

A comparative study of an aqueous grass pollen extract and glutaraldehyde-treated grass pollen-tyrosine adsorbate in the treatment of pollenosis

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Summary

Hyposensitization therapy gave a subjective improvement in 84% of grass pollen-sensitive patients receiving an eighteen dose course of aqueous pollen extract (SDV) and in 69% of patients receiving a three dose course of glutaraldehyde-treated pollen-tyrosine adsorbate (Pollinex). Systemic reactions to treatment were considerably more frequent with the aqueous course, but virtually absent with the short adsorbate course, which seems therefore more convenient for the patient. The SDV treatment induced a more pronounced IgG antibody response both in patients and in guinea-pigs receiving the clinical dosage schedule. The Pollinex treatment showed a tendency to enhance the IgE antibody response in new patients.

Introduction

Attempts in recent years to improve the acceptability to the patient of hyposensitization vaccines have taken two main forms: the addition of sparingly soluble adsorbants, such as aluminium hydroxide or tyrosine, and the use of chemical modification of the allergenic components. Formulations employing these principles have the main objective of shortening the course of treatment without compromising either safety or effectiveness.

Assessment of achievement of these objectives is at best imprecise in the current state of understanding of the mechanisms of hyposensitization. Assessment must be based primarily upon clinical parameters. Assessment by measurement of an objective parameter, such as levels of IgG antibody production, must be secondary until precisely validated.

Chemically modified allergens have been studied in a number of laboratories, with a view to bringing about a reduced capacity of the allergen to react with cell-bound IgE and a substantially retained ability to induce an allergen-specific IgG antibody response. Formaldehyde (Marsh, 1971; Marsh, Lichenstein & Campbell, 1970;

Marsh, Lichtenstein & Norman, 1972) and glutaraldehyde (Patterson, Suszko & McIntire, 1973a; Patterson *et al.*, 1973b; Patterson & Suszko, 1974; Moran & Wheeler, 1976; Wheeler, Jenkins & Moran, 1976) treatment of pollen extracts has been shown to go some way towards satisfying these requirements.

The amino acid L-tyrosine has been assessed by Miller & Tees (1974) and Johansson *et al.* (1974b) as a depot adjuvant for hyposensitization vaccines, and as an adjuvant for the induction of pollen-specific antibody in experimental animals by Wheeler *et al.* (1974).

Clinical assessments of glutaraldehyde-treated tyrosine-adsorbed grass pollen extract have been reported by a number of investigators (Foucard & Johansson, 1976; Johansson *et al.*, 1974a; Miller, 1976b; Palmer & Balacescu, 1977; Purser, 1976; Sychlowy, 1977; Verstraeten *et al.*, 1975) and, where comparisons have been made (Symington, O'Neill & Kerr, 1977; Miller, 1976a), effectiveness of the three dose formulation (Pollinex) has in general been equated with that of alum-precipitated pyridine extract (Alavac-P), which has itself been established as an effective treatment when compared with placebo (Kerr & Murchison, 1963). IgG responses to tyrosine-adsorbed material have been reported by Johansson *et al.* (1974a) and by Foucard & Johansson (1976).

The main object of the current study was to extend clinical observations by a direct comparison of Pollinex with a full eighteen dose course of treatment with aqueous extract of mixed grass. A further objective was to compare IgG antibody responses to these treatment courses, and to compare them to responses in an animal model.

Materials and methods

Both formulations contained an extract of Bencard group B2 grass pollen mixture (bent, brome, cocksfoot, dogstail, fescue, foxtail, meadow, rye, vernal, Timothy, false oat and Yorkshire fog).

Glutaraldehyde-pollen-tyrosine adsorbate (Pollinex)

This was prepared in individual syringes of 0.5 ml with doses of 300, 800 and 2000 Noon units (NU). The injections were given at intervals of 1 week.

Aqueous extract (SDV)

This was given according to the manufacturer's recommendation on a twice weekly schedule over 9 weeks to a top dose of 10,000 NU.

Both treatments were administered pre-seasonally with the last injection prior to the end of April.

Patients

These were selected on the basis of symptom history and a positive prick test reaction to B2 extract. Sixty-four patients took part in the trial with a randomized distribution. A breakdown of the groups is shown in Table 1. Eight patients failed to report. Another five patients had to be excluded later because they were found to be habitual steroid users. Finally, fifty-one patients were taken into account for the analysis of the results. Patients were interviewed before and after treatment and during the first weeks of September. Blood samples were taken at each visit. At the second visit patients were questioned about reactions to the treatment: diary cards were issued to cover the 3 principal months of the grass pollen season.

Table 1. Patient groups

Patient details	SDV (n = 25)	Pollinex (n = 26)
Number of patients	31	33
Males	18	18
Females	8	12
Mean age (in years)	30	21
Excluded from final assessment:		
Failed to report	5	3
Habitual steroid users	1	4
Number of patients assessed	25	26
Treated before	22	12
Never treated	3	14
Duration of symptoms (in years)	14.4	9

Guinea-pigs

Groups of eight male Hartley strain guinea-pigs received the clinical dosage schedules by subcutaneous injection. Blood was taken by cardiac puncture under barbiturate anaesthesia before immunization, at various times during treatment and 10 days after the final injections.

Evaluation of assessment

This was carried out on those patients who finished the course of treatment, firstly by diary cards where symptoms were recorded on a three point scale (1 = mild, 2 = moderate and 3 = severe) respectively for eye running and itching, for nose running and blocking and for bronchial wheezing. The total score for each symptom was summed monthly.

Secondly, at the final interview, patients were subjectively assessed on a four point scale: symptom-free, much better, same and worse than the previous year.

IgG antibody

IgG antibody specific for a mixed grass pollen extract was measured in the sera of a number of patients and of guinea-pigs by the RCLAAR method, described originally by Hunter & Coombs (1969). Briefly, antigen-sensitized erythrocytes were prepared by the addition of rabbit anti-red cell γ globulin linked to the mixed grass extract by photo-oxidation with Rose Bengal.

IgE antibody

This was measured in the same human sera by the RAST, and expressed as a percentage of a standard serum.

Results*Effectiveness*

Subjective assessments of effectiveness are recorded in Table 2. 84% of the patients receiving aqueous therapy and 69% of those receiving Pollinex recorded improvement. A careful analysis of the patients' files showed that five out of seven patients receiving

steroids (triamcinolon) had to be considered as habitual steroid users; these five patients were excluded from the final analysis. However, the remaining two patients, in the Pollinex group, were considered as treatment failures, because steroid usage was necessary.

Table 2. Subjective assessment of effectiveness of treatment

Patients	SDV (<i>n</i> = 25)	Pollinex (<i>n</i> = 26)
Free of symptoms	6	3
Much better	13	12
Better	2	3
Same	3	2
Worse	1	4
Steroid users	—	2 (Failures)

Table 3. Mean symptom scores (patients on steroids excluded)

Symptom	SDV (<i>n</i> = 25)			Pollinex (<i>n</i> = 26)		
	May	June	July	May	June	July
Eyes running	2.2	3.8	1.4	2.6	7.6	0.6
Eyes itching	1.1	4.3	1.5	4.2	9.7	1.5
Nose running	4.2	15.0	4.8	10.8	23.6	6.3
Nose blocking	4.5	17.4	3.1	9.4	19.2	4.3
Bronchial wheezing	2.2	1.3	2.4	7.8	2.8	1.4

Mean symptom score: total score for each symptom summed monthly for all patients together divided by the number of patients (habitual steroid users are excluded).

Table 4. Reactions to treatment

Reactions	SDV (<i>n</i> = 25)	Pollinex (<i>n</i> = 26)
Systemic reactions		
Total number of patients with systemic reactions	11	1
Total number of systemic reactions	21	1
Asthma	10	—
Hay fever	1	—
Quincke oedema	2	—
Urticaria	8	1
Local reactions		
Number of patients with local reactions	10	2

Symptom scores during the season are summarized in Table 3. In both groups, peak scores were reached in June and the main symptoms were nose running and blocking. Eye running and itching, as well as nose running, showed lower scores in patients receiving SDV than in those receiving Pollinex.

Reactions

Systemic reactions. Systemic reactions to treatment are shown in Table 4. Eleven out of twenty-five patients receiving the aqueous treatment suffered a high level of asthmatic and urticarial reactions: several patients presented different types of allergenic reactions, twenty-one reactions in all. Only one urticarial reaction was reported by a patient receiving Pollinex.

Local reactions. Local reactions to treatment are shown in Table 4. These were also more frequent in patients treated with the aqueous solution: ten patients reported itching and local oedema at the injection site. Only two out of the twenty-six patients treated with Pollinex reported local reactions.

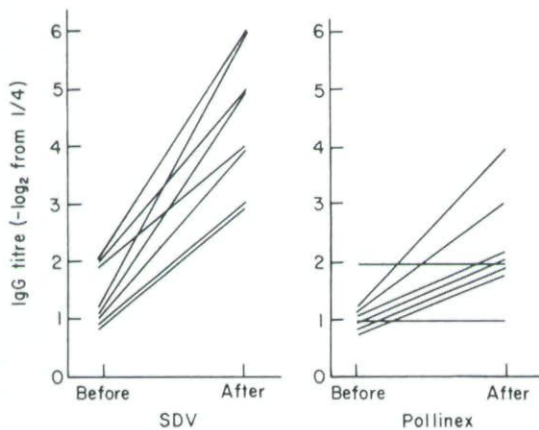


Fig. 1. IgG antibody responses (RCLAAR) to clinical dosage schedules in guinea-pigs. Eight out of eight raised for SDV; six out of eight raised for Pollinex.

Antibodies

IgG antibody responses before and after treatment for individual guinea-pigs are shown in Fig. 1; responses of patients are shown in Fig. 2 and Tables 5 and 6. Comparable results were obtained in both guinea-pig and human systems: the average and scatter of the IgG antibody levels in guinea-pigs (Fig. 3) and patients (Fig. 4) show that Pollinex stimulated the production of mixed grass-specific IgG antibody, but in fewer subjects and at lower levels than the aqueous SDV treatment.

There was a general tendency for IgG antibody levels in patients to fall off post-seasonally. It can be seen in Fig. 5 that previous treatment had little effect on the ability of either material to boost IgG antibody levels.

IgE antibody levels tended to be depressed by both treatments in patients who had received previous treatment. In previously untreated patients, Pollinex caused an increase, and SDV a decrease, in the small number of patients tested in this category.

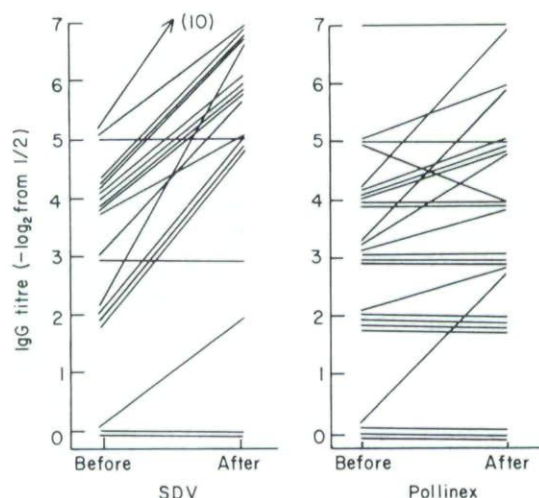


Fig. 2. IgG antibody responses (RCLAAR) in patients. Sixteen out of twenty raised for SDV, ten out of twenty-five raised for Pollinex.

Table 5. Antibody changes after treatment with SDV

Change	IgE			Total patients (SDV-treated)
	(+)	(0)	(-)	
IgG				
(+)	5	6	5	16
(0)	2	0	2	4
(-)	0	0	0	0
Total patients	7	6	7	20

Twenty SDV-treated patients had a complete set of samplings. (+) Increase; (-) decrease; (0) no change.

Table 6. Antibody changes after treatment with Pollinex

Change	IgE			Total patients (pollinex-treated)
	(+)	(0)	(-)	
IgG				
(+)	7	3	0	10
(0)	6	4	4	14
(-)	0	0	1	1
Total patients	13	7	5	25

Twenty-five Pollinex-treated patients had a complete set of samplings. (+) Increase; (-) decrease; (0) no change.

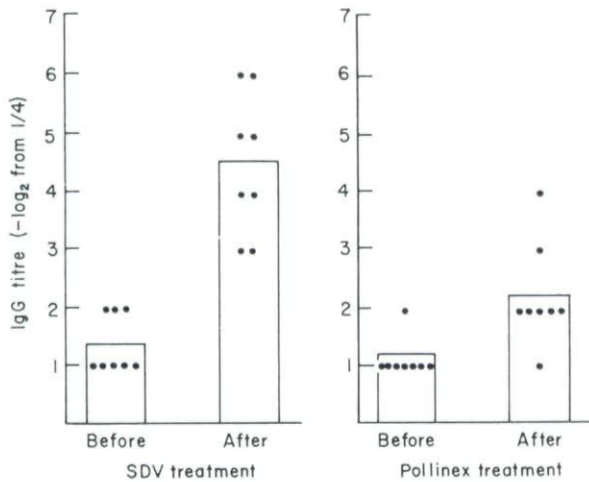


Fig. 3. Scatter and average of specific IgG titres in guinea-pigs.

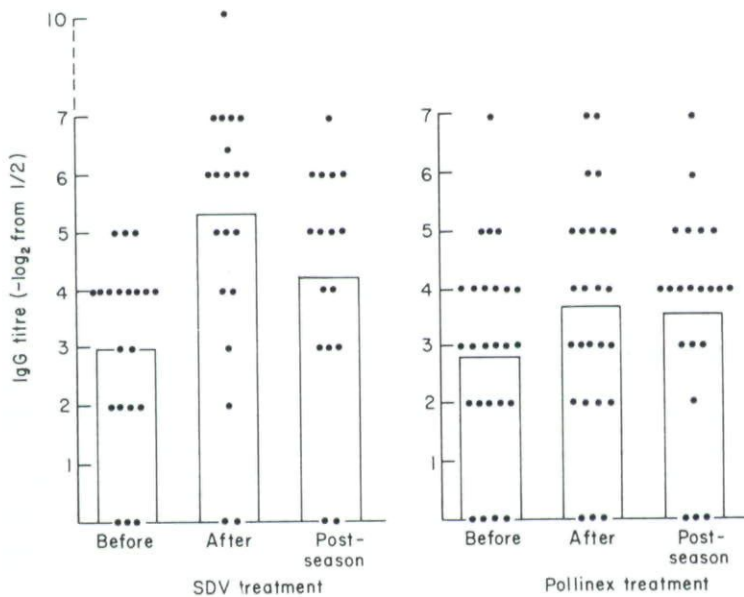


Fig. 4. Scatter and average of specific IgG titres in patients.

No correlation for individual patients could be detected between degree of clinical improvement in either group and IgG or IgE antibody changes.

Discussion

Hyposensitization treatment with the aqueous extract of grass pollen (SDV) gave a high level of clinical improvement associated with a general rise in IgG antibody levels: precise correlation of these two parameters, however, was not possible. No consistent effect on IgE antibody levels could be detected.

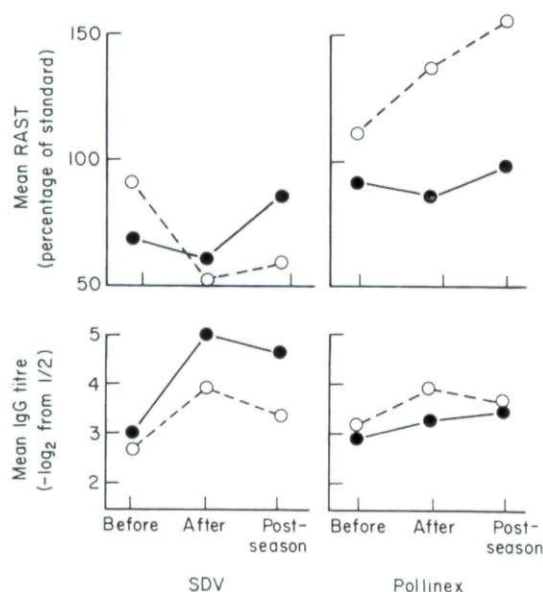


Fig. 5. Antibody response and effect of previous treatment. (O) Never treated; (●) treated before.

Glutaraldehyde-treated pollen-tyrosine adsorbate (Pollinex) gave a lower level of clinical improvement associated with smaller rises in IgG antibody levels in a smaller proportion of patients: again, no precise correlation of these parameters was possible. In patients who had not previously been treated there was a tendency for IgE antibody levels to rise; this effect on IgE antibody levels has been observed previously (Johansson *et al.*, 1974a) both for tyrosine and for alum adsorbates.

The level of reactions to treatment was markedly higher for the aqueous schedule than for the tyrosine adsorbate. It would seem that the Pollinex dosage schedule gives a high level of safety, with the consequent convenience to the patient of a short course of treatment, but associated with a lessening in the level of clinical efficacy—69% compared with 84% with SDV—and with a reduced IgG antibody response.

In the absence of any precise correlation of IgG antibody responses with clinical efficacy it is clearly impossible to use production of IgG antibody in an animal model in a predictive manner. It is of interest, however, that similar levels of IgG antibody response to the two clinical schedules were obtained in guinea-pigs and in patients.

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