

Safety of inhalant allergen immunotherapy with mass units-standardized extracts

E. Nettis, D. Giordano, A. Pannofino, A. Ferrannini and A. Tursi

Department of Medical Clinic, Immunology and Infectious Diseases, Division of Allergy and Clinical Immunology, University of Bari, Italy

Summary

Background Allergen-specific immunotherapy (SIT) is an effective treatment for patients with respiratory allergies. However, subcutaneous injection of allergens can provoke systemic side-effects.

Objective This study was carried out to determine the incidence and risk factors of systemic reactions caused by SIT treatment, using extracts of different inhalant allergens, adsorbed in aluminium hydroxide and biologically standardized with the major allergens quantified in mass units.

Methods Five hundred and fifty-five subjects with allergic rhinitis and/or asthma were evaluated on clinical history and skin prick test (SPT) reactions to common inhalant allergens. Subcutaneous SIT was administered to all patients, according to the suggested precautionary guidelines and administration schedule. Patients were treated with house dust mite, grass pollen, *Parietaria judaica* pollen and olive pollen extracts, each receiving one or two extracts.

Results A total of 36 359 injections were administered in the 555 patients. We observed 34 episodes of serious systemic side-effects (0.093% of all injections), in 29 patients (5.2% of all patients), and no fatalities. About 55% of patients reported mild rhinitis and asthma. The majority (59%) of the serious systemic reactions (SSR), and all the anaphylactic reactions, were immediate (i.e. occurred within 30 min after the injection). Asthmatic subjects were at higher risk of SSR than patients with rhinitis ($P=0.01$). Most of the side-effects observed occurred during the dose-increase phase ($P<0.05$). There was no association of SSR with age, gender, SPT reactivity or allergen type.

Conclusion SIT performed in patients with respiratory allergies by specialized staff with the allergen extracts studied, standardized in mass units, provoked a low rate of SSR. The significant risk factors for systemic reactions were asthma and the build-up period.

Keywords allergen-specific immunotherapy, serious systemic reactions, asthma, dose-increase, mass units

Submitted 16 October 2001; revised 17 April 2002; accepted 8 September 2002

Introduction

Almost a century has passed since the pioneering work by Noon [1] and Freeman [2], who successfully treated hayfever patients using a low-dose incremental schedule of pollen injections. Nowadays, allergen-specific immunotherapy (SIT) is accepted as an effective treatment for relieving the clinical symptoms of respiratory allergic diseases, provided high-quality extracts are used. The allergen extracts have up to now been characterized in different ways: weight/volume (w/v), protein nitrogen units (PNU), and biologic units (BU).

Nevertheless, SIT poses the risk of side-effects such as reactivation of the disease, and even anaphylactic shock and death [3–5]. In a study carried out in the USA between 1945 and 1985 Lockey et al. [6] reported 24 cases of fatal shock occurring during immunotherapy and in a further study between 1985

and 1989, Reid et al. [7] described 17 cases. In a similar study undertaken in the UK between 1957 and 1986, the Committee on the Safety of Medicines [8] reported 26 cases of fatal shock and advised that subcutaneous immunotherapy ‘should be used only where facilities for full cardiorespiratory resuscitation are immediately available, and patients should be kept under medical observation for at least two hours after treatment’. In these reports, most deaths were due to ignoring safety instructions and insufficient early treatment of anaphylaxis; safety monitoring is extremely important.

The efficacy of SIT depends on adequate dose administration, since low-dose treatment was found to be ineffective [9]. The ‘highest tolerated dose’ features more efficacious immunotherapy [10, 11], but it also causes a higher rate of adverse reactions.

The aim of this study was to assess the incidence and risk factors of systemic reactions in patients with respiratory allergies during immunotherapy treatment, considering four different allergen extracts: dust mites, grass pollen, *Parietaria judaica* pollen, and olive pollen, adsorbed in aluminium hydroxide and standardized in mass units.

Correspondence: Dr Eustachio Nettis, Cattedra di Allergologia e Immunologia Clinica, Padiglione Chini – Policlinico, Piazza Giulio Cesare, 70124 BARI, Italy. E-mail: e.nettis@allergy.uniba.it

Materials and methods

Between December, 1995 and June, 2001, a total of 555 patients (266 males and 289 females, mean age $\pm$ SD: 27.2 ± 10.9 years, age range 7–64 years), referred to our outpatient department to receive immunotherapy for respiratory allergies, were enrolled in the study. Allergic rhinitis and/or asthma were assessed by a careful clinical history, *in vivo* and *in vitro* tests. Immediate-type hypersensitivity skin prick tests (SPT) were carried out with common inhalant allergens including *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, *Parietaria judaica* pollen, grass pollen, olive pollen, cypress pollen, *Artemisia vulgaris*, *Alternaria tenuis*, *Aspergillus*, dog and cat dander. Of the 555 patients, 336 had seasonal or perennial allergic rhinitis and 219 allergic asthma with or without allergic rhinitis.

The SPT were performed using histamine (10 mg/mL) and normal saline as positive and negative controls, respectively. Weals ≥ 3 mm were considered positive in the absence of a reaction to normal saline [12]. Responses were classified according to the mean weal diameter, as negative < 1 mm or $1+ (1-2 \text{ mm})$, and positive $2+ (3-5 \text{ mm})$, $3+ (6-9 \text{ mm})$, or $4+ (> 9 \text{ mm})$. In 81 patients, laboratory evaluations including measurements of specific serum IgE (Pharmacia CAP system, RAST FEIA, Uppsala, Sweden) were performed under standardized conditions, as recommended by the manufacturer. When required, a bronchial challenge was performed. After obtaining written informed consent, all subjects were treated with SIT according to the guidelines established by international expert panels [4].

Four different aluminium hydroxide-adsorbed extracts were used: house dust mites (*D. pteronyssinus* and *D. farinae*), grass, *Parietaria judaica* and olive pollens (Pangramin[®] BU-UM, ALK-ABELLO[®] S.A., Madrid, Spain). All extracts had been standardized by biological methods, with the major allergens quantified in mass units [13–17]. Three hundred and forty-seven patients received one extract and 208 patients received two extracts. The patients received each allergen in a different syringe. Immunotherapy was administered according to the dose schedule reported in Table 1. Injections were administered weekly at increasing doses (vial I, II and III) until the maintenance dose of 0.80 mL vial III (1000 STU/mL) was reached, corresponding to 8 BU for house dust mites and *Parietaria judaica* pollen extracts (equivalent to 3.2 μg of Der I and 1.6 μg of Der 2

and 0.48 μg of Par j 1) and 20 BU for grass and olive pollen extracts (equivalent to 2 μg of Group 5 and 12 μg of Ole e 1).

The interval between injections was then increased to four weeks. During the relevant pollen season, the immunotherapy dose for pollen allergies was reduced to 50% of the maintenance dose [4]. Before each injection, all patients were asked about any occurrence of local and systemic reactions after their last injection. Each patient was kept in hospital under medical control for at least 30 min after the injections and was asked to return or telephone after 24 and 48 h so as to determine whether any reaction had occurred later. Equipment and personnel for emergency resuscitation were present during the therapy.

As advised by the international guidelines [4], the following data were collected from the medical records and personal interviews with the investigators: the patient's clinical conditions; the concentration, volume, and dose of each allergen administered; any side-effects (including nature, timing, severity and treatment given); recent exposure to allergens. In addition, data related to the patient's demographic characteristics, allergic disease, and degree of skin test positivity were recorded.

Serious systemic reactions (SSR) induced by immunotherapy were divided into three grades [4]: mild rhinitis or asthma (grade 2); urticaria, angioedema or severe asthma (grade 3); anaphylactic shock (grade 4). All systemic reactions were also divided into two categories [18]: (1) immediate reactions appearing within 30 min after injection; (2) late reactions occurring after 30 min.

Data tabulations as well as comparison of results were performed using EPI INFO 6 software programs. Statistical analysis was performed with the chi-square test and, if there were few cases, Fisher's exact test. A *P*-value < 0.05 was considered significant for both tests.

Results

A total of 36 359 injections were administered in the 555 patients. Twenty-nine patients (5.2%) developed SSR, of whom five subjects had suffered two SSR, making up a total of 34 injection reactions (0.093%).

SSR did not correlate with sex (19/289 females [7%] vs. 10/266 males [4%]; *P* > 0.05). The age of patients experiencing reactions ranged from 10 to 41 years. The number of SSR was

Table 1. Treatment schedule

	Vial	Doses (mL)				
<i>D. pteronyssinus</i> + <i>D. farinae</i> (Der 1 and 2)	I (10 STU/mL) 0.04 and 0.02 μg /mL	0.1	0.2	0.4	0.8	
	II (100 STU/mL) 0.4 and 0.2 μg /mL	0.1	0.2	0.4	0.8	
	III (100 STU/mL) 4 and 2 μg /mL	0.1	0.2	0.4	0.6	0.8
Olive pollen (Ole e 1)	I (10 STU/mL) 0.15 μg /mL	0.1	0.2	0.4	0.8	
	II (100 STU/mL) 1.5 μg /mL	0.1	0.2	0.4	0.8	
	III (1000 STU/mL) 15 μg /mL	0.1	0.2	0.4	0.6	0.8
Grass pollen (Group 5)	I (10 STU/mL) 0.025 μg /mL	0.1	0.2	0.4	0.8	
	II (100 STU/mL) 0.25 μg /mL	0.1	0.2	0.4	0.8	
	III (1000 STU/mL) 2.5 μg /mL	0.1	0.2	0.4	0.6	0.8
<i>Parietaria judaica</i> pollen (Par j 1)	I (10 STU/mL) 0.006 μg /mL	0.1	0.2	0.4	0.8	
	II (100 STU/mL) 0.06 μg /mL	0.1	0.2	0.4	0.8	
	III (1000 STU/mL) 0.6 μg /mL	0.1	0.2	0.4	0.6	0.8

greater in young adults aged between 21 and 40 (52%) but this was not statistically significant ($P > 0.05$) in comparison to the other age groups (Table 2).

Analysis of the severity of SSR at the time of onset is shown in Table 3. Grade 2 SSR were recorded in 16 patients (55%), grade 3 in 10 (34.5%), and grade 4 in three (10.5%). Two grade 4 systemic reactions started within 10 min and the other one started 30 min after the injection. Immediate treatment with adrenaline was only needed in these three patients developing grade 4 SSR. No fatalities were observed. Twenty (59%) SSR occurred in the first 30 min after injection and 14 (41%) SSR developed after 30 min.

SSR were observed after allergen doses ranging from 0.2 mL vial I of *Parietaria judaica* extract (0.02 BU) to 0.8 mL vial III of grass extract (20 BU) and *D. pteronyssinus* + *D. farinae* extract (8 BU); analysis of the severity of SSR according to vial administered is shown in Table 4. The rate of SSR (number of reactions/number of allergen doses given) was significantly higher during dose escalation treatment compared to the maintenance phase (17/11 946 [0.14%] vs. 17/24 413 [0.07%]; $P < 0.05$). Correlation between the allergic disease and SSR showed that there were significant differences between the group of asthmatic patients with or without allergic rhinitis and the group with rhinitis (Table 5).

One-hundred and ninety-one subjects were treated with house dust mites, 224 with grass pollen, 215 with *Parietaria judaica* pollen, and 133 with olive pollen extracts. We found that 9/11 112 (0.08%) grass pollen injections, 8/10 093 (0.08%) *Parietaria* pollen injections, 6/6456 (0.09%) olive pollen injections, and 11/8698 (0.13%) mite injections elicited SSR. There was no significant difference in the incidence of SSR in the four groups of allergic patients ($P > 0.05$).

The relation between the mean monthly pollen counts for southern Italy [19] and the incidence of SSR in the patients who received pollen allergens was evaluated. The main pollen season in southern Italy starts with *Parietaria judaica* pollen in February and persists throughout the spring and summer months. The *Parietaria* pollen period lasts less long: from the end of August to October. Grass pollen reaches its peak in April

and early June. Olive pollen peaks in May. During SIT with pollen allergens, SSR occurred mainly out of season (82.6%).

Finally, a comparison of the skin test reactivity to allergens at the initial allergy evaluation vs. the incidence of SSR was made. All 555 patients were positive to SPT with the allergens used in the SIT. In 28/29 (96.5%) cases of SSR, prick tests with inhalant allergens were graded 3+ or higher, i.e. there was a large local reaction. In only one case of SSR was the skin test result graded 2+. However, prick tests graded 3+ or higher did not correlate with the development of SSR in 657/734 (89.5%) cases. Therefore, comparison between these two rates was not statistically significant ($P > 0.05$).

Discussion

In the present study of the safety of immunotherapy, the frequency of adverse reactions and the percentage of systemic reactions were both lower than those previously reported [18, 20, 21]. Differences in the reaction rate may depend on manufacturing differences in allergen standardization and potency, on differences in the formulation of the allergen extract, on the grading of systemic reactions, and on the setting (including adequate training of the staff and appropriate patient selection, according to suitable protocols).

In this study, we used adsorbed extracts. These were introduced many years ago to address problems of poor tolerance of aqueous extracts, which, because of their rapid absorption, cause a higher number of systemic reactions compared to depot preparations. Since the advent of adsorbed extracts, systemic reactions have decreased significantly.

Most systemic reactions, including both immediate and late reactions, consisted of mild rhinitis or asthma (grade 2), requiring either no treatment or only minor treatment.

In contrast with the recommendation by the Committee on Safety of Medicine in England, to observe a 2-h waiting period [8], our data indicates that most reactions and all grade 4 reactions occurred within 30 min after the injection. No fatalities were reported in our study: patients were observed carefully

Table 2. Analysis of SSR according to age

Patient age (years)	No. of patients	No. of patients with SSR	% of SSR
1–20	160	13	8
21–40	317	15	5
> 40	78	1	1

Table 3. Grading of SSR in relation to time of onset

	Grade 2				Grade 3				Grade 4				Total			
	Patients		SSR		Patients		SSR		Patients		SSR		Patients		SSR	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
30 min	8	(27.5)	10	(29.5)	6	(20.5)	7	(20.5)	3	(10.5)	3	(9)	17	(58.5)	20	(59)
> 30 min	8	(27.5)	10	(29.5)	4	(14)	4	(11.5)	0	(0)	0	(0)	12	(41.5)	14	(41)
Total (%)	16	(55)	20	(59)	10	(34.5)	11	(32)	3	(10.5)	3	(9)	29	(100)	34	(100)

Table 4. Analysis of the severity of SSR according to vial administered

Vial	mL (STU)	Grade 2 (n = 20)	Grade 3 (n = 11)	Grade 4 (n = 3)	Total SSR (n = 34)	(%)
I	10	1	0	0	1	(3)
II	100	2	2	0	4	(12)
III	1000	17	9	3	29	(85)

Table 5. Incidence of SSR according to diagnosis

	Rhinitis	Asthma with or without rhinitis	P-value
Patients with SSR/patients	11/336 (3%)	18/219 (8%)	0.01
No. of SSR/injections	14/21 484 (0.06%)	20/14 875 (0.13%)	0.03

during the first 30 min after the allergen injection, with fully equipped resuscitation facilities ready to hand to cope with possible anaphylaxis. Late reactions were only grade 2 or 3, and occurred after the patient had left the outpatient department (between 45 min and 23 h after injection). Some patients return to the clinic for treatment while other SSR were managed by the patient without medical help.

With regard to the potential risk factors for systemic reactions, some studies have demonstrated a higher risk for females than for males [22, 23], whereas other studies did not [18, 24]. Our statistical analysis did not support a higher risk for females nor an increased incidence of systemic reactions in young subjects, confirming that any age may be affected.

Many studies [6, 20] have reported that patients with asthma have more systemic reactions to SIT. Our study confirms this observation, as asthmatic patients had a higher incidence of SSR compared with patients with isolated rhinitis. This led us to take even greater precautions in treating asthmatic subjects, with close patient observation to evaluate the severity of their disease before allergen administration [4]. Fortunately, most of our patients were not asthmatic.

In contrast to other studies, the rate of SSR was not related to the size of the SPT [6, 22]. This finding may be because 90% of our patients scheduled to receive SIT were highly allergic (SPT reactions greater than or equal to 3+), thereby reducing the power of our study to address this relationship.

Although the possibility of an increased risk of systemic reactions during the relevant pollen season [7] has been suggested, our study failed to demonstrate this correlation and, if anything, we saw more reactions when the pollen counts were low. This may have been due to reduction of the SIT dosage during the pollen season. In our study we only saw pollen season-associated reactions in those receiving *Parietaria* immunotherapy. This may relate to the very long *Parietaria* pollen season in southern Italy, typically 8–9 months running from February to October [19].

We found a similar frequency of SSR for the four inhaled allergens studied, and thus no risk related to the allergen used for SIT.

In keeping with previous reports [7, 22], the rate of reactions was significantly greater during the initial build-up phase than during maintenance therapy (1 in 703 injections vs. 1 in 1436 injections). Therefore we recommend that the clinician should be especially vigilant during the dose-escalation phase. Such reactions were also more frequent when using the highest concentration vial.

In conclusion, the low rate of SSR in patients receiving immunotherapy for respiratory allergies in our clinic suggests that the appropriate use, according to manufacturers instructions, of aluminium hydroxide-adsorbed extracts has a low and acceptable rate of side-effects. The majority of SSR were mild and immediate with no cases of fatal anaphylaxis. The two main

high risk factors for SSR were asthma and the dose-increase phase, while we found no association with gender, age, SPT size or the type of allergen.

Acknowledgement

The authors gratefully acknowledge the help of the Scientific Department of Alergia Inmunologia Abelló S.A. in preparing the manuscript.

References

- Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911; 1:1572–3.
- Freeman J. Vaccination against hay fever: report of results during the first three years. *Lancet* 1914; 1:1178.
- Malling H-J. Immunotherapy as an effective tool in allergy treatment. *Allergy* 1998; 53:461–72.
- Malling H-J, Weeke B. EAACI position paper. *Immunotherapy Allergy* 1993; 48 (Suppl. 14):9–35.
- Bousquet J, Lockey RF, Malling H-J, eds. WHO Position Paper Allergen Immunotherapy: Therapeutic Vaccines for Allergic Diseases. *Allergy* 1998; 53 (Suppl. 44):1–42.
- Lockey RF, Benedict LM, Turkeltaub PC, Bukantz SC. Fatalities from immunotherapy (IT) and skin testing (ST). *J Allergy Clin Immunol* 1987; 79:660–77.
- Reid MJ, Lockey RF, Turkeltaub PC, Platts-Mills TAE. Survey of fatalities from skin testing and immunotherapy 1985–89. *J Allergy Clin Immunol* 1993; 92:6–15.
- Committee on Safety in Medicine. Desensitizing vaccines. *Br Med J* 1986; 293:948.
- Norman PS. An overview of immunotherapy implications for the future. *J Allergy Clin Immunol* 1980; 65:87–96.
- Van Metre TE, Adkinson NF Jr, Amodio FJ et al. A comparative study of the effectiveness of the Rinkel method and the current standard method of immunotherapy for ragweed pollen hay fever. *J Allergy Clin Immunol* 1980; 66:500–13.
- Norman PS. Immunotherapy. *Prog Allergy* 1981; 32:318–55.
- Dreborg S, Backman A, Basomba A et al. Skin tests used in type I allergy testing. Position paper prepared by the Subcommittee on Skin Tests of the EAACI. *Allergy* 1989; 44 (Suppl. 10):1–59.
- Lombardero M, González R, Duffort O et al. Evaluación de la actividad biológica total y composición alérgica de extractos alérgicos. *Allergol Immunophatol (Madr)* 1986; 14:189–98.
- Ventas P, Lombardero M, Duffort O, Carreira J. Cuantificación de los alérgenos Der p 1 y Der f 1 de los ácaros del polvo doméstico y Fel d 1 de gato, mediante un ELISA en fase sólida. *Rev Esp Allergol Immunol Clin* 1990; 5:71.
- Ramírez J, Obispo TM, Duffort D et al. Group 5 determination in *Pooideae* grass pollen extracts by monoclonal antibody-based ELISA. Correlation with biologic activity. *Allergy* 1997; 52:806–13.
- Lauzurica P, Maruri N, Galocha B et al. Olive (*Olea europea*) pollen allergens – II. Isolation and characterization of two major antigens. *Mol Immunol* 1988; 25:337–44.

- 17 Corbi AL, Ayuso R, Lombardero M, Duffort O, Carreira J. A competitive solid-phase radioimmunoassay for quantitation of the major allergen of *Parietaria* pollen. *J Immunol Meth* 1985; 83:83–8.
- 18 Møllerup MT, Hahn GW, Poulsen LK, Malling H-J. Safety of allergen-specific immunotherapy. Relation between dosage regimen, allergen extract, disease and systemic side-effects during induction treatment. *Clin Exp Allergy* 2000; 30:1423–9.
- 19 D'Amato G, Lobefalo G. Allergenic pollens in the southern Mediterranean area. *J Allergy Clin Immunol* 1989; 83:116–22.
- 20 Tabar AI, Garcia BE, Rodriguez A, Olaguibel JM, Muro MD, Quirce S. A prospective safety-monitoring study of immunotherapy with biologically standardized extracts. *Allergy* 1993; 48:450–3.
- 21 Greenberg MA, Kaufman CR, Gonzáles GE, Rosenblatt CD, Smith LJ, Summers RJ. Late and immediate systemic allergic reactions to inhalant allergen immunotherapy. *J Allergy Clin Immunol* 1986; 77:865–70.
- 22 Lin MS, Tanner E, Lynn J, Friday GA Jr. Nonfatal systemic allergic reactions induced by skin testing and immunotherapy. *Ann Allergy* 1993; 71:557–62.
- 23 Nelson BL, Dupont LA, Reid MJ. Prospective survey of local and systemic reactions to immunotherapy with pollen extracts. *Ann Allergy* 1986; 56:331–4.
- 24 Rieckenberg MR, Khan RH, Day JH. Physician reported patient response immunotherapy: a retrospective study of factors affecting the response. *Ann Allergy* 1990; 64:364–7.