Effect of application of benzyl benzoate on house dust mite allergen levels

Jung H Chang,* Allan Becker,† Alexander Ferguson,‡ Jure Manfreda,§ Estelle Simons,† Henry Chan,* Kukuh Noertjojo,* and Moira Chan-Yeung*

Background: Several acaricides have become available for reducing house dust mite allergen levels.

Objective: The purpose of this study was to assess whether the use of benzyl benzoate (Acarosan) provides additional benefit to the usual mite control measures including encasement of mattress and pillows with vinyl covers.

Methods: A randomized controlled trial was carried out in 26 homes (14 control versus 12 treatment) of asthmatic patients in two cities (Vancouver and Winnipeg). The control group had the usual house dust mite control measures including the use of vinyl covers for mattresses and pillows while the treatment group had application of benzyl benzoate to mattresses and carpets in the bedroom and the most commonly used room, in addition to the above control measures. Mite allergen levels were measured 3 months and immediately before, 1 week, and 1 and 3 months after the application of house dust mite control measures. Patients kept diary cards on asthma symptoms and peak expiratory flow rates morning and evening one month before and three months after the onset of mite allergen control measures.

Results: A reduction of mite allergen level was found in mattress samples in both groups, statistically significant at all times in the treatment group and at one and three months in the control group. Mite allergen levels on floor carpets also showed progressive reduction in both groups, but were significantly different in the treatment group (compared with controls) at 1 week, and were lower compared with baseline in the treatment group up to 3 months. No significant changes in asthma symptoms, peak expiratory flow rates, spirometric measurements, or bronchial hyperresponsiveness were observed among treatment or control group subjects.

Conclusion: The addition of benzyl benzoate to conventional house dust mite control measures resulted in a significant reduction in floor carpet dust mite levels that persisted for 3 months. The results of this study should be confirmed in a larger and longer study.

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INTRODUCTION

House dust mite is one of the major allergens responsible for asthma.[1] Clinical studies that include measurements of mite allergens in homes have established their important role in asthma.[2] There are many methods directed at reducing exposure to these allergens. Enclosing mattresses and pillows with vinyl covers has been shown to reduce the mite allergen levels.[3–5] The efficacy of acaricides for reducing dust mite exposure has been reviewed recently.[6] Most of the previous studies compared the efficacy of either the use of acaricides alone or the use of enclosing mattresses together with other methods of dust removal. The goal of this study was to determine whether the use of an acaricide (Acarosan) provides additional benefit to the usual mite control measures, including encasement of mattresses with vinyl covers, in reducing mite allergen levels in the homes of patients with asthma and in reducing asthma severity.

MATERIALS AND METHODS

Study Design

Twenty-six patients with asthma and house dust mite sensitivity, 12 from Vancouver and 14 from Winnipeg, were invited to take part in the study. They were selected from a cohort of 120 patients with asthma (about half from each city), who took part in a panel study on environmental risk factors in asthma,[7] on the basis that mite allergens (either Der p 1 or Der f 1) in their homes were found to be higher than 1 μg/g of dust in one of the samples collected from either the mattress or the bedroom floor during the previous year and that they had positive skin tests to either mite allergen. The remaining patients in the cohort did not satisfy the selection criteria. In general, the mean mattress and bedroom floor mite allergen levels in the homes of this cohort of asthmatic patients were low. The geometric mean mattress and bedroom mite allergen levels (sum of Der p 1 and Der f 1) were 1.73 ± 3.33 and 1.33 ± 2.24 μg/g dust respectively for homes in Vancouver and 0.94 ± 2.38 and 0.76 ± 2.12 μg/g dust respectively for homes in Winnipeg.[7] The homes of the 26 patients who took part in this study were randomly assigned into the control group and the treatment group.

This study was started in December, 1993, for a period of 4 months to avoid the confounding effect of exposure to another allergen. All patients had observations 1 month before and 3

* Department of Medicine, University of British Columbia.

† Division of Allergy, Department of Paediatrics, University of Manitoba.

‡ Division of Allergy and Clinical Immunology, Department of Paediatrics, University of British Columbia.

§ Department of Medicine, University of Manitoba.

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months after treatment. After the initial assessment which included spirometry, methacholine inhalation test, and allergy skin tests, patients were asked to keep diary cards until the end of the study. House dust samples were collected on five occasions: 3 months and immediately prior to treatment, 1 week, 1 month, and 3 months after treatment. Three months after the institution of house dust mite avoidance measures, the patients were reassessed with spirometry and methacholine inhalation test.

House dust samples were collected using a portable vacuum cleaner with a special sock attachment. The pore size of the material used to make the socks varied from 5 to 10 microns in diameter. The patient's bedroom floor was vacuumed once covering all areas. The bed mattress was vacuumed for two minutes. The socks were placed in ziplock bags and stored at −20 °C until being analyzed. Large particles were removed by sieving through a 300-μm mesh sieve before processing. Samples of dust were extracted in borate buffered saline in the ratio of 100 mg to 2 ml, left overnight and filtered. Extracts were stored at −20 °C until being analyzed for mite allergen level.

The house dust mite avoidance measures were as follows: both groups were asked to vacuum their homes at least once a week and to wash the bedding in hot water (>58 °C) and to encase mattresses and pillows with vinyl covers. In the treatment group, benzyl benzoate (Allergopharma, Hamburg, Germany) was applied to the carpet in the bedroom and the most commonly used room; the mattress was also sprayed with benzyl benzoate and dried before encasing with vinyl covers.

Diary Card

The patients were asked to keep diary cards of their chest symptoms, medication requirement and morning and evening peak expiratory flow rates (PEF) measured by a mini-Wright peak flow meter. The symptoms included the following: cough, wheeze, and breathlessness during day/night and the activity level during the day. Each symptom was scored nominally (0 to 3) as follows: 0 - no symptoms; 1 - mild; 2 - moderate; 3 - severe. The daily symptom score was calculated from the sum of the scores of each symptom. Anti-asthma medications, bronchodilators and steroids, were recorded as the total number of tablets or puffs used in 24 hours. The actual dose used in 24 hours for each medication was calculated. Peak expiratory flow was calculated as the mean of morning and evening readings. The monthly mean symptom score, medication requirement and PEF were calculated for each patient.

Spirometry and Methacholine Inhalation Test

Forced expiratory volume in one second (FEV_1) and maximal mid-expiratory flow rate ($FEF_{25–75\%}$) were measured by spirometry according to the method recommended by the American Thoracic Society.[8] Methacholine challenge test was carried out using a method described previously.[9] The provocative concentration of methacholine that induced a 20% fall in FEV_1 from the lowest post saline level (PC_{20}) was determined.

Allergen Assay

Mite allergens were assayed using monoclonal antibodies against purified Der p 1 and Der f 1 by the enzyme linked immunosorbent assay (ELISA) method as previously described.[10] These antibodies and standard purified Der p 1 and Der f 1 antigens were kindly supplied by Dr. Martin Chapman of the University of Virginia. All analyses were carried out in duplicate and the means of the results were taken for statistical analysis.

Statistical Analysis

The data were analyzed using the SPSS and SAS statistical package on an IBM 486 personal computer. Logarithmic transformation was done on mite allergen levels and PC_{20} in order to normalize the distribution. The mite allergen levels used for analysis were the sum of Der p 1 and Der f 1. The baseline levels of mite allergens used in this analysis were the mean of the results of samples collected 3 months and immediately before the study. There was no significant difference between the mite allergen levels of the samples collected three months before and the samples collected immediately before the study. The floor mite allergen levels were the mean of the samples collected from the bedroom floor and the floor of the most commonly used room. Both parametric and nonparametric analyses were used to compare differences between groups in pulmonary function parameters and PC_{20} before and after institution of house dust mite avoidance measures. General linear model procedures for repeated measurements[11] were used to examine changes in mite allergen levels, symptom score, and PEF in both groups. For analyses of symptom score and PEF, the use of bronchodilators and steroids was included as covariates.

This study was approved by the Committees on the Use of Human Subjects in Research of the University of Manitoba and the University of British Columbia, and an informed written consent was obtained.

RESULTS

There were 14 patients in the control group and 12 in the intervention group. Of the 26 patients, 11 were children and 15 were adults. There were no differences between the two groups in terms of distribution of age and smoking status (data not shown). In addition to the mite allergens, 24 (92.3%) of 26 patients also reacted to at least one other common allergen and 19 (73.1%) to cat dander. There was no difference in the proportion of patients with positive skin test reactions between the control and the treatment group.

Table 1 shows mite allergen levels before and after treatment. In the treatment group, mite allergen levels in mattress samples decreased significantly from the baseline within 1 week of treatment and remained significantly lower at 1 and 3 months after treatment. Floor mite allergen levels also decreased significantly from baseline level at one week and persisted for three months after treatment. In the

Table 1. Mite Allergen (Der p 1 + Der f 1, μg/g dust) Levels Before and After Treatment (geometric mean ± SD)

	Baseline	1 Week After	1 Month After	3 Months After
Control group				
Mattress	1.68 ± 2.22	0.93 ± 1.90	0.56 ± 1.46*	0.28 ± 1.32*
Floor	2.05 ± 2.05	2.02 ± 2.21	1.71 ± 2.41	1.10 ± 2.17
Treatment group				
Mattress	2.17 ± 2.64	0.40 ± 1.57*	0.21 ± 1.27*	0.06 ± 1.12*
Floor	2.38 ± 2.24	0.68 ± 1.47*†	0.74 ± 1.91*	0.50 ± 1.71*

* $P < .05$ compared with the baseline value, GLM procedure for repeated measures.
† $P < .05$, comparison between treatment group and control group, GLM procedure for repeated measures.

control group, mite allergen levels in mattress samples showed a more gradual reduction and were significantly lower than baseline at 1 and 3 months. Although there was a trend for floor mite allergen levels to decrease with time, the differences from the baseline were not significant at any time. There was no overall significant difference in mattress mite allergen levels between the control group and the treatment group; however, significant differences in floor mite allergen levels were found between the control group and the treatment group.

Table 2 shows the results of symptom score, lung function tests and PC_{20} before and after the institution of house mite avoidance measures. The mean baseline values were not different between the two groups. The mean total symptom score and the mean PEF after treatment at all times were not significantly different from baseline in both groups adjusted for the use of bronchodilators and steroids. The mean lung function and PC_{20} were not different at 3 months after treatment compared with baseline in both groups.

DISCUSSION

Since Heller-Haupt and coworkers[12] first introduced the use of acaricide 20 years ago, several chemicals have been studied for the control of house dust mites. These include benzyl benzoate, tannic acid, natamycin, and organophosphates. The effectiveness of these chemicals is controversial. Several studies have shown that benzyl benzoate is more effective when applied on carpets than on mattresses[6,13–16] and can reduce mite allergen levels from 50% to 90%.

In this study we found that encasement of mattresses and pillows with vinyl covers in addition to hot water wash of bedding lowered mite allergen levels gradually over a period of 3 months, a finding similar to Owen et al.[5] The use of benzyl benzoate before encasing mattresses and pillows decreased mite allergens more rapidly and to a significantly low level within 1 week. It is not clear why application of benzyl benzoate would reduce mite allergen levels more quickly than the use of vinyl covers. It could be due to the lowering of mite allergen levels in the bedroom as a result of application of benzyl benzoate to the bedroom floor as well. Benzyl benzoate also decreased mite allergen levels in carpets; the reduction was statistically significant within 1 week after the application.

Seasonal variations in mite allergen concentrations, lowest in April through June, have been reported.[17] Another study of dust mite levels in homes in the midwestern United States demonstrated a seasonal cycle of mite abundance, with the greatest density occurring from June to October and the lowest density in January to April.[18] We attempted to avoid seasonal variation by recruiting all subjects at the same time and conducting the study during winter months from December, 1992 to March, 1993. Despite this, there was a trend for the mite allergen in floor samples to decrease as the study progressed in the control group, although the reduction was not significant. The more rapid reduction in mite allergen levels in mattress and floor samples in the treatment group suggests that seasonal variation cannot explain the decline in mite allergen levels we observed in this study.

Table 2. Severity of Asthma Before and After Treatment

	Symptom Score*	Pulmonary Function Parameters*			PC_{20}†, mg/mL
		PEFR, L/min	FEV_1, %	$FEF_{25–75\%}$, %	
Control group					
Baseline	0.6 ± 0.8	381 ± 97	88 ± 11	55 ± 18	0.47 ± 5.62
1 month	1.2 ± 1.6	379 ± 99	—	—	—
2nd month	0.7 ± 1.7	378 ± 104	—	—	—
3rd month	0.4 ± 0.5	383 ± 100	90 ± 15	57 ± 19	0.82 ± 3.84
Treatment group					
Baseline	1.5 ± 2.1	402 ± 69	85 ± 21	48 ± 20	0.76 ± 1.93
1st month	1.5 ± 1.9	399 ± 75	—	—	—
2nd month	1.6 ± 2.2	405 ± 69	—	—	—
3rd month	1.1 ± 1.7	411 ± 75	87 ± 20	48 ± 22	0.87 ± 2.29

* Values expressed as arithmetic mean ± SD.
† Values expressed as geometric mean ± SD.

There is controversy about the duration of action of benzyl derivatives as acaricides. Some investigators reported only short-term effectiveness from 3 days to 2 months[6,15,16]; Kalra et al[19] observed the loss of effectiveness after 3 months. The lack of long-term effectiveness appears to be due to recolonization from other sites or multiplication of the remaining mites. Marks and colleagues[20] found that simple chemical treatment and bedding encasement was not sufficient to promote a sustained, beneficial reduction in allergen levels in homes in Sydney, Australia. A more recent study from Charlottesville, Virginia,[21] showed that although application of chemical treatments can reduce mite allergen levels in carpet dust, the effects do not appear to be maintained for long periods, and are not dramatic.

In this study, we found that the effect of benzyl benzoate on carpet mite allergen persisted for at least 3 months. It should be noted that mite allergen levels in these two Canadian cities are low compared with other parts of the world where the relative humidity levels are high.[7] The reason for the discrepancy in the findings of this study and others could be due to the relatively low baseline mite allergen level and the small number of homes in this study.

We failed to show significant improvement in any clinical variables up to 3 months after the institution of avoidance measures. The failure to document improvement could be due to the short duration of follow-up. Walshaw and Evans[4] examined several objective parameters including pulmonary function and PC_{20} at 4 months and 1 year after similar house dust mite avoidance measures. The improvement was found only at 1 year, not 4 months. Exposure to other aeroallergens to which many of our patients also had allergy may explain our failure to document improvement in clinical variables.

We conclude that the application of benzyl benzoate to carpets resulted in a reduction of floor mite allergen level lasting for at least 3 months. A larger study of longer duration is necessary to confirm this observation.

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Moira Chan-Yeung
Respiratory Division
Vancouver Hospital & Health Sciences Ctr.
University of British Columbia
2775 Heather St
Vancouver, BC
Canada V5Z 3J5