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Changes in nasal lavage fluid due to formaldehyde inhalation

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Summary. The aim of the study was to characterize the nature of the formaldehyde-induced nasal response consisting in symptoms of rhinitis and changes in nasal lavage fluid. Eleven healthy subjects and nine patients with specific skin sensitization were provoked in a toxicological chamber with formaldehyde at a dose of 0.5 mg/m^3 over 2 h. Nasal lavage was performed prior to and immediately after provocation and 4 and 18 h later. Provocation with formaldehyde caused transient symptoms of rhinitis and prolonged changes in nasal washings. There were increases in the number and proportion of eosinophils and elevated albumin and total protein levels in nasal lavage fluid 4 and 18 h after provocation. No difference in the nasal response to formaldehyde was found between patients with skin sensitization and healthy subjects. These data confirm the irritative effects of formaldehyde and are also suggestive of nonspecific proinflammatory properties when formaldehyde is inhaled at a low (0.5 mg/m^3) dose.

Key words: Formaldehyde – Nasal lavage – Eosinophils

Introduction

During the past few years, controversy has arisen over the possible risk of airway allergization posed by exposure to formaldehyde (F). In contrast to its known capacity to cause allergic sensitization in the skin (Maibach 1983), this route has not been sufficiently confirmed to produce a similar effect on the respiratory tract. Immunological evaluation of workers with respiratory symptoms due to F exposure did not reveal any correlation with specific antibodies (Grammer et al. 1990). Moreover, provocational studies on F-induced bronchospasm or rhinitis symptoms have not excluded the possibility of an irritative mechanism (Hendrick et al. 1982).

The aim of this study was to determine the nature of the nasal response to 2 hours' inhalation of F in a toxicological chamber. Since most of the inhaled F is retained in the upper respiratory tract (Chang et al. 1983), the clinical effects of exposure relate mainly to this area. The techniques of controlled inhalational challenge combined with the measurement of symptoms and the performance of nasal lavage make it possible to elucidate the symptoms of rhinitis induced via immunological (Bascom et al. 1988; Raphael et al. 1989, 1991) as well as nonimmunological pathways. Previous studies have revealed some morphological and biochemical differences in nasal lavage fluids following specific allergen and nonspecific irritant provocational challenge (Brofeldt et al. 1986). In our study, the changes in the cell number, differential count, and protein levels in the nasal lavage fluid were determined immediately prior to and after the provocation and 4 and 18 h later. Additionally, the tryptase concentration as an indicator of mast cell degranulation was measured after the provocation. A high correlation between the tryptase levels in nasal washings and the symptoms during the course of the immediate allergic response appears to be a specific indicator of an ongoing allergic disorder (Castells and Schwartz 1988). However, elevated tryptase levels in bronchoalveolar lavage fluid have also been recorded in tobacco smokers without any symptoms of atopy (Kalendarian et al. 1988). Since F is one component of tobacco smoke, the question of the nature of clinical symptoms due to gaseous F exposure has still to be answered.

Materials and methods

Subjects. Two groups of volunteer nonsmokers aged 26–52 were analyzed. The first group comprised nine subjects (seven male and three female) occupationally exposed both to gaseous F and to formalin solutions. All of them suffered from skin hypersensitivity to F, as indicated by their histories and the results of skin patch tests with 0.5% solution of the chemical. They had negative skin prick tests to common allergens (Beecham Bencard) and a normal serum IgE level (below 100 kU IgE/l, Pharmacia). One patient had a positive Phadezym RAST to F. None of the the subjects suffered from rhinitis although all of them complained of transient eye irritation in the workplace. The second, control group comprised 11 healthy males with no history of allergic diseases, normal serum IgE level, negative skin tests to common allergens (Beecham Bencard), and a negative patch test to F. All the subjects had normal appearing nasal mucosa at inspection.

Patients with ongoing symptoms of other nasal diseases or ongoing drug treatment were excluded. All subjects were informed

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about the experiment and the tests were performed after obtaining their consent to participation in the study. The study was approved by the local medical ethical committee.

Study protocol. The study was designed as a two-stage, single-blind examination. The aim of stage 1 was simultaneous evaluation of symptoms and morphological and biochemical changes in the nasal washings after placebo and F inhalation. In stage 2 a comparison was made of the nasal mucosa response in patients with allergic skin sensitization and that in healthy subjects.

Nasal lavage an provocational challenge procedure. Saline washings were performed by means of the "nasal pool" technique (Greiff et al. 1990) immediately before and after provocation and 3 and 18 h later. The "nasal pool" device (10 ml syringe with closely fitting nostril), filled with 6 ml saline, was inserted in the nasal cavity for 5 min and then recovered. This technique allows application of agents at predetermined concentrations over a large and defined area of the surface of the nasal mucosa for extended periods. All saline washings were performed only on one side of the same cavity. One 5-s saline irrigation was performed before each saline washing. Provocational inhalation of F was carried out in a toxicological chamber with a capacity of 12 m^3. F concentration at the dose of 0.5 mg/m^3 was measured by means of the spectrophotometric method. Since the odor of F at a dose level of 0.5 mg/m^3 is beyond perceptibility for most humans, we used clear air as placebo. One-week intervals were allowed between provocations.

Clinical score and the processing of nasal washings. The number of sneezes, the degree of mucosal edema, rhinorrhea, and itching were evaluated. Total symptom scores ranged from 0 to 7 and represented the sum of the scores for sneezing (0 sneezes: 0 points; 1–4 sneezes: 1 point; >4 sneezes: 2 points), rhinorrhea (none: 0 points; mild: 1 point; abundant: 2 points), mucosal edema (none: 0 points; mild 1 point; nasal block: 2 points) and itching (none: 0 points; itchy eyes: 1 point). A positive clinical challenge was defined as >3 points.

Centrifugation (10 min at 1000 rpm) of saline washings separated cell pellet and supernatant. Biochemical assays were performed on the sol phase. The obtained sediment was washed in a sterile phosphate buffered saline (PBS, Sigma) and then suspended in 1 ml of RPMI (Sigma). After staining the cells (Hansel's method: Hansel 1954) the total number of eosinophils and basophils (metachromatic cells) was counted with the use of a Fuchs-Rosenthal chamber allowing determination of the number of cells in 1 ml of recovered fluid. The sample was further centrifuged at 600 rpm, transferred onto a slide, and allowed to air dry. Differential counts were performed on slides stained by the Giemsa method. We counted 200 cells on each slide and classified them as epithelial cells, eosinophils, neutrophils, basophils, and mononuclear cells – a category that included lymphocytes and monocytes.

Albumin concentration was measured by means of the single radial immunodiffusion method (the assay ranged between 20 and 2000 mg/l), and total protein was measured according to Lowry's method (Lowry et al. 1951).

Data analysis. The Wilcoxon matched pairs, signed rank test was used to determine the significance of the increase in total protein, albumin, and proportion and total number of cells. The data were expressed as means ± SEM. The results obtained after inhalation challenges in allergics were compared with those in healthy subjects using the Mann-Whitney U test. The value of $P<0.05$ was considered as statistically significant.

Results

None of the parameters determined in nasal washings after clear air inhalation differed as compared to the baseline levels (P ranged from 0.45 to 0.88), and no nasal symptoms were produced in the same analyzed patients from the two groups. Consecutive nasal lavages after placebo inhalation caused an insignificant decrease in the total number of determined cells and protein levels, which may have been due to the wash-out effect. The decrease was masked by the inflammatory response triggered when the subjects were challenged with F. The average amount of fluid recovered was 4.8 ml (SEM ± 1.0). These data confirm the usefulness of the "nasal pool" method for determining the morphological and biochemical changes in nasal lavage fluid. There was no statistical difference between analyzed groups with respect to baseline cell number and protein level in 1 ml of the recovered fluid.

Two hours' inhalation of 0.5 mg/m^3 F in the toxicological chamber caused itching, sneezing, and congestion. Immediately after inhalation, allergic and healthy subjects presented an increased number of eosinophils in nasal lavage fluid (Fig. 1) [from 39×10^3/ml (SEM ± 11×10^3) to 69×10^3/ml (SEM ± 20×10^3), $P<0.05$, and from 42×10^3/ml (SEM ± 12×10^3) to 72×10^3ml (SEM ± 23×10^3), $P<0.05$]. The eosinophil count continued to be elevated 3 and 18 h after provocation [counts (± SEM) at 3 h in allergic and healthy subjects, respectively: 59 ± 14×10^3/ml and 58 ± 10^3/ml; corresponding figures at 18 h: 59 ± 22×10^3/ml and 48 ± 12×10^3/ml). All of the subjects exhibited a significant influx of eosinophils into nasal washings at all times after provocation. However, no changes in the basophil number were observed following the inhalation.

The proportion of different cell types in the nasal washings of healthy subjects was modified following F inhalation and some variables were significantly different (Fig. 2). Immediately after provocation the proportion of epithelial cells decreased from 82% (SED ± 12%) to 53% (SEM ± 6.4%) ($P<0.05$); 4 and 18 h later the decrease in the epithelial cell percentage was still significant: 55% (SEM ± 14%) ($P<0.05$) and 62% (SEM ± 10%) ($P<0.05$), respectively. The eosinophil proportion increased from 8% (SEM ± 6.2%) to 38% (SEM± 12%) immediately after provocation ($P<0.05$); 4 and 18 h later the percentages were still higher than the baseline data: 36% (SEM ± 12%) ($P<0.05$) and 26% (SEM ± 8%) ($P<0.05$), respectively. The proportion of neutrophils, basophils, and mononuclear cells remained unchanged after F provocation. A similar morphological response was observed in the nasal washings of allergic patients. The proportion of epithelial cells decreased from 79% to 56% immediately after provocational challenge ($P<0.05$) and to 62% ($P<0.05$) and 57% ($P<0.05$) 4 and 18 h later; by contrast the proportion of eosinophils increased from 15% to 36% immediately after the challenge and remained increased, at 29% and 24%, 4 and 18 h later ($P<0.05$). Biochemical analysis of nasal washings showed an increase in the albumin level immediately after provocation both in allergics and in the control group: from 111 mg/l (SEM ± 25) to 221 mg/l (SEM ± 43) and from 101 mg/l (SEM ± 14) to 199 mg/l (SEM ± 52), respectively. The albumin level decreased to 169 and to 140 mg/l in allergics and to 152 mg/l and to 129 mg/l in controls 4 and 18 h later. However, 3 and 18 h

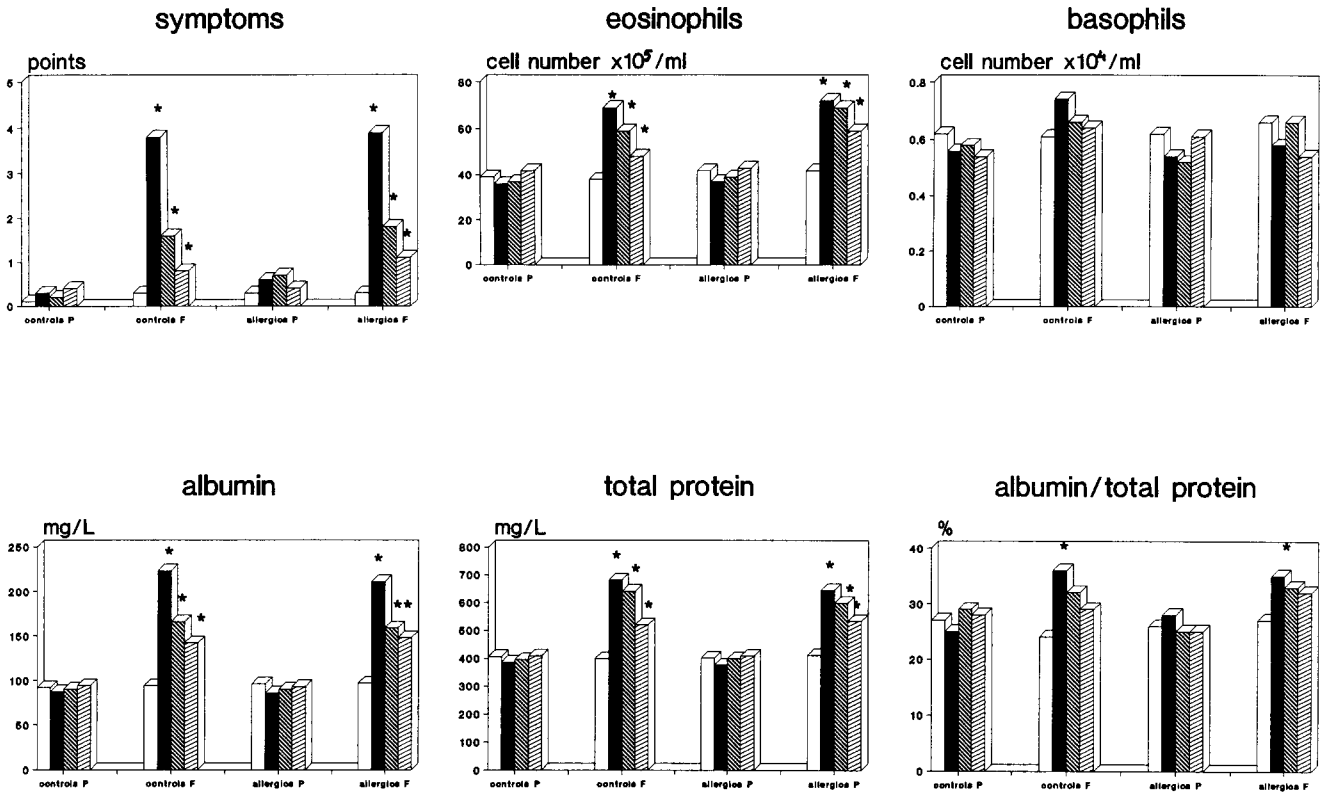

Fig. 1. Clinical symptoms and morphological and biochemical changes in nasal lavage fluid of allergics and healthy subjects due to F and placebo inhalation. * $P < 0.05$ vs baseline value (pre). □ Pre; ■ 10 min; ▨ 4 h; ▨ 18 h

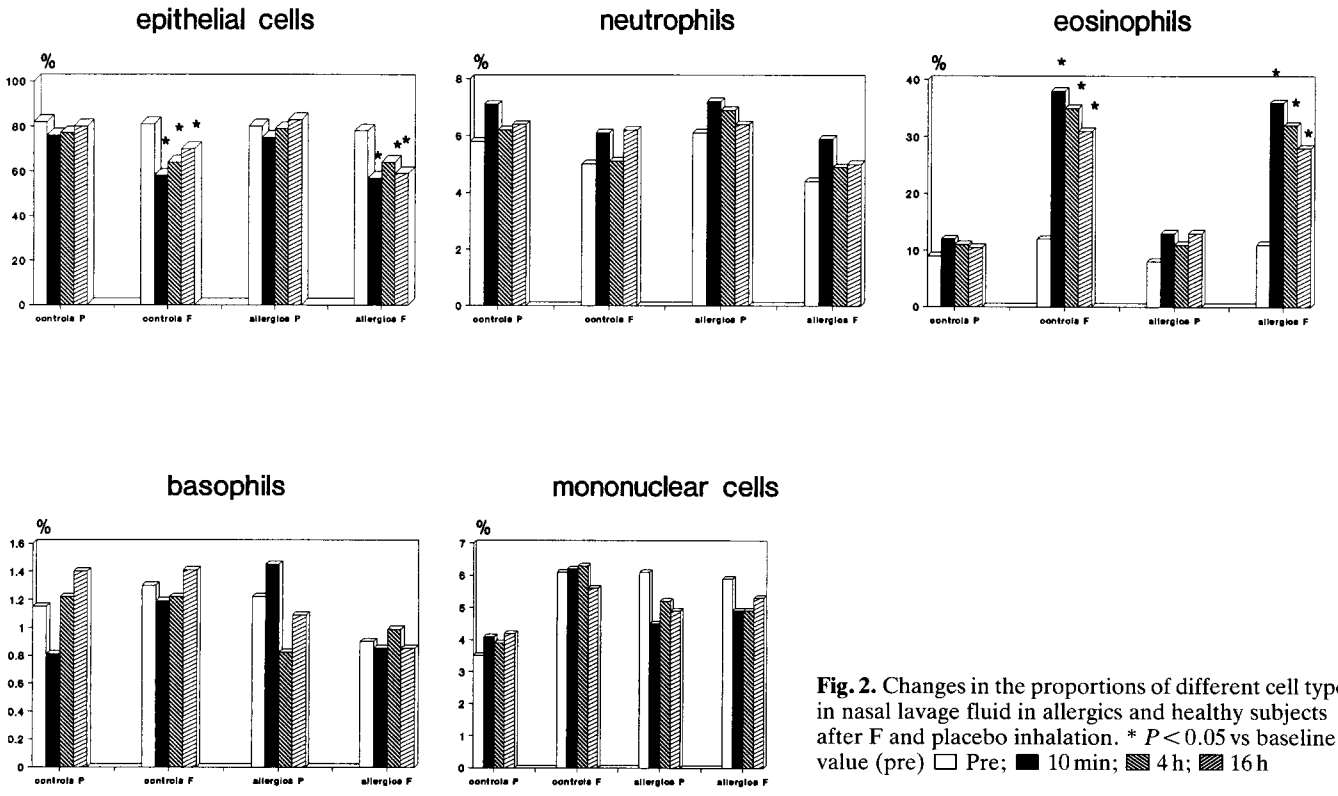

Fig. 2. Changes in the proportions of different cell types in nasal lavage fluid in allergics and healthy subjects after F and placebo inhalation. * $P < 0.05$ vs baseline value (pre) □ Pre; ■ 10 min; ▨ 4 h; ▨ 16 h

after exposure, an elevated level of total protein in nasal lavage fluid was observed in both groups: in allergics it rose from 396 mg/l to 621 mg/l and 486 mg/l, respectively, and in controls, from 369 mg/l to 586 mg/l and 466 mg/l, respectively. The albumin percentage as an index of vascular permeability (Raphael et al. 1989) was significantly elevated only for 10 min after provocation.

Statistical analysis of nasal washings before and after F provocation showed no difference in nasal response to the chemical between healthy subjects and patients with skin sensitization. Spearman's rank correlation test revealed a correlation only between eosinophil count and increase in albumin level in the nasal lavage fluid recovered immediately after F inhalation ($P < 0.0001$, $r_s = 0.85$). Determination of tryptase concentration in washings recovered prior to and immediately after provocation did not show any increase in protease above the detectability limit of 0.5 ng/l.

Discussion

The objective of this study was to examine the response of airway mucosa to F inhalation. Since most of the inhaled chemical is retained in the upper respiratory tract, our observations relate to that area. Two hours' inhalation of 0.5 mg/m^3 F produced transient burning sensations of the eyes and nasal passages, which may have been caused by the irritation of trigeminal and olfactory nerve endings. Although the clinical response to provocational inhalation was transient, the morphological and biochemical changes in the nasal washings were longer lasting. Prolonged influx of eosinophils, observed 4 and 18 h after provocation, could be explained in several ways. Firstly, it may have been associated with increased permeability; however, an increase in the numbers of the other cells was not observed. Secondly, eosinophils may be attracted by mediators released from other cells activated during inflammatory processes. Although we did not find any evidence of mast cell degranulation, the activation of monocytes, neurtrophils, and epithelial cells cannot be excluded. Thirdly, the observed sneezing, itching, and mucosal edema, as well as an increase in vascular permeability, suggest that some inflammatory mediators, among them histamine, were released during provocation. Since eosinophils have a well-recognized capability to neutralize histamine (Zeiger et al. 1976), we cannot completely exclude the possibility that they have a protective role in the inflammatory reaction induced by F.

Interesting data were obtained from protein analysis of the washings. An increase in albumin percentage was found immediately after provocation, and elevated levels of albumin and total protein in nasal washings were determined 4 and 18 h later. Previous studies have revealed that an increase in albumin and albumin percentage resulted in increased vasopermeability, while elevated total protein without any changes in albumin percentage indicates glandular secretion (Raphael et al. 1989). In the present study, an increase in total protein with unchanged albumin percentage was recorded at 4 and 18 h after provocation. Other clinical observations indicated that nasal reactions to irritants such as cigarette smoke, sulfur dioxide, and F may cause stimulation of trigeminal sensory nerves as well as induction of axon responses and parasympathetic reflexes. Trigeminal sensory nerve depolarization manifests as an itchy nose and sneezing – the symptoms observed after F provocation. Sensory nerves trigger parasympathetic reflexes that lead to glandular secretion. Activation of axon responses is suggested by the release of neuropeptides (Walker et al. 1988), especially substance P (SP), calcitonin gene-related peptide (CGRP), and gastrin-related peptide (GRP). These substances may induce epithelial permeability and prolonged vascular secretion (SP, GRP) and arteriolar vasodilatation (CGRP, SP), and may also act as chemoattractants and inflammatory cell activators (Barnes 1987). Although all these effects were observed in the present study, more evident is necessary to confirm this hypothesis.

To conclude, the lack of evidence for mast cell degranulation, the unchanged number of basophils, and the similarity of the responses to provocation in healthy subjects with specific skin sensitivity would indicate the occurrence of nonspecific, nonallergic inflammatory processes in the nasal mucosa. Moreover, apart from supporting the irritational properties of gaseous F at doses between 1 and 11 ppm (Bardana and Montanaro 1991), the present study suggests proinflammatory properties of the chemical for nasal mucosa when it is absorbed at low dose of 0.5 mg/m^3. In view of the fact that eosinophils may have the potential to liberate mediators that damage epithelial surfaces, and given the histological changes in nasal mucosa resulting from exposure to F at 0.1–1.1 mg/m^3 (Elding et al. 1988), it seems an important conclusion that inhalation of the chemical at low doses over 2 h may have caused a prolonged response in the upper respiratory tract.

Summing up, the nasal lavage technique appeared to be a very useful tool for evaluating morphological and biochemical changes in the nasal washings. Further studies are required to explain the nature of the response of the nasal mucosa to F, and the described technique may be used to determine more specific mediators released during chemical-induced inflammatory reactions.

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