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D. PTERONYSSINUS-TYROSINE ADSORBATE: BIOLOGICAL AND CLINICAL PROPERTIES

By

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The involvement of *Dermatophagoides pteronyssinus* as a major allergen in allergic sensitivity to house dust is well established (4, 9), to the extent that hygiene and avoidance measures are an important part of therapy (10). Hyposensitisation with extracts of this mite have also been shown to lead to clinical improvement (2, 3, 11), although problems of correlating subjective assessments with objective criteria are apparent in these studies. Formulation in depot form offers advantages of safety and convenience for this form of therapy. Tyrosine has been developed as a metabolisable adsorbant base for grass pollen allergens (5, 7), and its use has now been extended to *D. pteronyssinus*. The current paper describes some biological properties of *D. pteronyssinus* adsorbed on tyrosine, together with results of initial clinical studies. This adsorbate has subsequently been marketed under the trade name Migen®; results of a trial in general practice have been published (6).

MATERIALS AND METHODS

D. pteronyssinus was cultured under controlled laboratory conditions. Mite bodies and excreta were separated from residual substrate and extracted in a medium containing 50 per cent glycerol and 1 per cent phenol.

L-tyrosine adsorbate was prepared by aseptic precipitation of *L*-tyrosine in a dialysed and sterile-filtered extract of *D. pteronyssinus*. The washed precipitate

was resuspended in phenol-saline to a concentration of 4 per cent w/v L-tyrosine. It was dissolved for assay by addition of ethanolamine (1 ml) to adsorbate (5 ml), and the solution was centrifuged through a column of Sephadex G-25 (fine grade) packed into a 20 ml syringe barrel to remove ethanolamine and tyrosine: control solutions were treated in a similar way. Aliquots (1 ml) of this solution were used for antibody assays.

Antiserum to D. pteronyssinus-tyrosine adsorbate was produced in guinea pigs by three subcutaneous injections each of 1 ml administered on days 0, 14, and 21 respectively. Sera were collected on day 28.

Antiserum to D. pteronyssinus was produced in rabbits by injection of mite extract in Freund's Complete Adjuvant.

7-S γ_1 antibody was determined by passive cutaneous anaphylaxis (8). Guinea pigs received intradermal injections (0.1 ml) of 1:5 serial dilutions of antiserum in the shaved backs and flanks. Eighteen hours later they received an intravenous injection of antigen solution, plus 0.3 ml of a 5 per cent solution of Pontamine Sky Blue. Reactions were recorded after 30 min, and titres were expressed as the highest serum dilution showing a response.

Residual tyrosine at the site of subcutaneous injection in the guinea pig was measured in the excised tissue by acid extraction and reaction with ninhydrin.

Patients

Twenty-eight patients who had suffered from perennial rhinitis or asthma for at least 1 year, and who showed a strong positive reaction (> 4 mm diameter) to prick test with *D. pteronyssinus* were selected for trial. Patients were not excluded on the basis of multiple sensitivities, but none had received desensitisation treatment in the previous 12 months, or were receiving drug maintenance therapy. During the period of treatment no patient experienced a change in environment which was thought likely to affect symptoms.

Injection Schedule

Incremental injections of 4, 10, 25, 60, 150 and 400 N.u. were given at weekly intervals to all patients. Varying numbers of subsequent maintenance doses of 400 N.u. were given to different patients, the first two at fortnightly, and the remainder at monthly intervals.

Assessments

Patients were assessed periodically, and their condition compared with their pre-treatment condition on a four point scale.

- 3 - very much improved
- 2 - moderately improved
- 1 - slightly improved
- 0 - not improved or worse

The type and duration of all reactions to treatment were recorded.

TABLE 1

Examples of PCA Titres in Sera from Guinea Pigs Injected with Mite-Tyrosine Adsorbate.

Batch	Concentration N.u./ml	No. of guinea pigs		<i>D. pteronyssinus</i> Challenge dose N.u.	Mean PCA titre-log _s
		Immunised	PCA		
C2098	400	2	5	6000	3.0
MA 46	1600	2	9	6000	2.1

TABLE 2

PCA Cross Reactions of Guinea Pig Antiserum to Mite-Tyrosine Adsorbate.

Antigen	Challenge dose	No. of guinea pigs	Mean PCA titre-log _s
<i>D. pteronyssinus</i>	2,500 N.u.	3	4.6
House dust	750,000 N.u.	3	1.0
B ₂ mixed grass	5,000 N.u.	3	0
<i>D. pteronyssinus</i> culture medium	2 mg (soluble)	3	0

RESULTS

Antisera raised to *D. pteronyssinus*-tyrosine adsorbate in guinea pigs reacted with *D. pteronyssinus* extract, as shown in Table 1.

Cross-reaction was observed with house dust extract, as shown in Table 2, but not with the mite culture medium, nor with the unrelated antigens from grass pollen. Recovery of the antigen from the adsorbate was effected by ethanolamine treatment as shown in Table 3.

Injected tyrosine was shown to remain at the site of injection for some days, but removal from the site was effected within 7 days, as shown in Fig. 1. At this time, residual amounts were barely detectable following initial injection of 40 mg.

Clinical results are summarised in Table 4. Twenty-four

TABLE 3

PCA Titre of Dissolved Adsorbate Against Rabbit Antiserum to D. pteronyssinus.

Antigen (ethanolamine-treated)	Initial concentration N.u./ml	No. of guinea pigs	Mean PCA titre-log _s
<i>D. pteronyssinus</i> tyrosine adsorbate	1,600	3	2.7
<i>D. pteronyssinus</i> extract	5,000	3	3.3
	1,666	3	2.3
	555	3	0.7

patients attended for a full treatment course. All but one of the patients suffered from rhinitis and nine from asthma. Seven had a single sensitivity to house dust, and of these, five (2, 8, 16, 17, 23) achieved a rating of 3 with no evidence of relapse, two (16, 17) achieving this rating after the basic course, two (8, 23) after 12 months, and one (2) after 15 months. The other two showed initial improvement, but later relapsed after cessation of therapy.

Of the remaining 17 patients four (3, 11, 15, 29) had completely unsatisfactory responses, and four (7, 14, 20, 28) showed indeterminate results. The remaining nine (1, 4, 5, 13, 18, 24, 25, 26, 27) achieved rating of 3 with no evidence of relapse. Of the nine patients suffering from asthma, five (23, 24, 25, 26, 27) achieved ratings of 3, with no evidence of relapse, three (20, 21, 28) gave indeterminate results, and one (29) did not improve. Seven patients (1, 4, 13, 16, 17, 18, 27) achieved ratings of 3 after the basic course alone, and those that were subsequently reassessed did not relapse.

Some local reactions were observed. Patients 1 and 2 complained of nodules, which resolved within a week: patients 2 and 4 complained of swelling at the site of injection.

General symptoms were reported as urticaria in patients 1, 5 and 18, commencing 3-4 days after injection and persisting for 2-10 days: as rhinitis in patients 11 and 14; and as breathlessness in patient 28. One reaction was at the 4 N.u. dose, one at 150 N.u. and the remaining four at 400 N.u.

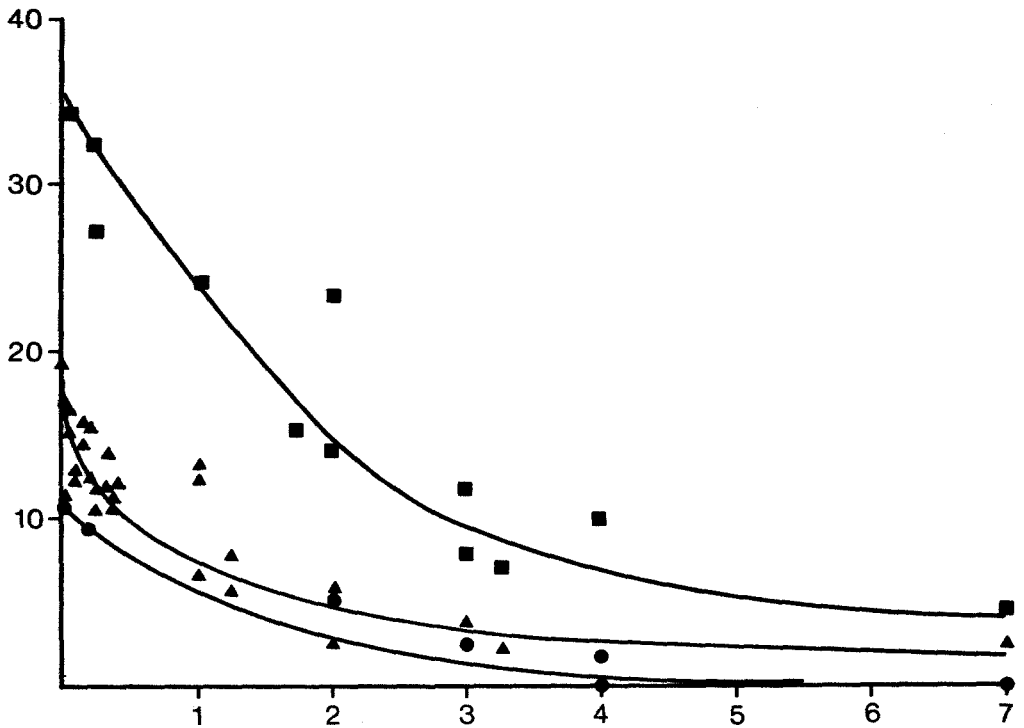


Fig. 1.

Removal of L-tyrosine from injection site. ■ 40 mg injected; ▲ 20 mg injected; ● 10 mg injected. Ordinate tyrosine (mg) remaining at injection site. Abscissa days after injection.

DISCUSSION

Tyrosine suspension has been shown to be removed slowly from the site of injection, but no detectable residue is left after a week. The possibilities of permanent nodule formation which attend the use of some other adjuvants are thus minimised.

Selection of the dosage schedule for clinical use was based on preliminary studies in which reaction rates to increasing initial doses were noted. The general reactions noted in the currently reported study, although mild in nature, were of high enough incidence to confirm that dose levels higher than those selected might not be tolerated.

The clinical response was satisfactory, the majority of patients achieving good ratings in subjective assessments. The value of subjective assessments in an uncontrolled trial of this

TABLE
Clinical

Patient No.	House dust	<i>D. pteronyssinus</i>	Grass pollen	Moulds A13	Cat/dog
1	+	+	+	+	—
2	+	+	—	—	—
3	+	+	—	+	—
4	+	+	+	—	—
5	+	+	+	+	—
7	+	+	—	—	+
8	+	+	—	—	—
9	+	+	—	—	—
11	+	+	+	—	+
13	+	+	—	+	—
14	+	+	—	+	—
15	+	+	+	+	+
16	+	+	—	—	—
17	+	+	—	—	—
18	not recorded				
20	+	+	—	+	—
21	+	+	—	—	—
23	+	+	—	—	—
24	+	+	—	—	+
25	+	+	—	+	—
26	+	+	—	+	—
27	+	+	+	—	+
28	+	+	—	+	—
29	+	+	+	+	+

+ = positive skin test.

— = negative skin test, or symptoms absent.

type is always questionable in view of the improvement recorded for patients receiving placebo in some controlled trials (1). However, rates of improvement in patients receiving placebo during trials of *D. pteronyssinus* hyposensitisation vaccine (3, 11) using subjective assessments have generally been less than 40 per cent, and the current study showed con-

Assessments.

No. of maintenance doses	Assessment at time after completion of basic course (months) for asthma (A) and rhinitis (R)									
	0		3		6		12		15	
	A	R	A	R	A	R	A	R	A	R
0	-	3	-	N	-	N	-	N	-	N
7	-	2	-	2	-	2	-	1	-	3
4	-	2	-	0	-	N	-	0	-	0
7	-	3	-	3	-	N	-	3	-	3
11	-	2	-	3	-	N	-	3	-	3
7	-	0	-	2	-	N	-	N	-	N
7	-	1	-	2	-	2	-	3	-	3
7	-	2	-	2	-	2	-	0	-	1
8	-	1	-	0	-	0	-	0	-	0
8	-	3	-	3	-	3	-	3	-	3
6	-	0	-	N	-	2	-	2	-	-
7	-	0	-	0	-	1	-	0	-	-
7	-	3	-	N	-	3	-	3	-	-
7	-	3	-	N	-	3	-	3	-	-
6	-	3	-	N	-	3	-	3	-	-
7	1	-	2	-	2	-	2	-	1	-
9	2	2	3	3	1	1	0	0	N	N
6	2	1	2	3	N	N	3	3	3	3
7	1	1	2	0	2	2	2	2	3	3
7	0	0	1	1	0	0	3	3	3	3
6	2	1	3	3	3	3	3	3	3	3
0	3	3	N	N	N	N	N	N	N	N
7	0	2	1	3	3	2	1	1	N	N
7	0	0	2	2	0	0	1	1	N	N

N = not assessed.

siderably higher percentages of improvement, regardless of the criteria by which the patients were grouped.

It is to be concluded that use of mite-tyrosine adsorbate in immunotherapy of allergic subjects permits a reduction in the initial graded series of doses to six without apparent impairment of the previously determined effectiveness of this aller-

gen in treatment of house dust allergic conditions. Improvement was recorded in the majority of patients following the first six doses, but further improvement followed subsequent maintenance injections.

SUMMARY

Adsorption of *D. pteronyssinus* on tyrosine gives a depot formulation with retained immunogenicity. Preliminary clinical evaluation of this material showed improvement in the majority of allergic subjects after receipt of the initial six doses, improvement being continued during subsequent maintenance therapy.

ACKNOWLEDGEMENTS

We wish to thank Dr D. Bunsell for the biological evaluations, and members of the Beecham Pharmaceuticals Statistical Department for co-ordination of the clinical trial. We also thank Mrs E. J. Dickinson for her clerical help.

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