

Glutaraldehyde-pollen-tyrosine: clinical and immunological studies

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Summary

Grass pollen extract was reacted with glutaraldehyde, and used in the form of a tyrosine adsorbate for the treatment of hay fever. The incidence of systemic reactions was reduced by the chemical treatment to negligible levels, permitting the therapeutic schedule to be reduced to three doses without impairment of clinical effectiveness. The levels of pollen-specific IgG antibody induced by the chemically modified material were similar to those induced by pollen-tyrosine or by alum-precipitated pyridine extract. The increase in IgE antibody induced by glutaraldehyde-pollen-tyrosine was lower than that induced by pollen-tyrosine: only the latter achieved significance.

Introduction

The recent aims of our research into improved formulations for specific therapy have been to reduce the chances of an injection inducing a reaction in the patients. Such reactions, where they occur, are normally due to excessive release of allergen or to the presence of foreign materials at the injection site. The problem of foreign materials has been met by the use of a fully metabolizable formulation, using tyrosine as adsorbant for the allergen (Miller & Tees, 1974). This type of formulation has now been further developed so as to incorporate a cross-linking process for the allergen, which then has lowered immediate bioavailability, and is less likely to cause an anaphylactic response.

It has been shown that insoluble antigens are not biologically available to cause anaphylaxis in sensitized animals (Albuquerque, Perini & Mota, 1971), and that cross-linked proteins can induce antibodies which cross-react with the unmodified protein (Habeeb, 1969). Since cross-linking of proteins can lead to decreased solubility, we have attempted to develop a cross-linking procedure for allergens to give a formulation combining in an optimal manner both of these properties.

A number of cross-linking reagents have been investigated (Overell, 1968; Mullan & Overell, 1969), and glutaraldehyde was selected for further study. During the course

of the work a study of the cross-linking of ragweed allergen with glutaraldehyde was published. (Patterson, Suszko & McIntire, 1973a; Patterson *et al.*, 1973b).

A formulation containing cross-linked grass pollen adsorbed to tyrosine was developed, and following preliminary clinical studies to confirm the improved safety of these materials (Lessof, 1971), a full trial was carried out in 1972 to compare its effectiveness with that of pollen-tyrosine and of an established alum-precipitated pyridine extract. Levels of antibodies induced by the experimental formulation were compared with those induced by the formulations previously studied.

This paper describes the results of the clinical and immunological study of the use in the treatment of hay fever of cross-linked pollen allergen adsorbed to tyrosine. The fully developed formulation has now been marketed by Bencard under the name 'Pollinex.'

Materials and methods

Allergen potencies are expressed throughout in Noon Units (NU).

Mixed grass pollens

All formulations were prepared from Bencard Group B₂ 12-grass mixture, comprising equal amounts of the following grass pollens. Bent, Brome, Cocksfoot, Dogstail, Fescue, Foxtail, Meadow, Rye, Vernal, Timothy, False Oat and Yorkshire Fog.

Pollen-tyrosine adsorbate

Doses were prepared in volumes of 0.5 ml in individual vials as shown in Table 1.

Glutaraldehyde-pollen-tyrosine-adsorbate

The same doses were prepared for the cross-linked formulation as for pollen-tyrosine.

Alum-precipitated pyridine extract

Bencard Alavac-P in standard 3 vial formulation.

Selection of patients

Patients were diagnosed as suffering from seasonal grass-pollenosis on the basis of symptom history and a positive weal reaction to prick test with Bencard B₂ pollen extract (25,000 NU/ml.)

Table 1. Injection schedule for pollen-tyrosine and for glutaraldehyde-pollen-tyrosine

Vial no.	Dose (NU)
1	20
2	50
3	125
4	320
5	800
6	2000

Treatment groups and injection schedules

One hundred and twenty-nine patients took part in the trial. The first forty new patients presenting and the first thirty who had previously received hyposensitization therapy were randomly allocated to Groups 1, 2 or 3. A further fifty-nine patients were successively allocated to groups receiving progressively shorter courses of glutaraldehyde-pollen-tyrosine (Groups 4, 5, 6 and 7). All received injections at approximately weekly intervals. Groups are summarized below.

Receiving pollen-tyrosine: Vials 1–6: Group 1, twenty-three randomly selected patients, comprising thirteen new patients (Group 1a), ten previously treated (Group 1b).

Receiving Alavac: Standard courses of treatment: Group 2, twenty-six randomly selected patients, comprising eleven new patients (Group 2a) and fifteen previously treated (Group 2b). Nine patients were assessed as mildly sensitive in accordance with manufacturer's printed instructions and received an 8-dose schedule terminating in 25,000 NU; three moderately sensitive patients received a 9-dose schedule terminating in 10,000 NU; fourteen very sensitive patients received a 9-dose schedule terminating in 5000 NU.

Receiving glutaraldehyde-pollen-tyrosine: Vials 1–6: Group 3, twenty-one randomly selected patients, comprising fourteen new patients (Group 3a), and seven previously treated (Group 3b).

Vials 1–6, Group 4—seventeen patients

Vials 2–6, Group 5—eighteen patients

Vials 3–6, Group 6—twelve patients

Vials 4–6, Group 7—twelve patients.

Patients in Groups 4–7 had almost all received previous hyposensitization treatment. Group 2 mostly commenced treatment early in March. Groups 1 and 3 commenced in late March or early April and terminated in late April or early May. Groups 4–7 terminated mainly at the same time, having commenced at the appropriate later date.

Clinical assessments

Effectiveness. The patients were assessed during the first 2 weeks of September, and their responses to treatment were graded on a three point subjective scale. (1) Not good (NG), no noticeable improvement of symptoms over previous seasons; (2) good (G), noticeable improvement of symptoms over previous seasons; and (3) very good (VG), minimal symptoms.

Adverse reactions. Systemic reactions were recorded as rhinitis, urticaria, wheeze, asthma or other, and were graded as mild, moderate, severe or very severe. Local reactions were recorded as nodule, swelling or itch.

Blood samples

Six samples of blood were taken from patients in Groups 1a, 2a and 3a who had not previously received desensitization treatment. Numbers of such patients giving complete sets of samples were: Group 1a, 11, Group 2a, 8, Group 3a, 11. Timings of the bleedings are summarized in Table 2.

Antibody assays

The RCLAAR (red cell linked antigen-antiglobulin reaction) and RAST (radio-

Table 2. Timings of bleedings

Bleeding no.	Occasion	Group 1		Group 2		Group 3	
		No. patients	Date	No. patients	Date	No. patients	Date
1	1st injection	10	Mar. 26	8	Mar. 5	9	Mar. 26
		1	Apr. 2			2	Apr. 2
2	End of course			3	Mar. 26		
	Groups 1 and 3	9	Apr. 30	1	Apr. 2	7	Apr. 30
	Mid-course	2	May 6	3	Apr. 9	4	May 6
	Group 2			1	Apr. 16		
3	One week	8	May 6	3	Apr. 30	7	May 6
	Post-course	3	May 13	5	May 6	4	May 13
4	Mid-pollen season	10	June 11	8	June 11	2	June 4
		1	June 25			8	June 11
						1	June 25
5	Immediate post-season			2	July 23		
		2	July 2	2	July 30	3	July 23
		6	Aug. 6	2	Aug. 6	7	Aug. 6
		2	Aug. 27	1	Aug. 27	1	Sept. 30
		1	Sept. 10	1	Sept. 10		
6	Late post-season	6	Oct. 1	7	Oct. 1	5	Oct. 1
		3	Oct. 8	1	Oct. 15	2	Oct. 8
		2	Not recorded			1	Oct. 15
						3	Not recorded

allergo-sorbent test) procedures were used as described previously (Johansson *et al.*, 1974).

Results

Clinical response

Results of clinical assessments are summarized in Table 3.

Three patients were not included for reasons discussed below. There were no significant differences between the effectiveness rates of the three treatments; the previously demonstrated effectiveness of pollen-tyrosine when compared with Alavac was thus confirmed (Miller & Tees, 1974). The glutaraldehyde-pollen-tyrosine showed the same rate of effectiveness, and this was not significantly diminished by elimination of the first three doses.

Adverse clinical reactions

The numbers and severity of generalized reactions are summarized in Table 4.

Severe or very severe reactions were experienced by five patients receiving pollen-tyrosine. Three of these reacted to the top dose: one who reacted to the penultimate dose did not complete treatment: one who reacted to the first dose was withdrawn from the trial, and was not assessed. Alavac caused one severe reaction of urticaria and

Table 3. Clinical assessment of results of treatment

Treatment	Group	Response					
		VG		G		NG	
		No	%	No	%	No	%
Pollen-tyrosine	1a	4	31	6	46	3	23
	1b	3	33	5	56	1	11
	Total 1	7	32	11	50	4	18
Alavac	2a	3	27	5	46	3	27
	2b*	6	43	7	50	1	7
	Total 2	9	36	12	48	4	16
Glutaraldehyde-pollen-tyrosine	3a	6	43	7	50	1	7
	3b	2	29	3	43	2	29
	Total 3	8	38	10	48	3	14
	4	11	69	5	31	0	0
	5	7	39	8	44	3	17
	6	3	25	6	50	3	25
	7	7	58	3	25	2	17
	Total 3-7	36	46	32	40	11	14

* One patient withdrawn from trial—see text.

Table 4. General reactions experienced during treatment

Treatment	Group	Patients showing reactions									
		None		Mild		Moderate		Severe		Very severe	
		No.	%	No.	%	No.	%	No.	%	No.	%
Pollen-tyrosine	1a	8	61	1	8	—	—	1	8	3	23
	1b	8	80	1	10	—	—	—	—	1	10
	Total	16	70	2	9	—	—	1	4	4	17
Alavac	2a	3	27	5	46	2	18	1	9	—	—
	2b	14	93	1	7	—	—	—	—	—	—
	Total	17	65	6	23	2	8	1	4	—	—
Glutaraldehyde-pollen-tyrosine	3a	12	86	1	7	1	7	—	—	—	—
	3b	5	71	2	29	—	—	—	—	—	—
	4	13	76	4	24	—	—	—	—	—	—
	5	17	94	1	6	—	—	—	—	—	—
	6	10	84	1	8	1	8	—	—	—	—
	7	12	100	—	—	—	—	—	—	—	—
	Total	69	86	9	11	2	3	—	—	—	—

Table 5. IgG antibody: initial titres

Patient group	No. of patients for each titre ($\log_3$)					
	1	2	3	4	5	6
1a	1	2	5	1	—	2
2a	—	5	1	—	1	1
3a	3	3	2	2	1	—

asthma at the penultimate injection, and treatment was stopped: three patients suffering mild or moderate reactions continued treatment after modification of the dosage schedule: and one was withdrawn from the trial for this and other medical reasons. Glutaraldehyde-pollen-tyrosine caused no severe or very severe reactions, and only one patient (Group 3a) required modification of the schedule in the form of a repeated second dose following a mild asthma attack. One patient (Group 4) commenced showing symptoms of hay fever in early May before treatment had finished, and was withdrawn from the trial. Local reactions were recorded in similar proportions of patients on each treatment—Group 1, 37%; Group 2, 42%; Groups 3–7, 31%. In no case was the reaction of a serious or persistent nature.

Antibody assays

IgG antibody. The range of initial RCLAAR titres is shown in Table 5. There was no significant difference between the ranges of initial levels of patients on the different treatments. Figure 1 shows the mean IgG antibody response of the three groups.

For statistical evaluation titres of bleedings from each patient were compared as shown in Table 6. An increase in titre from bleedings 1–3 was demonstrated, but thereafter changes were not significant.

IgE antibody. The mean initial RAST titre for patients receiving glutaraldehyde-pollen-tyrosine was higher than that for patients receiving pollen-tyrosine.

Both groups showed an increase during treatment to bleeding 3 (Fig. 2) but only that for the pollen-tyrosine group was significant (paired *t* test, pollen-tyrosine $P < 0.05$, glutaraldehyde-pollen-tyrosine $P < 0.10$). A slight decrease from bleedings

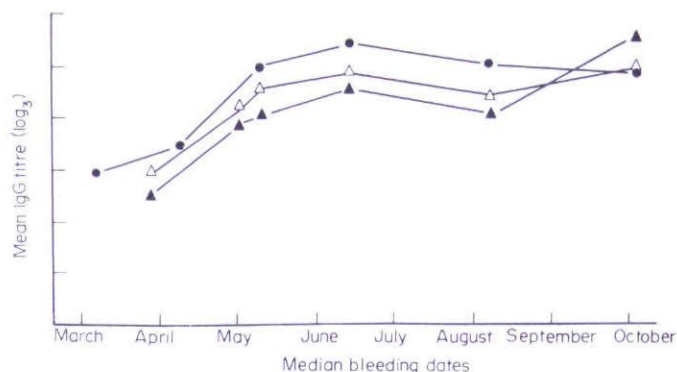


Fig. 1. IgG antibody response. ●, new patients receiving Alavac; ▲, new patients receiving pollen-tyrosine; △, new patients receiving glutaraldehyde-pollen-tyrosine.

Table 6. IgG antibody response

	Raised	No change	Lowered	Change*
Bleeding 3: Bleeding 1				
Group 1a	9	2	—	++
Group 2a	7	1	—	+
Group 3a	10	1	—	++
Bleeding 4: Bleeding 3				
Group 1a	3	7	1	0
Group 2a	3	5	—	0
Group 3a	2	9	—	0
Bleeding 5: Bleeding 4				
Group 1a	1	5	5	0
Group 2a	1	3	4	0
Group 3a	1	6	4	0
Bleeding 6: Bleeding 5				
Group 1a	5	4	2	0
Group 2a	2	2	4	0
Group 3a	6	1	4	0

* Binomial sign test. +, increase significant at 5% level; ++, increase significant at 1% level; 0, no significant change.

3-4 was followed by an increase, significant for both groups, following the pollen season (pollen-tyrosine $P < 0.05$, glutaraldehyde-pollen-tyrosine $P < 0.01$). The mean differences between levels in pollen-tyrosine patients and glutaraldehyde-pollen-tyrosine patients were not significant (t test) at any bleeding.

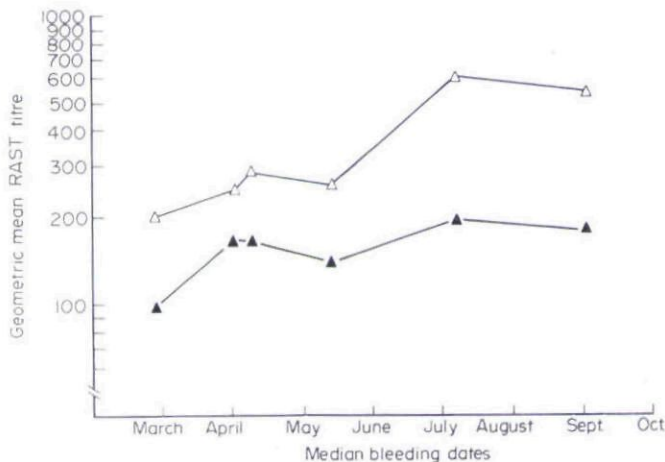


Fig. 2. IgE antibody response. ▲, new patients receiving pollen tyrosine; △, new patients receiving glutaraldehyde-pollen-tyrosine.

Discussion

Our previous studies (Johansson *et al.*, 1974) have shown that hyposensitization therapy with alum-precipitated pyridine extract of pollen (Alavac) or with tyrosine-adsorbed aqueous extract of pollen (pollen-tyrosine) causes a rise in IgG antibody levels over 2–3 units of titre, and these findings have been confirmed in the current study.

Treatment with glutaraldehyde-pollen-tyrosine has now been shown to induce similar levels of IgG antibody as measured by the same RCLAAR technique. The RCLAAR reagent used was prepared from crude grass extract, and thus contained non-allergenic specificities: as such the reagent did not provide a precise measure of blocking antibody. Nevertheless it would seem probable that any marked loss of specificity caused by glutaraldehyde would be detected by this technique. Patterson *et al.* (1973a) found that glutaraldehyde treatment of ragweed antigen E caused no antigenic deletion, nor were new antigenic determinants shown. Our own experiments using antisera raised in animals, to be published separately, have shown only marginal losses of specificity, and no acquired cross-reaction with other glutaraldehyde-treated proteins. It would seem probable, therefore, that the RCLAAR titre may be used as a measure of blocking antibody levels.

The marginal diminution in the IgE antibody response to glutaraldehyde-pollen-tyrosine when compared with that of pollen-tyrosine could be explained by a slight reduction in the immunogenicity of the allergen preparation used. However, more obvious is the reduction of the allergenicity as can be seen from the significant reduction of reactions in the treated patients. The significance of raised levels of IgE antibody in treated patients is in any case not easy to understand since the majority of patients show reduced allergic sensitivity.

Thus glutaraldehyde treatment of the allergen did not produce a marked alteration in the immunological response. The rate of patient reactions to treatment, however, was markedly reduced. Patients treated with pollen-tyrosine in the 1971 studies, using 8- or 9-dose schedules, showed rates of reaction comparable with those caused by the established treatment, Alavac. In the current studies, shortening of the pollen-tyrosine schedule to a 6-dose course has led to an increase in the rate of severe reactions to unacceptable levels. But the same dosage schedule of glutaraldehyde-pollen-tyrosine caused no severe reactions, and reduced the rate of mild reactions below the level of that caused by Alavac. This low level of reaction was maintained even when the first three doses were omitted.

There was no evidence from these studies that the clinical effectiveness of the pollen antigen was significantly altered by the glutaraldehyde treatment. The percentage of patients falling into the three assessment categories were very similar both for glutaraldehyde-pollen-tyrosine and for the established treatment Alavac. These studies have thus demonstrated that cross-linking of pollen allergen does not lead to any marked difference in the immune response it induces, but increases the safety to such an extent that a similar level of effectiveness can be achieved with three doses. Further trials are now under way to confirm and extend these findings.

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