had received immunotherapy against DPT or a cross-reactive allergen in the 5 years prior to the study. Patients with severe asthma, a forced expiratory volume in first second of less than 70%, or whose asthma required treatment with inhaled or systemic corticosteroids in the 8 weeks prior to the study were ineligible. Patients were also excluded if they met any of the following criteria: a history of anaphylaxis; chronic urticaria; moderate-to-severe atopic dermatitis; immunological, cardiac, renal or hepatic diseases; upper respiratory tract malformations; current treatment with tricyclic antidepressants, psychotropic drugs, beta-blockers or angiotensin-converting enzyme inhibitors; and women who were pregnant or breastfeeding, or of child-bearing age and not using adequate contraception.

Study treatment

The allergenic product used in the study was a sterile depot suspension of DPT native extract (Allergovac® depot, Bial-Aristegui, Zamudio, Spain) for subcutaneous administration, which was adsorbed to 0.33% aluminum hydroxide to produce prolonged release of the allergen from the injection site. Patients were assigned to active-treatment or placebo groups using computer-generated permuted-block randomization, with equal numbers assigned to each group.

During the 5-week initiation phase, patients received increasing concentrations of DPT extract at intervals of 1 week (± 2 days) in order to reach their target maintenance dose, which was 0.0625, 0.125, 0.25, 0.5 or 0.75

skin prick test (SPT) units for each of the five active-treatment arms. The doses were achieved using DPT extract Vial 2 (volumes 0.1, 0.2, and 0.5 ml) during weeks 1, 2 and 3, and Vial 3 (volumes 0.1, 0.2 and 0.5 ml) during weeks 4, 5 and 6. During the subsequent 12-week maintenance phase, patients received three maintenance doses at intervals of 4 weeks (± 1 week). Patients in the placebo group received an inactive substance that was physically indistinguishable from the active drug and administered using the same treatment schedule. All study medication was administered to patients by trained nurse, under the supervision of an allergist, at the hospital study sites.

Dose modifications were allowed in the event of adverse reactions, and followed the recommendations of Alvarez-Cuesta *et al.* [22].

Outcome measures

The primary efficacy endpoint was the change in the concentration of DPT extract needed to produce a positive nasal provocation test at the final visit compared with baseline. Secondary (surrogate) endpoints included changes in serum immunoglobulin levels (allergen-specific IgE, IgG and IgG_4) and change in cutaneous reactivity. The main safety endpoints were the number and severity of adverse events.

Nasal provocation tests, immunological assessments and skin prick tests were performed at baseline (prior to the initiation phase) and at the final visit (after 17 weeks of treatment). Patients were assessed for adverse events at each hospital visit for treatment administration. Patients used diaries to record any delayed reactions to injections they may have experienced after leaving the hospital following treatment administration, and any other adverse events that occurred between visits.

Nasal provocation testing was carried out in accordance with guidance from the Spanish Society of Allergy and Clinical Immunology [23]. Testing was performed using a negative control and then increasing doses of allergen extract (0.005, 0.05, 0.5 and 5.0 SPT units) until a positive response was elicited. After each test administration, the number of sneezes in the subsequent 15 min was counted, the patient rated his/her nasal itchiness and secretions using a 4-point scale (0 = no symptoms, 1 = mild, 2 = moderate and 3 = severe), and nasal inspiratory flow was measured with the highest of three measurements being used to assess response. A positive response was defined as ≥5 sneezes or a decrease of >50% in nasal inspiratory flow in the 15 min interval between each administration. Patients who did not develop a positive response at the final visit were assigned a concentration of 5.0 SPT units/ml for calculation purposes.

Skin prick testing was performed using four increasing concentrations of DPT extract (0.005, 0.05, 0.5 and 5 SPT units/ml) as well as positive (histamine 10 mg/ml) and negative (saline) controls. The change in cutaneous reactivity (wheal area in mm^2) from baseline to the final visit was assessed.

Specific immunoglobulin levels (IgE, IgG and IgG_4) were determined blindly in serum samples obtained at baseline and final visit. Samples were frozen and sent to BIAL central laboratory for analysis.

Adverse events recorded during the study were evaluated by investigators and graded for their severity and relationship with the investigational product. Adverse reactions related to the study treatment were classified as local or systemic. Local reactions were contained at the injection site, and were classified as immediate (within 30 min after the injection) or delayed (>30 min after the injection). Systemic reactions were classified according to the EAACI classification [22] and the Medical Dictionary for Regulatory Activities (MedDRA) Version 15.1.

Statistical methods

Descriptive efficacy analyses were performed using the full analysis set (randomized patients who met all inclusion/exclusion criteria, received at least one dose of active treatment or placebo and had available data on the primary variable) and the per-protocol population (randomized patients who achieved their target maintenance dose and completed the study without any major protocol deviations). Formal statistical analyses were performed using the per-protocol population. The safety analysis included the intent-to-treat population, which comprised all randomized patients who received at least one dose of treatment.

The differences between groups, including the placebo group, for the mean change from baseline to final visit in the concentration of DPT extract needed to produce a positive nasal provocation test, mean change in immunoglobulin levels and mean change in skin prick test values were analyzed by analysis of variance (ANOVA) or the Kruskal–Wallis test. Comparison of the results between groups two by two was performed using the Mann–Whitney test or post hoc tests. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 17.0 (SPCC Inc., Chicago, IL, USA).

A formal sample size calculation was not performed as it is considered unnecessary to obtain statistically significant differences between dose groups in dose-finding studies, provided that a clear trend across doses can be established [24]. Moreover, prior efficacy data were not available to establish the necessary assumptions. It was considered that 25 patients per

group would provide adequate data on the primary endpoint.

Results

A total of 136 patients were randomized into the study between May and November 2012. Six patients were screening failures because of not having met the inclusion/exclusion criteria relating to polysensitization and were considered nonevaluable, leaving 130 patients in the safety analysis set. After exclusion of another nine patients (two lacked efficacy data due to a positive nasal provocation test with the negative control at the final visit; two withdrew from the study because of an adverse reaction; three were lost to follow-up; one did not meet the inclusion criteria, but received treatment; and one withdrew prematurely for a nonspecified reason), the full analysis set comprised 121 patients. A further 26 patients were excluded mainly as a result of protocol deviations (major or minor; n = 15) or early discontinuation because of an adverse event (n = 8), leaving 95 patients in the per-protocol population. Patient disposition is shown in Figure 1.

The mean age of the study population was 29 years and the number of males and females was 59 and 62, respectively. The baseline demographics and clinical characteristics of patients were generally similar among the dose groups (Table 1). Overall, 104 patients (86%) had persistent allergic rhinoconjunctivitis (26 mild; 78 moderate to severe) and 17 (14%) had intermittent allergic rhinoconjunctivitis (2 mild; 15 moderate to severe). Approximately 30% of patients had concurrent asthma and 13% had received previous immunotherapy with DPT or cross-reacting allergens.

Nasal provocation test

An increase in the mean allergen concentration needed to induce a positive response in the nasal provocation test was observed at the final visit in all active treatment groups compared with baseline, achieving statistical significance in Group 4 (0.990 vs 0.035, $p < 0.01$); see Table 2. The difference between baseline and final visit concentrations needed to induce a positive response was generally greater with higher doses of depot DPT, suggesting a dose-dependent effect (primary endpoint, Figure 2), although comparisons between groups were not statistically significant ($p = 0.828$; ANOVA). The mean allergen concentrations needed to induce a positive response at the final visit did not differ significantly across groups ($p = 0.399$; ANOVA); see Table 2.

Immunoglobulin levels

Mean changes in immunoglobulin levels from baseline to final visit were generally greater with higher doses of depot DPT and differed significantly across dose groups (Figure 3). Increases in serum-specific IgG and IgG_4 levels were observed with active treatments and were significant compared with placebo (both $p < 0.001$; ANOVA); Figure 3. Serum-specific IgE levels decreased in all groups, although statistical significance versus placebo was achieved only for Group 4 ($p = 0.002$; Mann–Whitney) and Group 5 ($p = 0.037$; Mann–Whitney); see Figure 3.

Cutaneous reactivity

Duplicate dose–response skin prick tests showed that cutaneous reactivity to the allergen decreased at the final visit compared with baseline in all groups, although statistical significance was achieved only with active treatment and not with placebo. Wheal area was significantly reduced at the final visit compared with baseline using at least one of the four test vials in all active-treatment groups except Group 3 (0.25 SPT); see Figure 4.

Safety

During the study, 106 patients (81.5%) reported at least one adverse event with the most frequent being headache (126 events), delayed local reaction (50 events) and local reaction (28 events). Most of these events were nonserious and mild or moderate in severity. As expected, patients in Groups 3, 4 and 5, the highest treatment concentrations, experienced a greater number of adverse events.

Thirty-four patients (36.6%) experienced adverse reactions related to study medication. This average is derived from percentages per active-treatment group which ranged from 22.7% in Group 1 to 54.5% in Group 5, versus 9.5% in the placebo group. Out of 1147 dose administrations in total, 243 (21.2%) were associated with adverse drug reactions, including 170 local reactions (14.8%) and 73 systemic reactions (6.4%); Table 3. Across active-treatment groups, 10.4–26.0% of administrations were associated with local adverse drug reactions, compared with <5% in the placebo group. Only a few of the local reactions (n = 13; 1.1% of administered doses) were considered clinically significant, leading to dose adjustment at the next dose administration; at least one such reaction occurred in each active-treatment group, except Group 1. The majority of systemic adverse drug reactions were EAACI grade I (3.9% of dose administrations), followed by grades II (1.2%), III (0.6%), 0 (0.3%) and IV (0.3%) (Figure 5). There was a trend for the number, and also the severity, of adverse reactions to increase with increasing doses of DPT, particularly in Groups 3–5 (0.25–0.75 SPT); see Figure 5 and Table 3.

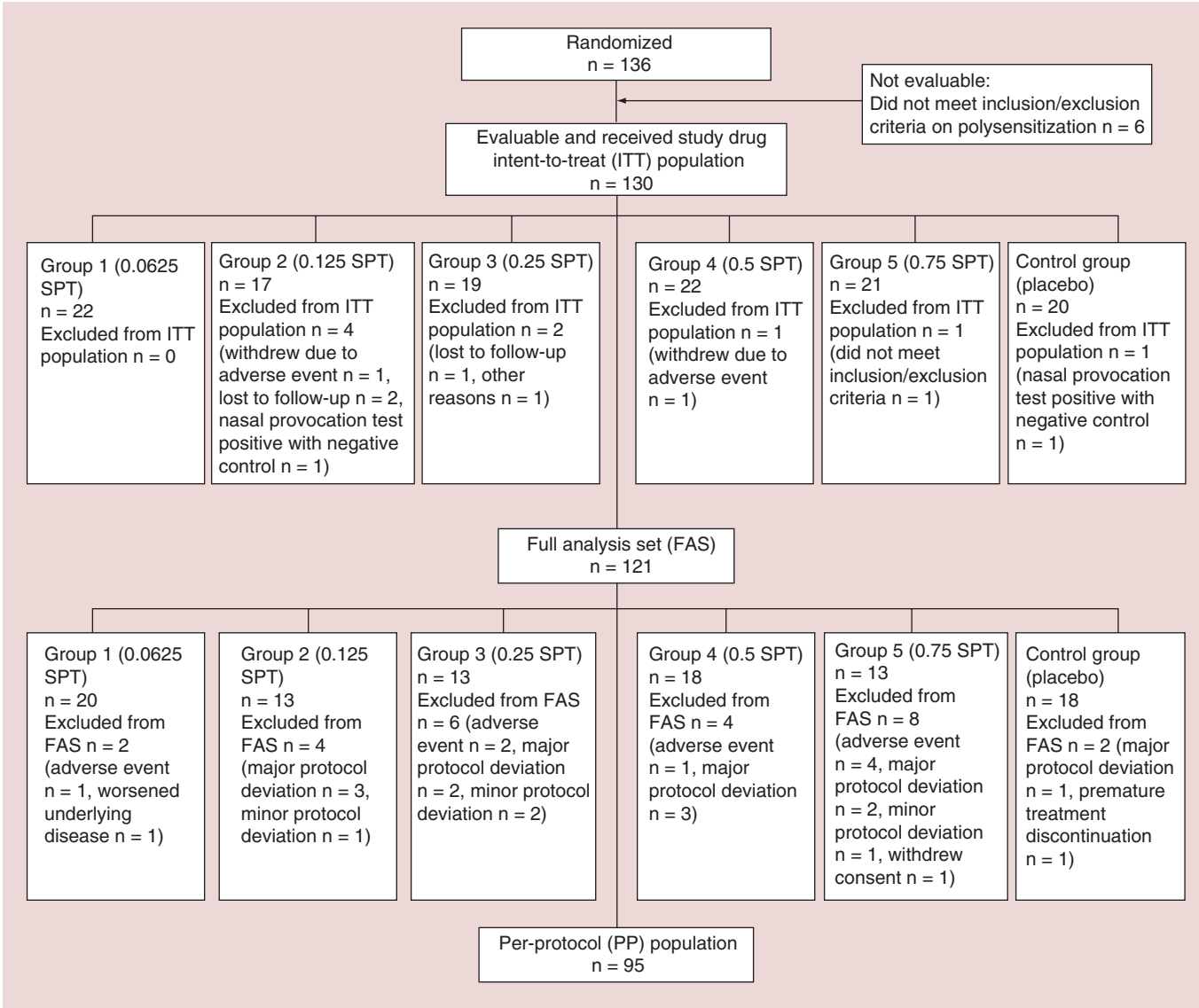

Figure 1. Patient disposition.

A grade III reaction occurred in each of Groups 1 and 2 and a grade IV anaphylactic reaction occurred in each of Groups 3, 4 and 5. All reactions were treated with corticosteroids and adrenaline until complete recovery. Ten patients (7.7%) withdrew from the study because of adverse drug reactions. At least one patient withdrew from each active-treatment group, although the majority of withdrawals (four patients) occurred in Group 5 (Table 3). All adverse reactions leading to withdrawal were grade III or IV systemic reactions, 50% of which were late reactions and occurred after patients had received between four and ten doses of study medication. All patients recovered from these events after receiving adrenaline, corticosteroids, antihistamines or bronchodilators.

There was no clear difference in the safety profile of the DPT extract between Group 1 and Group 2 (depot DPT 0.0625 and 0.125 SPT, respectively). Group 1 had more systemic reactions but of lower grade, whereas Group 2 had fewer systemic reactions but of generally higher grade. Based on the incidence of adverse drug reactions per total number of treatment administrations, Group 2 had the most favorable safety profile.

Discussion

The current dose–response study was designed in line with the EMA guideline on development of immunotherapy [19] and the position paper of the EAACI taskforce on 'dose–response relationship in allergen-specific immunotherapy' [21]. Although the latter, which is newer, recommends using symptoms/medication scores as the main outcome index, between-group differences in these outcomes are difficult to demonstrate

Table 1. Baseline demographics and clinical characteristics per group, with target maintenance doses of DPT depot extract in skin prick test units.

Characteristics	Group 1	Group 2	Group 3	Group 4	Group 5	Placebo
	SPT 0.0625	SPT 0.125	SPT 0.25	SPT 0.5	SPT 0.75	SPT 0
Number, n	22	17	19	22	21	20
Age (years), mean ± SD	31.3 ± 9.2	27.0 ± 8.5	29.5 ± 9.7	29.1 ± 7.3	28.3 ± 6.8	27.6 ± 8.4
Sex, n (%)						
– Female	12 (54.5)	9 (52.9)	11 (57.9)	9 (40.9)	11 (52.4)	10 (50.0)
– Male	10 (45.5)	8 (47.1)	8 (42.1)	13 (59.1)	10 (47.6)	10 (50.0)
Type of rhinitis, n (%)						
– Mild intermittent	–	2 (11.8)	–	–	–	–
– Moderate/severe intermittent	3 (13.6)	1 (5.9)	2 (10.5)	2 (9.1)	6 (28.6)	1 (5.0)
– Mild persistent	3 (13.6)	3 (13.6)	6 (31.6)	7 (31.8)	3 (14.3)	4 (20.0)
– Moderate/severe persistent	16 (72.7)	11 (64.7)	11 (57.9)	13 (59.1)	12 (57.1)	15 (75.0)
Asthma comorbidity, n (%)	8 (36.4)	5 (29.4)	5 (26.3)	9 (40.9)	5 (23.8)	4 (20.0)
Previous immunotherapy to DPT or cross-reacting allergens, n (%)	4 (18.2)	2 (11.8)	2 (10.5)	2 (9.1)	3 (14.3)	3 (15.0)
Specific immunoglobulin E level (ng/ml), mean ± SD	21.4 ± 36.0	21.6 ± 24.4	46.5 ± 58.6	30.0 ± 32.8	27.0 ± 33.7	34.1 ± 45.8
Positive NPT response with Vial 4 (5 SPT units), n (%)	1 (4.5%)	1 (5.9%)	1 (5.3%)	2 (9.1%)	3 (14.3%)	0 (0%)

DPT: Depot ***Dermatophagoides pteronyssinus*** extract; NPT: Nasal provocation test; SD: Standard deviation; SPT: Skin prick test.

during short-term treatment. The nasal provocation test provides a clinical endpoint that can be modified in different study groups during short-term immunotherapy.

The aim of the study was to compare the efficacy and tolerability of five different doses of a depot subcutaneous immunotherapy preparation directed against the most common species of house dust mite, DPT, also including a placebo control group, in patients with allergic rhinoconjunctivitis due to this allergen.

Treatment with the depot DPT extract for 17 weeks reduced reactivity to the DPT allergen, based on results of the nasal provocation test, immunoglobulin levels and skin prick test. The threshold concentration of allergen needed to produce a positive response in the nasal provocation test increased after treatment with all tested doses of the depot DPT extract (0.0625, 0.125, 0.25, 0.5 and 0.75 SPT units), with evidence of a dose–response relationship, although a statistically significant difference from baseline was seen only in Group 4 (depot DPT 0.5 SPT units).

The clinical efficacy of another DPT subcutaneous immunotherapy product (DPT allergoid adsorbed to $_{L}$-tyrosine) was demonstrated using a similar nasal provocation test in patients with allergic rhinitis and/or asthma secondary to DPT hypersensitivity [25]. The study evaluated two different dosing schedules of DPT extract: a conventional escalating dose regimen over 3 weeks and a cluster schedule (more than 1 dose/visit) over 4 weeks. While direct comparisons between this study and our own work are precluded by differences in formulations, dosing schedules, allergen challenge dosages and outcomes reporting, the significant decreases in clinical symptoms during nasal challenge recorded after treatment with the DPT extract complement our results.

Few dose–response studies of subcutaneous DPT immunotherapy have been published. Among two studies in patients with allergic asthma, one reported a dose-related increase in tolerance to bronchial challenge [26] whereas the other found a dose–response relationship for IgG levels but not for clinical symptoms [27]. Evidence of a dose-dependent effect on clinical symptoms has been reported for sublingual house dust mite immunotherapy in patients with allergic rhinoconjunctivitis (with or without asthma) [28,29]. Direct comparisons with the current study are not possible because of the different doses used for each preparation and because different methods were used to assess response. In one study, improvements in the total combined rhinitis score (nasal symptom score) of 17.4, 26.0 and 28.8% relative to placebo were seen with doses of 1, 3 and 6 SQ-HDM [29]. In a study in which the total nasal symptom score was evalu-

Table 2. Nasal provocation test before and after treatment with DPT depot extract measured in skin prick test units per milliliter needed to achieve a positive response.

Group (dose)	N	Allergen concentration (mean ± SD)		p-value*
		Baseline	Final visit	
Group 1 (DPT 0.0625 SPT units)	20	0.442 ± 1.097	1.166 ± 1.978	0.115
Group 2 (DPT 0.125 SPT units)	13	0.483 ± 1.369	1.282 ± 2.129	0.103
Group 3 (DPT 0.25 SPT units)	13	0.514 ± 1.364	0.936 ± 1.817	0.372
Group 4 (DPT 0.5 SPT units)	18	0.035 ± 0.022	0.990 ± 1.857	<0.01
Group 5 (DPT 0.75 SPT units)	13	0.874 ± 1.839	1.978 ± 2.490	0.114
Placebo	18	0.133 ± 0.203	0.440 ± 1.158	0.241
p-value**		0.444	0.399	

*Wilcoxon test.
**ANOVA (Kruskal Wallis).
DPT: Depot ***Dermatophagoides pteronyssinus*** extract, with target maintenance dose in skin prick test units; N: Number of patients; SD: Standard deviation; SPT: Skin prick test.

ated using the Vienna Challenge Chamber, improvements of 26.6 and 48.6% were seen with doses of 6 and 12 developmental units of MK-8237 [28]. The current study used nasal provocation testing to evaluate the response to depot DPT extract under controlled conditions, combining symptom scores with nasal inspiratory flow measurements to provide a more objective assessment of changes, as described by Dordal *et al.* [23]. The results suggested a trend toward a dose-dependent increase in efficacy with increasing doses from 0.0625 SPT units to 0.75 SPT units, with the exception of Group 3 (0.25 SPT units). Although the dose of immunotherapy was higher than the dose administered to Group 2, the allergen concentration needed to induce a positive response in the nasal provocation test was similar to that in Group 2. As the baseline characteristics of Group 3 were comparable to those of the other groups, the reason for this discrepancy is not clear. Possible explanations are that some patients needed more treatment doses, an

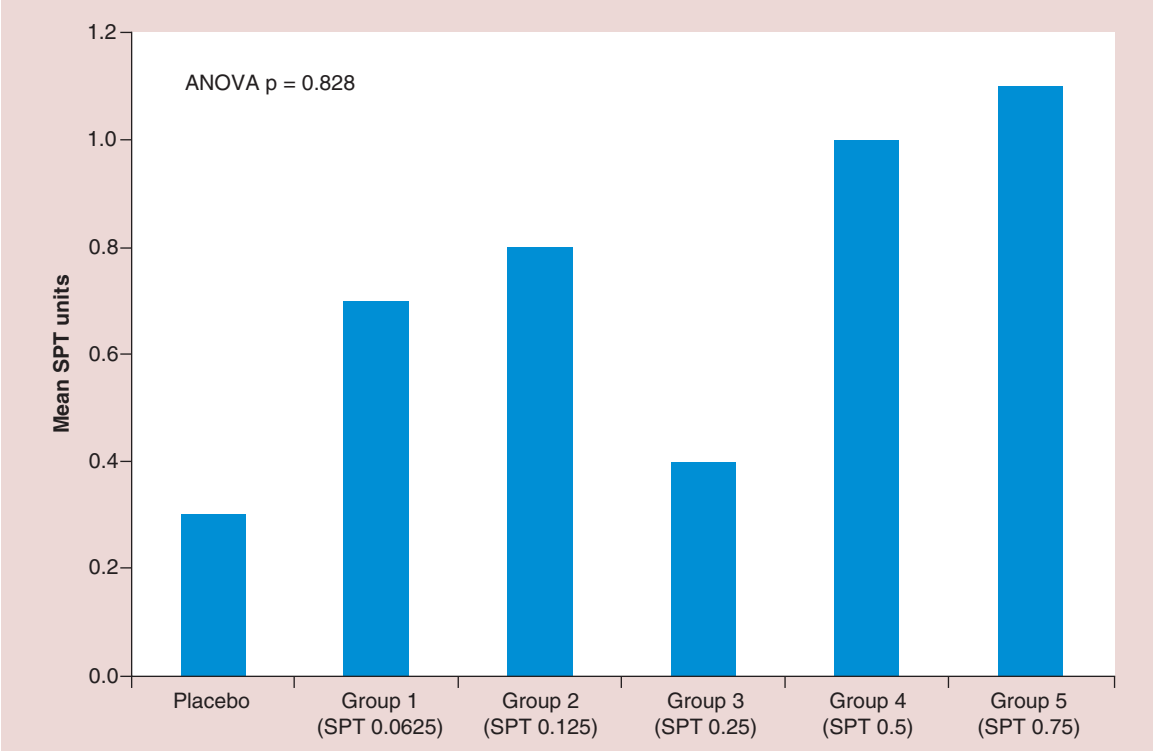

Figure 2. Changes in allergen concentration (mean skin prick test units/ml) needed to induce a positive nasal provocation test between final visit and baseline.

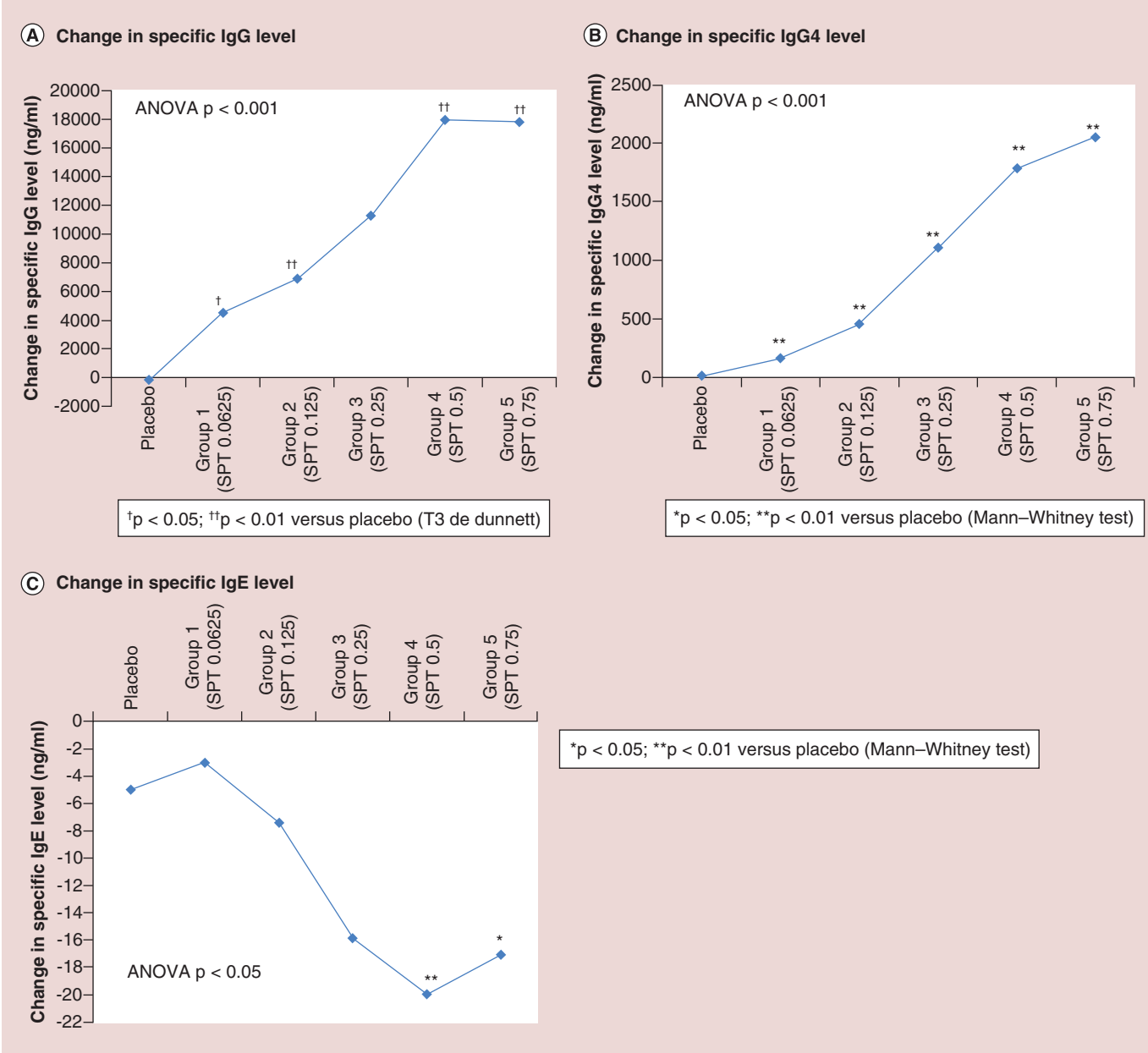

Figure 3. Change in mean immunoglobulin levels at final visit compared with baseline, between treatment groups versus placebo.
SPT: Skin prick test.

increased dose or concentrations above 5 SPT units/ml in the nasal provocation test to achieve a positive response. The last option is not feasible, however, as the percentage of patients in Group 3 with a positive response in the nasal provocation test with Vial 4 (5 SPT units) was similar to that in Groups 1 and 2 and smaller than that in Groups 4 and 5.

The study also found evidence of a dose-dependent decrease in skin reactivity after treatment with the depot DPT extract. Furthermore, a significant increase in the serum level of allergen-specific IgG and IgG_4 was seen in all groups, indicating that an immunological response was induced even at the lowest dose level of DPT extract and not with placebo. This dose-dependent effect is consistent with a previously published report [30]. There has been suggestion that successful specific immunotherapy is associated with an increase in IgG blocking activity that is not solely dependent on the quantity of IgG antibodies. It appears to be relevant, rather, to measure the blocking activity or affinity of allergen-specific IgG subsets, particularly IgG_4, instead of their total levels in sera [31]. However, measuring IgG total levels is a simple test that provides additional data and the increase in IgG_4 levels is also reported.

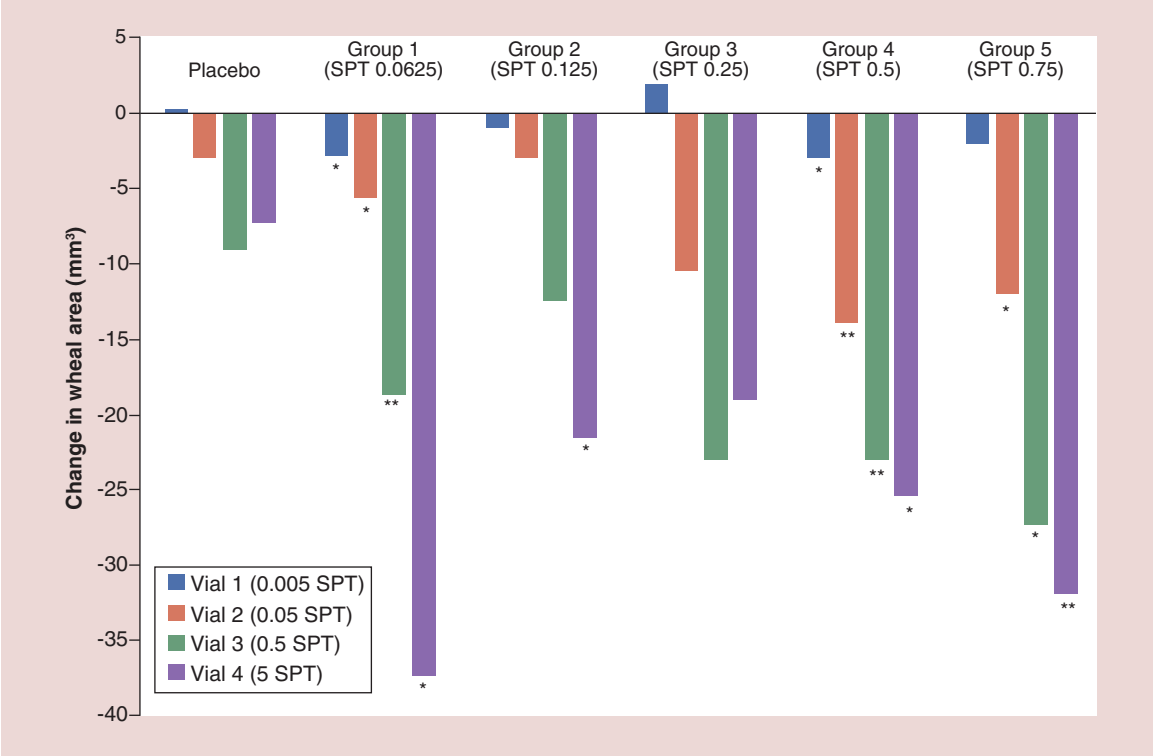

Figure 4. Reduction in skin reactivity: change in wheal area at final visit compared with baseline.
*p < 0.05; **p < 0.001 for final visit versus baseline.
SPT: Skin prick test.

Allergen-specific immunotherapy can be associated with local and systemic adverse reactions, although in the majority of cases symptoms are readily reversible [15,32]. In the current study, most adverse drug reactions were local reactions (14.8% of 1147 dose administrations) and only a fraction of them (1.1%) led to dose adjustments. Local reactions are known to be a common occurrence during subcutaneous allergen immunotherapy, affecting 5–82% of patients depending on the definition used; however, they are usually of little concern to patients and are not predictive of future systemic reactions [33]. Among the systemic reactions that occurred in the current study, most were EEACI grade I. Both the incidence and severity of adverse reactions tended to increase with increasing dose, particularly at doses of 0.25 SPT and above. There was no clear difference in the safety profile of DPT extract between the two lowest dose groups. Other studies of house dust mite immunotherapy have also found evidence of a dose response in terms of adverse event rates [26,27,29].

The main limitation of the study was the small sample size in each group, which may have contributed to the lack of statistically significant differences in efficacy parameters in all groups. Furthermore, as the maximum dose that could be studied in the nasal provocation test was 5 SPT, it was not possible to demonstrate an improvement at the final visit in patients who had had a positive response with Vial 4 (5 SPT units) at baseline.

In summary, there was evidence of a dose-related effect with the DPT extract, with an overall tendency towards a greater efficacy response at higher doses, but at the expense of an increased risk of adverse drug reactions. The risk–benefit profile appeared to be most favorable in Group 2 (DPT 0.125 SPT units), although this needs to be confirmed in larger trials.

The administration of immunotherapy, particularly subcutaneous formulations, directed at house dust mite allergens is known to reduce symptoms and associated medication use in patients with allergic rhinitis/rhinoconjunctivitis [16–18]. Although the evidence available to date on this newer subcutaneous DPT depot immunotherapy can only be considered preliminary, it indicates that the preparation shows promise and that it warrants further evaluation for the treatment of allergic rhinoconjunctivitis.

Conclusion

In vivo and *in vitro* data have demonstrated the efficacy of five different doses of DPT extract administered as a depot preparation for 17 weeks, showing

Table 3. Adverse reactions.

	Number of adverse reactions (% of total dose administrations)					
	Group 1	Group 2	Group 3	Group 4	Group 5	Placebo
	SPT 0.0625	SPT 0.125	SPT 0.25	SPT 0.5	SPT 0.75	SPT 0
Number of dose administrations	185	183	180	216	194	189
Adverse reactions	32 (17.3)	26 (14.2)	39 (21.7)	71 (32.9)	66 (34.0)	9 (4.8)
Local†	21 (11.4)	19 (10.4)	25 (13.9)	56 (26.0)	42 (21.6)	7 (3.7)
– Immediate	1 (0.5)	2 (1.1)	4 (2.2)	13 (6.0)	1 (0.5)	1 (0.5)
– Delayed	20 (10.8)	17 (9.3)	21 (11.7)	43 (20.0)	41 (21.1)	6 (3.2)
Local reactions (clinically significant)‡	0 (0)	5 (2.7)	1 (0.5)	4 (1.9)	3 (1.5)	0 (0)
Systemic§	11 (6.0)	7 (3.8)	14 (7.8)	15 (6.9)	24 (12.4)	2 (1.1)
– Grade 0	1 (0.5)	0 (0)	0 (0)	3 (1.4)	0 (0)	0 (0)
– Grade I	9 (4.9)	3 (1.6)	11 (6.1)	6 (2.8)	14 (7.2)	2 (1.1)
– Grade II	0 (0)	3 (1.6)	1 (0.5)	4 (1.9)	6 (3.1)	0 (0)
– Grade III	1 (0.5)	1 (0.5)	1 (0.5)	1 (0.5)	3 (1.5)	0 (0)
– Grade IV	0 (0)	0 (0)	1 (0.5)	1 (0.5)	1 (0.5)	0 (0)
Serious adverse reactions	1 (0.5)	4 (2.2)	3 (1.7)	6 (2.8)	10 (5.2)	0 (0)
Adverse reactions leading to withdrawal	1 (0.5)	1 (0.5)	2 (1.1)	2 (0.9)	4 (2.1)	0 (0)

†Local immediate (occurred in the first 30 min after administration) and local delayed (occurred more than 30 min after administration).
‡Local reactions that induce a dose modification.
§Classified according to the European Academy of Allergy and Clinical Immunology (EAACI) guideline.

a superior effect with higher allergen concentrations. Evidence of a dose–response effect was observed for both efficacy and safety parameters. On account of the clear tendency toward an increase in the number of serious adverse events from Group 3 onward, the Group 2 dosage (DPT 0.125 SPT units) was selected as appropriate for future large clinical trials. Compared with Group 1, the Group 2 dosage provided the optimal balance in terms of requiring efficacy with an acceptable safety profile.

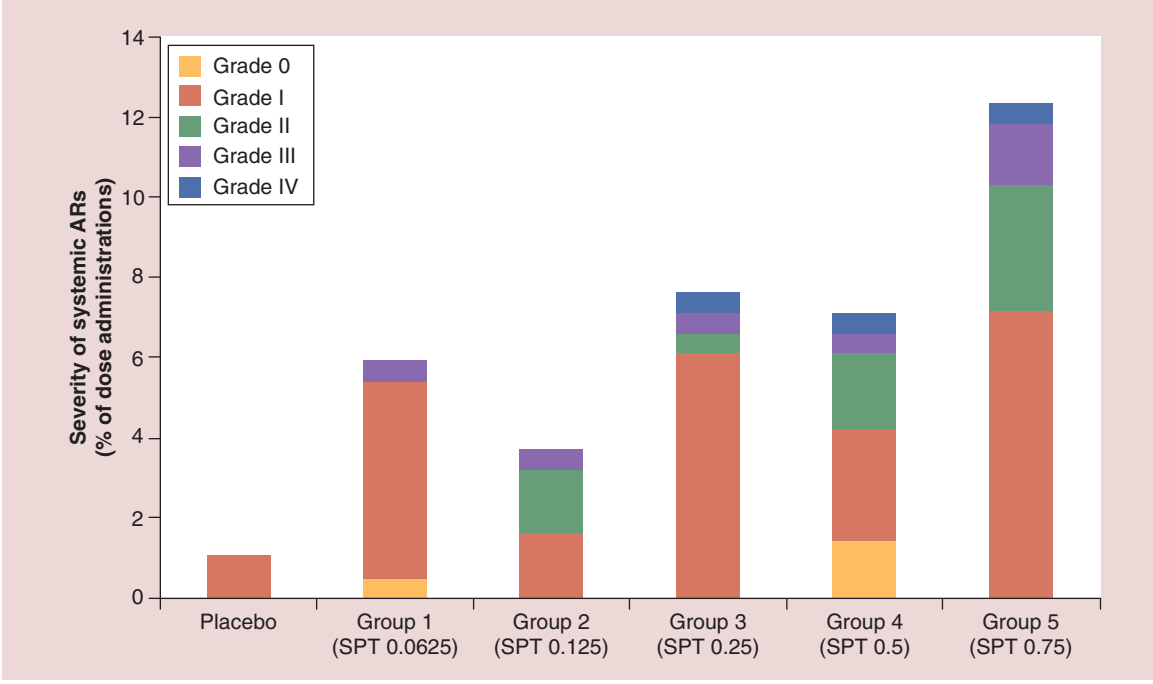

Figure 5. Severity of systemic adverse reactions related to study medication.

Executive summary

- A 17-week, randomized, double-blind, placebo-controlled, dose–response study of a *Dermatophagoides pteronyssinus* (DPT) extract for subcutaneous immunotherapy was performed in patients with allergic rhinoconjunctivitis (with or without asthma) due to this allergen.
- The DPT extract reduced sensitization to the allergen, as indicated by nasal provocation tests and skin prick tests (SPTs), and induced an immunological specific response with an increase in IgG level and decrease in IgE level.
- There was evidence of a dose–response effect for both efficacy and safety parameters.
- Most systemic reactions that occurred were EEACI grade I. Both the incidence and severity of adverse reactions tended to increase with increasing dose, particularly at doses of 0.25 SPT and above.
- The risk–benefit profile appeared to be most favorable with a DPT dose of 0.125 skin prick test units.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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