

Original article

Inhaled formaldehyde exposure: effect on bronchial response to mite allergen in sensitized asthma patients

Background: Formaldehyde, an indoor air pollutant, is known to be an irritant and an etiologic factor in occupational asthma. An epidemiologic study suggests that it may also increase the risk of childhood asthma for concentrations above $60 \mu\text{g}/\text{m}^3$.

Aim: To evaluate the influence of pre-exposure to low-dose formaldehyde ($100 \mu\text{g}/\text{m}^3$ in 30 min according to the World Health Organization's recommended maximum value for indoor environments) on bronchial response to *Dermatophagoides pteronyssinus*.

Method: Nineteen asthmatic subjects were included. Each subject underwent a mite allergen bronchial challenge test immediately after a standardized exposure in a chamber to formaldehyde or air (random order). Induced sputum were collected 24 h before and after mite challenge.

Results: After formaldehyde inhalation, patients developed an immediate bronchial response at a significantly lower dose of mite allergen than after air exposure (the geometric mean PD_{20} for Der p 1 was 34.3 ng after formaldehyde and 45.4 ng after placebo, $P = 0.05$). The late-phase reaction, expressed as the maximum fall in forced expiratory volume in 1 s (FEV_1) from baseline, was significantly higher after formaldehyde (15% vs 11%, $P = 0.046$).

Conclusion: Our study demonstrated that exposure to low levels of formaldehyde significantly enhanced bronchial responsiveness to mite allergen in mite-sensitized subjects with asthma.

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Key words: asthma; bronchial responsiveness; Der p 1; formaldehyde; house dust mite allergen.

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Accepted for publication 15 April 2006

Exposure to indoor air pollutants may affect respiratory health as town-dwellers spend about 65% of their lives indoors (1, 2). Indoor air may contain biologic air pollutants, such as allergens and microorganisms, and chemical pollutants, including nitrogen oxides and volatile organic compounds (VOCs). Of the indoor air chemical pollutants, formaldehyde has been identified as the most ubiquitous. Indoor concentrations range from 1 to several dozen $\mu\text{g}/\text{m}^3$ and depend on the materials and furnishings present (3, 4). Besides its well-established irritant effects on the upper respiratory tract, specific immunoglobulin (Ig)E sensitization to formaldehyde has been suggested (5–10). Another mechanism for formaldehyde's effects may be an increased risk of allergic diseases. Experimental studies have demonstrated such an effect in mice and guinea-pigs exposed to formaldehyde. Immunoglobulin E antiovalbumin production increases in sensitized animals (11, 12). In human children, exposure exceeding

$50 \mu\text{g}/\text{m}^3$ may increase the risk of allergic sensitization to common aeroallergens (13). An epidemiologic study suggests that formaldehyde exposure may increase the risk of childhood asthma (14). However, the interaction of this chemical pollutant with allergens in allergic bronchial symptoms has never been confirmed by a clinical study among subjects with asthma.

Mite allergen is the most frequent in allergic asthma (15), and group I mite allergen is identified very frequently in the dwellings of mite-allergic patients (16). Concomitant exposure to house dust mites (HDM) and formaldehyde in indoor air is undoubtedly common. The World Health Organization recommends that formaldehyde levels in nonindustrial indoor environments not exceed $100 \mu\text{g}/\text{m}^3$ in 30 min (17). The aim of this study was to evaluate the influence of pre-exposure to formaldehyde at a concentration just below the WHO recommended value on immediate and late-phase bronchial response to HDM allergen.

Methods

Patients

We studied 19 subjects with mild asthma, a positive skin prick test to *Dermatophagoides pteronyssinus* extract and specific IgE to *D. pteronyssinus* allergen higher than 0.7 kU/l. They were allowed to use only short-acting β -agonists, as needed during the study period. No patient had a respiratory tract infection in the 2 weeks before the test. Patients sensitized to seasonal allergens (pollens) were studied outside the relevant pollen season, and those sensitized to cat or dog allergens were included only if they had no pet at home. No patient was occupationally exposed to formaldehyde.

Assessment of domestic exposure to Der p 1 and formaldehyde

Mattress and floor dust were collected according to international guidelines. Der p 1 concentration was evaluated by enzyme-linked immunosorbent assay (ELISA; Indoor Biotechnology, Charlottesville, VA). We assessed household formaldehyde exposure during the study period. After windows were closed for 24 h, air from the bedroom and the living room was sampled during 30 min and measured in cartridges with 2,4-dinitrophenylhydrazine (2,4-DNPD; Sigma-Aldrich, St Louis, MO, USA).

Study design

The local ethics committee approved this double-blind, randomized, crossover protocol (Fig. 1). Twenty-four hours before each HDM bronchial challenge test (BCT), each subject had a methacholine BCT; induced sputum was collected an hour later. The HDM-BCT then took place immediately after exposure to formaldehyde or air (order randomly selected at the first BCT). Exposure took place in a chamber under standardized conditions for 30 min with a formaldehyde concentration just below $100 \mu\text{g}/\text{m}^3$. Pulmonary function tests preceded and followed exposure to formaldehyde or air and followed each HDM-BCT (every hour for 6 h), under medical supervision. Appropriate medication (β -agonist metered dose inhaler [MDI] or aerosol, oral corticosteroid) was given if necessary. The Borg scale results for dyspnea were also collected before and after formaldehyde or air exposure and after each HDM-BCT: at the end of the BCT and 6 and 24 h later. Induced sputum was collected 24 h after each HDM-BCT. These were separated by at least 3 weeks.

The methacholine BCT assessed nonspecific bronchial hyperreactivity (NSBHR), as previously described (18). A doubling cumulative dose of methacholine solution (1%), from 25 to 3200 μg , was delivered with a dosimeter (Mediprom FDC 88, Paris, France). The

test was stopped when forced expiratory volume in 1 s (FEV_1) decreased by at least 20% of the baseline value. The dose inducing a 20% decrease in FEV_1 (PD_{20}) was calculated by linear interpolation on the dose-response curve.

Formaldehyde exposure took place in an experimental chamber (7.26 m^3) under standardized conditions, as previously described (19). Background formaldehyde levels were measured before each experiment and during placebo exposure. Formaldehyde was generated from an aqueous medicinal solution of formaldehyde (titer = 35%) diluted with milli-Q water ($18.2 \text{ m}\Omega/\text{cm}$) at a ratio of 1 : 20. A weighed volume of this solution was placed into an airtight pyrex boiler, heated to 85°C , vaporized, and transferred into the chamber with a medical-quality air flow of 3 l/min for 10 min. For placebo exposure, milli-Q water alone was placed in the boiler. Air was mixed by use of two fans. The concentration of formaldehyde was measured in an air sample (60 l) pumped at a flow rate of 4 l/min for 15 min (Brooks Instrument, 5850S, Hatfield, PA, USA) through a cartridge filled with silica (45–60 mesh; Aldrich) impregnated with acidified solution of 2,4-DNPD (Aldrich), according to the method described by Zhou and Mopper (20). After reaction the derivative solutions extracted by elution with 20 ml acetonitrile were weighed and analyzed with high-performance liquid chromatography (reversed-phase column C-18, wavelength 360 nm, isocratic mobile phase 1 ml/min of acetonitrile-water 60/40) coupled to UV detection (Kontron, Herts, UK). The detection limit of the formaldehyde concentration in the chamber was estimated to be on the order of $2.4 \mu\text{g}/\text{m}^3$. The reproducibility of formaldehyde generation was always $< 10\%$. Homogeneity according to the mean intra-assay variation coefficient was $< 10\%$ ($n = 98$ measurements; 19).

During the formaldehyde generation, patients sat on a chair in the chamber and wore masks. Their noses were clipped during exposure, after the mask was removed. Two consecutive air samples were taken during the 30-min exposure to verify the formaldehyde level inside the chamber, which remained just below $100 \mu\text{g}/\text{m}^3$. After exposure, patients completed a questionnaire to determine their clinical symptom score (0, no symptoms; 1, mild symptoms; 2, medium symptoms; 3, severe symptoms) based on the ocular (watering, itching, and redness), nasal (running, itching, and sneezing), and respiratory (cough, dyspnea, and wheezing) symptoms described by subjects during exposure. The total possible clinical score ranged from 0 to 27.

House dust mite BCTs were performed with an automatic inhalation-synchronized dosimeter jet nebulizer (Spira Elektro2, Hamenlinna, Finland), as previously described (21), and an air-driven nebulizer (Pari Tia by the Pari Aerosol Research Institute, Munich, Germany) after checking its mass median aerodynamic diameters (MMAD) by laser particle size analysis (MasterSizer X; Malvern Instruments Ltd, Worcestershire, UK). The MMAD was $11.12 \mu\text{m}$ with a geometric standard deviation of 1.97 ($n = 15$). A *D. pteronyssinus* extract (Allerbio®, Varennes, France; lot 352 B 03) with a known concentration of Der p 1 ($11.08 \mu\text{g}/\text{ml}$) was used for this purpose. The starting concentration for HDM-BCT was one-tenth of the cutaneous end point titration of each subject's skin prick test. An increasing number of inhalations (2, 4, 8, and 16 breaths) were administered at each dilution (10^{-4} to 100 RI of mite allergen). The late-phase reaction (LPR) was defined as a reduction $\geq 15\%$ in the FEV_1 between 4 and 6 h after the end of BCT (22).

Sputum was induced and collected according to the method described by Pin et al. (23). Cell plugs were selected from the sample, separated from saliva, weighed, and dispersed by addition of 4 vol of 0.1% dithiothreitol (DTT; Sigma Pharmaceuticals, Saint Quentin Fallavier, France) and processed as already described (23). Filtered sputum was then centrifuged at $1370 g$ for 15 min to separate cells from the fluid phase. Fluid phase was stored at -80°C until the eosinophil cationic protein (ECP) assay.

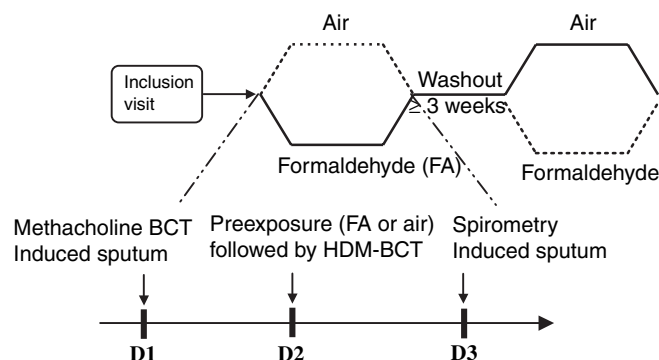


Figure 1. Study design.

ECP assays of sputum

Eosinophil cationic protein levels in fluid phase-induced sputum were determined with a commercially available specific double-antibody radioimmunoassay (Pharmacia Diagnostics, Saint-Quentin, France), as previously described (18). The interassay coefficient was 2.3% for 5 µg/l and 5.85% for 15 µg/l.

Statistical analysis

Repeated measures ANOVA compared with the baseline FEV₁, forced expiratory flow between 25% and 75% of the forced vital capacity (FEF₂₅₋₇₅), peak expiratory flow (PEF), and dyspnea scale before the HDM-BCTs and the spirometric parameters, ECP concentration, and peripheral eosinophil variation before and after exposure (SPSS, version 11.01; SPSS Inc., Chicago, IL). A Wilcoxon test compared with the postexposure clinical symptom scores. Student's paired *t*-test was used to compare the maximum FEV₁ decrease after HDM-BCT as well as the PD₂₀ for methacholine and Der p 1 after logarithmic transformation.

Results

Patients

The study included 19 subjects with mild asthma and allergic to *D. pteronyssinus*: 12 women and 7 men; ages ranged from 19 to 35 years. They all provided written informed consent. No subjects smoked, but five were

exposed to second-hand smoke at home (Table 1). They did not differ from the others for pulmonary functions (FEV₁, *P* = 0.474), NSBHR (*P* = 0.180) or Borg scale (*P* = 0.593).

Domestic exposure to Der p 1 and formaldehyde

Of 38 dust samples collected for assessment of patients' home exposure to HDM, the mean values of Der p 1 in floor and mattress dust were 1.1 ± 3.9 µg/g and 1.0 ± 3.1 µg/g, respectively (Table 1). Mean formaldehyde exposure at home was 36.7 ± 3.8 µg/m³. The five subjects exposed to smoke at home were not exposed to higher formaldehyde levels there than the other volunteers (*P* = 0.952).

Baseline dyspnea Borg scale, lung function, and methacholine BCT

Patients did not differ significantly for any of the baseline parameters before the formaldehyde or air exposure: dyspnea (Borg scale), PEF, FEV₁, FEF₂₅₋₇₅ before the two HDM-BCT (*P* = 0.515, 0.862, 0.725, and 0.882, respectively). Nonspecific bronchial hyperreactivity evaluated with the PD₂₀ of methacholine was not different (*P* = 0.821): geometric mean values of 296.3 µg of methacholine and 312.5 µg, before formaldehyde and placebo exposure, respectively.

Table 1. Characteristics of study subjects

Subject	Prick tests to common aeroallergens	Preinclusion FEV ₁ (% of predicted value)	Preinclusion FEF ₂₅₋₇₅ (% of predicted value)	Der p 1 (floor dust*; µg/g)	Der p 1 (mattress dust*; µg/g)	FA (living room; µg/m ³)	FA (bedroom; µg/m ³)
1	Dp, Df, cat, grass pollens	101	100	17.13	10.21	42.75	57.84
2	Dp, Df, tree pollens	100	96	0.18	<0.03	37.30	41.90
3	Dp, Df, grass pollens	109	87	<0.03	<0.03	20.99	29.06
4	Dp, Df, cat, dog, grass pollens	87	76	0.56	IV	38.68	29.88
5	Dp, Df, grass pollens	102	75	IV	<0.03	16.40	13.00
6	Dp, Df, cat, dog, grass pollens	93	66	IV	IV	50.76	50.17
7	Dp, Df, tree pollens	99	68	0.62	<0.03	17.05	27.46
8	Dp, Df	83	75	0.16	<0.03	29.59	36.54
9	Dp, Df	106	76	IV	IV	30.95	36.07
10	Dp, Df	73	55	<0.03	IV	41.25	39.62
11	Dp, Df	95	84	IV	IV	44.52	42.07
12	Dp, Df	99	70	IV	IV	18.63	19.20
13	Dp, Df, cat, dog	79	50	<0.03	0.09	63.67	72.24
14	Dp, Df, cat	92	66	IV	<0.03	77.83	80.64
15	Dp, Df, cat	111	92	1.29	9.44	37.65	45.61
16	Dp, Dft	93	67	<0.03	<0.03	16.44	16.90
17	Dp, Df, cat, dog, grass and tree pollens	80	50	0.72	<0.03	27.01	29.91
18	Dp, Df, cat, dog, grass and tree pollens	101	74	0.38	0.06	30.01	35.08
19	Dp, Df, cat, dog, grass pollens	104	89	<0.03	<0.03	26.40	22.00
Mean (±SEM)		95.1 (±2.43)	74.5 (±17.09)	1.11 (±0.89)	1.05 (±0.71)	35.2 (±3.75)	38.2 (±4.06)
Median		99.0	75.0	<0.03	<0.03	30.95	36.07

Dp, *Dermatophagoides pteronyssinus*; Df, *Dermatophagoides farinae*; IV, insufficient volume of dust to carry out Der p 1 dosage; FA, formaldehyde; FEV₁, forced expiratory volume in 1 s.

*0.03 µg/g was the detection limit for dust Der p 1 concentration.

Formaldehyde exposure in the experimental chamber

Patients in the chamber were exposed to mean formaldehyde concentrations of $92.2 \mu\text{g}/\text{m}^3$ (SEM: $1.8 \mu\text{g}/\text{m}^3$; median: $95.0 \mu\text{g}/\text{m}^3$) for 30 min. The mean formaldehyde background level during placebo exposure was $32.0 \mu\text{g}/\text{m}^3$ (SEM: $2.2 \mu\text{g}/\text{m}^3$; median: $31.0 \mu\text{g}/\text{m}^3$; Table 2). Respiratory functions and symptoms did not differ according to pre-exposure to formaldehyde or placebo (FEV_1 , FEF_{25-75} , PEF , and Borg scale with $P = 0.470$, 0.397 , 0.884 , and 0.329 , respectively), nor did clinical symptom scores ($P = 0.414$, 0.565 , and 1.000 , for the ocular, nasal, and respiratory symptom scores, respectively).

Early-phase bronchial response: PD_{20} Der p 1

The geometric mean values of the Der p 1 PD_{20} were 34.3 ng after formaldehyde exposure and 45.4 ng after placebo (Table 2). The dose inducing an immediate response decreased significantly in subjects exposed to formaldehyde ($P = 0.05$). After the HDM-BCT, none of the pulmonary function tests differed between formaldehyde and placebo exposure (FEV_1 , $P = 0.772$; PEF , $P = 0.835$; and FEF_{25-75} , $P = 0.610$).

Late-phase bronchial response

The maximum percentage FEV_1 reduction observed during the 6 h of follow up was significantly higher after formaldehyde than after air exposure (mean $\pm$ SEM of $15 \pm 1.6\%$ and $11 \pm 1.6\%$, respectively, $P = 0.046$; Fig. 2).

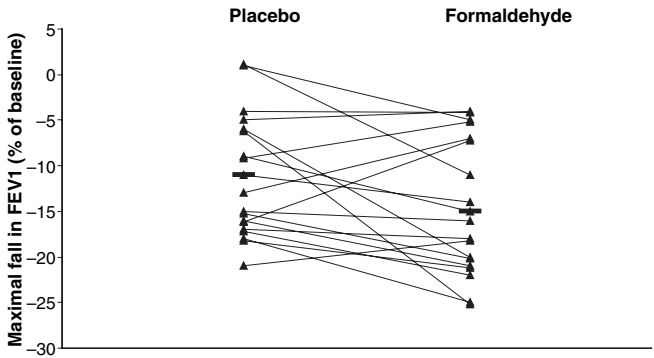


Figure 2. Late-phase response to mite allergen reported as the maximal fall in forced expiratory volume in 1 s (FEV_1) during the 6 h of follow up after house dust mite bronchial challenge test (HDM-BCT) according to the pre-exposure (arithmetic mean value).

Table 2. Background formaldehyde level measured during placebo exposure, formaldehyde concentration generated in the exposure chamber, and doses of mite allergen (PD_{20} Der p 1) provoking the immediate bronchial response during BCT after formaldehyde or air exposure

Subject	Formaldehyde exposure			Air exposure		
	FA (chamber; $\mu\text{g}/\text{m}^3$)	Total score symptom (/27)	PD_{20} Der p 1 (ng)	FA (chamber; $\mu\text{g}/\text{m}^3$)	Total score symptom (/27)	PD_{20} Der p 1 (ng)
1	96	0	97.1	22	0	186.5
2	85	2	23.2	39	0	87.2
3	75	0	19.3	14	0	19.8
4	81	0	195.0	28	0	125.0
5	98	0	40.0	26	2	63.3
6	94	0	54.0	30	0	27.9
7	84	1	86.3	49	1	57.5
8	99	0	26.1	36	0	22.5
9	93	0	18.3	27	0	21.1
10	85	7	12.5	27	3	32.1
11	99	4	57.2	22	2	297.9
12	96	5	71.3	19	6	78.8
13	99	2	17.8	48	5	18.3
14	81	1	186.7	46	2	174.6
15	99	0	28.1	39	0	39.7
16	99	2	26.9	33	1	29.6
17	99	3	2.0	33	2	3.4
18	95	2	65.0	31	2	85.6
19	95	4	11.9	41	7	19.2
AM ($\pm$ SEM)	92.2 (± 1.8)	2 (± 0.5)	54.7 (± 12.6)	32.0 (± 2.2)	2 (± 0.5)	73.2 (± 17.3)
Median	95.0	1	28.1	31.0	1	39.7
GM			34.3			45.4

PD_{20} , provocative dose of a 20% fall in FEV_1 ; FA, formaldehyde; AM, arithmetic mean; GM, geometric mean; FEV_1 , forced expiratory volume in 1 s; BCT, bronchial challenge test.

Cell counts and ECP levels in blood and sputum

The peripheral blood eosinophil count increased after allergen challenge ($P < 0.001$). In patients pre-exposed to formaldehyde, the mean variation of eosinophil counts after the inhalation of the last dose of allergen was higher at 3, 6 and 24 h. At 6 h after HDM-BCT, we observed a trend toward higher mean increases in eosinophil counts ($\pm$ SEM) after formaldehyde than after air ($37.94\% \pm 9.20$, and $11.04\% \pm 2.68$ after formaldehyde and air exposure, respectively, statistically not significant). In induced sputum the ECP levels were (mean $\pm$ SEM) 439.42 ± 171.45 $\mu\text{g/l}$ after formaldehyde inhalation and 156.23 ± 58.09 $\mu\text{g/l}$ after air.

Discussion

This double-blind study assessed the effect of standardized exposure to low levels of formaldehyde on airway response to inhaled allergen. Formaldehyde exposure for 30 min did not cause any changes in lung function and did not induce symptoms. It did, however, significantly enhance immediate and late airway responses to inhaled mite allergen in these subjects with mild asthma.

Our model of standardized exposure to < 100 $\mu\text{g/m}^3$ of formaldehyde for 30 min in an experimental chamber mimics the peak formaldehyde exposure possible at home (long period with no open windows, cigarette smoke, etc.) rather than chronic exposure. However, environmental exposure to formaldehyde in dwellings can best be considered as chronic exposure. Mean published levels were ranged from 27 to 44 $\mu\text{g/m}^3$ (3, 4). Jenkins et al. demonstrated for exposure to ozone and nitric oxide that the most relevant variable in exposure is the concentration of the peak rather than the total dose inhaled over a period of time (24).

During pre-exposure in the chamber, patients were asked to breath through their mouths to standardize formaldehyde inhalation and avoid possible interference with nasal flux because of associated rhinitis. Moreover, because of its high solubility in water, formaldehyde was thought to be essentially absorbed in the upper respiratory tract when breathed through the nose. The development of dosimetric models of inhaled formaldehyde determined that the formaldehyde flux is very large in the first bronchial generations of the tracheobronchial region and then decreases rapidly to no flux in the alveolar region (25). Accordingly, we chose to use an aerosol with large particles (MMAD: 11.1 μm) for allergen challenge. Because these are mainly deposited in the large airways, as reported by Heyder et al. (26), we believe that we have targeted the same region of the respiratory tract with formaldehyde and mite allergen. This decision is reinforced by our previous demonstration that large particles are more appropriate for studying immediate response during allergen BCTs (21, 27).

Lung function in our volunteers did not change after the formaldehyde pre-exposure. This is consistent with previous experimental findings that exposure to higher levels of formaldehyde does not affect bronchoconstriction in subjects with asthma (6, 8) and that a 10-min exposure of up to 3600 $\mu\text{g/m}^3$ only rarely induces changes in airway reactivity (7, 9). On the other hand, patients in those studies reported irritation symptoms (throat and eyes) at those high concentrations (6, 7). In our study, formaldehyde exposure to 100 $\mu\text{g/m}^3$ did not induce any symptoms, as shown by mean total symptom scores of 2/27 after formaldehyde and after air exposure. This confirms that the concentration used had no independent clinical effect.

Selection of the order of exposure to formaldehyde and air was randomized, and the exposures took place at least 3 weeks apart to decrease the risk of carryover effects from the first exposure. There was no statistical order effect. Our patients' home exposure to formaldehyde was evaluated at 36.7 $\mu\text{g/m}^3$, similar to concentrations obtained in other studies (3, 4). This level is also very close to the basal formaldehyde concentration in the exposure chamber (32.0 $\mu\text{g/m}^3$) and lower than the mean exposure concentration during the experiment (92.2 $\mu\text{g/m}^3$). Therefore, neither a carryover effect nor formaldehyde exposure at home should have influenced the effect of experimental formaldehyde inhalation in our study.

The allergen provocation dose differed significantly when subjects were pre-exposed to formaldehyde and to air. This finding indicates that formaldehyde affected the early asthmatic reaction ($P = 0.05$). We did not obtain a doubling dose difference, perhaps because of the low dose of formaldehyde we used. But the clinical effect of formaldehyde in mite-sensitized subjects with asthma was confirmed by the late-phase response. In the 6 h of follow up after the BCT, FEV₁ fell more markedly in patients who had inhaled formaldehyde than when they were exposed to air (mean maximal fall of FEV₁ 15% and 11%, respectively, $P = 0.046$). Four patients developed a late-phase response after formaldehyde but not after air, and eight had a more severe LPR. The clinical relevance of our results is supported by the good reproducibility of LPR found in several studies (28, 29).

Experimental results have shown that formaldehyde exposure enhances eosinophilic airway inflammation by increasing local concentrations of interleukin (IL)-5 and RANTES (30). We observed a trend after formaldehyde toward higher ECP concentration in sputum supernatant at 24 h. We also observed a greater increase in blood eosinophils at 3 and 6 h after allergen challenge, with a tendency at 6 h.

Several studies have shown that ECP levels in blood and in sputum increase after BCT (18, 31). We observed a higher increase when patients were pre-exposed to formaldehyde. One mechanism for this increase might be the denaturation of the respiratory barrier. Any environmental factor that perturbs the structure of

airway epithelium could facilitate transepithelial delivery of allergenic proteins. Exposure to NO₂ or O₃ increases permeability of cultured epithelial cells (32). MacNee et al. suggested that oxidant stress, such as O₃, and the consequent changes in intracellular glutathione levels may be the mechanism for this increased permeability (33). As formaldehyde inhalation enhances glutathione depletion and oxidative stress, this mechanism also appears applicable for this pollutant (34).

The indoor environment is a mixture of many chemical and biologic pollutants and formaldehyde may be one of the factors that could affect asthma severity. Langley et al. observed that, within the atopic asthmatic population, indoor exposure to high levels of dust mite and dog allergen is associated with increased airway reactivity even in subjects not sensitized to these allergens (35). These results suggest an increase in nonspecific reactivity because of nonallergic factors coming from the allergens or other factors, such as formaldehyde.

In conclusion, pre-exposure to the maximum recommended formaldehyde concentrations in mite-sensitized subjects with intermittent asthma decreased the dose of mite allergen inducing the immediate reaction and provoked a more severe LPR.

Acknowledgments

The authors thank A. Dazy, F. Frey, M. J. Hoffner, J. Linder, L. Mahr, B. Sbinne, A. Vérot, and R. Wrobel for their excellent technical assistance. Also thank Allerbio and Pharmacia Laboratories for providing the reagents.

This project has been supported by the French Ministère de l'Aménagement du Territoire et de l'environnement (Programme de Recherche Interorganisme pour une MEilleure Qualité de l'Air à l'échelle Locale 2002). Anne Casset was supported by the French Society of Allergology and Clinical Immunology (Grant 2003).

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