Short communication

Effect of continuous allergen challenge on clinical symptoms and mediator release in dust-mite-allergic patients


This study investigated the early, prolonged immediate, and late-phase reactions of dust-mite-sensitive subjects undergoing long-term challenge in the Vienna challenge chamber (VCC) in terms of clinical symptoms and inflammatory mediator level patterns in nasal lavage fluids. A concentration of 70 ng Der p 1/m³ of air (feces of Dermatophagoides) was maintained over 8 h in the VCC. To show the clinical impact of this challenge model, the effect of a histamine H₁-receptor antagonist that also has some antiallergic properties (loratadine) was also investigated. The study followed a double-blind, placebo-controlled, crossover design. Medication was given orally over 7 days before the provocation at a dose of 10 mg once daily. All 12 patients, whose dust-mite sensitivity was confirmed by disease history, skin prick test, and RAST, completed the challenge session. The documentation of the chosen parameters was performed every 30 min. Subjective nasal and ocular symptoms were assessed via a visual analog scale of 100 mm, nasal flow was recorded by active anterior rhinomanometry, and mediator release was evaluated with nasal lavages. Clinical aspect: the whole sample population showed a rise of nasal and ocular symptom severity and a nasal flow reduction, which were perceptibly, but not significantly attenuated by active drug treatment. Mediator pattern: in each patient, prostaglandin (PG)D₂ and leukotriene (LT)C₄ levels peaked within the first 2 h of provocation, PGD₂ then moving toward baseline levels, and LTC₄ then again rising continuously. Eosinophil cationic protein (ECP) exhibited a constant level increase over the whole provocation period, and tryptase levels did not change significantly. Whereas the area under the curve values of tryptase and ECP were higher in drug-treated patients than the placebo group, the early PGD₂ peak occurring during the first two challenge hours seemed to be mitigated by loratadine. These results reveal that there is no link between the clinical symptoms, the drug efficacy, and the released mediators (LTC₄, PGD₂, ECP, and tryptase).

House-dust mite (HDM) allergy is of increasing epidemiologic importance (1). Usually, the allergen content of a room is defined by carpet and floor dust samples (2). However, dust-mite allergens may become airborne; therefore, the levels of Dermatophagoides pteronyssinus 1 (Der p 1) per cubic meter of air are of essential interest for the sensitive patient, and not the allergens hidden in the carpet. It has been demonstrated that the airborne concentration of Der p 1 in a bedroom is within a wide range of 0.03 to more than 30.0 ng per cubic meter of air (3). However, little is known about the correlation between the concentration of airborne mite allergens (Der p 1, Der p 2) and the clinical impact.

We have developed a laboratory system for allergen provocation, the Vienna challenge chamber (VCC) (4), which enables us to challenge up
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Allergic patients simultaneously under controlled conditions with stable concentrations of different airborne allergens even to HDM (5). This is the first study to evaluate the mediator release of mast-sensitive subjects to continuous, controlled, long-term dust-mite challenge.

The pilot study aimed to clarify the relation between clinical symptoms and mediator release, especially in the course of the early, prolonged immediate, and late-phase reaction under the conditions of long-term HDM provocation. (“Prolonged immediate” refers to the reaction within 8 h of continuous allergen challenge under natural-like conditions.) The points of immediate interest were the release of the mediators prostaglandin (PG)D2, leukotriene (LT)C4, tryptase, and eosinophil cationic protein (ECP) and clinical symptoms such as nasal blockage, nasal secretion, nasal itching, sneezing, eye tearing, and eye itching. Furthermore, we used this model to evaluate the impact of loratadine on these parameters. Loratadine is a non-sedating antihistamine (6, 7) that is reported to have antiallergic properties in addition to the histamine receptor blockade (8–11), including the inhibition of eosinophil infiltration (12). There is in vitro evidence of downgrading of adhesion molecule expression by loratadine (13, 14). Although the efficacy and safety of loratadine have been thoroughly investigated in perennial allergic rhinitis (PAR) (15, 16), no specific data on the activity of this drug in specific dust-mite-induced allergy have been reported.

Material and methods

Subjects/eligibility criteria

Patients aged 20–35 years with HDM allergy of at least 1 year’s duration were included. The subjects had to show moderate symptoms only at the time of recruitment and after the washout of the first treatment period. For evidence of HDM allergy, positive case history, a positive skin prick test, and RAST classification ≥3 were required. Female patients had to be using adequate birth control. Written informed consent according to GCP and Austrian Drug Act regulations was obtained from the patients. The study was reviewed by the ethics committee of Vienna University.

Excluded were pregnant or breast-feeding women, subjects with ocular diseases or any other rhinologic disorders besides PAR, and patients with urticaria, atopic eczema, bronchial asthma, severe acute diseases, infections of the respiratory tract, known allergy to antihistaminic and anti-allergic substances, and known allergy to benzalkonium chloride. Sufficient washout times for any antihistamines, nasal steroids, anticholinergics, decongestants and cromolyn sodium, and any ophthalmic medication were requested. Other exclusion criteria were alcohol abuse, drug addiction, inability to comply with the protocol, and participation in other clinical studies during the last 4 weeks.

Study design

The study followed a double-blind, placebo-controlled, crossover design. Twelve patients were included. Two challenges were carried out, separated by an interval of 3 weeks (washout phase). The daily dosing of the study medication (10 mg loratadine or placebo) started 7 days before each study visit. The last medication was taken on the day of the provocation, 1 h before the start of the allergen challenge.

Supplies

Capsules containing 10 mg loratadine and identical placebo capsules were supplied by Schering Plough Austria, and feces of D. pteronyssinus were supplied by Allergon AB, Engelholm, Sweden.

Concomitant therapy

No concomitant therapy, except oral contraception and bronchodilator medication for severe bronchial reactions during allergen provocation, was allowed.

Allergen provocation

The continuous long-term allergen provocation was performed in the VCC (4, 5). Up to 12 patients can be challenged simultaneously under controlled conditions in the VCC. Constant humidity (30%), temperature (24°C), and allergen load can be maintained. The allergenic particles are dispensed by an automatic supply unit into a constant turbulent flow of air. The allergen concentration was kept at 70 ng Der p 1 per cubic meter of air, thus being definitely over the threshold of reaction but not leading to enforced to severe late-phase reactions. The allergen load was monitored by counting the airborne particles (2500 per cubic meter) at 5-min intervals. In addition, a second sampler was used to quantify the amount of Der p 1 allergen by means of a monoclonal antibody ELISA assay (HAL, Harlem NL) hourly. The challenge duration was 8 h.
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Procedures
At the beginning of each challenge, a baseline documentation of symptoms and descriptive parameters was made. Then the allergen provocation with HDM feces was started and lasted for 8 h. Nasal lavage for mediator level determination, and objective and subjective parameters were obtained in each patient at the beginning of the provocation, and every 30 min during the 8 h of provocation.

Nasal lavage
The following mediators were assayed: PGD₂ (RIA, Amersham, UK), LTC₄ (RIA, Amersham, UK), mast-cell tryptase (ELISA, Pharmacia, Sweden), and ECP (ELISA, Pharmacia, Sweden). Mediator baseline levels were achieved by double saline lavage procedures before fluid collection. Lavage was performed as microlavage with a 10-ml syringe and nostril adapter using 6 ml of warm 0.9% saline. The patient’s head was tilted forward during the washing procedure. The recovered lavage fluid was collected from both nostrils, pooled on an ice-water bath, and then centrifuged at 4300 rpm at 4°C for 10 min. The supernatant was dispensed in Eppendorf tubes and stored at –30°C for further investigation.

Rhinomanometry
The nasal flow was recorded by active anterior rhinomanometry by the method of Bachert & Feldmeth (17) with computer-aided rhinomanometry equipment (RhinoTest MP441, EVG Elektronik Vertriebs GmbH, Ludwigshafen, Germany). Recordings were obtained at the pressure difference of 150 Pa between anterior and posterior nares. The target variable was the change from baseline in total nasal airways flow at a pressure difference of 150 Pa.

Symptoms
The following symptoms were assessed by the patients at 30-min intervals with a visual analog scale (VAS) of 0–100 mm: itching of the nose, sneezing, running nose, itching of the eyes, and tearing of the eyes.

Statistics
A statistical hypothesis was not determined a priori due to the pilot nature of the study and the lack of data from comparable studies in this setting. Therefore, no sample calculation was possible and descriptive analysis was performed. Statistical comparisons were carried out by ANOVA for a crossover design. If normal distribution and homogeneity of variances could not be accepted, a nonparametric test method (Wilcoxon matched pairs signed rank test) was used. For safety analysis, the data of all patients were used. Adverse events were coded according to the WHO/BGA classification, and evaluated globally by the body system and preferred term evaluation.

Results
Twelve patients with confirmed dust-mite allergy and fulfilling all other entry criteria were enrolled. The group consisted of five women and seven men, mean age 27 years, range 24–34 years. All were able to complete challenges 1 and 2.

Generally, a rise in symptom scores could be demonstrated over the whole challenge period. Two patterns were found: 1) a steep rise, followed by a plateau phase until the end of provocation in nasal itching and running nose; 2) a constant rise during the whole test period in sneezing, blocked nose, and ocular itching. No rise was found for eye tearing. Objective measurement of nasal blockage showed a continuously decreasing amount of nasal flow at 150 Pa, which was moderate, but not significant.

The courses of inflammatory mediator levels in nasal lavage fluids followed different patterns: PGD₂ concentration in nasal lavage fluid peaked at 90 min after allergen challenge start, and dropped to baseline levels afterward, staying there until the end of allergen exposure. LTC₄ displayed a biphasic pattern: two equivalent peaks were set at 30 and 90 min, dropping then to baseline levels and rising again continuously from 240 min until the end of the challenge session. ECP concentrations traced a continuous increase throughout the whole challenge session. Changes in production of mast-cell tryptase could scarcely be shown. No correlation was found between clinical symptoms and mediator release.

Active treatment in comparison with placebo showed no conclusive picture. The course of symptoms in the active group was smaller than in placebo-treated patients. Statistical significance was neared but not reached, probably due to the small sample size of this pilot study. The rhinomanometrically measured nasal blockage was not less in the actively treated patients than in the placebo-treated.

Mediator release: whereas tryptase and ECP values were higher in patients treated with loratadine than in patients treated with placebo, loratadine slightly mitigated the PGD₂ release occurring during the first hours of challenge (Fig. 1). However, there was no significant difference.
Pilot study on mediator release

![Graph of Mediator Release](image1)

*Fig. 1.* Mean mediator levels in nasal lavage fluid (100 μl) in HDM-challenged patients, during challenge period of 8 h.

![Graph of Clinical Symptoms](image2)

*Fig. 2.* Mean subjective symptoms (VAS in mm), during challenge period of 8 h.

### Adverse events

No adverse events occurred except slight bronchoconstriction in two cases; this was found to be provocation-related.

### Discussion

HDM-allergic patients are permanently exposed to allergens in their homes. Therefore, it seems to be difficult to design an investigational course demonstrating the allergic reaction under these conditions. There are two reasons for this: first one must determine the “physiologic” concentration of the allergen, and, second, how to apply this concentration to the patient for a longer time than a conventional nasal provocation test (NPT) can do. To resolve these problems, we developed the HDM model of the VCC (5). The VCC enables us to challenge HDM-allergic patients with regard to the airborne character of HDM feces continuously over hours with stable concentrations of the allergen. Taking the work of Sakaguchi et al. (3) as the guiding principle, we discerned the optimal concentration necessary for this provocation model in several former investigations. We now dispose of a challenge system that imitates the natural conditions of HDM-allergic patients in a more authentic way than the usual locally applied NPT.

Because repeated biopsies are not feasible, the nasal lavage technique provides a practicable way to investigate the time course of mediator release in allergic reactions. In the paper of Castells & Schwartz (18), tryptase especially proved a useful indicator of mast-cell degranulation and correlated strictly with the course of clinical symptoms. Furthermore Rasp & Hochstrasser (19) found, in comparison with the results of Castells & Schwartz (18), that the release of tryptase under natural allergen exposure is even higher than in single allergen challenge, a finding which confirms the practicability of our challenge model. Indeed, we found no rise in the concentration of tryptase in our trial, in correspondence with the results of Wang et al. (20). No correlation was found between the subjective and objective clinical symptoms and the mediator release in either Wang et al.’s results (20) or ours.

However, under active treatment with lornadine, clinical symptoms are mitigated, but the mediator release is virtually unaffected, a fact which also shows no possible correlation. In view of the absolute lack of relation between clinical symptoms, mediator release, and drug efficacy, it must be considered whether the chosen mediators are the best to reflect the expected allergic reaction and the drug’s efficacy, respectively.

### Acknowledgment

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Horak F, Toth J, Hirschwehr R, Marks B, Stubner UP, Jager S, Ber U, Schleinitzer K, Gunczler P.

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