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Specific subcutaneous immunotherapy with recombinant grass pollen allergens: first randomized dose-ranging safety study

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Clinical & Experimental Allergy

Summary

Background Allergen-specific immunotherapy (SIT) with native allergen extracts and allergoids has been performed successfully for decades. Preliminary studies revealed the use of recombinant allergen-preparations as a promising option for SIT.

Objective The present study was designed to investigate the dose-ranging safety in SCIT with a mixture of five recombinant grass pollen allergens containing equimolar amounts of rPhl p 1, rPhl p 2, rPhl p 5a, rPhl p 5b and rPhl p 6, adsorbed to aluminium hydroxide. **Methods** A randomized, double blind, placebo-controlled, dose-ranging safety study (EudraCT number 2007-002808-18) was performed in 50 patients with allergic rhinoconjunctivitis, with or without asthma. Patients were randomized to groups of 10 to receive maximum doses of 20, 40, 80 or 120 µg of total grass pollen recombinant protein or placebo.

The primary end-point of this trial was the number of patients with at least one systemic reaction with possible, probable or definite relationship to the study medication determined at the end of the up-dosing phase. Secondary end-points included titrated intracutaneous test with natural six-grass pollen extract, allergen-specific conjunctival provocation test as well as IgG and IgE-levels throughout the study.

Results Eight of the 50 patients revealed systemic reactions grade 1 or 2 corresponding to the primary end-point definition. No systemic reactions grade 3 or 4 occurred in any dosage group. The systemic reactions were well distributed among the active groups. Results of secondary end-points imply that the study medication is effective and provokes immunological effects.

Conclusions and Clinical Relevance The first DBPC SCIT-DRF with a mixture of recombinant Phleum allergens (Phl p 1, 2, 5a, 5b, 6) in patients with rhinoconjunctivitis plus/minus asthma showed no major side effects in very high doses up to 120 µg.

Keywords allergy, dose-ranging safety study, Phleum pratense, recombinant allergens, subcutaneous immunotherapy

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Introduction

Allergic diseases, especially immunoglobulin E (IgE)-mediated inhalative allergies, have increased, especially during the last few decades, and are ranked amongst the most important chronic diseases in Europe [1]. Furthermore, an increased prevalence of allergic rhinitis (AR) has been observed during the last 40 years [2]. Subcutaneous immunotherapy (SCIT) has been implemented effectively and safely to reduce both symptoms of allergy and the use of anti-symptomatic medication

[3, 4]. Furthermore, clinical studies have proved an appropriate long term effect [5, 6], and suggested that SCIT prevents the development of new sensitizations and asthma [7, 8]. So far native allergen extracts and hypoallergenic derivatives (allergoids) have been used for SCIT. Despite the good experience with these preparations, they nevertheless have some disadvantages. For example, natural allergens have to be collected and may be influenced by geographical and environmental factors; extracts contain non-allergenic components, the effects of which are largely unknown; protein

interactions and degradation may occur; standardization of extracts may be problematic with uncertain comparability between those of different manufacturers; and content as well as dose of the different allergens cannot be influenced.

The nucleotide sequences of the genes encoding the most important allergens from mites, pollens, insects, animal dander, fungi and foods have been derived from cloning programmes, and these together with the amino acid sequences of the corresponding proteins are deposited in public databases (e.g. www.allergen.org). The cloned genes are the basis for producing recombinant allergen molecules which are chemically defined and which can be precisely characterized using physico-chemical and immunological techniques. It is thus possible to produce proteins with highly reproducible quality and purity, and allergen concentrations can be precisely defined and expressed in mass units. The further development of the recombinant allergen concept can lead to hypoallergenic variants with reduced allergenicity and retained immunogenicity or individualized immunotherapy tailored to an individual patient's sensitization pattern [9–11].

Grass pollen is one of the most important causes of allergic sensitization in the temperate climate of central Europe. *Phleum pratense* (Phl p, timothy grass), belonging to the subfamily Pooideae, is representative of temperate grasses, and its allergens show high allergenic cross-reactivity with other Pooideae species. The group 1 and 5 allergens in particular are highly conserved between the species, and are associated with the highest IgE-reactivity, and sensitization rates of 90–95% for Phl p 1 and 65–85% for Phl p 5a/b, whilst rates of 75% and 60% are attributed to Phl p 6 and Phl p 2. [12–16]. A large proportion of the grass pollen specific IgE could be shown to be directed against the group 1 and 5 allergens, and Phl p 1, Phl p 2 and Phl p 5 showed substantially higher skin prick test (SPT) potency than for example Phl p 4 and Phl p 13 [17]. Therefore it was decided to develop a mixture containing five of the most important *Phleum pratense* allergens (Phl p 1, Phl p 2, Phl p 5a, Phl p 5b and Phl p 6) for SCIT. A randomized, double-blind, placebo-controlled pilot study using this mixture with 40 µg total protein showed promising results with a favourable safety profile and improvements in both symptom-medication score and immunological parameters [18]. The relevant European Medicines Agency (EMA) Guideline requires that dose finding studies are conducted to establish a tolerated dose range and a dose-response relationship for clinical efficacy [19]. The present trial was therefore designed to evaluate safety and efficacy of four dosage schedules with different maximum doses over a short treatment period of 2–4 months. The primary objective of the study was to assess safety. Efficacy was judged by

secondary end-points. The same *Phleum* allergen cocktail as used previously was tested with maximum total protein concentrations of 20, 40, 80 or 120 µg.

Methods

Study design

A randomized, double-blind, placebo-controlled, single-centre, phase II SCIT study (EudraCT number 2007-002808-18) was performed with 50 patients with allergic rhinoconjunctivitis, with or without asthma. It was conducted in the Center for Rhinology and Allergology, Wiesbaden, Germany in accordance with the declaration of Helsinki and the ICH guideline for Good Clinical Practice. The local ethics committees approved the study and all patients provided informed written consent before entering the study. After screening, patients were randomized block wise by means of a computer generated randomization sequence using the randomization tool of Excel 2003. Randomization was performed in the quality management department of the sponsor which is a separate department to clinical research and separate from investigators' site. For stratification, patients were asked about severity of their symptoms and usage of anti-allergic medication during last pollen season before treatment. Patients with on average only moderate symptoms and low consumption of anti-allergic medication as well as relevant FEV₁ > 90% were stated as non-severe. Severity was used as strata during randomization process. Each group consisted of seven patients with moderate symptoms and three patients with severe symptoms. Finally, each of the five groups contained 10 patients receiving respectively maximum doses of 20, 40, 80 or 120 µg recombinant *Phleum* or a placebo (Fig. 1). For blinding the placebo contained histamine-dihydrochloride. All vials containing study medication were identical in outer appearance, packaging and labelling. Emergency envelopes, which were pasted and sealed could be opened in cases of emergencies which would lead to a direct drop out of the corresponding patient. All study personal in clinical research department and at investigator's site, the safety review board as well as any other personnel potentially involved in the study patients' management were blinded at any time from randomization (blinding) until unblinding.

The SCIT was started 5 months prior to the expected start of the grass pollen season. The primary end-point of this study was the number of patients with at least one systemic reaction (according to Tryba, general reactions grade I–IV [20]) with possible, probable or definite relationship to the study medication determined at the end of the up-dosing phase. Secondary end-points included titrated intracutaneous test (ICT) with natural six-grass pollen extract, allergen-specific conjunctival

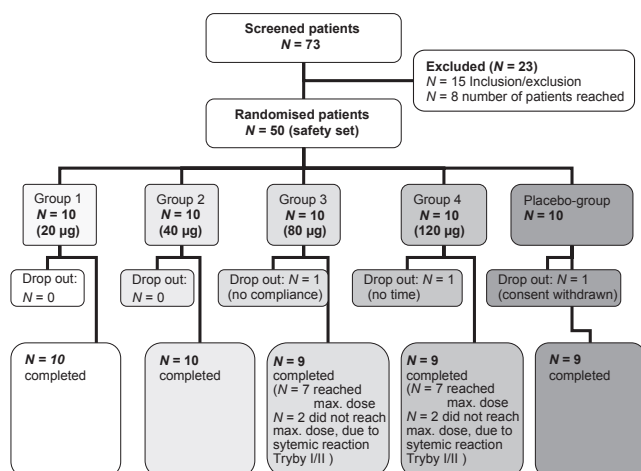


Fig. 1. Flow chart showing distribution between the groups. (Randomized patients, $n = 50$).

provocation test as well as allergen specific IgG and allergen specific IgE-levels throughout the study.

Patients

Seventy three patients between 18 and 50 years were screened to recruit 50 patients for this study conforming to following inclusion criteria: patients with history of moderate or severe IgE-mediated seasonal AR with controlled asthma [forced expiratory volume in 1 s (FEV₁) > 80% predicted according to GINA 2006] induced by grass pollen; main symptoms during grass pollen season; positive conjunctival provocation (simultaneous hyperaemia and irritation of conjunctiva after application of a six-grass pollen extract with a maximal concentration of 5000 SBU/mL (standardized biological units) or lower, (more detailed information in section of secondary end-points); positive SPT (weal $\geq$ positive control 0.1% histamine and ≥ 5 mm) with a six-grass pollen extract (*Phleum pratense*, *Holcus lanatus*, *Dactylis glomerata*, *Lolium perenne*, *Poa pratensis*, *Festuca pratensis*), test results have been documented 20 min after application of prick test solution; and positive EAST (Allergopharma Joachim Ganzer KG) for grass pollen $\geq$ class 2. Patients were excluded if they had previously received SIT to grass pollens or any unknown allergens, undergone an ineffective SIT, suffered from allergies other than grass pollen allergy, have been diagnosed with rhinoconjunctival or asthmatic diseases of other pathogenesis, chronic, inflammatory or systemic diseases. Pregnancy or planned pregnancies were exclusion criteria for female patients.

Study preparation

The five grass pollen allergens were cloned from cDNA expression library derived from pollen of *Phleum*

pratense (timothy grass). The respective cDNAs were subcloned into appropriate expression vectors and expressed in *Escherichia coli*. Recombinant allergens were purified by various chromatographic techniques including hydrophobic interaction, ion exchange and size exclusion. Physico-chemical and immunological properties were analysed with tests including densitometry, SDS-PAGE, reversed phase HPLC, IgE-reactivity-tests or RAST-/EAST-inhibition tests etc. Total endotoxin content of the maximum dose of the study preparation corresponded to 0.43 endotoxin units, 20–180 times less than mean corrected values of natural timothy extracts. Proteins were adsorbed to aluminium hydroxide to form depot preparations and these were mixed in appropriate proportions to produce the final product with approximately equimolar amounts of the five allergens (weighting Phl p 1/5 to Phl p 2/6 in relation of 2 : 1) [18, 21, 22].

The placebo vials contained Al(OH)₃-placebos with histamine-dihydrochloride analogue to the allergen-adsorbate rPhleum.

Immunotherapy

After each SCIT injection patients were observed for at least 30 min. Local and systemic reactions were documented according to the classification of Tryba [20]. In the case of any adverse event (AE) the observation period was prolonged and the dose for the next injection had to be adjusted according to the following rules: local reaction > 10 cm diameter: repeat the last well-tolerated dose; mild systemic reaction graded as general reactions grade I or II: reduce the dose for 2–3 steps; systemic reaction graded as general reactions grade III or IV: patient had to drop out of the trial. For safety reasons a peak flow measurement was performed in patients with asthma before and 30 min after each injection.

For blinding purposes all patients got the same volume and number of injections. Patients with a lower dose were given further treatment on the reached maximum dose (dosage schedule, Table 1). The first injection in all groups contained 0.156 µg protein, and the dose was doubled at each injection up to 20 µg at the eighth injection. Subjects in group 1 then received five further 20 µg doses, whilst those in groups 2, 3 and 4 progressed stepwise to 40, 80 and 120 µg respectively. The first nine injections with up to 40 µg protein content were administered with an interval of 4–14 days. The application of 60, 80, 100 and 120 µg protein was performed at 7–14 days intervals and patients were surveyed afterwards under partial intensive care unit conditions for up to 3 h. During the complete period of the trial patients were provided with emergency kits. In total, up-dosing consisted of 13 injections up to the

Table 1. Dosage schedule

Injection No.	Date	Individual Dose Dose per Injection				
		Arm 1 (max. dose 20,0 µg)	Arm 2 (max. dose 40,0 µg)	Arm 3 (max. dose 80,0 µg)	Arm 4 (max. dose 120,0 µg)	Arm 5 (placebo)
1		0.156 µg	0.156 µg	0.156 µg	0.156 µg	0 µg
2	4–14 days later	0.312 µg	0.312 µg	0.312 µg	0.312 µg	0 µg
3	4–14 days later	0.624 µg	0.624 µg	0.624 µg	0.624 µg	0 µg
4	4–14 days later	1.25 µg	1.25 µg	1.25 µg	1.25 µg	0 µg
5	4–14 days later	2.5 µg	2.5 µg	2.5 µg	2.5 µg	0 µg
6	4–14 days later	5.0 µg	5.0 µg	5.0 µg	5.0 µg	0 µg
7	4–14 days later	10.0 µg	10.0 µg	10.0 µg	10.0 µg	0 µg
8	4–14 days later	20.0 µg	20.0 µg	20.0 µg	20.0 µg	0 µg
9	4–14 days later	20.0 µg	40.0 µg	40.0 µg	40.0 µg	0 µg
10	7 (+ 7) days later	20.0 µg	40.0 µg	60.0 µg	60.0 µg	0 µg
11	7 (+ 7) days later	20.0 µg	40.0 µg	80.0 µg	80.0 µg	0 µg
12	7 (+ 7) days later	20.0 µg	40.0 µg	80.0 µg	100.0 µg	0 µg
13	7 (+ 7) days later	20.0 µg	40.0 µg	80.0 µg	120.0 µg	0 µg

maximum dose of the respective arm. If the maximum individually-tolerated dose or the maximum dose of 20, 40 and 80 µg in the respective patients of group 1, 2 and 3 was reached, this dose was repeated until the end of the study. The dosage guideline: recommendations for supervision of the patient and for dosage modification were the same in all active groups and placebo.

Secondary end-points were changes in titrated specific conjunctival provocation test (CPT), titrated ICT with natural six-grass pollen extract and specific antibody responses.

The CPT was performed before and after therapy. Initiating with the negative control solution (NaCl) followed by a concentration of 5 SBU/mL (standardized biological units) of a standardized six-grass pollen extract (Allergopharma Joachim Ganzer KG). The concentration would be augmented step by step from 5 SBU/mL, 50 SBU/mL, 160 SBU/mL, 500 SBU/mL, 1600 SBU/mL up to a maximal dose of 5000 SBU/mL until a clinical reaction appeared. The doses would be increased after time intervals of 10 min alternating the conjunctival sac until a simultaneous hyperaemia and itching/irritation of one conjunctiva appeared. After the active phase of immunotherapy a second CPT was performed with the individual positive allergen concentration of each patient. If no reaction was noticed at that point the result of CPT would be judged as improved.

The ICT was performed with five different concentrations (0.25, 2.5, 25, 250, 2500 SBU/mL) of natural six-grass pollen extract (Allergopharma Joachim Ganzer KG), positive control (0.165 g/L histamine dihydrochloride) and negative control (physiological NaCl-solution) before and after the treatment phase. After 15 min the early reaction and after 6 h the late-phase reaction was evaluated taking into account the medium diameter of the erythema and the wheal measured.

During the study the immunological parameters: allergen-specific IgE, IgG1 and IgG4 specific to native grass pollen were determined at five time-points of the trial. The first blood sampling took place at screening visit, the second blood sampling before regular 9th injection, the third blood sampling before regular 10th injection, the 4th blood sampling before regular 12th injection and the 5th blood sampling at the final visit after treatment. (The behaviour of immunoglobulines specific to the individual components of the cocktail was investigated and documented in the previous study [18]).

Statistical Analysis

It was planned to screen 70 subjects to include 50 patients finally. With a sample size of 50 subjects it was assumed a ratio of 38% of patients with relevant systemic reaction. In detail this assumption would correspond to an incidence of systemic reactions according to the first point criteria's at the end of up-titration phase of 20% in placebo, 20% in the treatment group with a maximal dose of 20 µg, 30% in the group with 40 µg, 40% in the group with 80 µg and 80% in the group with 120 µg. The study would have a power of 78% to yield a statistically significant result. The study data were evaluated by means of non-parametric methods. The homogeneity between the five treatment groups was tested using Fisher's Exact Test or Kruskal–Wallis test. The level of significance determined for the primary end-point and all other parameters was $\alpha = 0.05$. All statistical analyses were performed according to the protocol using SPSS for Windows version 8 and later. The statistical results were quality controlled and validated by an independent second statistician.

Results

Primary end-points

A total of 73 patients were screened and 50 patients were enrolled to the five treatment groups (Fig. 1). All 50 patients were evaluated in the ITT (Intention to treat) set. The ITT set consisted of 27 (54%) males and 23 (46%) females with an overall mean age of 32 years (mean age per arm ranged between 29 and 37 years). All patients suffered from symptoms of allergic rhinoconjunctivitis caused by grass pollen. Overall seven patients had a history of allergic asthma (FEV₁ > 80) induced by grass pollen. Group 2 and placebo-group did not contain any patients with allergic asthma. Positive prick test reaction to natural grass pollen allergen demonstrated by allergen weal at least as large as histamine control reaction and ≥ 5 mm diameter and a negative control test (saline solution) was shown for all 50 patients. In total 38 of the 50 patients received previous or concomitant medication. The distribution between the treatment groups ranged from six to nine patients per treatment group and can thus be seen as equally distributed. The most frequent ATC class medication that was administered for was the respiratory system with 33 cases. Mainly antihistamines were taken by all together 30 patients well distributed to the different treatment groups. Some patients needed to take drugs for obstructive airway disease or nasal symptoms (Table 2). In group 1 (maximum protein dose 20 µg) and 2 (40 µg) all patients completed the trial. One patient of group 3 (80 µg) dropped out by reason of poor compliance without having an AE relevant to the primary outcome. One patient of group 4 (120 µg) stopped the trial having no time anymore and one patient of placebo-group withdrew his consent. In

group 1 and group 2 all patients reached the top dose of 20 µg, respectively 40 µg. In group 3 eight of ten patients received the planned top doses of 80 µg (one subject received a dose of 20 µg and one subject a dose of 40 µg). Seven of ten patients received the planned top doses of 120 µg in group 4 (one subject was applied a top dose of 40 µg, one subject a top dose of 80 µg and one subject a top dose of 100 µg).

All together 14 of the total 661 injections caused systemic reactions (2.21% of injections) with at least possible relationship to treatment (8 grade I and 6 grade II). Systemic reactions were confined to 8 (16%) patients on active treatment. No systemic reactions graded as general reactions grade III and IV were observed. The systemic reactions were well distributed among the active groups: two patients with AEs in group 1, one patient in group 2, two patients in group 3 and three patients in group 4 (Table 4). The two-sided Fisher's Exact test does not show a significant difference in the number of patients experiencing systemic reactions in the different groups ($P = 0.591$). The moderate sample size of this dose-range finding trial limits the validity of the calculations above. The first systemic reaction with at least possible relationship in each of the eight patients occurred between the 1st and 11th injection at different doses.

In planning the study certain frequency of systemic reactions was assumed according to observations made in the pilot study where a grass pollen cocktail with 40 µg protein content was used [18]. The results of this study showed that the frequency of systemic reactions was lower than expected. The number of systemic reactions did not increase as strongly as expected with increasing doses. Only three patients receiving the highest dose of 120 µg experienced AEs, as opposed to the eight patients assumed.

Table 2. Baseline demographic data

	Study groups					Total
	Group 1 (max. 20 µg)	Group 2 (max. 40 µg)	Group 3 (max. 80 µg)	Group 4 (max. 120 µg)	Placebo-group	
<i>n</i>	10	10	10	10	10	50
Age (mean)	33.2	28.6	36.7	31.4	30.3	32.0
Non-/ex-/current smoker	5/4/1	3/3/4	6/2/2	8/1/1	8/2/0	6/2.4/1.6
Allergic rhinoconjunctivitis	10	10	10	10	10	50
Allergic asthma	1	0	3	3	0	7
Previous and concomitant medication	6	7	5	8	7	33
for respiratory system:						
Antihistamines for systemic use	4	7	4	8	7	30
Obstructive airway disease drugs	1	1	3	3	3	11
Nasal preparations	0	0	3	3	2	8
Positive Prick test reaction to natural grass pollen allergen	10	10	10	10	10	50
Positive EAST for grass pollen ≥ 2	10	10	10	10	10	50

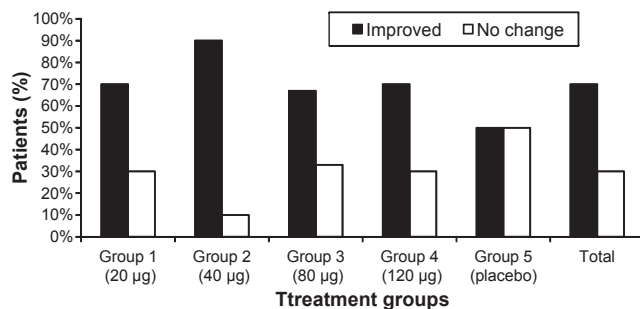
Table 3. Local and systemic adverse event (AEs) according to Tryba (0: local reaction, grade I: mild, grade II: moderate, grade III: severe, grade IV: vital organ failure) with or without probable connection to study medication

	Pro-band	Number of injections until AE occurred	Single dose (µg) before AE occurred	Cumulative dose (µg) until AE occurred	Description	Tryba-grades
Group 1 (20 µg)	1	1	0, 16	0, 16	Local swelling/itching (15 cm), headache	I
		10	20	40,31	Local swelling (15 cm)	
	2	8	20,00	39,84	Urticaria	I
Group 2 (40 µg)	1	3	0,62	1,09	1) mild dyspnoea	II
		6	2,50	5,46	2) mild dyspnoea	II
	2	13	40,00	239,84	Eosinophilia (without probable connection to study medication)	
Group 3 (80 µg)		3	10	40,00	Local swelling (15 cm)/itching	0
	1	11	80,00	219,84	1) local swelling (8 cm) cough, dizziness, fatigue	I
		13	80,00	339,84	2) local swelling (8 cm) flush, pruritus,	II
		15	40,00	419,84	3) dizziness, flush, mild dyspnoea	II
	2	6	5,00	9,84	1) urticaria, eye swelling	I
		13	40,00	89,21	2) urticaria, eye swelling	I
	3	6	5,00	9,84	1) local swelling (20 cm)	0
		13	80,00	227,34	2) local swelling (15 cm)	0
Group 4 (120 µg)	1	8	20,00	39,84	1) orthostatic dysregulation (without probable connection to study medication)	
		9	40,00	79,84	2) urticaria, pruritus	I
		11	40,00	139,84	3) flush, dizziness	I
	2	10	60,00	139,84	1) local itching	II
					Mild dyspnoea	II
		14	100,00	409,84	2) bronchospasm, flush	
	3	4	1,25	2,34	Local swelling (6 cm), pruritus	I

Secondary end-points

The main objective of this study was safety considerations. Nevertheless the study contained three different efficacy parameters. CPT was performed before and after therapy (see methods above). In total 70.2% of the patients improved in CPT after treatment (70% of group 1, 90% of group 2, 67% of group 3, 70% of group 4 and 50% of group 5, see Fig. 2). Due to the small sample size Fisher's Exact Test indicated no differences among the treatment groups ($P = 0.466$).

Intracutaneous testing was performed in all 50 patients at screening, but only in 1–4 patients per

**Fig. 2.** CPT results (before and after therapy, see Methods).

group at the final visit because it was stopped due to systemic reactions in 4 patients. (After therapy three patients in group 1, three patients in group 2, one patient in group 3 and respectively four patients in group 4 and 5 were retested with ICT again). After unblinding it was found that all systemic reactions had occurred in the placebo-group. Skin reactivity increased for early and late-phase reaction when augmenting doses at both visits. After treatment it could be seen tentatively that reactions of the placebo patients seemed to be greater than those of the actively treated patients (results for late-phase reaction, Fig. 3).

Allergen-specific IgG4 or IgG1 values did not change for the placebo-group during the study. Approximately a 100- to 135-fold increase could be seen for allergen specific IgG4 antibodies in all actively treated groups from the beginning to the end of the trial (after 13 injections). There were no significant group differences at screening, but all other blood samples after therapy begin showed significant group differences (Kruskal–Wallis test, $P < 0.01$). The treatment arms were not tested pair wise, so that pair wise significances are not given. The graph (Fig. 4) shows, that the placebo-group stays the same throughout the study, were as all the verum groups show increasing IgG4 values. The same was true for allergen-specific IgG1. Allergen specific

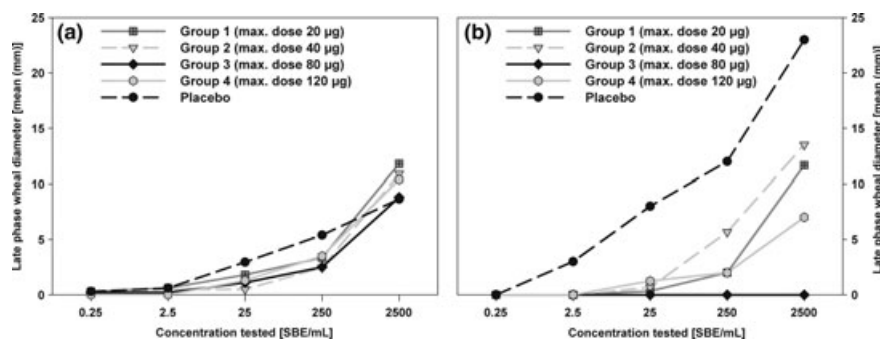


Fig. 3. Intracutaneous test: late-phase wheal reactions a) before and b) after treatment. Dose responses to five concentrations (0.25, 2.5, 25, 250, 2500 SBU/mL) of grass pollen extract standardized in biological units (BU). At final visit only in 1–4 patients per group could be tested due to safety reasons (see Results).

IgG1 concentrations ascended about 15- to 30-fold after 13 injections at the end of the study (Fig. 4). Allergen-specific IgE values of the placebo-group remained nearly unchanged whereas they seemed to decrease slightly in actively treated groups during the study (Fig. 4).

Discussion

Primary end-points

To our knowledge, this was the first dose-ranging safety trial ever performed with recombinant allergens for subcutaneous immunotherapy. It showed that in SCIT with even very high doses (80 µg, 100 µg, 120 µg) of a cocktail of recombinant major allergens of timothy grass no major side effects (grade III/IV) appeared. The choice of allergens in the trial medication includes grass pollen allergen groups that belong to the most prevalent and clinically relevant groups. They exhibit a high cross-reactivity with pollen allergens from a wide range of grasses [12, 14]. Efficacy and safety of the grass pollen cocktail could be proven in a previous clinical study [18]. The rate of systemic reaction (2.21% of injections) is slightly higher than that observed in the previous trial with a maximum dose of 40 µg protein of the same *Phleum pratense* allergen cocktail (0.96% of injections) when most of the systemic reactions (71%) occurred during the up-dosing phase [18]. Since the present study was restricted to an up-dosing phase and the fact that the number of adverse reactions generally decreases during maintenance treatment [23, 24], it is possible that the rate of systemic reactions would have been even lower with a maintenance phase. The rate of systemic reactions in this study (16% of patients or 2.21% of injections) is similar to results of other clinical trials with commercially available SCIT preparations that often contain much lower allergen contents [25]. A Cochrane meta-analysis for SCIT concluded that systemic reactions occurred in 19% of patients [4]. Other SCIT safety studies have reported

systemic reactions after 4.4 or 2.50% of injections [26, 27]. Due to the moderate sample size of a dose-range finding trial the calculated group differences only can show a trend: The two-sided Fisher's Exact test did not show a significant difference in the number of patients experiencing systemic reactions in the different groups ($P = 0.591$). This might indicate that even doses of 120 µg can be well tolerated.

Results of these studies suggest that this recombinant *Phleum* allergen preparation can be potentially viewed as a promising safe alternative for SCIT, even at very high dosages. Larger clinical phase – III – studies should follow in order to confirm the safety-profile.

Secondary end-points

Conjunctival provocation has been used in several immunotherapy studies as indicative of changes in sensitivity [28, 29]. This trial showed that over two-thirds (74.1%) of the patients following active treatment had improved tolerance in CPT. In contrast only 50% of placebo-patients improved in test results of CPT. While planning the study it was decided pragmatically to re-challenge all subjects with the dose that elicited symptoms. By using this approach the re-challenge might be done with a lower dose, because of cumulated dose preceded by apparently several steps perhaps inducing subclinical inflammation prior to the dose eliciting symptoms, and the fact that 50% of the placebo patients showed a negative CPT result fits to it.

The temporal relationship of the magnitude of early- and late-phase responses to intracutaneous testing with the duration of SCIT was shown for a natural *Phleum pratense* extract [30]. Late skin response was significantly reduced already after 2 weeks of treatment while it took 12 weeks for an effect to be seen on the early-phase reaction. Changes in the late-phase reaction were preceded by increased IL-10 production and in early-phase reaction by increased allergen-specific IgG4 [31]. In the trial on hand changes in the ICT response can be

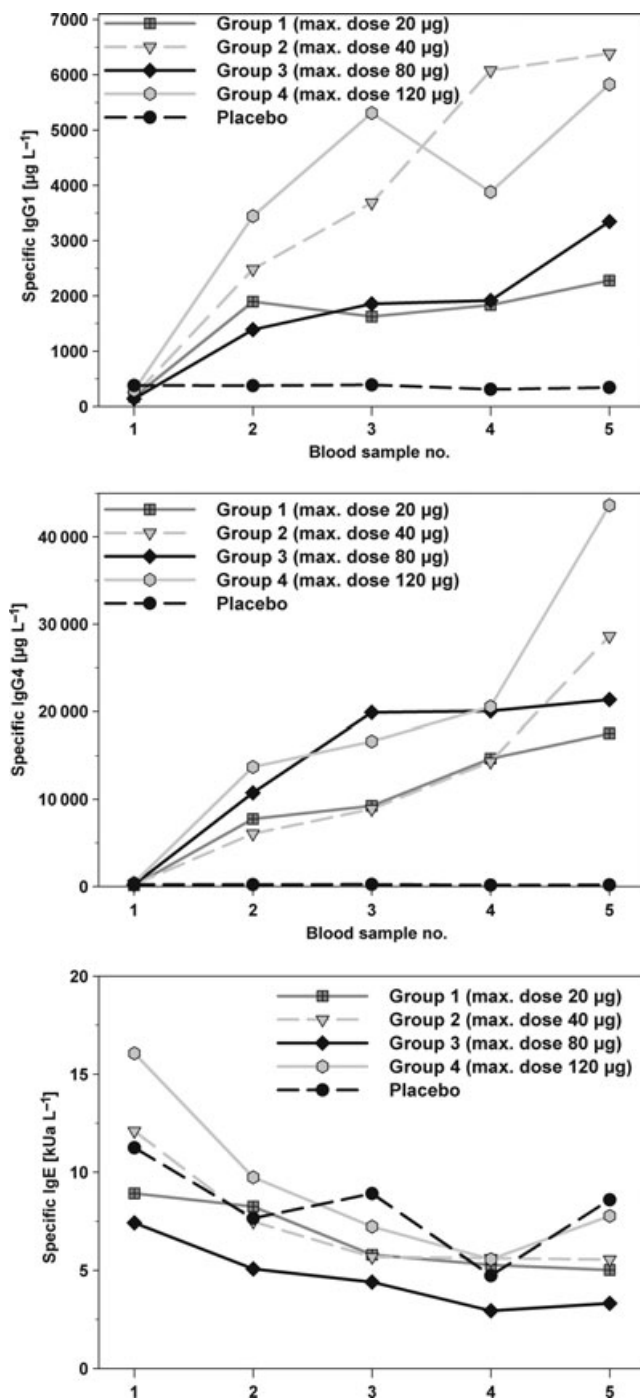


Fig. 4. *Phleum pratense* specific IgG1-, IgG4- and IgE-levels (means). Blood samples were collected (1) before immunotherapy, (2) before regular 9th injection, (3) before regular 10th injection, (4) before regular 12th injection; and (5) at final visit after the therapy.

interpreted only tentatively because of the small numbers of patients tested at final visit (10 subjects at screening vs. 1–4 patients per group at final visit) and because ICT was stopped prematurely due to systemic reactions. Analysing the systemic reactions during ICT

interesting observations can be revealed. Systemic reactions occurred only in each of the placebo patients at final visit before ICT was stopped. It was followed up that only one batch of pollen extracts was used excluding material differences. Furthermore, only one study nurse carried out the ICTs. As all actively treated patients had no systemic reactions after therapy we assume a benefit of SCIT with the recombinant *Phleum pratense* cocktail.

Increases of allergen-specific IgG4 and IgG1 levels during the study underline the immunological effect of the recombinant grass pollen cocktail. It was described that the rise in allergen-specific IgG4 antibodies is indicative of immunogenic activity and could be an objective marker for efficacy of a therapeutic preparation [32–34]. In this study allergen-specific IgG 1 level (15- to 30-folds) and allergen specific IgG4 level (100- to 135-folds) were rising during active treatment supporting the strong immunological effect of the study medication. The results of allergen specific IgG level correspond to the experiences having been made in the previous study with the same cocktail of recombinant grass pollen [18]. Furthermore, the measured allergen specific IgG levels cause by this study preparation are comparable or even higher than allergen specific IgG levels measured in conventional SCIT regimens [35, 36]. Overall results of secondary end-points imply that the study medication is effective.

Limitations of this study are the limitations of a dose range finding/tolerability trial that normally looks at trends only with a short treatment phase of 2–4 months and a limited number of patients. The selection and results of secondary parameters might be discussed as well. ICT and provocation test have been shown to be a useful parameter for efficacy in studies with low number of patients and short treatment duration, but for re-challenge after treatment the same procedure as before treatment might give even better results. The immunological parameters were very useful as well because they showed a clear immunological effect of the study medication. Nevertheless the main objective of this study was to investigate safety in different doses.

In favour for this study speaks that overall only two patients dropped out of the study. In total 35 of the 40 actively treated patients reached the planned maximal dose. As far as known this was the first dose-ranging safety trial ever performed with recombinant allergens for subcutaneous immunotherapy. It showed that SCIT with even very high doses (80, 100, 120 µg) of a cocktail of recombinant major allergens of timothy grass was safe in 50 patients suffering from grass pollen allergy. Severe systemic reactions (grade III or grade IV) could not be observed in any treatment group.

The 'Guideline on the development of products for specific immunotherapy for the treatment of allergic

diseases' of the EMA advises to show efficacy by means of symptom severity and intake of rescue medication as a primary end-point in phase III trials [19].

Therefore, larger clinical studies are planned in our centre aiming to confirm the safety-profile found in this study and to investigate the risk/benefit ratio that can be achieved with 80 and 120 µg maximum doses of this cocktail of recombinant major timothy grass pollen.

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