

A metabolizable adjuvant: clinical trial of grass pollen-tyrosine adsorbate

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Summary

Grass pollen extract adsorbed on to a suspension of L-tyrosine has been tested over 2 years as a treatment for hay fever. Eight- or nine-dose courses terminating at doses of between 1000 and 10,000 Noon units were equally effective when compared either with a placebo or with a pyridine-extracted alum precipitate.

Introduction

Hyposensitization therapy becomes more acceptable to the patient if the number of injections can safely be reduced. Formulations developed for this purpose compare well for effectiveness with conventional aqueous extracts (Knights & Tees, 1968; McAllen, 1969).

A new approach to this problem was developed by Brown (1969) who investigated the possibility of occluding antigens within crystals of amino acids and of inorganic salts: L-tyrosine was found to have particular advantages in the formulation of allergen vaccines. L-tyrosine is fully metabolizable, being a normal constituent of blood, and it demonstrates not only a low solubility at physiological pH's, but also strong powers of adsorption of proteins. When it is precipitated in the presence of allergen, a suspension is formed of allergen adsorbed on to tyrosine crystals, and this can be formulated in an injectable form.

Clinical trials of grass pollen-tyrosine adsorbate were carried out in 1970 and 1971. The aims of the trials were, in the first year, to establish effectiveness of treatment using this formulation; for this purpose as large a dose as was practicable was used: and, in the second year, to reduce the total dosage as far as possible without impairing the effectiveness.

Materials and methods

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

Mixed grass pollen extract. Bencard Group B₂ 12-grass mixture in glycerinated extraction medium, 6% w/v (= 60,000 NU/ml). The mixture comprises equal amounts

Table 1. 1970 dosage schedule

Vial no.	Dose	
	ml	NU
1	0.05	25
	0.1	50
	0.2	100
	0.5	250
	1.0	500
2	0.1	1000
	0.25	2500
	0.5	5000
	1.0	10000

of the following grass pollens. Bent, Brome, Cocksfoot, Dogstail, Fescue, Foxtail, Meadow, Rye, Vernal, Timothy, False Oat and Yorkshire Fog.

Pollen-tyrosine adsorbate

All formulations were in multiple-dose vials containing 50 mg/ml L-tyrosine, with adsorbed B₂-grass pollen extract in various concentrations as follows.

1970, vial 1: 500 NU/ml; vial 2: 10,000 NU/ml.

1971, vial 1: 100 NU/ml; vial 2: 500 NU/ml; vial 3: 5000 NU/ml.

Placebo

1970, vials 1 and 2 containing tyrosine suspension without allergen.

Table 2. 1971 dosage schedules

Vial no.	Dose			
	Sensitive		Very sensitive	
	ml	NU	ml	NU
1	0.20	20	0.10	10
	0.50	50	0.20	20
			0.40	40
2	0.20	100	0.15	75
	0.50	250	0.30	150
			0.60	300
3	0.10	500	0.10	500
	0.20	1000	0.20	1000
	0.40	2000	0.40	2000
	0.80	4000		

Alum-precipitated pyridine extract

1971, Bencard 'Alavac'-P, containing the same B₂ grass pollen mixture, and administered as an 8-dose schedule terminating in 25,000 NU to sensitive patients, and a 9-dose schedule terminating in 5000 NU to very sensitive patients.

Table 3. All patients: results of treatment

Results of treatment	1970				1971			
	Pollen-tyrosine (Groups 1 and 2)		Placebo (Group 3)		Pollen-tyrosine (Groups 4 and 5)		Alavac (Group 6)	
	No.	%	No.	%	No.	%	No.	%
VG	34	52	5	23	64	56	11	39
G	22	34	2	9	36	31	10	36
NG	9	14	15	68	15	13	7	25

Table 4. Pollen-tyrosine patients: results of treatment

Results of treatment	1970				1971			
	No previous treatment (Group 1)		Previous treatment (Group 2)		No previous treatment (Group 4)		Previous treatment (Group 5)	
	No.	%	No.	%	No.	%	No.	%
VG	15	43	19	63	23	47	41	62
G	14	40	8	27	18	37	18	27
NG	6	17	3	10	8	16	7	11

Selection of patients

The patients were diagnosed as suffering from seasonal grass pollenosis on the basis of symptom history and a positive weal reaction to prick test with B₂ pollen extract.

In 1970 all patients who had not previously received treatment were randomly allocated either to pollen-tyrosine (Group 1) or to placebo (Group 3). In addition, patients who had previously received desensitization treatment were treated with pollen-tyrosine (Group 2) except for two who received placebo and were included in Group 3.

In 1971 previously untreated patients were randomly allocated to pollen-tyrosine (Group 4) or Alavac (Group 6). All patients who had received previous treatment were treated with pollen-tyrosine (Group 5); of these, some received pollen-tyrosine over 2 years (Group 5a).

Table 5. 1970 pollen-tyrosine patients (Groups 1 and 2): relation of sensitivity to effectiveness

Results of treatment	No. sensitive patients	No. very sensitive patients
VG	17	17
G	10	12
NG	5	4

Table 6. 1971 pollen tyrosine patients: (Groups 4 and 5): relation of sensitivity to effectiveness

Results of treatment	New patients (Group 4)				All patients (Groups 4 and 5)			
	Sensitive		Very sensitive		Sensitive		Very sensitive	
	No.	%	No.	%	No.	%	No.	%
VG	10	42	13	52	45	57	19	53
G	13	54	5	20	28	35	8	22
NG	1	4	7	28	6	8	9	25

Table 7. 1971 Pollen-tyrosine patients (Groups 4 and 5): final dose levels reached by patients in different sensitivity and response groups

Final dose (NU)	VG		G		NG	
	Sensitive	Very sensitive	Sensitive	Very sensitive	Sensitive	Very sensitive
150	—	1	—	—	—	1
300	—	1	—	1	—	—
500	1	2	1	1	—	2
1000	1	3	—	1	—	1
2000	7	12	4	5	—	5
4000	36	—	23	—	6	—

These patient groups are summarized below:

1970: Group 1, thirty-five new patients receiving pollen-tyrosine.

Group 2, thirty previously treated patients receiving pollen-tyrosine.

Group 3, twenty new patients (Group 3a) and two previously treated patients (Group 3b) receiving placebo.

1971: Group 4, forty-nine new patients receiving pollen-tyrosine.

Group 5, sixty-six previously treated patients receiving pollen-tyrosine.

Group 5a, twenty-four patients from Group 1 who received pollen-tyrosine in the second year.

Group 6, twenty-eight new patients receiving Alavac.

Table 8. 1970 Pollen-tyrosine patients (Groups 1 and 2) correlation of effectiveness with final dose

Results of treatment	Final dose (NU)			
	1000-2500		5000-10,000	
	No.	%	No.	%
VG	6	40	28	56
G	6	40	16	32
NG	3	20	6	12

Dosage schedule

In 1970 the schedule shown in Table 1 was recommended as a guide: in practice the initial and final doses used depended upon the assessment of the patient's sensitivity. Courses were terminated by the middle of May.

In 1971, on the basis of the experience gained in 1970, different schedules were recommended for 'sensitive' and for 'very sensitive' patients, as shown in Table 2.

Clinical assessment

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; (3) Very good (VG), minimal symptoms.

Results

In 1970 sixty-five patients received pollen-tyrosine, and assessments of clinical effectiveness, summarized in Table 3, were significantly different from those obtained with placebo ($\chi^2 = 24.24$, $P < 0.001$). In 1971 115 patients received pollen-tyrosine, and results were marginally, but not significantly, better than those obtained with Alavac ($\chi^2 = 3.404$, $P > 0.05$).

When the pollen-tyrosine-treated patients were differentiated into those who were previously untreated and those who had had previous vaccine treatment (Table 4), results were marginally, but not significantly, better in the previously treated group (1970, $\chi^2 = 2.726$, $P > 0.05$; 1971, $\chi^2 = 2.707$, $P > 0.05$). In 1971 results with new patients receiving pollen-tyrosine were not significantly different from those with patients receiving Alavac, all of whom were new patients ($\chi^2 = < 1$, $P > 0.05$).

Before treatment, patients were divided into sensitive and very sensitive classes. In 1970 no difference in response could be detected (Table 5) ($\chi^2 = < 1$, $P > 0.05$). In 1971, a better response was detected in sensitive patients when compared with the very sensitive, and this difference held whether previously untreated patients alone were considered ($\chi^2 = 8.424$, $P < 0.02$) or all the patients ($\chi^2 = 7.207$, $P < 0.05$) (Table 6). Some patients did not complete the recommended course for various reasons (Table 7), and the level of significance is lower if only those patients who completed treatment are considered ($\chi^2 = 3.152$, $P > 0.05$) (i.e. the numbers underlined in Table 7).

Since the dosage schemes in 1971 differed for sensitive and very sensitive patients, it is not possible with the relatively small numbers available to separate the effects of final dose level from the effects of patient sensitivity. In the 1970 trial no difference in response could be detected between patients who terminated treatment with a dose of 1000–2500 NU and those reaching a dose of 5000–10,000 NU (Table 8, $\chi^2 = 1.307$, $P > 0.05$).

Table 9. Numbers of pollen-tyrosine patients in each response category over 2 years' treatment (Group 5a)

		1971		
		NG	G	VG
1970	NG	0	2	1
	G	1	3	6
	VG	0	2	9

Some patients received pollen-tyrosine in both years, and their response gradings are shown in Table 9. There was a trend towards further improvement in the second year, but this did not reach significance. (Binomial test $P = 0.145$.)

The reduction in increments and in final dose levels used in the second year was guided by patients' reactions experienced in the first year. During the second year's treatment mild general reactions, in the form of urticaria, wheezing, or rhinitis, were experienced by nine of the twenty-nine patients receiving Alavac, and by thirteen of the fifty previously untreated patients receiving pollen-tyrosine. One patient receiving Alavac suffered a severe urticaria; one patient inadvertently received pollen-tyrosine injection while virus pneumonia was developing, and suffered a generalized reaction. Both these patients were withdrawn from the trial.

Local reactions defined as swelling, itch, and/or nodule, were experienced to some degree by 54% of all the 1971 patients receiving pollen-tyrosine, and 48% of those receiving Alavac. None of these local reactions persisted.

Discussion

The establishment of a dosage schedule for a new allergy vaccine formulation involves no clearly established principles. It is thought that effectiveness of therapy with aqueous vaccines may depend upon the administration of the maximum tolerated dose, and this was one consideration in the design of the 1970 schedule terminating in a dose of 10,000 NU. The results obtained in 1971, however, show that such a high dose is not necessary to achieve effectiveness with the tyrosine-adjuvanted vaccine. Eighty-six per cent of patients receiving pollen-tyrosine in 1971 had a good or very good season, and results obtained with final doses of either 2000 or 4000 NU could not be clearly differentiated. The potentiating effect of tyrosine on the hyposensitization process was thus clearly demonstrated.

It is possible that the work reported here has not yet fully exploited the potential of tyrosine in permitting dosage reduction, and further trials are in progress to explore this possibility.

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