

Effectiveness of benzyl benzoate in elimination of house dust mites

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Received 13 December 1994; revised 29 September 1995; accepted 15 December 1995

Abstract

The effect of elimination treatment with benzyl benzoate was examined in 30 adults with asthma caused by sensitivity to house dust mites. The patients were randomised into a control and an active group, who treated their mattresses with benzyl benzoate (Acarosan[®]). The study lasted 12 months and the effect of the treatment was monitored by monthly dust sampling, analyzed for major allergens from *Dermatophagoides pteronyssinus* and *farinae* by the ELISA method and for guanine by the Acarex[®] method. The clinical effect was assessed by measuring lung function, daily peak flows, symptoms and medicine consumption as well as skin prick tests, and specific IgE to mite allergens. For both groups, a significant decrease was observed in house dust mite allergens, but there was no significant difference between the groups. No considerable differences were observed in clinical parameters within or between the groups. A good correlation was observed between ELISA and Acarex[®], but the latter showed a major variation in the different classes. In conclusion, treatment of mattresses with benzyl benzoate had no effect in a group of patients with asthma due to house dust mite allergy. Regular vacuum cleaning of the bed may reduce house dust mite allergen exposure.

Keywords: House dust mites; Allergy; Asthma; Benzyl benzoate; Guanine; ELISA; Acarex[®]; Acarosan[®]; Aerobiology

1. Introduction

Allergic symptoms due to house dust mite allergy are commonly observed (Korsgaard, 1983a). In Denmark approximately 1% of the total population is affected (Korsgaard and Iversen, 1991). Dust mites are found in almost all dwellings, and in much larger concentrations in mattresses than on floors (Maunsell et al., 1968). Avoidance of house dust mites (especially in the bed) is therefore of crucial clinical importance for house dust mite allergic patients. Many techniques have been proposed for reducing dust mite allergens in homes. In countries where there is a relatively low outdoor humidity in 3 months or more per year, it is sometimes possible to reduce the indoor humidity just by ventilation through the windows, so the house dust mite population decreases (Korsgaard, 1983b). However, even in such countries it is not always possible to reduce the house dust mite exposure and it has been proposed to install mechanical ventilation systems in

order to reduce the indoor humidity (Harving et al., 1988). This method is expensive and other techniques should be tried. Acarosan[®] is an acaricide, the main active substance being benzyl benzoate, which is known from the treatment of scabies and considered to be without any unexpected side effects (Landegren et al., 1979). Treatment with benzyl benzoate has been shown to reduce the number of house dust mites (Hayden et al., 1992), and thereby improve rhinitis (Kniest et al., 1991), but not asthma due to house dust mite allergy. Other authors did not find a significant reduction in house dust mite allergen levels after benzyl benzoate (Ehnert et al., 1992).

Quantifying of house dust mites is routinely performed by either mite counting under microscope or by analysis of house dust mite specific allergens by the ELISA method (Lind, 1986). Acarex[®], another test for quantifying of dust mites, is based on the detection of guanine, a major metabolic product from house dust mites (Le Mao et al., 1989).

The aim of this study was primarily to determine the house dust mite allergen reduction capability of treatment with Acarosan[®]; secondly to evaluate the potential subsequent clinical effect in patients with asthma

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due to house dust mite allergy. Finally, the study aimed to evaluate the usefulness of single observations with the Acarex® method by comparison to the well documented ELISA method (Lind, 1986).

2. Materials and methods

2.1. Patients

Thirty patients of both sexes, aged 18–65 years, were selected according to (1) a clinical history indicating asthma due to house dust mite allergy i.e. exacerbation of the perennial, asthmatic symptoms in the autumn, and wheezing after dust exposure such as cleaning, (2) a positive skin prick test with allergen extracts of house dust mites, (3) increased levels of specific IgE antibodies to *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in sera, (4) a dust sample from the patient's bed, which should be positive to house dust mite allergen (> 500 ng Der p I and f I/g dust). Patients with major medical diseases, low compliance, patients who have had immunotherapy with extracts of house dust mites within 2 years, and patients who received actual treatment with systemic corticosteroids were excluded. Patients with other perennial allergies were also excluded, if they were currently exposed to the specific allergen to which they were allergic (e.g. dog and cat). During the study, the patients' homes and especially their bedrooms were not allowed to be changed with respect to the indoor environment during the study.

2.2. Study design

The study was performed as an open, controlled study lasting for 12 months. The patients were examined in the clinic before and after a run-in period (visit 1 and 2), and after 3 (visit 3), 6 (visit 4), 9 (visit 5) and 12 (visit 6) months. At visit 2, after the run-in period, the patients were matched in pairs based on sex and age and subsequently randomized to either active treatment with benzyl benzoate (group 1) or no treatment, controls (group 2).

2.3. Elimination treatment

Cleaning the bedroom was part of the recommendation for the acaricide treatment. In order to minimize differences between the groups in procedures, which theoretically could eliminate mites, all patients were asked at visit 2 to clean their beds carefully. This meant vacuuming their mattresses for 5 min, vacuuming under their beds and in the bedroom. The bed linen should be laundered at least at 60°C. The patients in group 1 were then instructed to clean their mattresses with Acarosan® (Werner and Metz, Mainz, Germany) foam.

Bedroom carpets were treated with Acarosan® powder. Cleaning was performed by the patients, according to both the producers recommendations, and a careful verbal and written instruction by the investigators.

2.4. Dust samples

Dust samples were collected by the patients twice before elimination treatment and thereafter once a month during the study. Dust samples were collected standardized with ALK filter mouthpieces (Mosbech et al., 1988), which are mouthpieces for household vacuum cleaners with exchangeable collection units with filter papers. The patients were instructed to vacuum clean the dust samples from the surface of their mattresses. Collection time was 2 min/m². On collection day all the dust samples were mailed to the allergy clinic where they were analyzed immediately by the Acarex® test (Werner and Metz, Mainz, Germany). The Acarex® test is a semiquantitative test based on the detection of guanine, a major metabolic product from house dust mites (Le Mao et al., 1989). The results were expressed in classes from 0 (negative) to 4 (highly positive). All analyses were performed by the same clinician (the corresponding author). The dust samples were, on the same day they were received, forwarded to ALK Laboratories without indicating the Acarex® test result, and were analyzed there for specific Der p I and f I respectively including all subspecies from the *farinae* group by a sandwich ELISA technique (Lind, 1986). The results were expressed in ng/g dust. Dust samples were analyzed for *Dermatophagoides pteronyssinus* in one run and *farinae/microceras* in another run. The figures presented as results in this paper are the total exposure of house dust mite allergens. All dust samples were analyzed immediately at the laboratory following the recommended procedures (Lind, 1986).

2.5. Skin prick test

At inclusion and after 1 year, skin prick tests with a standard panel of inhalant allergens (Soluprick® SQ, ALK laboratories), including *Betula verrucosa*, *Phleum pratense*, *Artemisia vulgaris*, horse dander, cat dander, dog dander, *Dermatophagoides pteronyssinus* and *farinae*, *Alternaria* and *Cladosporium herbarum* were performed. Diluents with or without histamine dihydrochloride (10 mg/ml) were used as positive and negative controls, respectively. Quantitative skin prick test titrations with extract of *Dermatophagoides pteronyssinus* with 1, 3, 10, 30 and 100 HEP dilutions were performed at inclusion, after 6 and 12 months. All skin prick tests were performed using double-estimations, and evaluated according to Nordic guidelines (Aas et al., 1978). The sensitivity of the skin was estimated by skin index (SI), defined as:

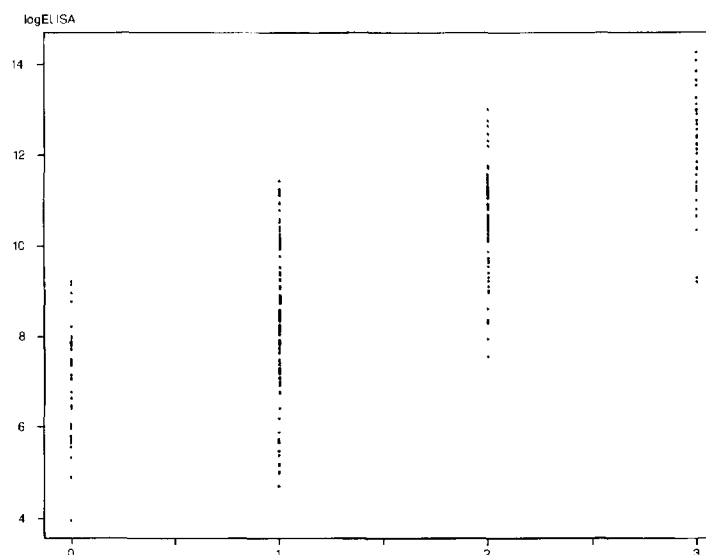


Fig. 1. Distribution of actual content of ng Der p I and f I/g dust (ELISA) in dust samples for the different Acarex[®] classes.

$$SI = D(A) + d(A) / D(H) + d(H)$$

where $D(A)$ = largest diameter of allergen wheal, $d(A)$ = midorthogonal diameter of allergen wheal, $D(H)$ = longest diameter of histamine wheal and $d(H)$ = midorthogonal diameter of histamine wheal.

2.6. Specific IgE

Sera collected at inclusion, after 6 and 12 months were analyzed for the levels of specific IgE to *Derma-tophagoides pteronyssinus* and *farinae* (Magic Lite[®] SQ Allergy System, ALK Laboratories).

2.7. Lung function

Lung function (FEV₁ and FVC) was measured on a dry spirometer (Vitalograph[®]) at visit 1, 3, 4, 5, and 6.

2.8. Daily record cards

Before elimination treatment with benzyl benzoate (between visit 1 and 2), after 5, and 11 months the patients kept daily record cards in periods of 1 month, recording morning and evening peak flows, (best of three measurements, Mini Wright[®] peak flow meter[®], Airmed, Harlow, UK), symptom score on a scale from 0 (no symptoms) to 4 (very severe symptoms), and daily medicine consumption.

2.9. Statistical analysis

All calculations were performed with SAS. The statistical differences were calculated by use of statistical methods indicated in Section 3. When analyzing data by *t*-test, the following model was used: Observa-

tion = level + contribution from treatment + error. To allow a more complicated structure, the following model was used by ANOVA: Observation = level + contribution from treatment + contribution from sex + interaction between treatment and sex + error. When analyzing data where observations are taken from the same subjects at points of time, one must take the possible presence of a timewise correlation between measurements into account. This may be done by a repeated measurements analysis of variance and for this, the following model was used: Observation = level + contribution from *i*'th treatment + contribution from *j*'th patient in *i*'th treatment + contribution from *k*'th timepoint + interaction between treatment and time + error. The level of significance was 5%.

3. Results

Twenty-six patients completed the study. Fourteen in the active group (five women and nine men, aged 16–56 years) and 12 in the control group (five women and seven men, aged 16–55 years). Four patients dropped out either due to lack of compliance or change of home. No significant difference between the groups were found according to sex, age, height or weight. None of the patients had bedroom carpets, so no Acarosan[®] powder was used.

In total, 278 dust samples were collected, and 257 dust samples were analyzed by the Acarex[®]-test as well as the ELISA-method. By an ordinary least squares regression, the squared multiple correlation coefficient was 60.03, i.e. more than 60% of the variation in the ELISA values may be explained by the variation in the Acarex[®] values. Fig. 1 gives the distribution between the two tests using the logarithm of the total Der p I

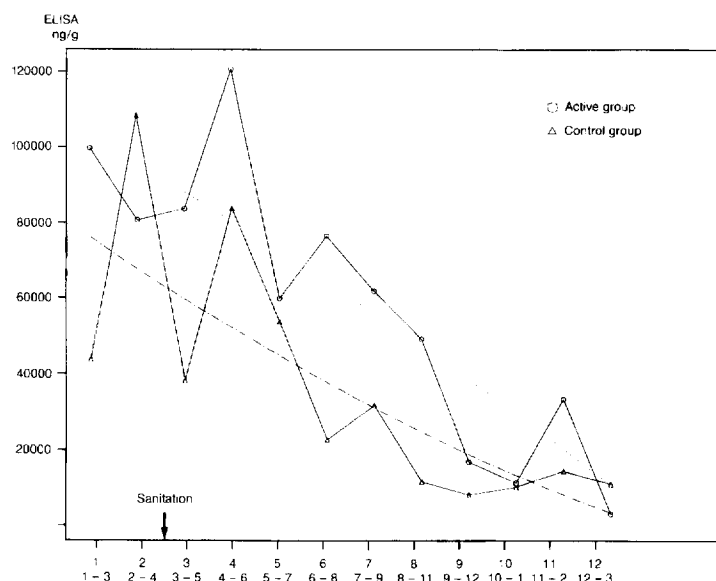


Fig. 2. House dust mite allergen levels, Der p I and f I, in dust samples expressed as mean values in ng/g dust (ELISA) for the active group and the control group during the study. Time for benzyl benzoate sanitation is indicated between no. 2 and no. 3 dust sample set. The calendar months in which the samples were collected are indicated below each sample no.

and f I measured by ELISA ($\log(\text{ELISA})$) and the corresponding Acares[®] classes.

During the study, fluctuations in house dust mite allergen levels were observed in the active group as well as the control group. However, a significant gradual decrease was found (by repeated measurement analysis) in the level of Der p I and f I (ELISA, $P < 0.001$) and guanine (Acares[®], $P < 0.05$) within both groups, but no significant difference was observed between the groups. Fig. 2 shows the mean values for house dust mite allergen content in dust samples (ELISA) for the active and control group. Data from one patient in the active group is excluded in the figure due to unexplained fluctuations in allergen content from < 5000 ng/g to $> 1\,400\,000$ ng/g. Calculations performed without this data did not change the statistical results. Benzyl benzoate elimination treatment is indicated between sample no. 2 and 3. After performing benzyl benzoate sanitation in dust sample no. 3, we found a non-significant decrease in the Der p I and f I levels, as compared to the mean before elimination treatment level ($P > 0.05$). The same observation was found in the control group.

No significant changes in FEV₁ and FVC were observed within or between the groups during the study. Peak flow levels (Table 1), were relatively stable during the three registration periods for the active group, whereas there was a decrease during the study for the control group. There was a significant difference between the active and control groups in the third period for evening peak flow ($P = 0.0355$) by simple t -tests and ANOVA ($P = 0.0404$).

The mean values for the symptom scores are shown in Table 1. There was no significant difference during the study for the groups or between the groups.

The patients' consumption of inhaled beta-2-agonists and inhaled steroids are shown in Table 1 expressed as the mean value for each group per day. There were no significant changes during the study or differences between the two groups.

Only a few patients used peroral beta-2-agonists and theophyllines. For the active group the consumption of oral beta-2-agonists was quite stable, but for the control group there was a significant increase in the third period, and the difference between the two groups was shown to be significant ($P = 0.0214$, t -test). The consumption of oral theophyllines showed no significant difference during the study or between the two groups.

Within the treated group we found a significant decrease between 0 and 12 months testing with 3 and 30 HEP *Dermatophagoides pteronyssinus* dilutions ($P = 0.009$ and 0.028 , respectively, Wilcoxon). No other significant changes in skin prick tests within or between the groups were found (Mann-Whitney). Specific IgE to *Dermatophagoides pteronyssinus* and *farinae* showed no significant differences within or between the groups.

4. Discussion

We investigated the levels of house dust mite allergens in two groups of subjects over 12 months. Baseline mean values as well as values obtained during the first months showed relatively high measurements, approxi-

Table 1

Mean values of inhaled beta-2-agonists and inhaled steroids, the symptom score and morning/evening peak flow measurements from patient diaries during the three registration periods (before elimination treatment and after 5 and 11 months)

Period	Inhaled beta-2-agonist (mg/day)		Inhaled steroids (μ g/day)		Symptom score (points/day)		Peak flow morning/evening (l/min)	
	Active	Control	Active	Control	Active	Control	Active	Control
I	0.45 (0.49)	0.51 (0.77)	260 (440)	358 (446)	3.86 (2.68)	1.88 (1.28)	500/526 (98)/(88)	452/465 (118)/(107)
II	0.42 (0.43)	0.39 (0.34)	153 (346)	374 (543)	2.86 (2.71)	1.44 (1.55)	523/551 (111)/(90)	449/461 (125)/(118)
III	0.55 (0.61)	0.47 (0.34)	197 (366)	511 (491)	3.66 (3.00)	2.90 (3.05)	508/527 (94)/(91)	416/432 (125)/(110)

Standard deviation in parentheses.

mately 60 000–100 000 ng Der p I and f I/g dust. However, the selected patients were house dust mite allergics, who consequently have higher levels of house dust mites in their homes than controls (Korsgaard, 1983b). Additionally, we measured not only Der p I but also f I, and used the total value for our calculations. This may contribute to the relatively high values we obtained compared to studies using only the Der p I value. Surprisingly, in one single test among one patient's samples we found an extreme value, namely 1 400 000 ng/g dust. The result was confirmed by performing repeated dilution estimation. We are not aware of similar findings in other studies.

The house dust mite allergen content in the patients' beds decreased during the study in *both* groups and there was no significant difference between the groups, which indicates that benzyl benzoate treatment *in vivo* has no effect on the house dust mite allergen levels, as compared to vacuuming. On the other hand, the results may suggest that a regular cleaning of the bed reduces the allergen content — both groups vacuum cleaned their mattresses monthly when they prepared dust samples. In contrast to these findings, no clinically significant differences were observed within or between the groups. The skin prick tests, specific IgE and lung function measurements, symptom scores, or medicine consumption did not show any clinically significant differences. No contrast in these parameters were expected between the groups due to the lack of difference in Der p I/f I antigen in the two groups. However, a clinical response *within* the groups could have been the result, because of the significant decrease in mite exposure in both groups, but still after this reduction, the patients are exposed to high levels of house dust mites, much higher than the risk levels.

We found a good correlation between the allergen content expressed as ng/g Der p I and f I by ELISA, and the semiquantitative guanine content by Acarex[®] expressed in classes. However, there was a large variation in the single Acarex[®] classes. Out of 39 samples

classified as Acarex[®] class 0, (negative), 13 (33.3%) had an allergen content below 500 ng/g dust. However, 26 (66.7%) samples in class 0 had a content of house dust mite allergen above 500 ng/g dust which is above the risk level. In contrast, 12 (10.9%) samples were classified as positive in class 1 by Acarex[®], and these were below 500 ng/g dust. Guanine is produced by birds and other mites, e.g. storage mites, which could explain false positive results, but not false negative results. Testing with Acarex[®] is therefore not reliable for single testing, which is why the clinical value is restricted. It may be useful for screening populations since there is a good correlation with the ELISA method.

Acknowledgements

We thank Professor Knut Conradsen, Institute of Mathematical Statistics and Operation Analyses, The Danish Technical University, Copenhagen, for performing the statistical analyses.

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