

ORIGINAL ARTICLE

A double blind, placebo-
controlled cross over trial of
cellulose powder by nasal
provocation with Der p1 and
Der f1

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ABSTRACT

Objective: The purpose of this study was to assess whether inert cellulose powder would reduce the response to nasal challenge with house dust mite antigens. The study aimed to investigate the efficacy of inert cellulose powder applied to the nose for the control of persistent allergic rhinitis in adults due to house dust mite allergy. The powder has been registered as a medical device since 1994 and is available in many countries as a remedy for hay fever. Anecdotal evidence reported that it reduced symptoms of persistent rhinitis but no scientific evidence exists for this.

Research design and methods: A double blind, placebo-controlled cross over trial was conducted on 15 adult persistent rhinitis sufferers (diagnosed positive to Der p1 and/or Der f1 by SPT) and who had symptoms over the previous 2 years. The placebo was lactose powder. Challenge was by measured dose of homogenised allergenic dust. The study took place in the spring of 2006 before

the main pollen seasons.

Main outcome measures: The primary outcome measures were observed severity scores for 3 symptom categories and the amount of ECP in nasal secretions. The secondary outcome measures were symptom scores by subject report (nasal blockage, itching of nose, throat and eyes), nasal peak inspiratory (PIFn) and expiratory flow (PEFn).

Results: The results show significant differences for sneezing, itchy nose, runny nose and ECPs in nasal secretions. Some results are also significantly different between placebo and active for PIFn and for PEFn (all at $p = 0.05$). There were no adverse reactions.

Conclusions: The inert cellulose powder can have significant effects in reducing some symptoms of persistent rhinitis due to house dust mite allergy.

Introduction

The trial reported in this paper aimed to investigate the efficacy of inert cellulose powder applied to the nose for the control of symptoms of persistent allergic rhinitis in

adults due to house dust mite allergy. Allergic rhinitis is a heterogeneous disorder that has high prevalence but is often undiagnosed¹. Symptoms include one or more of sneezing, itching, nasal congestion and rhinorrhoea and can have a notable impact on quality of life as

well as exerting a high economic cost. In a two step cross-sectional population based study in 6 European countries the prevalence of subjects with clinically confirmable allergic rhinitis ranged from 17% in Italy to 29% in Belgium with an overall value of 23%². This large scale study confirmed that allergic rhinitis has a high prevalence in Western Europe and is frequently undiagnosed. In addition, within the population with allergic rhinitis, 29% had persistent rhinitis. Bauchau and Durham report that this group had more severe symptoms and a greater self awareness³. In the USA, allergic rhinitis affects approximately 20–40 million people⁴ of whom 20% are intermittent, 40% persistent and 40% of cases are mixed⁵.

Inert cellulose powder (Nasaleze*) has been registered with the Medicines and Healthcare Products Regulatory Agency (formerly Medical Devices Agency) since 1994 and is on sale in many countries, including the UK, as a remedy for hay fever. It is applied to the inside of the nose where it forms a gelatinous coating. It is thought that this layer prevents the allergens from reaching the mast cells in the nasal mucosa. Two previous trials have been conducted by the authors to investigate the efficacy of the powder in the control of hay fever^{6,7}. The results showed that the product reduces some symptoms. No adverse reactions were reported and the trials confirmed the powder as a low cost remedy with no known side effects.

Anecdotal evidence suggested that the powder also reduces symptoms of persistent rhinitis but scientific evidence was lacking. The principal objective of the current trial was to determine if the inert cellulose powder has a significant effect in the control of symptoms of persistent allergic rhinitis due to house dust mite allergy when no other medication was being taken.

Patients and methods

Basic study design

A double blind placebo controlled cross over trial with allergen provocation was conducted on 15 adult persistent rhinitis sufferers who had symptoms over the previous 2 years. The placebo was lactose powder. The study took place in the spring of 2006 before the main pollen seasons.

Ethical considerations

Ethical approval was given by NHS Hereford and Worcester local ethics committee REC number

05/Q2801/104. The study complies with the declaration of Helsinki.

Patients

Fifteen adult patients were recruited both male and female during the month before the trial. The sample size was set by a power calculation which indicated that the probability was 90% that the study would detect a treatment difference at a two sided 1.00% significance level if the true difference between the treatments is 1.609 units. This is based on the assumption that the within patient standard deviation of the response variable is 1.000.

Potential volunteers were sent a Baseline questionnaire 1–2 months before the trial which included questions on monthly occurrence and severity of symptoms, treatments used etc. People who seemed to be suitable were invited for a skin allergy test if they had not had one in the previous year. Selection was then based on the following. Inclusion criteria were subjects who have had a positive skin prick test for house dust mite allergen (Der p1 and/or Der f1) performed within the previous 12 months (wheal diameter at least 75% as large as histamine control), subjects who have had persistent rhinitis symptoms for a minimum of 2 years and were being treated by a doctor for this and who have no history of asthma. Principal exclusion criteria were people with asthma, upper respiratory viral infections, nasal deformities, pregnant women and people who have any other adverse medical conditions. Subjects must not have taken antihistamines in the preceding week or have used corticosteroids within the preceding 30 days.

Study design

Before the start of each trial the subjects were interviewed to explain the procedures in detail and to check for the presence of the symptoms that would be monitored, namely, sneezing, runny nose, itchy eyes, running eyes, itchy nose, itchy throat and itchy throat/palate. The symptoms were assessed by observation and by questionnaire which took approximately 30 min. If the subject had these symptoms that day they were excluded from the trial and another appointment was made. No medication was permitted to be taken by the subjects during the trial.

A pre wash (2.5 mL sterile saline) was given to each nostril and allowed to dry for 15 min. The saline wash was retained for analysis for ECPs (Eosinophil cationic proteins)⁸. Baseline PIFn (Nasal peak inspiratory flow) and PEFn (Nasal peak expiratory flow) were taken

* Nasalez is a registered trade mark of Nasaleze International Ltd

(best of three noted) using a computerised system (Vitalograph 2120 operated with the Vitalograph Spirotrac 4.20 software). Baseline symptom scores were also recorded.

Powder (real or placebo labelled as A or B, order randomised by blind draw) was put into each nostril as two applications per nostril from a plastic bottle with a patented valve applicator and was allowed to settle for 15 min. During the application the subjects were asked to breathe in but were told not to sniff. The powder was applied by a trained member of staff (either a nurse or a post doctorate researcher). The placebo was a lactose powder of similar particle size to the cellulose and in identical plastic bottles. New bottles were used for each application.

An allergen challenge was delivered to the nostrils by a Morrow Brown microspoon⁹, equivalent to 5 µg of Der p1 and 5 µg Der f1 per g of inert carrier fine particle dust (particles 15–100 µm). The dust was prepared in house and the allergen content was checked by 3 separate repeats of ELISA for Der p1 and for Der f1 each with 5 replicates at 4 serial dilutions. The dose given was 0.01 µg of homogenised dust mix in each nostril.

At baseline and at regular intervals after challenge, scores were taken for sneezing, itchy eyes, running eyes, itchy nose, running nose, itchy throat and itchy throat/palate graded as symptoms 0 = absent, 1 = very mild, symptoms hardly noticeable, 2 = mild, symptoms noticeable intermittently but do not interfere with any normal daily activities, 3 = moderate, symptoms noticeable all the time but do not interfere with any normal daily activities, 4 = severe, symptoms interfere with normal activities some of the time, 5 = very severe, symptoms interfere with normal everyday activities constantly. They were taken 5 min after challenge, every 15 min for the first hour after challenge then at 30 min intervals until 4 h after challenge, then at 6 h and at 24 h to observe any late phase reactions.

Nasal secretions were sampled for ECPs and measures were taken of PIFn and PEFn at 5 min after challenge, 15 min later, then at 30 min intervals for a further 2 h, then again at 4 h. At 6 h and 24 h after challenge peak flow readings were taken.

Nasal secretions were taken by inserting pre-weighed Whatman number 1 filter strips (Whatman Ltd, England) into the nostrils (left and right separately) following the methodology described by Knowles *et al.*¹⁰ which is acceptable to subjects and minimises stimulation that could lead to extra sneezing or secretions. The strips were stored in eppendorf tubes (pre-weighed), re-weighed then frozen until analysis. Any with blood stains were discarded. The nasal wash taken at baseline before the start of each session and the samples of nasal secretions taken through the trials

were analysed for ECPs using the Pharmacia Unicap system by the Department of Immunology, Northern General Hospital, Sheffield.

The second visit for each subject for the trial with the alternative powder was at least 7 days after the first visit.

The primary outcome measures were observed severity scores for symptoms (sneezing, nasal secretion and runny eyes) and the amount of ECP present in nasal secretions. The secondary outcome measures were symptom scores by subject report (nasal blockage, itching of nose, itching of throat, itching of eyes), PIFn and PEFn.

The participants and researchers did not know the identity of the powders until after the analysis was completed.

Subjects

Seven female and eight male subjects were recruited ranging in age from 18 to 60 years (modal age range 38–47 years). All had consulted their doctors about 'year round' symptoms of rhinitis. The recruits were selected on the basis of replies to a baseline questionnaire, an interview covering the baseline questions and the results of a skin allergy test. The baseline questionnaire and interview included questions designed to investigate whether patients had symptoms due to their house dust mite allergy. For example, subjects were questioned with regards to the severity of their symptoms and when and where they experienced these symptoms. Only those patients who had a suitable symptom history were recruited. The possibility of sensitisation to other allergens which may have been present in the home during the time the trial was undertaken was not taken into account. Subjects were symptom free at the start of the trial.

Some differences in the severity of rhinitis symptoms experienced over the previous 2 years were noted in the recruitment questionnaire (Table 1) but all of the recruits had persistent symptoms of frequent sneezing and runny nose to some degree. All of the subjects were allergic to Der p1 and 10 were also allergic to Der f1. The differences in subject profile were considered in the interpretation of the results. For example, the results of the skin allergy tests and the range and severity of symptoms reported in the baseline questionnaire were considered.

Statistical analysis

In most cases nonparametric tests of significant difference (Mann–Whitney and Wilcoxon tests) were applied as these do not assume normality and can be used to test ordinal variables. Student *t*-tests were used where possible

Table 1. Profile of the 15 participants

(a) Age, gender and symptom severity reported on recruitment questionnaire for the 15 participants

Participant No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Gender M/F	F	M	F	F	M	M	M	M	F	F	F	F	M	M	M
Age range	38-47	58+	38-47	38-47	58+	28-37	58+	58+	38-47	58+	28-37	18-27	58+	48-57	38-47
Symptom scores as estimate for severity over the last 2 years; symptoms 0 = none, 1 = slight, 2 = moderate, 3 = severe															
Sneezing	2	2	3	2	3	3	1	2	2	1	3	2	3	3	3
Itchy eyes	2	0	3	2	2	3	1	3	2	0	2	1	1	3	2
Running eyes	0	1	2	2	3	1	1	2	0	2	2	0	1	3	3
Running nose	2	2	2	2	3	2	2	2	1	2	3	1	3	3	3
Blocked nose	0	2	2	3	3	3	0	3	3	0	3	2	0	3	3
Headache/tiredness	0	1	2	2	2	3	0	3	2	2	1	1	1	2	3
Itchy throat/mouth	2	2	2	2	2	1	0	2	2	0	2	0	3	3	2
Total symptom score	8	10	16	15	18	16	5	17	12	7	16	7	12	20	19
Seasonality of symptoms over last 2 years															
All year	All	All	All	All	All	All	All	All	All	All	All	Any time of year	All year	All year	All year
but less in winter												but not constantly			

(b) Skin test results and medication used routinely for rhinitis

Participant No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Skin test result*															
Der f1 (mm)	No	No	4.0	4.0	No	No	4.0	7.0	6.0	No	5.5	6.5	6.0	5.0	3.5
Der p1 (mm)	6.0	4.0	5.0	5.0	3.5	5.5	5.0	6.0	5.0	5.5	5.5	6.5	4.0	7.5	5.6
Medication used for symptoms of rhinitis**															
NS, A, S	P	P	A	NS	NS	N	N	N	ST	N	A	N	A	NS	NS, A

*Y = yes then diameter of wheal (all at least 75% diameter of histamine control)

No = negative test result

Medication used**: A = antihistamine tablets; NS = nasal spray; P = pseudoephedrine hydrochloride; S = steroids; ST = sudafed tablets; NS = not specified; N = none

in cases of interval scale data. The level of probability was set at 0.05 or higher for acceptance. In many cases the data sets were highly skewed so descriptive statistics such as standard deviation are not useful. However, these have been included for comparison in some cases.

Results

No adverse reactions occurred. All 15 participants completed both stages of the trial.

In the cases of several of the symptom categories the scores were low or zero for many of the subjects. This may have been because the patients did not have allergic sensitivity to house dust mite. The low scores or absence of some symptoms could have been due to the relatively low amount of the challenge given and to the subjects' tendency to get only certain types of symptoms rather than all of the ones being monitored.

Primary outcome measures

Symptom scores

In the results for symptoms of runny nose (Figure 1), the differences overall are significant at the $p \leq 0.05$ level. Means are 8.8 cellulose powder and 15 placebo with SD 8.4 and 11.5, respectively. The confidence intervals of the means are 4.0 and 6.1 at $p \leq 0.05$.

In the case of sneezing, the difference in symptom scores is significant at $p \leq 0.05$ (means were 3.5 cellulose powder and 9.5 placebo, SD 3.6 and 10.2, confidence interval of the mean 1.4 and 5.2, respectively at $p \leq 0.05$). However, 4 subjects did not have symptoms.

Six subjects did not have any symptoms of running eyes on either stage of the study. A further 5 had only

very low scores and spasmodic symptoms. Of the other 4, three had zero scores with the cellulose powder but had total scores for the placebo of 17, 10 and 10, and one subject had a total score of 6 with the placebo against 1 with the cellulose powder.

Eosinophil cationic proteins

The results show a wide variance between ECP; however, there is a general trend towards the presence of larger amounts of ECP after the first 20 min following challenge (Table 2). In the samples taken 5 min after challenge (numbers 1–15), 7 of the participants had higher levels of ECP when the placebo was used than when the cellulose powder was used. This is compared with 3 subjects who had lower levels of ECP when on placebo than when on cellulose powder. The remaining 5 subjects had negligible differences. The same pattern is evident in the next set of readings, taken at 20 min after challenge. At 60 min after challenge, the level of ECP in some of the samples increased notably. Eight of the subjects had higher ECP levels with the placebo and in 5 cases, the differences were very large (over 100%). In 3 cases this difference occurred the opposite way round (results for cellulose powder were > 100% more than placebo). In the other four cases the results for the two wings were similar. Considering the magnitude of readings, for those at or over 2000 $\mu\text{g/L}$, 13 are with the placebo and 9 with the cellulose powder. For those at or over 4000 $\mu\text{g/L}$ seven are with the placebo and 4 with the cellulose. For readings at or over 6000 $\mu\text{g/L}$ four are with the placebo and only 1 is with the cellulose. The readings for the placebo are significantly different from those for the cellulose powder ($p \leq 0.5$), for the readings taken at 60 min and at 90 min after challenge.

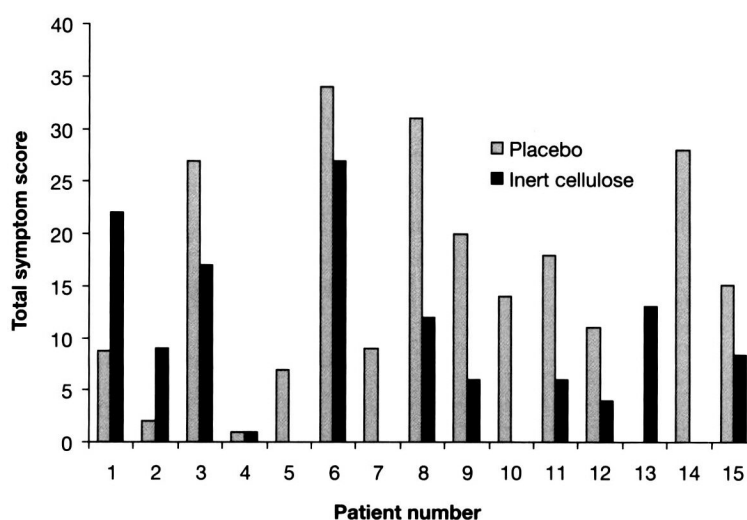


Figure 1. Total symptom scores for running nose from challenge to 4.5 h later. Means are 15 for placebo, SD 11.5 and 8.8 for cellulose powder, SD 8.4. Differences are significant at $p \leq 0.05$. Vertical axis is total symptom score, horizontal axis is patient number

Table 2. ECP results per participant at 5 min, 20 min, 60 min and 90 min after challenge

Time after challenge in min	5		20		60		90	
	C	P	C	P	C	P	C	P
Participant								
1	64	1174	250	240	250	940	2072	487
2	136	80	77	304	288	303	54	200
3	271	278	59	64	825	315	220	500
4	1020	546	943	1212	2033	2313	1232	2548
5	250	100	375	645	176	120	250	863
6	284	304	902	111	833	946	1024	131
7	120	1008	300	1100	158	3540	300	249
8	860	675	N	307	5025	441	4085	185
9	968	2678	392	2033	3038	1050	736	1125
10	381	450	580	429	461	255	317	1011
11	3906	436	549	215	3061	10857	422	4500
12	248	5882	1606	2562	1916	7317	832	804
13	1025	1503	1954	710	7500	331	731	15575
14	1130	786	429	354	2919	284	1000	895
15	1136	990	1622	6383	4110	952	1485	5000
Totals	11799	16891	10038*	16667	32592	29966	14760	34072

C: cellulose powder; P: placebo; N: no data; *One missing reading

ECP shown as µg/L. Differences are significant at $p \leq 0.05$ between placebo and cellulose treatments for the readings taken at 60 min and at 90 min after challenge

Secondary outcome measures

For symptoms of itchy nose (Figure 2) the difference in the results overall is significant at $p \leq 0.05$. Means are 17.2 with placebo and 7.8 with cellulose powder with SD 14.1 and 10.2, respectively. Confidence intervals of the means are 7.1 and 5.2 at $p \leq 0.05$. However, one subject had no symptoms and in 4 cases the total symptom scores were higher with the cellulose powder than with the placebo. In three of these cases the differences are not large but in one case there is a very marked difference.

For the other two symptom categories there are no significant results. For itchy mouth/palate, eight of the subjects had zero scores on both stages of the trial. For itchy throat, 5 subjects did not have any symptoms on either stage of the trial. The results for the other ten subjects are very similar for placebo and cellulose powder apart from one subject who had a total symptom score of 36 for the placebo and 24 for the cellulose powder.

Nasal flow readings

In the majority of cases mean PIFn readings were notably better compared to baseline, after challenge when the inert cellulose powder was used, compared to the results when the placebo was used (Figure 3). The mean difference is significant at $p \leq 0.05$. Means are -25 (placebo) and -3 (cellulose powder) with SD

-7 and -29. The confidence intervals of the means are 50 and 14.7, respectively.

The mean peak PEFn results were significantly better ($p \leq 0.05$) after challenge when the inert cellulose powder was used compared with when the placebo was used (Figure 4). However, in three cases (numbered 1, 3 and 8 on the figure) the results were better with the placebo than with the inert cellulose powder. The means were -43 (placebo) and 22 (cellulose) with SD 96 and 95. The confidence intervals of the means were 48 and 47, respectively.

In several subjects there were marked differences with a clear pattern of decreased PEFn when using the placebo. These general patterns both for overall results and for individual subjects were apparent through the time course of the study.

Discussion

Although there was only a 1 week wash out period, no order effect was noted between wash out with active and placebo treatment. Also Baseline nasal resistance was noted to have returned to baseline after a minimum of a 1 week wash out period.

The results display considerable variance but this is not unexpected in trials such as this. A lot of factors may influence the outcomes, including thresholds of

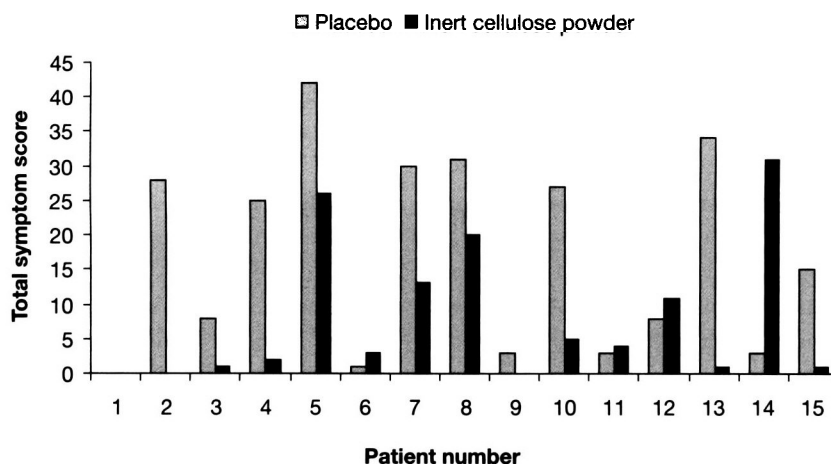


Figure 2. Total symptom scores for itchy nose from challenge to 4.5 h later. Mean for placebo scores =17.2, SD 14.2, mean for cellulose powder scores is 7.8, SD 10.2. Differences are significant at $p \leq 0.05$. Subject one had no symptoms. Vertical axis is total symptom score, horizontal axis is patient number

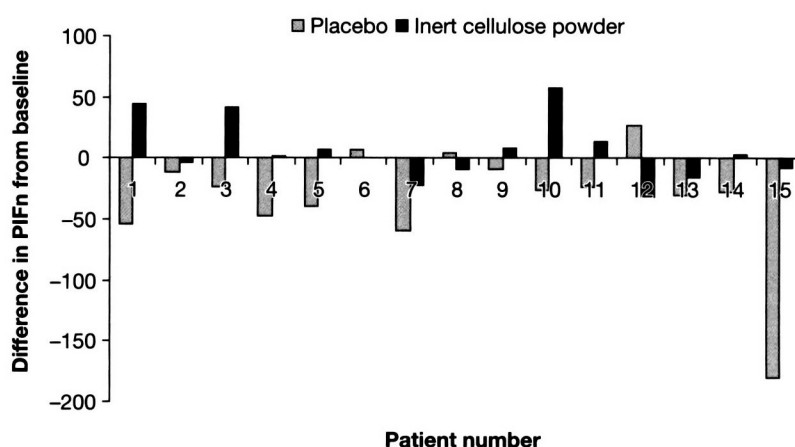


Figure 3. PIFn mean of differences from baseline from immediately after challenge to 4.5 h later. Overall mean for placebo is -25, SD 47, overall mean for cellulose powder is -3, SD 25. Difference is significant at $p \leq 0.05$. Readings are in L/min. Vertical axis is difference in PIFn from baseline, horizontal axis is patient number

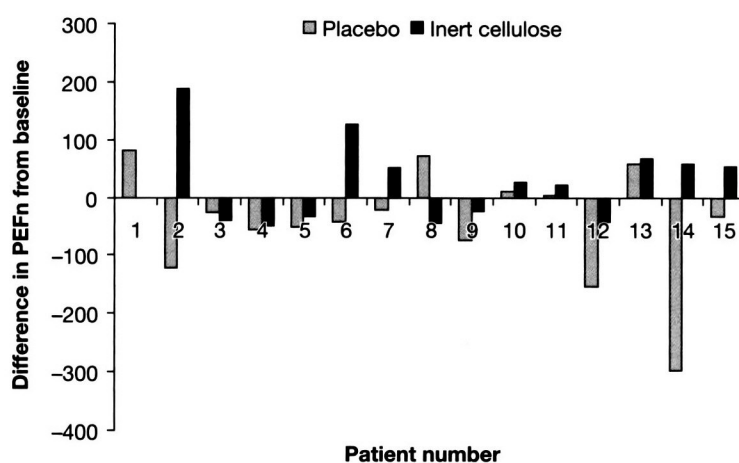


Figure 4. Mean PEFn readings for individuals as differences from baseline. Mean is for 7 readings taken from after challenge to 4.5 h later. Overall mean for placebo is -43, SD 96. Overall mean for cellulose powder is 24, SD 69. For participant one there was no difference from baseline to mean PEFn when using the cellulose powder. Readings are in L/min. Vertical axis is difference in PEFn from baseline, horizontal axis is patient number

sensitivity. Although all of the subjects had positive skin prick tests results to Der p1 and 10 were also positive to Der f1, the reaction to the challenge could differ notably. The challenge was set at a fairly low level even though the subjects had no history of asthma. Crude reference points for risk levels for asthma due to Der p1 and Der f1 exposure have been cited as for sensitisation > 2 µg/g dust, and for symptoms > 10 µg/g dust^{11,12}, but comparable figures for rhinitis are lacking. These thresholds would apply to a continuous exposure and little information is available to predict reactions when the allergen is applied in one dose. Previous studies have indicated that nasal provocation by house dust mite allergens may also provoke concomitant asthmatic symptoms during the late phase reaction¹². This is not exclusive to people with a history of asthma and we wanted to minimise the risk of this complication in the study whilst having the possibility of invoking some symptoms of rhinitis.

Some previous research projects have used nasal spray with phosphate buffered saline (PBS) with concentrations of house dust mite allergen extracts¹³. This approach was not considered suitable for this trial as we were interested in achieving conditions which were as close as possible to those in real life. The challenge was, therefore, delivered as a homogenised and standardised dust rather than as a spray.

Currently the powder is supplied in plastic containers and is dispensed through a patented valve system by a squeezing action. The amount of powder delivered per puff is approximately the same but it may not be constant. This aspect was minimised by having a standard technique and only two people making the applications.

The challenge was given as one delivery of allergen to the nose. Other work has examined the effect of continuous allergen challenge on clinical symptoms and mediator release in dust mite allergic patients¹⁴ over 8 h. In this double blind placebo controlled cross over study the whole sample population showed a rise of nasal and ocular symptoms which were perceptibly but not significantly attenuated by active drug treatment. ECPs exhibited a constant level over the whole provocation period.

The study was designed to detect both early and late phase reactions. Early phase response occurs within minutes of the allergen challenge and tends to produce sneezing, itching and clear rhinorrhoea. Late phase response occurs 4–8 h after allergen challenge and is characterised by congestion, fatigue, malaise, irritability and possibly neurocognitive deficits. However, in this study the development of late phase

reactions was not evident, possibly due to the low level of the challenge.

Eosinophil cationic proteins in nasal fluid have been used frequently as a marker for local inflammation^{15,16}. Previous research has established that this marker is an effective measure of degranulation and thus activation of the eosinophils¹⁷. In order to maintain the blind nature of the trial the collation and analysis of the results was done by different people from those who assisted with the challenge tests.

Conclusion

The results of the trial indicate that the inert cellulose powder can have significant effects in reducing some symptoms of rhinitis due to house dust mite allergy. The results show significant differences between the placebo and active treatments for sneezing, itchy nose and runny nose, and ECPs in nasal secretions. Also the results indicate that when the inert cellulose powder is used the mean peak nasal expiratory and inspiratory flows are higher than when the placebo is used.

Further research is in progress to determine the mode of action of the cellulose powder, particularly its capacity to act as a barrier to the passage of allergens.

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References

1. Strachan D. In: Epidemiology of rhinitis in asthma and rhinitis, 2nd ed. Vol. 1, chapter 5, pp. 33–42, 2000
2. Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur Respir Journal* 2004;24:758–64
3. Bauchau V, Durham SR. Epidemiological characterization of the intermittent and persistent types of allergic rhinitis. *Allergy* 2005;60:350–8
4. Skoner DP. Allergic rhinitis: definition, epidemiology, pathophysiology, detection, and diagnosis. *Allergy Clin Immunol* 2001(Suppl 1):S2–S8
5. Bachert C, van Cauwenberge P. The WHO ARIA (allergic rhinitis and its impact on asthma) initiative. *Chem Immunol Allergy* 2003;82:119–26
6. Emberlin J, Lewis R. A double blind placebo controlled trial of inert cellulose powder for the relief of symptoms of hay fever in adults. *Curr Med Res Opin* 2006;22:275–85
7. Emberlin J, Lewis R. A double blind placebo controlled trial of inert cellulose powder by nasal provocation with grass pollen to assess the efficacy of the product in controlling the symptoms of hay fever. EACCI 2006 Abstract book ISBN 3-9810999-0-7;370

8. Wojnarowski C, Studnicka M, Kuhr J, et al. Determinants of eosinophil cationic protein in nasal lavages in children. *Clin Exp Allergy* 1998;28:300-5
9. Morrow-Brown H. In: Shripad Agashe, editor. The relationship between aerobiology and allergology. In recent trends in aerobiology, allergy and immunology – a collection of plenary lectures and contributory articles. New Hampshire Publishers; 1994. Chapter 1, p. 1-31
10. Knowles G, Townsend P, Turner-Warwick M. A standardized filter paper technique for assessing nasal secretory activity. *Clin Allergy* 1981;11:287-92
11. Ross MA, Curtis L, Scheff PA, et al. Association of asthma symptoms and severity with indoor bioaerosols. *Allergy* 2000;55:705-11
12. Nitschke M, Pilotto LS, Attewell RG, et al. A cohort study of indoor nitrogen dioxide and house dust mite exposure in asthmatic children. *J Occup Environ Med* 2006;48:462-9
13. Wang DY, Goh DY, Ho AK, et al. The upper and lower airway responses to nasal challenge with house-dust mite *Blomia tropicalis*. *Allergy* 2003;58:78-82
14. Horak F, Toth J, Hirschwehr R, et al. Effect of continuous allergen challenge on clinical symptoms and mediator release in dust-mite-allergic patients. *Allergy* 1998;53:68-72
15. Rasp G, Thomas P, Bujia J. Eosinophil inflammation of the nasal mucosa in allergic and non allergic rhinitis measured by eosinophil cationic protein in native nasal fluid and serum *Clin Exp Allergy* 1994;24:1151-6
16. Klimek L, Rasp G. Norm values for eosinophil cationic protein in nasal secretions: influence of specimen collection. *Clin Exp Allergy* 1999;29:367-74
17. Wand D, Clement P, Smits J, et al. Monitoring nasal allergic inflammation by measuring the concentration of eosinophil cationic protein and eosinophils in nasal secretions. *Allergy* 1995;50:147-51

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