

Placebo-controlled study of the mite allergen-reducing effect of tannic acid plus benzyl benzoate on carpets in homes of children with house dust mite sensitization and asthma

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We studied the effect of a spray containing 1% benzyl benzoate, an acaricide, and 1% tannic acid ('Lowal'; a protein-denaturing substance), on concentrations of major allergens from house dust mite (HDM) species *Dermatophagoides pteronyssinus* and *D. farinae* (*Der p 1* and *Der f 1*, respectively) in carpets. In a double-blind, placebo-controlled study with crossover design, 30 homes of children with HDM sensitization and asthma were included. All houses showed ≥ 400 ng/g of *Der p 1*+*Der f 1* in carpet dust. The first treatment was performed on day 0 (group 1 active treatment, $n=15$; group 2 placebo treatment, $n=15$). After 2 and 8 weeks, dust samples were collected for quantification of mite allergens. After a 2-week washout period, the second treatment was performed (group 1 placebo treatment; group 2 active treatment). Again, carpet dust was collected after 2 and 8 weeks. Twenty-two of 30 families completed the trial: 14/15 in group 1 and eight of 15 in group 2. On day 0, there was no significant difference in mite allergen exposure between group 1 and group 2 (1,498 vs. 2,239 ng/g of *Der p 1*+*Der f 1*, respectively). In group 1, the geometric mean for the difference of mite allergen concentration comparing day 0 and week 6 was 196 ng/g (95% CI: –7,161 and 8,401) for the first treatment (active) and 15 ng/g (95% CI: –1,079 and 1,292) for the second treatment (control). In group 2, the difference was 66 ng/g (95% CI: –398 and 1,515) for the first treatment (control) and 609 ng/g (95% CI: 186 and 9,264) for the second treatment (active). Comparing placebo and active treatment in total, there was a significant decrease following placebo treatment after 14 days ($p = 0.026$). After 8 weeks, active treatment was superior to placebo treatment ($p = 0.049$), but the allergen reduction achieved was $<20\%$ (median 1,500 ng/g on day 0 vs. 1,250 ng/g after 8 weeks). We conclude that the slight mite allergen reduction on carpets achieved by the treatment with 'Lowal' is unlikely to achieve worthwhile clinical benefit either in the treatment of mite-sensitive patients or in primary or secondary prophylaxis.

**Susanne Lau, Julia Wahn,
Gabriele Schulz,
Christine Sommerfeld
and Ulrich Wahn**

University Children's Hospital Charité,
Humboldt University, Berlin, Germany

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Dr Susanne Lau, Charité Campus Virchow,
Kinderklinik mit S. Pneumologie und
Immunologie, Augustenburger Platz 1, 13353
Berlin, Germany
Tel.: +49 30 4505 66 182
Fax: +49 30 4505 66 931
E-mail: susanne.lau@charite.de

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In mite-sensitive patients with asthma, the reduction of mite allergen exposure is recommended as the first line of treatment (1,2). However, conflicting reports have been published about the long-term effect of chemical avoidance procedures (3–7). Studies of the short-term efficacy of benzyl benzoate treatment have

found that benzyl benzoate is not able to achieve a significant reduction of house dust mite (HDM) on mattresses (5). On carpets the effect was better, but many sites still showed allergen concentrations of >2 μg after application of benzyl benzoate. There are different reports on the effect of a combination of benzyl alcohol and

tannic acid. The degree of mite allergen reduction ranged from 90% on blankets (4) to 23% on carpets, upholstery, and beds (3). However, no study could show a significant reduction lasting for longer than 6 weeks. The present study was performed in order to analyze the mite allergen-reducing capacity of a newly introduced product on carpets over an 8-week period after one single application.

Materials and methods

Selection of households and application of treatment

Thirty households of children from the outpatient clinic of the University Children's Hospital Charité, Berlin, were initially included in the study. Children were selected who had bronchial asthma and HDM sensitization (CAP class ≥ 2). Inclusion criteria were as follows: a measured concentration of major HDM allergens *Der p 1*+*Der f 1* of >1000 ng/g in carpet dust in the 12 months prior to the start of the study; and the presence of a fitted carpet in the child's room. Exclusion criteria were *Der p 1*+*Der f 1* concentrations of <400 ng/g on day 1 before treatment, changes in carpeting and furnishing 12 months prior to the start of the study, and missing dust samples during the study period. The study period was March–October 1998.

In a double-blind placebo-controlled crossover design, 15 families were assigned to group 1 and 15 to group 2 by randomization (Fig. 1). After randomization and start of the trial, five families had to be excluded in group 2 because of a mite allergen concentration of <300 ng/g of carpet dust on day 0. In group 1, 14/15 children completed the trial and in group 2, eight of 10 children completed the trial. Three households were excluded (one in group 1 and two in group 2), because of unavailability of dust samples during the study period. Group 1 started with active treatment on day 0 and received placebo treatment after 10 weeks. Group 2 started with placebo treatment and received active treatment after 10 weeks. The crossover design was selected in order to reduce random errors and to motivate participation. A washout period of 2 weeks was selected as previous studies have shown that the effect of acaricides and tannic acid is not long lasting (3,8,9).

Active treatment was performed using a spray containing 3% benzyl benzoate and 1% tannic acid dissolved in isopropanol. Placebo treatment was performed using isopropanol alone as a spray. The manufacturer's instructions recommended the use of a bottle (≈ 375 ml) per 20 m^2

and a 4-h period to react and dry. Vacuuming was recommended after treatment in order to remove dead mites. Therefore, according to the manufacturer's instructions, 20 ml/m^2 was applied to the carpets in the child's bedroom. After 3–4 h, the carpets were dry and patients were allowed to use the room again. Active and placebo sprays were provided by Trimedal (CH-8306, Brüttisellen, Switzerland). Blinding of the study was achieved by the identical design of the bottles and an identical smell of the contents. Bottles were marked with different numbers; the codes were known only by the manufacturer.

Dust sampling

Dust samples were collected in a standardized manner by the same persons (S.L. and J.W.). The same 1-m^2 area of carpet surface was vacuumed for 3 min using the patients' vacuum cleaner ($>1000 \text{ W}$) and a new dust bag. The dust bag was put into a plastic bag and stored at 4°C until extraction and analysis of the dust.

Dust samples were collected on day 0, and then 2 and 8 weeks after treatment.

Analysis of indoor allergens

Allergen extraction and analysis of *Der p 1* and *Der f 1* in carpet dust samples were performed, as previously described (10), by using a sandwich enzyme-linked immunosorbent assay (ELISA; ALK-Abello, Copenhagen, Denmark). Allergen quantification was performed by Anja Holm and Susanne Thyssen (ALK-Abello, Horsholm, Denmark). The assay sensitivity was 2 ng/g of dust. Previous experiments had demonstrated an inter-assay coefficient of variation of 17–25% and an intra-assay coefficient of variation of 5–10% (10,11).

Statistical analysis

Data were analyzed by using the summary measures approach suggested by Matthews et al. (12). Analysis of covariance (ancova) was used to test for the difference between intervention groups at 8, 10, and 18 weeks, with the reading at the start of the intervention (day 0 and week 10) treated as covariate. However, data were not normally distributed, therefore the data are not shown.

Within-group comparisons were performed using the Wilcoxon matched pairs test, and between-group comparisons were performed using the Mann–Whitney *U*-test (SPSS version 8). Statistical significance was assumed at $p < 0.05$.

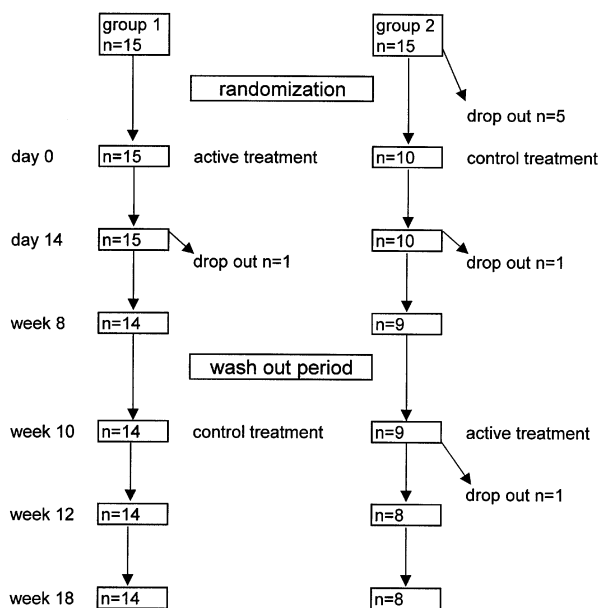


Fig. 1. Study design.

Results

Median allergen concentrations were slightly lower on carpets on day 0 than in the 12 months prior to the study. However, all households showed *Der p 1 + Der f 1* concentrations of >400 ng/g on carpets. Median concentrations on mattresses were 7,630 ng/g (range: 1,460–50,000 ng/g). Between group 1, starting with active treatment, and group 2, starting with placebo treatment, there was no statistically significant difference in the initial mite allergen concentrations on carpets (median 1,489, range: 519–9,664 vs. 2,239, range: 422–13,150 ng of *Der p 1 + Der f 1* per g of dust). After the washout period, the mite allergen concentration tended to be lower in

Fig. 2. Mite allergen concentrations (*Der p 1 + Der f 1* ng/g) in group 1 (n=14) starting with active treatment and in group 2 (n=8) starting with control treatment. Median concentrations, 25th and 75th quartiles and minimum/maximum values are marked.

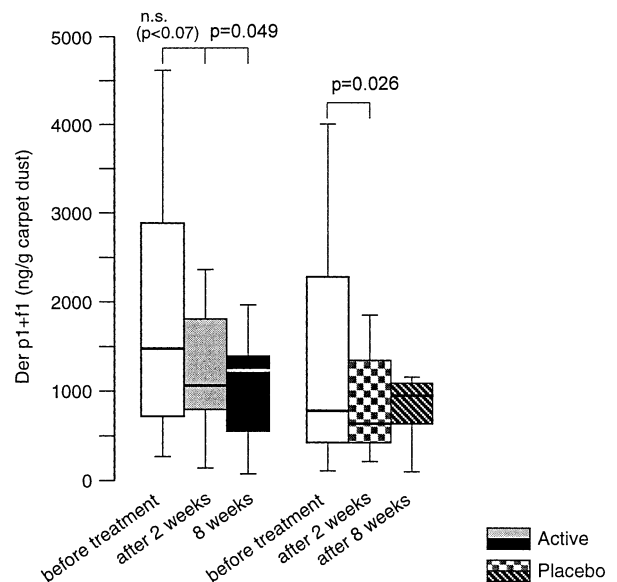
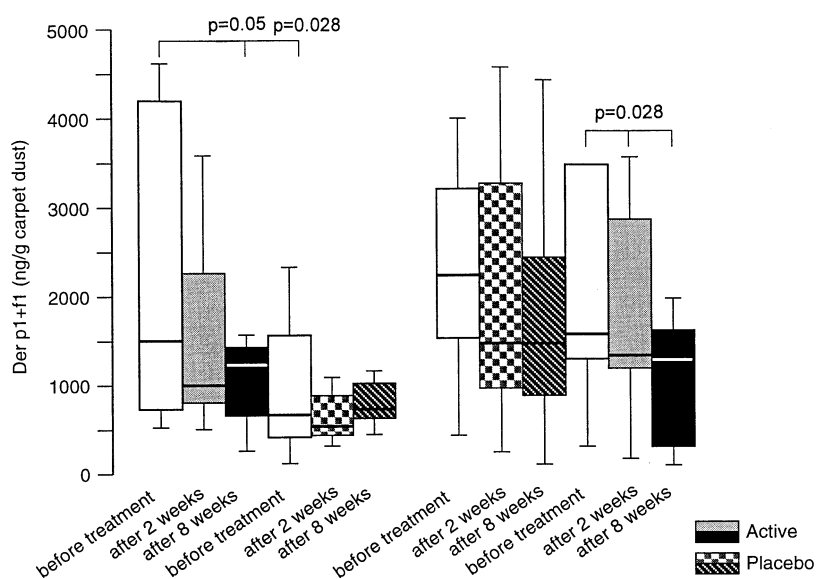


Fig. 3. Mite allergen concentrations (*Der p 1 + Der f 1* ng/g) in the active and placebo groups (n=22). Median concentrations, 25th and 75th quartiles, and minimum/maximum values are marked.

group 1 compared to group 2; however, this trend was not significant (median 658, range: 124–6,085 vs. 1,665, range: 288–9,383 ng of *Der p 1 + Der f 1* per g of dust) ($p = 0.159$). In group 1, there was a significant decrease in mite allergen concentrations after active treatment comparing day 0 (before treatment) and weeks 8 and 10 (median 1,489 vs. 658 and 546 ng/g, respectively; $p = 0.05$ and $p = 0.028$). After 10 weeks, an allergen reduction of $\approx 55\%$ was observed. In group 2, there was a significant decrease in allergen concentration after active treatment comparing the day before treatment, week 2 and week 8,

respectively (median 1,665 vs. 1,331 and 1,251 ng/g; $p=0.028$) (Fig. 2).

In group 1, the geometric mean for the difference of mite allergen concentration comparing day 0 and week 6 was 196 ng/g (95% CI: 7,161 and 8,401) for the first treatment (active) and 15 ng/g (95% CI: 1,079 and 1,292) for the second treatment (control). In group 2, the difference was 66 ng/g (95% CI: 398 and 1,515) for the first treatment (control) and 609 ng/g (95% CI: 186 and 9,264) for the second treatment (active).

Comparing placebo and active treatment in total (Fig. 3), there was also a significant decrease in mite allergen exposure after placebo treatment comparing day 0 and day 14 ($p=0.026$). After 8 weeks, active treatment was superior to placebo treatment ($p=0.049$). However, the allergen reduction achieved was only $\approx 20\%$ (median 1,500 ng/g on day 0 vs. 1,250 ng/g after 8 weeks). As the mite allergen level in group 1 did not return to the same level after the first (active) treatment, group 1 and group 2 differed at the start of the second period (difference not significant; $p=0.159$). Therefore, data were log-transformed and an ancova was performed (although the values were not normally distributed), which did not alter the results (data not shown). Data were also analyzed using the summary measures approach (12). There was only a significant decrease in the mite allergen levels for group 1, who had started with active treatment ($p=0.007$). When the two groups were combined according to active and placebo treatment, the decrease in mite allergen levels after active treatment was no longer significant.

One family complained of slight brownish stains on the carpet.

Discussion

Treatment of carpets with 'Lowal' achieved a significant mite allergen reduction after 14 days in group 2 and after 8 weeks in both groups. However, the decrease was small, which might be attributable to low initial concentrations of mite allergen.

The reduction of mite allergen in patients' homes represents the first line of treatment as a tertiary preventive measure in mite-sensitive patients with bronchial asthma (2,7,13,14). Removing sensitized asthmatic subjects to areas with very low mite allergen exposure, i.e. mountain institutions or hospitals, results in prolonged improvement in symptoms and bronchial hyper-responsiveness (15,16). Primary preventive strategies have been targeted at infancy (17) because most cases of asthma arise then.

However, there are complex interactions between the various factors involved in the development of allergic disease. Moreover, in the case of asthma we face a heterogeneous entity with various phenotypes.

So far, primary prevention studies, such as the Isle of Wight study, have demonstrated a decrease of sensitization to HDM and a non-significant trend in the reduction of current wheezing in 8-year-old children as a result of mite and food allergen elimination in early childhood (18–20). The relationship between exposure and sensitization has been demonstrated in numerous studies (7,10,21,22), but there seems to be no safe minimum level of exposure appropriate to all susceptible individuals. In turn, the relationship between the development of childhood asthma and mite allergen exposure appears to be less clear (22,23). While some authors speculate that the reduction of exposure would cause a reduction of asthma prevalence (24), prospective data from a German birth cohort study were unable to prove a direct association between asthma and mite allergen exposure, although sensitization to *Dermatophagoides* was a risk factor for the evolution of allergic airway disease (22).

Numerous acaricides and protein-denaturing substances, such as tannic acid, have been introduced (8,9,25). However, although in the case of benzyl benzoate the *in vitro* data looked promising (9), the effect on mattresses was found to be poor and on carpets is not long lasting (5,8,26). Often only a 20–50% reduction of mite allergen is achieved, which may not be biologically relevant – especially if patients are exposed to concentrations of $>10 \mu\text{g/g}$ of solid dust (5,6). The effect of tannic acid on mite allergen exposure may have been overestimated in the past owing to interference with ELISA systems (8).

The treatment of carpets, mattresses, and furniture upholstery with a combination of tannic acid and benzyl alcohol has been performed in controlled trials in Australia (3) and the UK (27). In the Australian study, the initial reduction 3 days from baseline was 23–28%; after 4 weeks there was no significant reduction. The mite allergen reduction on blankets was better (4). In the present study the treatment with 'Lowal' achieved only a slight reduction in allergen concentrations on carpets, but the difference between the active and placebo groups was still evident after 8 weeks. This might be attributable to the lower exposure in Germany (10,22), as concentrations of the benzyl derivative and tannic acid, and the applied

quantity on carpets in the Australian study, were comparable with our study. However, the application rate may have been insufficient to penetrate more than 2–3 mm below the surface of the carpet. The depth of carpet pile is commonly 8–12 mm.

Mite group 1 (like group 3 and 6) allergens have enzymatic proteolytic activity (1) and are not resistant to denaturing substances. As over 80% of mite-sensitive patients are found to produce immunoglobulin E (IgE) to mite group 1 allergens, these allergens are of major clinical relevance. However, it cannot be excluded that epitopes other than those binding to the monoclonal antibodies of the ELISA were destroyed to a greater extent by the tannate. The effect of tannic acid on mite group 1 epitopes has been demonstrated *in vitro* and *in vivo* (25,27).

The magnitude of the mite allergen-reducing effect of tannic acid can be decreased in homes with cats (8); however, pet ownership did not influence the outcome in our study as only two families kept a cat and one family a dog.

Moreover, seasonal variation, i.e. an increase of allergen load in autumn at the end of the study period, was not responsible for the poor effect of Lowal. In the Berlin area, like in other studies, a seasonal increase of mite allergen levels is only rarely observed (28).

As the groups in the present study were small, one could argue that the lack of significance was because the study was under-powered. Power calculations showed that 23 homes would be required in each group to detect a 50% reduction in mite allergen concentrations at a power of 85%. However, other studies on mite allergen-reduction procedures detected a significant decrease in mite numbers if the humidity was reduced (29), and a reduction of mite allergen concentrations and bronchial hyper-responsiveness was observed when using mite-impermeable encasings (14).

In group 1, which started with the active treatment, the strongest decrease of allergen exposure was observed 10 weeks after the wash-out period (55%). The washout period of 2 weeks was probably too short, as the mite allergen levels in group 1 at the beginning of the second treatment tended to be lower than those in group 2; however, this trend did not reach statistical significance. It seems possible that the allergen-reducing effect of benzyl benzoate became apparent after a longer period owing to mite killing and a slower decrease of allergen. Unfortunately, no dust samples of group 2 were collected 10 weeks following active treatment.

The slight allergen decrease after placebo treatment might be a result of the fact that isopropanol is also able to denature proteins. However, it cannot be excluded that once patients have been included in a study, they change their cleaning habits, although the participants of the present study were instructed not to do so. A number of families were disturbed by the strong smell of isopropanol, although the alcohol evaporated quickly if windows were open. However, patients with bronchial hyper-reactivity may experience irritation and start coughing. In conclusion, the results of this trial, performed in accordance with the manufacturer's instructions, do not recommend 'Lowal' as a single tertiary, secondary or primary measure of mite allergen avoidance.

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