

Oral Administration of Grass Pollen to Hay Fever Patients

An Efficacy Study in Oral Hyposensitization

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Oral hyposensitization is still widely used in the treatment of allergic diseases, but controlled studies proving a beneficial effect are lacking. Fifty-eight hay fever patients were admitted to a double-blind placebo efficacy study in oral hyposensitization. An enterosoluble tablet containing timothy whole pollen or placebo was taken daily. Preseasonally, the actively treated patients received 4,315,000 PNU (880,260 AUR) and totally for 6 months 8,915,000 PNU (1,818,660 AUR). Such high doses have never been tried in similar studies. A new principle has been used – "the pollen count interval method" – in the evaluation of symptom and medication score. The study failed to prove any beneficial effect of oral hyposensitization measured by symptom score, medication score, nasal provocation test or skin prick test. There was no change in timothy specific IgE and IgG which could be caused by the treatment. The possibility that oral hyposensitization might be an effective treatment of hay fever in the future is discussed, but it is concluded that the present regimens cannot be recommended.

Key words: enterosoluble tablets; grass pollen; hay fever; hyposensitization; oral administration.

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CLINICAL ASPECTS

Oral hyposensitization is widely used in the treatment of allergic diseases (rhinoconjunctivitis and asthma). Nevertheless, it has not been proved beneficial in a single placebo controlled clinical trial. This is the first placebo controlled study using *in vitro* standardized enterosoluble grass pollen tablets for oral hyposensitization in hay fever. No beneficial effect was found despite a very high dosage schedule. It is concluded that oral hyposensitization cannot be recommended at present.

Since its introduction by Noon in 1911 (21) subcutaneous hyposensitization treatment in the management of hay fever has been used extensively. Because of side effects with aqueous extracts, new alum-precipitated (Allpyral[®]) extracts and alum-adsorbed (Alutard[®], Spectralgen[®]) extracts have been developed. This has reduced the number and severity of side effects, but the treatment is still very de-

manding on both patient and doctor (e.g. many visits, 1/2 h observation after each injection, and risk of systemic reactions). There has therefore been great interest in developing newer, safer types of immunotherapy involving fewer visits to the doctor. Modified allergens have been created for subcutaneous hyposensitization, and intranasal application of allergens has been tried.

Design

The study was double-blind and placebo controlled, starting in February 1982 and ending in September 1982. At the first visit to the clinic the following procedures were performed: skin prick test titration using lyophilised timothy alpha fraction ((Dome/Hollister-Stier), timothy pollen was extracted in Coca's solution and then dialysed in a hollow fibre system with a cut of 5,000 daltons), nasal provocation test using lyophilised timothy alpha fraction, blood samples taken, and Fisons Danish diary card issued. The patients were instructed in daily recording of nose, eye, skin or GI symptoms, and anti-allergic medication taken. The symptoms were recorded in arbitrary units: 0 = none, 1 = mild, 2 = moderate, 3 = severe. Recording of symptoms started 1 week before tablet dosing and continued throughout the study. Table 1 shows the times of the following four visits and the procedures used. At each visit diary cards were collected and checked and new ones issued.

Allocation of patients

Several factors are thought to be important in predicting the course of an individual patient's disease. These factors and the resulting groups into which the patients were divided are shown below in estimated order of importance:

Table 2

Balance of treatment groups concerning initial factors

Initial factor	Level	Active (n = 28)	Placebo (n = 26)
Severity	mild	13	12
	moderate	13	12
	severe	2	2
Nasal reactivity	low	15	10
	high	13	16
Age	younger	9	7
	older	19	19
Skin reactivity	low	19	18
	high	9	8

- 1) Initial severity of disease (mild, moderate, or severe). This was based on medication taken during the previous grass pollen season.
- 2) Result of initial nasal provocation test to timothy (50% least sensitive and 50% most sensitive).
- 3) Age $\geq$ and $<$ 18 years old.
- 4) Initial skin reactivity to timothy (50% least reactive and 50% most reactive) based on the individual concentration of lyophilized timothy alpha fraction expressed in AUR (activity units by RAST) that produced the same wheal area by skin prick test as histamine dihydrochloride 1 mg/ml (1 HEP).

To avoid the possibility of finishing with a gross imbalance in any of these factors, or in the number of patients in each treatment, the four groups were balanced when the patients entered the trial. The techniques used involved minimization (25, 29) and blocking. The patients were entered in pairs, both patients in a pair having the same initial severity. One patient was allocated to the active treatment and the other to the placebo so as to minimize the imbalance between treatment groups at this stage (Table 2). The calculations were performed using a programmable calculator.

Skin prick testing (SPT)

Skin prick testing was performed in duplicate using the volar side of both forearms. The skin prick test was performed according to the common Nordic guidelines (6) and using the skin prick test needle described by Malling et al. (19). The following 3-fold serial dilutions of lyophilized timothy alpha fraction in glycerol Coca's solution were used: 1 AUR/ml, 2 AUR/ml, 5 AUR/ml, 15 AUR/ml, 45 AUR/ml, 135 AUR/ml, and 400 AUR/ml. As positive controls histamine dihydrochloride 1 mg/ml (ALK) and 10 mg/ml in glycerol Coca's solution (Dome/Hollister-Stier), but only at the end, were used. Glycerol Coca's solution was the negative control. The use of antihistamine and topical/systemic steroid were avoided for at least 72 h before SPT. The wheals were outlined after 15

min, and by use of cello tape a print of each wheal was transferred to mm graph paper. All tests were performed by nurses who in preliminary trials had documented a reproducibility in SPT within the recommended $\pm 20\%$ from the mean. The wheal area was calculated by multiplying the largest diameter with the right angle diameter through the middle of the former. The wheal areas for each patient were plotted on a log-log graph paper as a function of allergen concentration, and the AUR value corresponding to the wheal area of 1 mg histamine dihydrochloride (= 1 HEP) and the wheal area of 10 mg histamine dihydrochloride ("10 mg histamine equivalent") were estimated from the graph. The allergen solutions were made just before the first skin prick session and used for 1 month. They were stored at 2–8°C when not in use. New allergen solutions were made just before the final skin prick session.

Lyophilised timothy alpha fraction from the same batch was used for SPT, nasal provocation and for all *in vitro* analyses.

Nasal provocation test (NPT)

The method described earlier (38) for NPT was carried out with lyophilised timothy alpha fraction, reconstituted in normal human serum albumin, which also was used as negative control. The following dilutions were used: 1 AUR/ml, 2 AUR/ml, 4 AUR/ml, 8 AUR/ml, 15 AUR/ml, 30 AUR/ml, 300 AUR/ml. Test solution 0.2 ml was atomized into the nose using a DeVilbiss® spray No. 15, three puffs into each nostril. Each provocation test started with the negative control and if there was no reaction within 5 min the first allergen provocation was given using 1 AUR/ml. Every 10 min an increasing allergen concentration was given until a positive reaction expressed in AUR and defined as the nose sensitivity to timothy. The reaction was defined positive if two of the following three criteria were fulfilled within 10 min of provocation: > 5 sneezes; > 0.5 ml secretion; > 20% decrease in nasal peak-flow.

The allergen solutions were freshly made every day and used for a maximum of 7 h.

Immunological blood tests

Blood samples were obtained at four visits as shown in Table 1. At the end of the study all four samples were assayed for total IgE, timothy specific IgE, and timothy specific IgG, and immune complexes.

Total IgE and timothy specific IgE. Total IgE was analysed using the paper radio immunosorbent test (PRIST) (24). Timothy specific IgE was analysed using the paper radio allergosorbent test (RAST) (3).

Timothy specific IgG. Serum IgG antibodies to timothy grass pollen extract were measured by an immunoradiometric assay as described in detail elsewhere (9). In brief, 0.2 ml serum diluted 1:80 was incubated for 2 h at 37°C with a nylon ball covalently coated with timothy alpha fraction. The balls were then incubated overnight with about 45 ng ¹²⁵I-labelled affinity-purified goat anti-human IgG, specific activity 6–7 mCi/mg, with equal reactivity against all four IgG subclasses (10). The results were expressed as binding-index, X:

$$X = \frac{\text{cpm}_S - \text{cpm}_B}{\text{cpm}_R - \text{cpm}_B}$$

where cpm_S is the bound radioactivity for the sample, cpm_B the bound radioactivity for pooled normal human serum, and cpm_R bound radioactivity for a human reference serum, obtained from a patient parenterally desensitized with grass pollen mixture for several years. All determinations were done in duplicate, and consecutive samples from the same patient were run on the same day. Inter-assay coefficient of variation was 12.3% for a control serum with binding index 40.9. The range of binding indices obtained with 20 normal human sera was –3.4 to 15.9. Based on this observation only patient sera with binding indices equal to or higher than 20 were considered positive. Arbitrarily, rises in binding indices were considered significant if the value was equal to or higher than 20, and at least twice the value found in the sample obtained before immunotherapy had begun.

Medians for serum IgG antibodies to a partially purified timothy pollen extract in non-allergic adults and in parenterally immunized patients were calculated on the basis of the results presented in Djurup et al. (9).

Circulating immune complexes (IC). The sera of the patients were examined for the presence of IC by two independent methods, a polyclonal rheumatoid factor assay (pRF) based on inhibitory activities of IC towards agglutination of IgG-coated latex particles by polyclonal rheumatoid factor (16), and a polyethylene glycol precipitation complement consumption assay (PEG-CC) (2).

Tablet dosing

Timothy whole pollen was administered orally in enterosoluble tablets. The pigmented enteric coat consisted of polyvinyl acetate phthalate applied in methanolic solution. The pigments were non-allergic iron oxides giving colours of pink and yellow for top and bottom strengths of tablets, respectively. A film thickness equivalent to an approximate tablet weight gain of 6–7% ensured that they withstood 2 h emersion in 0.1 molar HCl at 37°C in a tablet disintegration apparatus.

When 0.1 M HCl was replaced by mixed phosphate buffer at pH 6.8 tablet disintegration occurred in 3–7 min (European Pharmacopoeia).

Strengths of whole timothy pollen tablets were 1,000 PNU and 10,000 PNU and expressed in AUR's: 385 AUR, 2,040 AUR respectively. The placebo tablet resembled the pollen tablet, but instead of pollen approximately 90 mg lactose was used. The tablets were taken on a fasting stomach with 100 ml water, 15–30 min before breakfast as follows: 1st day: 1,000 PNU, 2nd day: 2,000 PNU, 3rd day: 4,000 PNU, 4th day: 8,000 PNU, 5th day: 10,000 PNU, 6th day: 20,000 PNU, 7th day: 30,000 PNU, 8th day: 40,000 PNU, 9th day: 50,000 PNU and then 50,000 PNU each day for the rest of the study. To secure optimal patient compliance the tablets were dispensed into tablet dosage boxes.

Each day the patients recorded on the diary card how many tablets he/she had taken.

Anti-allergic medication

The patients were allowed limited use of certain medication in order to control allergic symptoms on a daily basis. For nose and eye symptoms antihistamine eye and nose drops were allowed when needed, with a maximum dose of: Antistina Privin® 1 drop in each eye t.i.d., Antistina Privin Ciba® 2 drops in each nostril t.i.d. If this was not enough to control symptoms prednisone 5–10 mg was given as tablets. Other antiallergic hay fever medication was not used.

In order to perform comparisons between treatment groups a score system was used to produce an overall medication score for each day: antihistamine eye drops 1 drop = 1 point, antihistamine nose drops 2 drops = 1 point, prednisone 5 mg tablets, 1 tablet = 6 points.

Missing symptom and medication scores

Occasionally some patients forgot to record symptom and medication scores for several days, or left the pollen count area (defined as a radius of 30 km from the pollen spore trap) to go on holiday. On such days values were coded as a blank and later on replaced by calculating the average medication and symptom score for that particular patient from all days with the same pollen count.

Birch- and mugwort-sensitive patients

Any patient who was clinically or skin test positive and recorded symptoms during the birch pollen season was considered to have been made extra sensitive at the start of the grass pollen season. As a result, symptom and medication scores were replaced by blanks during the first 15 days of the grass pollen season.

Similarly, mugwort-sensitive patients had all positive symptom and medication scores replaced by blanks between July 20 and August 18.

Days recorded as blanks as described here

were replaced by individually calculated values in the same way as for missing values.

Grass pollen data

Symptom and medication scores were analysed and compared with the Copenhagen grass pollen count, lasting from May 30 to August 18, a total of 81 days (11½ weeks). Pollen grains were collected in a Burkard Recording Volumetric Spore Trap placed 15 m above ground in the centre of Copenhagen. Determinations and counting were carried out by the aerobiological group in Copenhagen and published in the Danish Pollen Bulletin. All patients lived within a 30 km radius of the spore trap.

In order to perform analyses of symptom and medication scores the grass pollen season was divided into four quartiles according to the grass pollen count, and the corresponding symptom score and medication score were calculated for each quartile as shown in Table 3. This method of comparing the medication and symptom score between two treatment groups is called the pollen count interval method and is discussed in detail elsewhere (36).

Statistics

Before it was possible to make group comparisons concerning medication and symptom scores it was necessary to perform analyses of covariance for the following reasons:

1) That the treatment groups had been ba-

lanced for the four initial factors meant that analyses of covariance had to be used in order to avoid distorting the *P*-values resulting from the analyses (25).

- 2) The balance in the treatment groups achieved by use of minimization techniques at the start of the trial was reduced by drop-outs.
- 3) A review of the nasal reactivity and skin sensitivity groupings produced some changes in the groupings.

The analyses of covariance included all four initial factors to begin with, after which the analyses were repeated with treatment groups only and any initial factor which was shown significant ($P < 0.10$) in the first analysis.

Symptom and medication totals formed distributions with a high degree of positive skewness. To reduce this as much as possible, so that analyses of covariance could be performed, a transformation had to be applied to the data. The transformation in terms of both skewness and kurtosis was $y = \log_{10}(10 + x)$.

Analyses of covariance were all performed on data thus transformed, and the results are presented after the inverse transformation had been performed. All analyses involving symptoms and medications were performed on an IBM 4331 computer using BMDP (a package of 40 statistical programmes. Statistical Software Department of Biomathematics, University of California, Los Angeles, U.S.A.). All other analyses were performed with the aid of a programmable calculator.

Treatment group comparisons concerning skin prick test and gastrointestinal symptoms were made using Kolmogorov-Smirnov Two Sample Test, a non-parametric test.

Calculations concerning HEP and "10 mg histamine equivalent" values were performed after logarithmic transformation to avoid problems of skewness.

The study was approved by the local ethics review committee and all patients gave their informed consent to participate. The patients were free to leave the study at any time.

Table 3
Composition of pollen count quarters

Quarter	Pollen level	Grass pollen count	Average pollen count in quartile	Number of days in quartile
First	"very low"	0-3	1.7	20
Second	"low"	4-8	6.0	20
Third	"medium"	9-20	15.5	20
Fourth	"high"	21-61	34.0	21

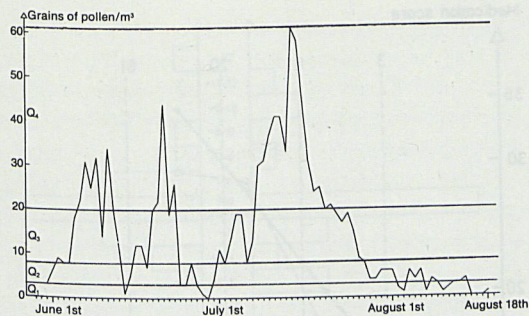


Fig. 1. The Copenhagen grass pollen count 1982. Area under curve is divided according to the pollen count quarter given in Table 3. Q_1 days having "very low" pollen count, Q_2 days having "low" pollen count, Q_3 days having "medium" pollen count, Q_4 days having "high" pollen count.

RESULTS

Fifty-four patients completed the study, 28 in the active and 26 in the placebo group. The actively treated patients received preseasonal 4,315,000 PNU (880,260 AUR) of timothy pollen and 8,915,000 PNU (1,818,660 AUR) during the whole trial (6 months). Concerning the initial factors for which the treatment groups were balanced, good comparability was obtained between the two groups, except for nasal reactivity (Table 2).

Grass pollen counts

In Fig. 1 the Copenhagen grass pollen count for 81 days during 1982 is shown with maxi-

Table 4

Total symptom and medication scores for the grass pollen season (81 days)

Treatment group	Symptom scores		Medication score
	Nose	Eye	
Active ($n = 28$)			
mean values	119.4	67.4	170.4
median values	62.0	39.5	84.5
range of values	8-463	0-227	0-1139
Placebo ($n = 26$)			
mean values	158.1	55.9	175.5
median values	99.5	38.0	58.0
range of values	0-511	0-190	0-754

mum pollen count reaching only 61 grains/ m^3 that year. Table 3 gives the pollen quartiles.

Symptom and medication score

For each day in the grass pollen season a nose symptom score was calculated for each patient by adding scores for sneezing, nose blocking and running (maximum $3 \times 3 = 9$ points). An eye symptom score comprised redness/swelling and itching/running (maximum $3 \times 2 = 6$ points). A medication score was also calculated for each day in the grass pollen season using the arbitrary medication points given previously.

For each of the symptom and medication scores a pollen season total was obtained for each patient in the trial by adding scores for all 81 days of the grass pollen season. These total symptom and medication scores are given in Table 4.

For reasons pointed out in the statistic section, analyses of covariance were performed after logarithmic transformation of symptom and medication scores. After reverse transformation the resulting total symptom and medication scores are given in Table 5.

Performing analysis of covariance, it was interesting to note that the only initial factor which had any significant influence on patient nose and eye symptoms was initial nasal reactivity ($P = 0.05$). It could be shown that patients who recorded high nasal reactivity in the pretrial NPT recorded higher levels of nose and eye symptoms than patients with low initial nasal sensitivity. This was equal in both groups.

The only factor having a significant influence on patient medication scores was initial severity

Table 5

Total symptom and medication scores during grass pollen season (81 days) after allowance for initial factors, i.e. after logarithmic transformation analysis of covariance and reverse logarithmic transformation has been performed presented as means

Treatment group	Nose + eye symptoms	Medication score
Active	126.9	82.7
Placebo	120.6	73.4

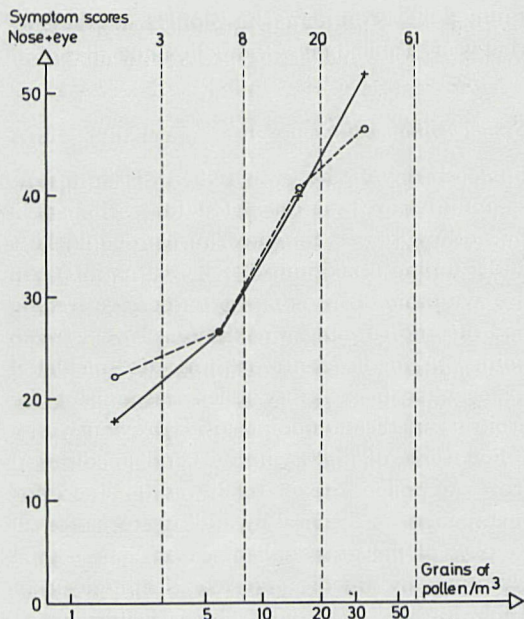


Fig. 2. Mean eye and nose symptoms are calculated for all days in each pollen quarter. Quarter eye + nose symptoms is expressed as a function of the average pollen count for each quarter (Table 3).

×: Active treatment; ○: placebo treatment.

($P = 0.015$).

To show the influence of pollen levels on symptom and medication scores an average mean score was calculated corresponding to the days in each of the four pollen quartiles shown in Table 3. In this calculation nose and eye symptoms were added. Also here the scores are recorded after analysis of covariance has been performed. In Figs. 2 and 3 it is shown how increasing pollen levels are followed by increasing symptom and medication scores in both groups. There is no significant difference between the groups.

Number of weeks coded as blank, i.e. number of weeks substituted by calculated individual mean symptom and medication score in relation to the pollen count, were in the active group median 2 weeks (range 1–7) and in the placebo group median 3 weeks (range 1–6). Such calculations were done on 16 active- and 17 placebo-treated patients.

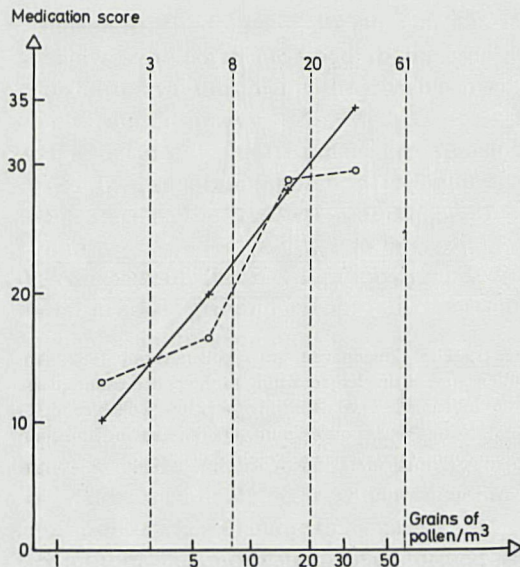


Fig. 3. Mean medication score is calculated for all days in each pollen quartile. The average pollen counts for each quartile are expressed as a function of quartile medication score.

×: Active treatment; ○: placebo treatment.

Nasal provocation test

Patients recording positive nasal symptoms on the diary card within 10 days of the provocation test were excluded from the NPT results. Patients claiming to have a cold or other nasal diseases on the day of the test were also excluded. Data from 45 (22 in the active versus 23 in the placebo group) were evaluated. The test concentration causing a positive reaction by NPT after treatment was compared with that causing a positive response before treatment. The "after treatment" value was divided by the "initial value" to give an index of change. Thus, a figure of 1 indicates no change, a figure of 2 indicates a twofold increase in concentration of solution required to produce a positive response, i.e. a twofold decrease in sensitivity to grass pollen. Decreases are explained in the same way (Fig. 4). There was no difference between the two groups. Patients with high initial reactivity were examined separately to see if active treatment had a greater effect than pla-

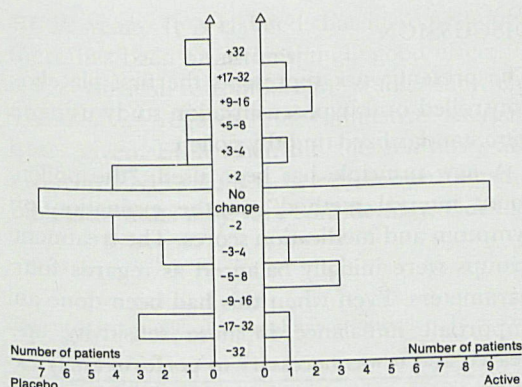


Fig. 4. The individual results of NPT tests in the active and placebo group are recorded. The "after treatment NPT result" was divided by the initial NPT result to give an increase/decrease figure.

cebo in reducing the reactivity of highly reactive patients. This was not so.

Skin prick test

Data from 52 patients were suitable for analyses. Table 6 gives the results of skin prick testing before and after treatment. The mean values in the first two columns should be treated with caution due to skewness of the underlying distribution involved. The ratio of median values after and before active treatment average a 2.1-fold increase in HEP values and for placebo a

Table 6

Skin prick test results expressed in AUR, before and after treatment

Treatment group	Individual patient HEP values		Ratio of AUR's after:before treatment
	before treatment	after treatment	
Active (n = 25)			
mean values	32.9	77.0	1.9*
median values	15	45	2.2
range of values	3-240	1-400	0.1-13.3
Placebo (n = 27)			
mean values	21.4	58.1	2.3*
median values	10	28	2.6
range of values	3-172	4-315	0.1-39.4

* Geometric mean value used due to skewness of distribution.

2.5-fold increase. This treatment group difference does not achieve statistical significance.

After treatment, HEP values and corresponding "10 mg histamine equivalent" values were plotted on double logarithmic paper and resulted in "10 mg histamine equivalent" = $4.7 \times$ HEP.

Wide variations were observed and it could be estimated that for patients with very low HEP or "10 mg histamine equivalent" values the transformation factor is nearer 10, and for patients with high HEP or "10 mg histamine equivalent" values the factor is nearer 3.

Immunological blood tests

Total IgE and specific IgE. Preseasonal values (3 samples) in both groups showed unchanged values and no change between the two groups was found. The after season values showed increased values of specific IgE as is normally seen after the grass pollen season, but no statistical difference between the two groups was observed.

Specific IgG. By inhibition experiments with timothy, birch and mite extracts (1st incubation), and IgG, IgA and IgM proteins (2nd incubation) the assay was found to be specific for IgG antibodies to grass pollen (9). Two patients treated actively and six patients given placebo tablets had significant amounts of IgG anti-timothy before treatment began. Two actively treated patients and one placebo-treated demonstrated significant rises in serum IgG antibodies to timothy during the therapy. The medians for binding indices ranged from 4.5-7.0 (active group) and 5.0-7.0 (placebo group).

Immune complexes (IC). By use of the pRF assay, 60% in the active group and 59% in the placebo group were positive for IC. By PEG-CC only one patient (from the active group) was positive for IC. Nineteen patients had a negative IC test before starting tablet dosing, and one or more of the following tests showed IC. Sixteen patients had a positive test for IC before starting tablet dosing, and one or more of the following tests showed increasing values for IC. Three

patients were positive at the beginning showing the same value during the trial, 19 were negative throughout the trial. Examination of data from the birch- and mugwort-sensitive patients showed that these allergies could not explain the positive IC values.

Side effects

No systemic side effects were seen. Gastrointestinal side effects were recorded from March 3 to August 15. The patients were asked to record if they had diarrhoea, abdominal swelling, pain or nausea. Actively treated patients recorded more of these symptoms than the placebo-treated patients. No statistically significant differences were found with analyses of individual side effect total scores. However, when all gastrointestinal symptom scores are added the actively treated patients recorded higher levels of symptoms than placebo patients ($P = 0.06$). From a clinical point of view it is more relevant to examine the first period, in which patients gradually increase the grass pollen dose to maintenance level. It is apparent that the actively treated patients had significantly more gastrointestinal symptoms during the first 4 weeks than in the following 4-week periods ($P = 0.05$).

Subjective assessment of efficacy

After the end of the grass pollen season the patients were asked whether there had been a symptomatic improvement/deterioration or no difference compared with the previous year. The difference between the groups is not statistically significant.

Power of the trial

The ability of the trial to detect possible treatment group differences was calculated (12).

The trial had an 80% chance of finding a significant difference between treatment groups if active treatment caused an average reduction in symptoms of 50% in comparison with placebo treatment.

DISCUSSION

The present work represents the first placebo-controlled oral hyposensitization study using *in vitro* standardized timothy pollen.

A new principle has been used, "the pollen count interval method", in the evaluation of symptom and medication scores. The treatment groups were initially balanced as regards four parameters. Even when this had been done an important imbalance in nose sensitivity appeared and it was necessary to perform analyses of covariance. Also this is a new methodological approach in such studies. The remaining three factors which make this study methodologically reliable are:

- 1) The use of standardized and recorded hay fever medicine.
- 2) The recording of missing data and the new, more optimal way of replacing such data.
- 3) For all *in vivo* and *in vitro* tests timothy alpha fraction from the same batch was used.

Clinical considerations

The question whether oral hyposensitization is effective or not has been discussed ever since it was introduced in 1900. The growing literature concerning this subject consists mostly of uncontrolled trials in children suffering from allergic rhinoconjunctivitis and/or asthma (5, 15, 17, 27, 34, 37). Most of the authors claim a good treatment effect with preseasonal oral hyposensitization when the allergen in question is administered as a powder or an aqueous solution. A few controlled trials have failed to prove any treatment effect superior to placebo or subcutaneous hyposensitization (7, 26, 31, 32). Table 7 gives details of some of the recent studies and, to our knowledge, includes all the controlled studies published. Wortmann has reviewed his long-term results in uncontrolled studies (37) of oral hyposensitization in children suffering from pollen hay fever and claims a treatment effect in 70% of the treated patients. Wortmann tried the same treatment back in the 1960's in older children (age > 12 years) and adults, but the results were generally not as good as in children under the age of

10–12 years. It is claimed that in some adults there has been an astonishingly good effect. It is very difficult to compare the studies shown in Table 7 as regards the cumulative allergen doses given. Even when the allergen doses are given in PNU values it is well known that the biological units may vary considerably. Our use

of *in vitro* standardized timothy pollen (expressed in AUR's) is a first approach towards more comparative studies of oral hyposensitization. In recent years *in vitro*/biological standardized extracts have been introduced for subcutaneous hyposensitization, and we would recommend that similar extracts should be

Table 7

Recent effect studies using oral hyposensitization. In two of the five controlled trials the control group has been treated with subcutaneous (s.c.) hyposensitization

Authors	Control group: placebo/s.c. hyposensitization	Children/adults	Allergic disease	Duration of oral hyposensitization (s.c. hyposensitization)	Allergen used for oral hyposensitization (s.c. hyposensitization)	Preseasonal dose and/or total dose	Effect of oral treatment
E. Wortmann (37)	uncontrolled	children	rhinoconjunctivitis/asthma	20 weeks preseasonal × 2–3 years	Purex® (6 grass) aqueous solution	oral 160,000 PNU preseasonal × 2–3 years	good in 70 %
W. Rebien et al. (26)	s.c. hyposensitization	children	do./do.	20 weeks preseasonal × 2 years	grass pollen aqueous extract for oral use	oral 140,000 PNU preseasonal × 2 years	oral treatment inferior to s.c. treatment
					grass pollen alum adsorbed extract	s.c. 40,000 PNU preseasonal × 2 years	
R. Urbanek et al. (31)	placebo	children	do./do.	42 weeks	house dust mite aqueous extract	36,000 PNU	none
J. Cooper et al. (7)	placebo	children	do./do.	26 weeks	SDV oral grass vaccine Bencard® aqueous extract	not given in PNU or biological units	none
R. Urbanek et al. (32)	s.c. hyposensitization	children	do./do.	7 weeks preseasonal × 2 years (oral)	oral: 75 % grass + 25 % wheat aqueous extract	oral: 1,750,000 PNU preseasonal × 2 years	oral treatment inferior to s.c. treatment
				s.c. hyposensitization 10 weeks preseasonal × 2 years	s.c.: grass allergoid (same composition as oral)	s.c.: 1,550 PNU preseasonal × 2 years	
E. Taudorf et al. 1984	placebo	adults (15–45)	do./do.	26 weeks	timothy whole pollen (dialysed) in enterosoluble capsules	4,315,000 PNU 880,260 AUR preseasonal 8,915,000 PNU 1,818,660 AUR total	none

used in future studies of oral hyposensitization. In one of the placebo controlled studies (7) no cumulative dose has been reported at all. When comparing the grass pollen dose in our study with the dose normally recommended by Wortmann it is seen that our patients received 25 times more allergen (in PNU values) preseasonally. Our administration of the allergen in enterosoluble tablets ensured a higher concentration of undigested allergen at the intestinal mucous surface than allergen administered in aqueous solution or as powder. This indicates that the real differences between cumulative allergen doses, i.e. allergen available for per-sorption, are probably much higher than recorded.

In the present study a great effort was made to secure comparability between the two groups of grass pollen patients. Due to group imbalance at the end of the trial it was necessary to perform analyses of covariance in order to make a correct comparison between the groups. Table 4 shows that the mean value of total nose symptoms in the active group is substantially lower (119.4) than in the placebo group (158.1). There are slightly more eye symptoms (mean value) and slightly reduced medication scores (mean value) in the active group than in the placebo group. This might indicate a treatment effect, but after transformation, as shown in Table 5, there is no difference between the two groups. This is also in agreement with the fact that there were more patients in the placebo group with high nasal reactivity than in the active group (Table 2).

The power of this trial has been calculated showing an 80% chance of finding a significant difference between treatment groups if active treatment causes an average reduction in symptoms of 50% in comparison with placebo treatment. Although statistically this does not appear to be very powerful it must be born in mind that results for individual patients cover a substantial range, and thus a 50% decrease in symptoms would still result in a very large overlap between treatment groups.

Performing clinical placebo controlled trials with hay fever patients in order to test a new treatment, e.g. hyposensitization with pollen,

one is faced with a very difficult problem. Which effect parameter is most relevant to use when comparing the groups? In a recent work (11) three different medications were compared in hay fever patients using a cross-over design. Three measurements were used to compare these treatments: a) post symptom questionnaires, b) daily analogue scale scores of symptoms, and c) nasal FEV₁.

Daily analogue scale diary scores with analyses which take account of pollen count variation proved to be the most sensitive of the three measurements.

In numerous controlled clinical trials including hay fever patients receiving immunotherapy, daily symptom score and pollen count have been used in the evaluation of treatment effect (13, 22, 23, 38, 39). However, it has not been generally accepted that it is necessary to record daily medication score standardized in relation to daily pollen count (22, 23). Norman (22) has shown that if daily symptom score is added to the daily medication score, analyses of results were the same whether the medication score was added or not. However, the maximum possible symptom score (eye + nose + lung) was 15 points whereas the maximum medication score was only 4. If medication score were weighted as much as symptom score it might have given another result. In the study by Østerballe (38) the maximum possible symptom score and medication score are more equal. Also in this study an overall daily symptom score is produced by adding daily total symptom score and total medication score. This score is then related to the daily pollen count. In producing daily symptom and medication scores an arbitrary scale was used even though the scores are qualitatively different. Therefore it is dubious to add symptom score and medication score. We suggest that medication score and symptom score in relation to pollen count must be presented separately. When making an overall clinical assessment to prove whether a new treatment is effective or not, symptom score differences and medication score differences can be calculated separately. A decrease in symptom score for one group can then only be accepted as a clinical improvement if the medi-

cation score has not increased. The same can be applied to medication score.

The way to handle missing data (symptom score/medication score/being outside the pollen trap area) in the literature concerning hyposensitization trials is only poorly, if at all, discussed. We find the method described in our trial convenient, providing the number of missing data (days) is not a critical proportion of the total numbers of days in the trial; this was not the case in our trial.

The pollen count interval method provides a much better understanding of the effectiveness of the treatment involved than the method normally applied, i.e. comparing daily symptom score/medication score to daily pollen count. Our method yields a dose response curve for these parameters. In Figs. 2 and 3 it is clearly shown, as expected, that increasing pollen count is followed by increasing symptom score and medication score. We are able to show clearly if there are differences between the treatment groups at four different pollen count levels. This is of interest, since a new treatment might only show effect at high or low pollen counts. With the conventional method it would be difficult to show such treatment effect since the pollen count day-to-day variation is often large.

Using analysis of covariance we found a significant relation between pretrial nasal reactivity (NPT result) and the total eye + nose symptoms during the grass pollen season, i.e. increased nasal reactivity (low NPT result) causing increased eye and nose symptoms. This is in agreement with Viander et al. (33), who reported the same findings in birch pollen allergies.

Paraclinical data

Oral administration of timothy-containing tablets did not cause a significant rise in the median of serum IgG antibodies to timothy. The median of serum IgG antibodies before and during therapy was higher in both placebo and actively treated patients than in non-allergic adults, in whom we found a median of 1.0 ($n = 20$) for IgG antibodies to partially purified timothy extract. On the other hand, the levels

of serum IgG antibodies found in this work was much lower than the level seen in parenterally immunized patients, in whom the median was 87.8 for IgG antibodies to partially purified timothy extract (9).

Since no timothy specific IgG response could be detected this is probably the main reason for not finding IC.

Side effects

Wortmann (37) has reported side effects in 4–8% allergic children receiving oral hyposensitization. Most frequent were conjunctivitis, rhinitis and mild asthma attacks. Gastrointestinal disturbances such as vomiting, stomach-ache and diarrhoea were less frequent, and urticaria was reported in a few cases. No systemic side effects were recorded in our trial. Overall, gastrointestinal side effects were more frequent during the initial 4-week period in the actively treated group, which may indicate that the grass pollen has initiated "some irritations" in the gastrointestinal mucosa, though not causing an immune response which could be detected by our measurements.

Theoretical considerations

Numerous animal experiments show that it is possible to induce tolerance to both soluble and particulate antigens by oral feeding in non-sensitized animals (1, 4, 30). Recently, it has been shown that antigen feeding is able to prevent secondary antibody response and interrupt reagenic (IgE and IgG₁) antibody production to the antigen in question (18). Gastrointestinal experiments show that intact macromolecules are able to cross the gastrointestinal epithelium in man (35). During oral immunotherapy local and systemic side effects may occur, indicating that molecules with allergenic properties are also able to cross the gastrointestinal epithelium.

Greater knowledge has been acquired in recent years about the mucosa defense system (14). A unique experiment in man has recently been performed showing selective induction of an immune response in human external secretions by ingestion of bacterial antigen (20). In-

gestion of capsules which contained killed *Streptococcus mutans* (a bacteria causing caries) resulted in the appearance of specific antibodies of immunoglobulin A class in saliva and tear fluid within 1 week. A second period of immunization resulted in a pronounced increase of specific antibody levels, which occurred earlier than in the primary immunization period and reached peak levels by day 10. No change could be shown in serum antibody levels. There is substantial evidence that the precursors of IgA-producing plasma cells in salivary and lacrimal glands originate from gut-associated lymphoid tissues and mesenteric lymph nodes. Thus there is experimental evidence that ingestion of particulate antigen may cause specific antibody formation in external secretions. The ingestion of the allergen in question by hay fever patients might cause the same specific antibody formation resulting in reduction of seasonal symptoms.

Conclusion

Our study of the efficacy of oral hyposensitization, using enterosoluble grass pollen tablets in the treatment of grass pollen hay fever has failed to show any treatment effect. For various reasons discussed above oral hyposensitization of allergics might later prove to be successful. It may only be a question of increasing the dose of potent allergens, adding adjuvants to the allergen powder or increasing the medication period, or perhaps ensuring delivery of active allergen to a specific, as yet unknown, site in the gastrointestinal tract.

Until it has been proved that oral hyposensitization has a beneficial effect it cannot be recommended.

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