

Formaldehyde exposure enhances inhalative allergic sensitization in the guinea pig

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Formaldehyde (FA), a common indoor air pollutant, has been associated with increased prevalence rates of asthmatic symptoms among exposed individuals in epidemiologic surveys. We studied the influence of FA exposure on inhalative allergic sensitization in the guinea pig. Three groups of guinea pigs ($n=12$ each) were exposed to clean air or two different FA concentrations (0.13 and 0.25 ppm) over 5 consecutive days. Exposure was followed by inhalation of 0.5% ovalbumin (OA) as sensitizing allergen. Three weeks later, specific bronchial provocation with OA was performed with body plethysmographic measurement of compressed air (CA). Furthermore, specific anti-OA-IgG1 (reaginic) antibodies were determined in serum. In a further six animals, the respiratory tract was examined histologically for signs of inflammation directly after the end of FA or clean air exposure. In the group exposed to 0.25 ppm FA, 10/12 animals were found to be sensitized to OA (positive reaction on specific provocation) vs. 3/12 animals in the control group ($P<0.01$). Furthermore, CA measurements of specific bronchial provocation and serum anti-OA-antibodies were significantly higher in the 0.25 ppm FA group than in controls (CA 0.35 vs. 0.09 ml median, $P<0.01$; anti-OA-IgG1 13 vs. <10 EU median, $P<0.05$), indicating enhanced sensitization. In the group exposed to 0.13 ppm FA, no significant difference was found compared to the control group. There was no sign of inflammation of the lower airways in FA-exposed guinea pigs other than mucosal edema, which was discovered by morphometry. We conclude that short-term exposure to a low concentration of FA (0.25 ppm) can significantly enhance sensitization to inhaled allergens in the guinea pig.

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Formaldehyde (FA) is a chemically reactive gas which is heavily used in industrial countries as a component of foam insulation material (1). Furthermore, it is part of cigarette sidestream smoke; therefore, it is one of the most ubiquitous airborne indoor pollutants. In view of the reduced air exchange in houses due to energy-conserving measures, this widely distributed air pollutant is now of public health concern (2). In the last decade, concern focused mainly on the potential role of FA as a chemical respiratory tract sensitizer. Recent studies, however, have found no consistent evidence that FA causes acute or chronic hypersensitivity disease (3), and the existence of immunologically mediated FA asthma has been questioned (4, 5).

On the other hand, air pollution may play a role in facilitating airway sensitization to common air-

borne allergens (6), as has been shown previously for sulfur dioxide and ozone in animal models (7–9). In a recent study, higher levels of specific IgE antibodies to intranasally applied antigens were produced in mice exposed to FA (10).

Therefore, we studied the influence of exposure to low FA concentrations on sensitization to inhaled ovalbumin (OA) in a guinea pig model, and we were able to demonstrate a significant enhancement of inhalative sensitization after short-term exposure to FA concentrations as low as 0.25 ppm.

Material and methods

Animals

Perlbright-white Dunkin-Hartley guinea pigs (female, specific pathogen-free, weight 240–320 g)

were purchased from the Winkelmann Co. (Borken, Germany) at 4–5 weeks of age and kept on an OA-free diet throughout the experiment to avoid sensitization by the oral route.

Study design

Guinea pigs ($n=12$ in each group) were exposed to FA in two different concentrations (0.13 and 0.25 ppm) or clean air (controls) for 5 days, followed by sensitization to inhaled OA. Sensitization to OA was investigated by specific bronchial provocation with OA and determination of specific reaginic serum antibodies to OA (IgG1 subclass). For protocol, see Fig. 1.

FA exposure

Groups of 12 animals were exposed to FA in two different concentrations (0.13 ± 0.04 ppm and 0.25 ± 0.06 ppm, mean $\pm$ SD) or to clean ambient air (control group, FA 0 ppm). The exposure took place in a 100-l gas-tight glass exposure chamber for 8 h on 5 consecutive days. FA from pressurized bottles (Messer-Griesheim, Düsseldorf, Germany) and ambient air were mixed to a constant flow of 3 l/min for chamber ventilation, thus allowing complete gas exchange in the exposure chamber nine times per hour.

FA concentrations were measured every 2 h by the chemical method described by Lyles et al. (11): FA reacts with Tetrachlor mercurate, sodium sulfite, and Pararos aniline to produce sulfonic acid, which can be measured photometrically. This method has a sensitivity of 0.008 ppm and a relative standard deviation (SD) of 2.6%.

Air sampling was carried out in the chamber with a tube close to the heads of the animals, and a fan was used to circulate air within the chamber.

The container and all the tubing exposed to FA were made of glass or had a nonstick coating. Humidity was measured by a hygrometer and kept constant in the exposure chamber at $58.5 \pm 4.1\%$ (mean $\pm$ SD) by adding drying beads to the chamber

as needed while the temperature in the chamber was at a constant level of $21.2 \pm 1.1^\circ\text{C}$ (mean $\pm$ SD).

Sensitization

After the last FA exposure (or clean air exposure in the control group) on day 5 (for protocol, see Fig. 1), the animals inhaled 0.5% OA (grade VII, Sigma Chemicals Co., Deisenhofen, Germany) as allergen, freshly prepared in isotonic saline, in an inhalation chamber specially designed for guinea pigs. In this chamber, the head of the animal was fixed in a 50-ml head box in front of a nebulizer outlet in order to optimize the density of the inhaled allergen. Inhalation was performed for 3 min with a compressor nebulizer (Pari Co., Starnberg, Germany, particle size 0.5–5.0 μm , output 0.75 ml/min). After 2 weeks (on day 19), the sensitizing inhalation was repeated as a booster inhalation with the same sensitizing protocol. During this booster inhalation, no anaphylactic reaction was noted.

Bronchial provocation

After another week (on day 26), a specific bronchial provocation test with OA was performed in order to test for specific bronchial sensitization to OA: after measurement of baseline pulmonary function (see below), inhalation of nebulized OA was carried out for 1 min with a 400-ml inhalation bag and compressor nebulizer (Pari Co., see above). For bronchial provocation, we used OA in a concentration of 1%, as this OA concentration is followed by reproducible bronchial obstruction in sensitized animals, as seen in previous studies (12). OA inhalation was followed by repeated lung-function measurements for 15 min to monitor bronchial obstruction (see below).

Pulmonary function measurements were performed in spontaneously breathing animals with a two-chamber body plethysmograph, as described in detail elsewhere (9). In brief, the animals were placed in the body chamber with the head in the respiratory chamber and a rubber cuff around the head separating the two chambers, in which gas conditions (identical volume, 37°C , 100% humidity) were kept constant. Pressure changes in the two compartments were recorded by sensitive pressure transducers (Svemor Co., Sweden, Type SP 2060, 0–6 Torr) and were volume-calibrated with a pump with defined volumes of 1–4 ml. The pressure changes in both chambers were continuously recorded, stored, and plotted against each other with an XY recorder (Philips Co., Kassel, Germany).

Without respiratory resistance between body

Protocol

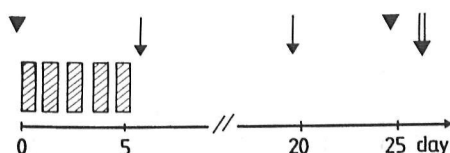


Fig. 1. Protocol: ▨ exposure to FA in two different concentrations, ↓ sensitizing OA inhalation, ▼ determination of anti-OA antibody in serum, ▴ specific bronchial provocation with OA.

chamber (lung) and respiratory chamber (mouth), a straight -45° line would result. With airflow obstruction, the end-expiratory turning point deviates to the left from the ideal -45° line, indicating compressed air (CA) in the lung, measured in milliliters, which cannot be expired. The normal range for CA in spontaneously breathing healthy animals was found to be 0.025 ± 0.012 ml (mean $\pm$ SD). Significant airway obstruction was defined as CA of more than 0.10 ml, representing more than five standard deviations above the mean.

Two consecutive respiratory loops were recorded for baseline pulmonary function and after antigen challenge every 2 min for a period of 11 min. The highest CA value recorded during this period (usually 3–5 min after antigen inhalation) was used for statistical analysis. This form of pulmonary function testing has been shown to be more sensitive than measurements of airway resistance in spontaneously breathing guinea pigs (13).

Antibody determination

For measurement of specific serum antibodies against OA, blood was obtained from all animals by puncture of the retro-orbital plexus before FA exposure (day 0) and on day 25, the day before the bronchial provocation testing.

Specific anti-OA IgG1 antibodies (the IgG1 subclass represents the main reaginic antibodies in the guinea pig) were determined by a direct ELISA, as described previously (14). Briefly, microtiter plates (Nunc Laboratories, Denmark) were coated with OA (grade VII, Sigma Co.) at a concentration of 10 mg/l and sealed with 0.1% gelatin. After addition of serial 1:5 dilutions of guinea pig serum, plates were incubated for 5 h. Plates were washed three times before the second antibody was added (rabbit anti-guinea pig IgG1 at a dilution of 1:10 000). All preparations were purchased from Miles Scientific Laboratories, Naperville, IL, USA. After incubation for 2 h at room temperature and three washes, alkaline phosphatase-conjugated goat antirabbit IgG (Medac Co., Hamburg, Germany) at a concentration of 1:3000 was added, and plates were incubated for 18 h at room temperature. Finally *p*-nitrophenylphosphate (1 mg/ml) was added. The reaction was stopped after 100 min with 1 N NaOH, and extinctions were measured at 405 nm in a multiscan photometer. Results were expressed in terms of the ELISA unit (EU), which was defined as the reciprocal value of the serum dilution which gave an optical density of 1.0. To ensure reproducibility, a known serum pool of guinea pig anti-OA IgG1 was run with each test as a standard. This standard serum was obtained by sensitizing guinea pigs to repeated s.c. injections of 0.5% OA with incomplete

Freund's adjuvant (Behring Co., Marburg, Germany) over a period of 12 weeks. In serum from nonsensitized animals on an OA-free diet, anti-OA antibodies were not detectable (<10 EU).

The specificity of the antibody had been demonstrated by complete specific inhibition.

Histology

Histologic examinations of lungs and tracheal tissues were performed on three animals after exposure to 0.20 ± 0.02 ppm FA and clean air, respectively. Immediately after exposure day 5, these animals were killed by a lethal intraperitoneal dose of Embutramide (T61, Hoechst Veterinär Co., Germany). To avoid injuries to blood vessels, the lungs were removed *in toto* from the chest cavity after thoracotomy.

The right pulmonary lobe was perfused with 4% buffered Formalin, sliced into blocks (approximately 1×1 cm), embedded in paraffin, cut at a thickness of 5 μ m, and stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS), and elastica-van Gieson's (EVG) stain.

Morphometry

The wall thickness of the respiratory bronchioles (T[br]) and alveolar septa (T[al]) was directly measured with a microscope-video-digitizing-table set (Ibas I, Kontron, Oberjochen, Germany). The thickness of at least 20 bronchial walls and alveolar septa was measured.

For measurement of alveolar walls, a line between the respiratory bronchioli and the next pleural surface or interlobular septum was drawn, and all alveolar walls cut by this line were assessed for thickness. This method of selection of alveolar walls was chosen in order to reduce systematic errors due to observer bias. The total wall thickness was measured in the middle between the junctions of alveolar walls, when both air-tissue interfaces could be discerned easily. Alveolar walls near larger blood vessels and airways were excluded from measurements. Cumulative mean plots were performed to ensure that the number of bronchial walls or alveolar septa was sufficient to reduce the standard error of the mean to values lower than 5%.

Statistics

For statistical analysis, lung-function parameters (CA in milliliters) were compared between each FA-exposed group and the control group with the two-way ANOVA and Student's unpaired *t*-test. Immunologic parameters (anti-OA IgG1 in EU, not normally distributed) were compared between the 0.25 ppm FA-exposed group and the control group

with the two-tailed Mann-Whitney U-test. The incidence of positive bronchial reactions on airway challenge was compared between the FA group and the control group with Fisher's exact test. A probability of 5% ($P < 0.05$) was considered to be significant.

This experimental design was approved and the care of the experimental animals supervised by Staatliches Veterinäramt, Regierungspräsident Giessen, Germany.

Results

Allergen challenge

After exposure to 0.25 ppm FA, 10 of 12 animals (83%) reacted with airway obstruction (CA > 0.1 ml) on OA challenge, indicating inhalative sensitization to OA, while only three of 12 animals reacted in the control group ($P < 0.01$). CA as a parameter of airway obstruction on the bronchial provocation test was significantly higher in the 0.25 ppm FA-exposed group (CA 0.35 [0.25–0.72] ml median with 95% confidence interval [CI]) than in the clean air-exposed control group (CA 0.088 [0.03–0.09] ml; $P < 0.01$, Fig. 2).

In the group with exposure to the lower FA concentration (0.13 ppm), the bronchial reaction on allergen challenge did not differ from the control group (Fig. 2).

Specific antibody determination

Specific anti-OA-antibody of the IgG1 subclass (major anaphylactic antibody of the guinea pig) was not detectable (<10 EU) in any of the animals at the beginning of the experiment. At the end of the sensitizing period (day 25), control animals again did not have detectable antibody titers,

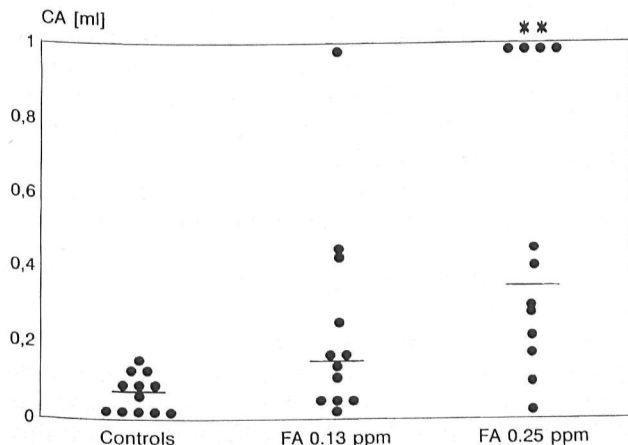


Fig. 2. Bronchial reaction on allergen challenge, compressed air (CA) in milliliters as parameter for airway obstruction in control group and two FA-exposed groups (— median, $**P < 0.01$).

whereas in the 0.13 ppm-exposed groups, 3 of 12 animals had increased specific antibody titers.

In the group exposed to 0.25 ppm FA, 6 of the 12 animals had increased antibodies to OA indicating sensitization, and the anti-OA-IgG1 antibody titer was significantly higher in this group than in the control group (13 [10–130] EU, median with 95% CI, vs. <10 EU, $P < 0.05$, Fig. 3).

Histology

Histologic examination revealed edema of the bronchial mucosa in animals exposed to FA without any sign of mucosal inflammation throughout the tracheobronchial system (Fig. 4). The thickness of the bronchial walls (T[br]) was significantly higher in the FA-exposed animals than in the controls (T[br] 40.9 ± 2.5 vs. 28.2 ± 1.2 μm , mean and SD, no overlap), while the thickness of alveolar septa was not different between these groups (T[al] 5.2 ± 0.2 vs. 5.3 ± 0.2 μm). This finding indicates bronchial wall edema due to FA exposure and lack of inflammation in lower airways.

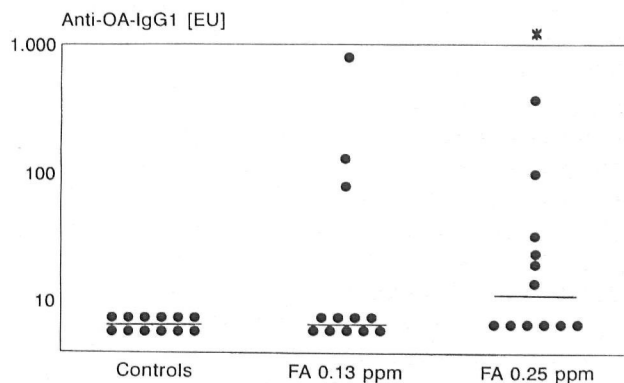


Fig. 3. Specific anti-OA antibody of IgG1 subclass (reaginic antibody) in ELISA units (EU) on day 25 in control group and two FA-exposed groups (— median, * $P < 0.05$).

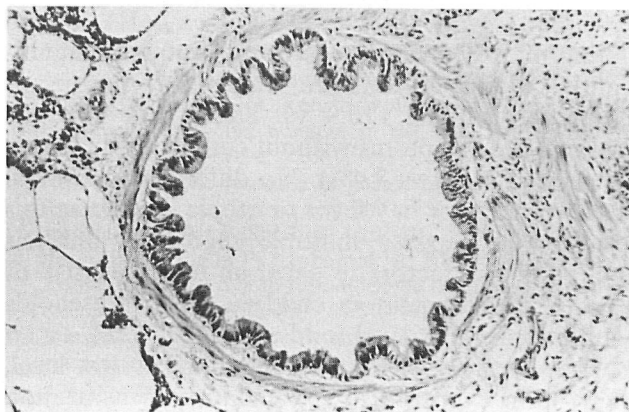


Fig. 4. Histologic examination of bronchus of FA-exposed guinea pig, demonstrating intramural edema without inflammatory reaction (HE $\times 160$).

Discussion

Formaldehyde, a ubiquitous air pollutant, is known to cause irritation of mucous membranes in low concentrations (15, 16). After short-term exposure, only mild airway dysfunction has been found in controlled clinical studies (17, 18). However, chronic FA exposure in occupational settings over many years may lead to lung-function impairment, as recently shown by Kilburn et al. (19), whereas, in earlier studies, an association between chronic occupational FA exposure and respiratory symptoms could not be demonstrated (20, 21).

Air pollution has been previously shown to be associated with allergic sensitization in epidemiologic studies (6). Furthermore, in animal experiments, exposure to sulfur dioxide and ozone significantly enhanced allergic sensitization to inhaled allergens (7–9), and environmental tobacco smoke, which contains FA in higher concentrations, has been shown to increase the sensitization rate in children (22, 23) and in experimental animal studies (21).

In a recent study, Tarkowski & Gorski (10) demonstrated increased IgE anti-OA antibodies in mice sensitized intranasally to OA, when exposed to 2 mg/m³ (1.6 ppm) FA for 10 days. In the present study, however, we have seen that FA exposure enhances inhalative allergic sensitization to inhaled OA in the guinea pig, even in concentrations often encountered in daily life.

Epidemiologic studies of FA exposure in children have shown that asthmatic symptoms are more prevalent in FA-exposed children than in non-exposed controls. Krzyzanowski et al. found higher prevalence rates in asthma among children living in homes with FA concentrations of 60–120 ppb than in less exposed households (24).

In a recent report, Czap et al. (25) compared prevalence rates for asthma in children from homes with higher indoor FA levels (mean 59.2 µg/m³ = 0.05 ppm) with low-level homes (FA mean 7.4 µg/m³). These authors found not only significantly higher prevalence rates for physician-diagnosed asthma (16.9 vs. 8.8%), but also for asthma-like symptoms without confirmed diagnosis of asthma (18.8 vs. 8.4%). No difference, however, was reported for hay fever or atopic eczema in this study, but stepwise multiple regression analysis including risk factors revealed an increased risk of asthma development in children from households with a higher FA level (odds ratio 3.3) (25).

However, in our guinea pig model, low-level, short-term exposure to FA enhanced sensitization depending on the FA concentration, thus indicating a dose-response relationship. In this animal model, we used CA as a sensitive body plethysmographic

parameter of airway obstruction (13) on specific bronchial provocation test with OA and therefore as a parameter of sensitization to OA. In the group exposed to 0.25 ppm FA, reactions to OA inhalation were significantly greater than in the control group, but not in the group exposed to 0.13 ppm FA.

Furthermore, in the 0.25 ppm FA group, specific antibodies of the IgG1 subclass were found to be significantly higher in serum before specific bronchial provocation than in the control group, indicating increased specific sensitization to OA in this FA-exposed group. We measured the specific IgG1, as this subclass has been shown to be the major reaginic antibody in the guinea pig (26).

In the 0.25 ppm FA group, only 6 of 12 animals had increased specific anti-OA IgG1 antibodies, while 10 of 12 animals reacted to the specific bronchial provocation test with airway obstruction, indicating sensitization. Thus, the specific bronchial provocation test may be more sensitive for airway sensitization than the determination of specific reaginic serum antibodies in our guinea pig model.

No signs of inflammation of the lower airways were found histologically in FA-exposed animals. This finding is in accordance with data from Swiecichowski et al. (27), who reported no evidence of inflammation after exposure to 0.3 ppm FA for 8 h in guinea pigs. The only significant difference seen histologically in our model was the thickness of bronchial mucosa, indicating bronchial epithelial edema in FA-exposed animals compared to clean air-exposed controls. This mucosal edema may be due to the effects of mediators from the upper airways where FA as highly water-soluble gas is nearly completely absorbed. In dogs, about 95% of FA has been shown to be absorbed in the upper respiratory tract, including the trachea (28). Vascular leakage causing mucosal edema is a possible explanation, but has not been investigated in this study.

We cannot completely exclude the possibility that the FA-induced upper airway changes influenced lung-function measurements of CA on specific bronchial provocation testing with OA in our model. However, as this provocation took place more than 2 weeks after the last FA exposure, this influence is supposed to be negligible.

We cannot comment on the relevance of this animal experiment to human beings. Guinea pigs might be more sensitive to FA than man, as exposure to concentrations as low as 0.6 ppm over 1 h has been shown to increase airway resistance in guinea pigs (29), but not in man.

Furthermore, the mechanism for increased sensitization to inhaled allergens due to FA exposure was not investigated in our study. Mucosal permeability might be increased in the upper airways, where inflammatory reactions have been demonstrated previously in guinea pigs (30). However, our study

did not investigate the permeability of mucous membranes.

Moreover, mucociliary clearance has been shown to be impaired at least after high concentrations of FA (31), leading to prolonged allergen exposure on concomitant allergen inhalation.

Finally, we do not know whether FA influences immunologic reactions to inhaled allergens in the guinea pig.

In conclusion, exposure to relatively low concentrations of FA, which are frequently encountered in the indoor environment, can enhance allergic sensitization in the guinea pig. Further studies on the relevance of this animal model to man are necessary because FA is a common air pollutant in the human environment.

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