

Potential of allergic bronchoconstriction by repeated exposure to formaldehyde in guinea-pigs *in vivo*

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Summary

Background Indoor formaldehyde (FA) might worsen allergies and be an underlying factor for the increasing incidence and severity of asthma; the exact mechanism, however, remains unclear.

Objective The present study examined the effects of repeated exposure to FA on methacholine- and antigen-induced bronchoconstriction in guinea-pigs *in vivo*.

Methods First, non-sensitized guinea-pigs were transnasally treated with 0.1 or 1.0% FA or saline three times a week for 6 weeks, and increasing concentrations of methacholine (50, 100, and 200 µg/mL) were inhaled at 5-min intervals. Second, guinea-pigs pre-treated with transnasal administration of FA or saline using the same protocol were passively sensitized with anti-ovalbumin (OA) serum 7 days before antigen challenge. Third, guinea-pigs were actively sensitized with OA and pre-treated with transnasal administration of FA or saline using the same protocol. The lateral pressure of the tracheal tube (P_{ao}) was measured under anesthesia and artificial ventilation.

Results The antigen-induced increase in P_{ao} in actively sensitized guinea-pigs was significantly potentiated by FA exposure in a dose-dependent manner. The dose-response curve of the methacholine-induced increase in P_{ao} in non-sensitized guinea-pigs or of the antigen-induced increase in P_{ao} in passively sensitized guinea-pigs was not altered by FA exposure. Transnasal administration of FA significantly increased the serum anti-OA homocytotropic antibody titre (IgG) as measured by the passive cutaneous anaphylaxis reaction in actively sensitized guinea-pigs.

Conclusion The results suggest that repeated exposure to FA worsens allergic bronchoconstriction through enhancing antigen sensitization.

Keywords antigen sensitization, bronchial asthma, formaldehyde, repeated transnasal exposure

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Introduction

The incidence of allergic diseases such as bronchial asthma, atopic dermatitis, and allergic rhinitis has recently increased [1]. Various antigens, including mite, fungus, and food, cause allergic diseases. Epidemiologic surveys indicate that formaldehyde (FA) is associated with the increasing prevalence rates of the allergic symptoms among exposed individuals. FA is widely used in the chemical, construction, textile, paper products, plastic, paint, adhesive, and cosmetic industries. Occupational asthma develops in workers in the tanning and rubber industries [2]. Hendrick and Lane [3] described a nurse working at a renal haemodialysis unit who developed asthma as a result of continuous exposure to the formalin used to sterilize artificial kidney machines. Therefore, previous exposure to FA in the workplace can cause acute adverse effects on respiratory health. FA, which is widely distributed as an air pollutant, is now a public health concern because of

the reduced air exchange in houses due to energy-conserving measures [4, 5].

In humans, acute exposure to 3 p.p.m. FA produces a small and transient decrease in pulmonary function and mild to moderate eye and upper respiratory tract irritation [6]. Chronic workplace exposure to FA and solvents at low doses reduced pulmonary function in 280 non-smoking white women working as histology technicians [7]. The correlation between chronic exposure to FA and respiratory symptoms, however, remains uncertain [8, 9]. The mechanisms of FA-induced asthma might be lower respiratory tract irritation from inhaled FA, immunologic reaction to FA, and/or FA-induced direct release of histamine [2].

Recent studies indicated that intranasally applied antigens produced higher levels of specific IgE antibodies in mice exposed to FA [10], and exposure to a low concentration of FA can enhance sensitization to inhaled allergens in the guinea-pig [11]. The correlation between the airway response and the immunologic response to FA exposure in guinea-pigs, however, remains uncertain.

The present study evaluated whether transnasal administration of FA enhances methacholine-induced and antigen-induced bronchoconstriction in guinea-pigs. Furthermore, the

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effect of repeated FA exposure on immunologic responses was evaluated by measuring homocytotropic antibody titres (IgG and IgE) of the serum as estimated by passive cutaneous anaphylaxis (PCA) reaction.

Materials and methods

Animals

Male, albino, Hartley strain guinea-pigs weighing 250–350 g (4 weeks of age) were obtained from Sankyo Laboratory Service (Toyama, Japan). After arrival at the Institute of Animal Experiments in our university, they were kept in conventional animal housing facilities for 1 week before use. Food and water were available *ad libitum*. The experiment was performed according to the Principles of Laboratory Animal Care formulated by the Institute of Animal Experiments at Kanazawa University.

FA exposure

Guinea-pigs were exposed to two different concentrations of FA solution (0.1% and 1.0%) or saline (control group) three times a week for 6 weeks (a total of 18 times). FA or saline solution (50 μ L volume) was administered unilaterally to the nasal cavity twice a day using a micropipette (100 μ L/body/d).

Measurement of bronchoconstriction

The guinea-pig was anesthetized with sodium pentobarbital (75 mg/kg *i.p.*) after repeated exposure to FA or saline for 6 weeks. The guinea-pig was placed in the supine position and the trachea was cannulated with a polyethylene tube (outside diameter 2.5 mm, inside diameter 2.1 mm). After surgery, the guinea-pig was artificially ventilated with a small animal respiratory pump (Model 1680, Harvard Apparatus, South Natick, MA) adjusted to a tidal volume of 10 mL/kg at a rate of 60 strokes/min. The changes in lung resistance to inflation and the lateral pressure of the tracheal tube (pressure at the airway opening; P_{ao} ; cmH₂O) were measured by the modified method of Konzett and Rössler [12] described by Jones et al. [13] using a pressure transducer (Model TP-603 T, Nihon Kogyo, Tokyo, Japan). Since we confirmed that the change in P_{ao} following inhalation of leukotriene C₄ and histamine represented the average of the changes in pulmonary resistance and reciprocal dynamic lung compliance ($1/C_{dyn}$) [14], P_{ao} was used as an overall index of bronchial response to bronchoactive agents [15]. To standardize the volume history of the lungs, the lungs were over-inflated by two times the tidal volume for two breaths by closing the outlet port of the respirator after completion of all surgical procedures.

Protocol

Methacholine challenges were administered only to non-sensitized animals, while OA challenges were administered only to animals that were actively and passively sensitized.

Methacholine-induced bronchoconstriction in non-sensitized animals Non-sensitized guinea-pigs ($n = 33$) were exposed to FA or saline for 6 weeks before methacholine inhalation. Fifteen minutes after the preparation of non-

sensitized guinea-pigs, when P_{ao} had stabilized, the animals inhaled a succession of doubling concentrations of methacholine from 50 to 200 μ g/mL at 5-min intervals under continuous ventilation. The aerosol was generated during a 30-s period by an ultrasonic nebulizer developed for small animals at our institution. The nebulizer generated an aerosol at a rate of 15.2 μ L/min during the 30-s period, and 46.4% of the generated aerosol was deposited in the lungs as measured using the radioaerosol technique [16]. The median aerodynamic diameter of the particles of normal saline was $3.59 \pm 1.96 \mu$ m (mean $\pm$ SD) [17].

Antigen (OA)-induced bronchoconstriction in passively sensitized guinea-pigs Non-sensitized guinea-pigs ($n = 20$) were exposed to FA or saline for 5 weeks before an intraperitoneal injection of anti-OA serum. The guinea-pig homocytotropic anti-OA serum was made according to the method of Santives et al. [18]. Briefly, 500 μ g of OA was emulsified in Freund's complete adjuvant and injected intradermally into each guinea-pig at multiple sites. Boosting was performed in the same manner 2 weeks later. Serum was collected from each animal 2 weeks after the booster, pooled, and kept frozen until use. The serum antibody titres were 1:12 800, 1:6400, and 1:512 as estimated by PCA at 4 h, 24 h, and 7 days, respectively. Normal guinea-pigs were passively sensitized with 1.0 mL anti-OA serum/kg intraperitoneally. After the intraperitoneal injection of anti-OA serum, they were exposed to FA or saline for 1 week until the OA challenge. Fifteen minutes after the preparation of passively sensitized guinea-pigs, animals were challenged with nebulized OA solution dissolved in saline (1.0 mg/mL) without interrupting the constant ventilation. The OA aerosol was generated for 30 s with the ultrasonic nebulizer. The P_{ao} was measured for 30 min.

Antigen (OA)-induced bronchoconstriction in actively sensitized guinea-pigs Guinea-pigs ($n = 33$) were actively sensitized according to the method of Tohda and co-workers [19]. Animals were intraperitoneally injected with 2000 μ g OA and 100 mg Al(OH)₃ 2 days after an intraperitoneal injection of 30 mg/kg cyclophosphamide (Fig. 1). Three weeks after the

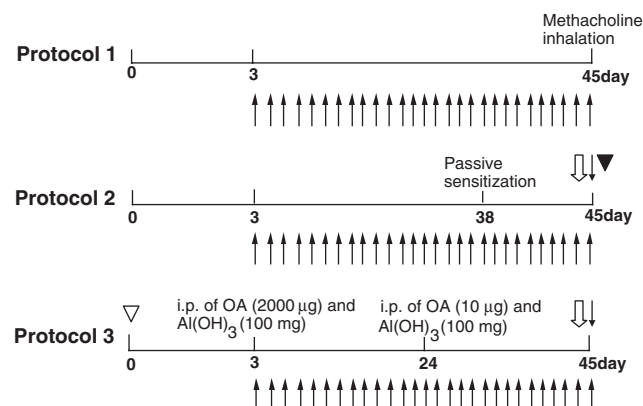


Fig. 1. Experimental protocol: ∇ intraperitoneal injection (*i.p.*) of cyclophosphamide (30 mg/kg), $\uparrow$ transnasal administration (50 μ L $\times$ 2) of formaldehyde in two different concentrations or saline three times a week for 6 weeks, $\downarrow$ OA inhalation, $\downarrow$ bronchoalveolar lavage, $\blacktriangledown$ collection of serum for determination of anti-OA antibody titre.

first injection of OA, they were intraperitoneally injected with 10 µg OA and 100 mg Al(OH)₃. The actively sensitized animals were used for experiments 3 weeks after the booster injection. Actively sensitized guinea-pigs were exposed to FA or saline three times a week for 6 weeks after the first sensitization. Fifteen minutes after the preparation of actively sensitized guinea-pigs, animals were challenged with nebulized OA solution dissolved in saline (1.0 mg/mL) without interrupting the constant ventilation. The OA aerosol was generated for 30 s with the ultrasonic nebulizer.

Analysis of bronchoalveolar cells

Airway inflammation was assessed by bronchoalveolar lavage (BAL) cell examination. BAL was performed 30 min after the OA challenge when measurement of antigen-induced bronchoconstriction was completed. Sterile saline (10 mL) at 37 °C was infused twice through the tracheal cannula, and the BAL fluid was recovered manually by gentle aspiration with a disposable syringe after each infusion. Total cell number was determined using Turk solution. Differential cell counts were performed on cytopspin preparations (Cytospin 2, Shadon, England) by counting 300 cells after staining by the May–Grünwald–Giemsa method.

Passive cutaneous anaphylaxis (PCA) reaction

The serum anti-OA IgE and IgG antibody titres were determined by PCA reaction. Blood was obtained from the cannulated left cervical artery of actively sensitized animals 30 min after OA challenge. Serum was collected from each animal, pooled, and stored at –80 °C until use. Each serum sample was diluted with saline to 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048, 1/4096, and 1/8192 that of the original concentration. Serial dilutions of the serum were injected intradermally in 0.1 mL quantities into the shaved back of normal guinea-pigs (300–400 g). The guinea-pig was challenged 4 h later by an intravenous injection of 5 mg OA and 0.5 mL of 1% Evans blue into the left jugular vein to measure the IgG antibody titre. In addition, 7 days after the intradermal injection of serum, another guinea-pig underwent the same OA challenge for the measurement of IgE antibody titre. Each guinea-pig was killed 30 min after the OA challenge, and the extent of extravasated dye was determined by grading the reaction according to the diameter of the blue spot [20]. We defined the reciprocal of the most diluted serum concentration having blue spot diameters of more than 5 mm as the PCA titre (IgG and IgE) [20].

Statistical analysis

All data are presented as mean ± standard error of the mean (SEM). Statistical differences were determined by analysis of variance (ANOVA) among three groups. Differences in the percentage increase in P_{ao} from the baseline value after OA provocation was analyzed among animals exposed to 0.1% FA, 1.0% FA, or saline using a two-factor repeated measures ANOVA. The dose–response curve for methacholine-induced bronchoconstriction was also analyzed by a two-factor repeated measures ANOVA. A *P*-value of 0.05 or less was considered to be statistically significant.

Chemicals

The following chemicals were used: OA (Sigma, St. Louis, MO), diphenhydramine hydrochloride (Sigma), sodium pentobarbital (Abbott Laboratories, North Chicago, IL), methacholine (Wako Pure Chemical, Osaka, Japan), FA solution (HCHO, Wako Pure Chemical), diethyl ether (C₂H₅OC₂H₅, Wako Pure Chemical), and Evans Blue (Wako Pure Chemical).

Results

Methacholine-induced bronchoconstriction

The mean baseline P_{ao} (±SEM) immediately before methacholine inhalation was 9.9 ± 0.3 , 9.7 ± 0.1 , and 9.6 ± 0.2 cmH₂O in guinea-pigs exposed to saline, 0.1% FA, or 1.0% FA, respectively. There were no significant differences among them. Exposure to FA did not alter methacholine-induced bronchoconstriction (Fig. 2).

Antigen-induced bronchoconstriction in passively sensitized guinea-pigs

The P_{ao} values before OA challenge were 10.2 ± 0.4 , 9.5 ± 0.2 , and 10.5 ± 0.2 cm H₂O in animals exposed to saline, 0.1% FA, or 1.0% FA, respectively, and these values were not statistically different. The percent increase in P_{ao} from the baseline value after OA challenge in the three groups is shown in Fig. 3. The increase was not significantly different between the groups.

Antigen-induced bronchoconstriction in actively sensitized guinea-pigs

The baseline P_{ao} values before OA challenge were 9.2 ± 0.2 , 9.1 ± 0.2 , and 9.1 ± 0.2 cm H₂O in saline-, 0.1% FA-, and 1.0% FA-exposed animals, respectively. There were no significant differences among them. The percent increase in P_{ao} from the baseline value after OA challenge in the three groups is shown in Fig. 4. Repeated FA exposure significantly

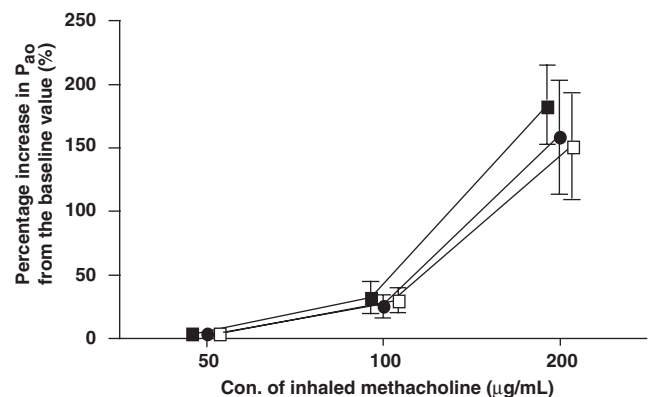


Fig. 2 Effect of repeated intranasal administration of formaldehyde (FA) on bronchial responsiveness to inhaled methacholine in non-sensitized guinea-pigs. Filled circles indicate response after administration of 0.1% FA ($n = 11$). Open squares indicate response after administration of 1.0% FA ($n = 11$). Filled squares indicate response after administration of saline ($n = 11$). Each point indicates mean ± SEM.

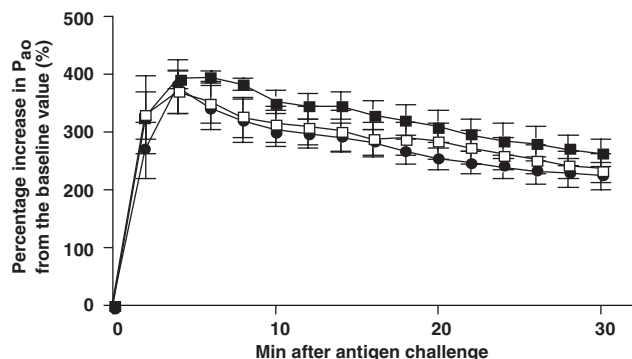


Fig. 3. Effect of repeated intranasal administration of formaldehyde (FA) on antigen-induced bronchoconstriction in passively sensitized guinea-pigs. Filled circles indicate response after administration of 0.1% FA ($n=6$). Open squares indicate response after administration of 1.0% FA ($n=8$). Filled squares indicate response after administration of saline ($n=6$). Each point indicates mean $\pm$ SEM.

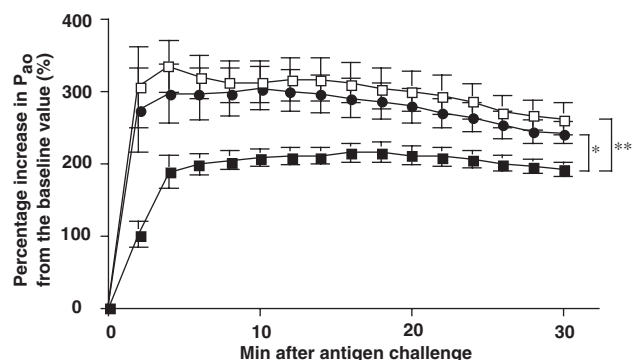


Fig. 4. Effect of repeated intranasal administration of formaldehyde (FA) on antigen-induced bronchoconstriction in actively sensitized guinea-pigs. Filled circles indicate response after administration of 0.1% FA ($n=11$). Open squares indicate response after administration of 1.0% FA ($n=11$). Filled squares indicate response after administration of saline ($n=11$). Each point indicates mean $\pm$ SEM. ** $P<0.01$, * $P<0.05$ compared with the saline treatment group analyzed by repeated measure analysis of variance (ANOVA).

($P<0.05$ by two-factor repeated ANOVA) potentiated the antigen-induced bronchoconstriction of actively sensitized guinea-pigs in a dose-dependent manner. The peak P_{ao} value after the OA challenge was 214.8 ± 13.6 , 302.5 ± 30.2 , and $333.6 \pm 35.9\%$ with saline, 0.1% FA, and 1.0% FA. The peak P_{ao} value of the FA-exposed animals was significantly greater than that of those exposed to only saline (0.1% FA: $P<0.05$ and 1.0% FA: $P<0.01$).

BAL cell counts

The BAL fluid recovery was 11.8 ± 1.1 , 13.2 ± 0.7 , and 13.3 ± 0.7 mL in passively sensitized animals exposed to saline, 0.1% FA, and 1.0% FA, respectively. The total cell numbers of BAL fluid were $(1.38 \pm 0.26) \times 10^5$, $(2.02 \pm 0.19) \times 10^5$, and $(1.73 \pm 0.49) \times 10^5$ cells/mL with saline-, 0.1% FA-, and 1.0% FA-exposed animals, respectively. BAL fluid cell differentiation was not significantly different between the three groups (Fig. 5).

In actively sensitized guinea-pigs, the BAL fluid recovery following OA challenge was 12.9 ± 0.7 , 13.0 ± 0.8 , and 13.4 ± 0.9 mL with exposure to saline, 0.1% FA, and 1.0%

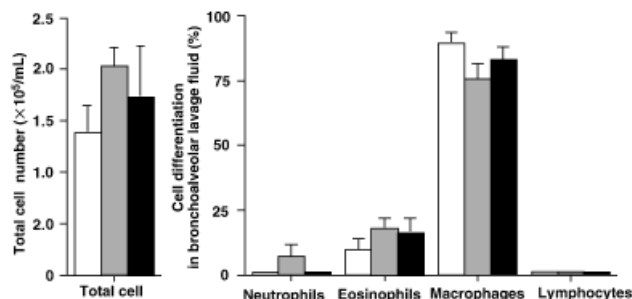


Fig. 5. Effect of formaldehyde (FA) on antigen-induced change of bronchoalveolar lavage cell component in passively sensitized guinea-pigs. Bronchoalveolar lavage was performed 30 min after antigen (OA) challenge. Guinea-pigs were divided into three groups. Hatched bars indicate response after administration of 0.1% FA ($n=6$). Solid bars indicate response after administration of 1.0% FA ($n=8$). Open bars indicate response after administration of saline ($n=6$). Each bar indicates mean $\pm$ SEM.

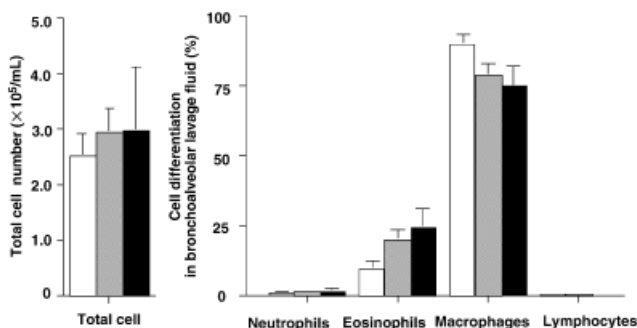


Fig. 6. Effect of formaldehyde (FA) on antigen-induced change of bronchoalveolar lavage cell component in actively sensitized guinea-pigs. Bronchoalveolar lavage was performed 30 min after antigen (OA) challenge. Guinea-pigs were divided into three groups. Hatched bars indicate response after administration of 0.1% FA ($n=11$). Solid bars indicate response after administration of 1.0% FA ($n=11$). Open bars indicate response after administration of saline ($n=11$). Each bar indicates mean $\pm$ SEM.

FA. The total cell number in BAL fluid was $(2.52 \pm 0.40) \times 10^5$, $(2.93 \pm 0.43) \times 10^5$, and $(2.97 \pm 1.15) \times 10^5$ cells/mL with saline-, 0.1% FA-, and 1.0% FA-exposed animals, respectively. BAL fluid cell differentiation was not significantly different between the three groups (Fig. 6).

PCA reaction

The PCA reaction 4 h after OA challenge was 471.3 ± 98.5 , 837.8 ± 142.7 , and 1256.4 ± 333.7 in guinea-pigs exposed to saline, 0.1% FA, and 1.0% FA, respectively. The value was significantly ($P<0.05$) higher with 0.1% FA and 1.0% FA than with saline. At 7 days, the PCA reaction was negative in all the three groups (Fig. 7).

Discussion

In the present study, we examined whether the transnasal administration of FA enhances methacholine-induced and antigen-induced bronchoconstriction in guinea-pigs *in vivo*. Our results clearly indicate that transnasal administration of

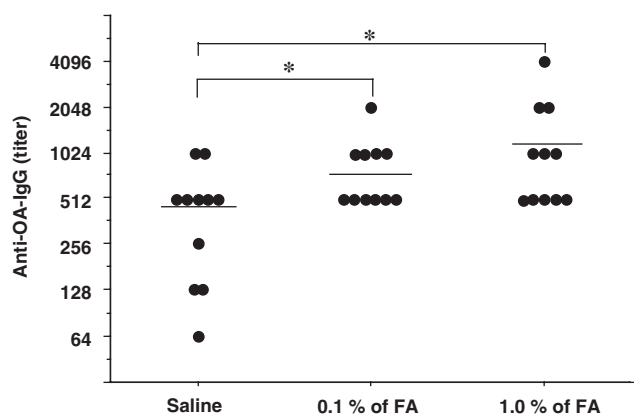


Fig. 7. Effect of formaldehyde (FA) on anti-OA IgG antibody production in actively sensitized guinea-pigs. The titre was assessed by passive cutaneous anaphylaxis reaction. Guinea-pigs were divided into three groups, one group treated with 0.1% FA ($n = 11$) intranasally, one treated with 1.0% FA ($n = 11$), and the other treated with saline ($n = 11$). Each horizontal bar indicates median value. * $P < 0.05$ compared with the saline treatment group analyzed by Mann–Whitney's U test. OA, ovalbumin

FA enhances antigen-induced bronchoconstriction in actively sensitized guinea-pigs in a dose-dependent manner. Transnasal administration of FA does not, however, alter non-specific bronchial responsiveness of non-sensitized guinea-pigs or antigen-induced bronchoconstriction of passively sensitized animals. Transnasal administration of FA significantly increased the anti-OA homocytotropic antibody titre (IgG) in the serum as estimated by PCA reaction in the actively sensitized guinea-pigs.

FA is a micromolecular substance with both irritating and sensitizing properties. FA-induced asthma usually involves an irritant effect, a classic IgE-mediated allergic event, or a pharmacologic abnormality (histamine release) [21].

In humans, FA is a well-recognized irritant to the respiratory system, and causes decrease in pulmonary function at concentrations greater than 5 p.p.m. [22]. It has been suggested that exposure to FA induces asthma symptoms [22]. Healthy laboratory workers did not experience any acute or delayed changes in lung function after exposure to 2.0 p.p.m. FA at rest or with exercise, and had few irritative symptoms [23]. In addition, there was no significant airway obstruction as measured by flow-volume parameters and airway resistance in 15 asthmatic volunteers during or immediately after the exposure [24]. On the other hand, normal subjects had statistically significant ($P < 0.02$) decreases in the following pulmonary functions after a 55 min exposure to FA as compared with clean air: 3.8% in forced expiratory volume in 1 s (FEV_1) and 2.65% in forced vital capacity (FVC) [25]. There were no statistically significant decreases in pulmonary function in asthmatic patients [25].

Chronic workplace exposure to FA (0.2–1.9 p.p.m.) and solvents at low concentrations reduced pulmonary function in 280 non-smoking white women working as histology technicians [7]. The major functional change in the histology technicians was a steeper decrease in vital capacity and flow rates from ages 20–60 years as assessed by regression analysis compared with that in the general Michigan population [7]. Harving et al. [26] reported that exposure to FA once a week for three consecutive weeks caused no significant change in FEV_1 , airway resistance, functional residual capacity, flow-

volume curves, or subjective ratings in participants with high histamine reactivity ($PC_{20} < 0.50$ mg/mL). They suggested that the lack of response of the lower respiratory system to low concentrations of FA might be explained by complete clearance of FA in the upper airways [26]. These reports suggest that bronchial response to short- or long-term exposure to FA is different between healthy and asthmatic subjects.

Swiecichowski et al. [27] reported that specific pulmonary resistance and bronchial responsiveness (to infused acetylcholine) increased with FA exposure *in vivo* and *ex vivo* in guinea-pigs. Exposure to more than 0.3 p.p.m. FA for 8 h produced a significant increase in bronchial responsiveness, while a similar effect occurred only after more than 9.4 p.p.m. FA for 2 h in guinea-pigs [27]. By extending the length of exposure, lower concentrations (< 1.0 p.p.m.) of FA caused bronchial hyperresponsiveness that was not observed after 2-h exposure to similar concentrations. Thus, the duration of exposure is important to the induction of bronchial hyperresponsiveness. *In vivo* bronchial responsiveness following FA exposure increased with time after exposure and became maximal 2–6 h post exposure. This effect was still evident 24 h after exposure [27]. It is unclear, however, how long the FA-induced bronchial hyperresponsiveness lasts. In the present study, transnasal exposure of FA did not alter the bronchoconstriction induced by inhalation of methacholine 48 h after the last exposure, suggesting that FA exposure has no direct effect on bronchial responsiveness 48 h post exposure.

During the last decade, concern has focused mainly on the potential role of FA as a chemical sensitizer of the respiratory tract. Dykewicz et al. [28], however, failed to find consistent evidence that FA causes acute or chronic hypersensitivity. The authors reported that IgE or IgG antibody to FA-human serum albumin was not associated with exposure to gaseous FA or with respiratory symptoms [28]. It has not been established whether FA inhalation causes any immunologically mediated disease [29].

Since the 1970s, FA has been thought to be an airway-sensitizing agent. Its ability to bind proteins and acquire immunogenicity suggests that FA has allergenic properties. Most of the clinical reports of an IgE response to FA, however, were based upon case studies [30]. Other studies indicate a very low incidence of FA-induced airway sensitization [31–33]. A recent study in humans reported a greater prevalence of asthma and chronic bronchitis in children living in homes with 0.06–0.12 p.p.m. FA than those living with < 0.06 p.p.m. FA [34]. FA might facilitate sensitization to high-molecular-weight allergens [10] by increasing the permeability of respiratory epithelium and the destruction of suppressive immunologic barriers. The upper respiratory tract epithelium constitutes a very important barrier that limits the effective contact between local immune cell populations and incoming antigens. The extraordinary solubility of some aeroallergens, however, allows them to penetrate the epithelial barriers even in healthy upper respiratory tract [35]. The high solubility of FA in the respiratory mucus as well as the formation of covalent adducts and the induction of increased cell turnover indicates that FA might directly influence the activity of suppressor cells that inhibit immunologic inflammatory responses at the fragile epithelial surfaces [36–38]. The present study suggests

that FA is an airway-sensitizing agent and might facilitate sensitization.

In a recent study, Tarkowski & Gorski [10] demonstrated an increase of anti-OA IgE antibody in mice sensitized intranasally to OA, when exposed to 2 mg/m³ (1.6 p.p.m.) FA for 10 days. Riedel et al. [11] reported that short-term exposure (5 days) to a low concentration of FA (0.25 p.p.m.) could significantly increase serum anti-OA-IgG1 antibody to inhaled allergens in the guinea-pig, indicating enhancement of sensitization by FA. In the present study, repeated transnasal administration of FA enhanced antigen-induced bronchoconstriction in actively sensitized guinea-pigs, and increased anti-OA homocytotropic antibody titre (IgG) in the serum. The repeated transnasal administration, however, did not alter antigen-induced bronchoconstriction in passively sensitized guinea-pigs. These results strongly suggest that repeated exposure to FA enhances immediate asthmatic response through enhancement of antigen sensitization, but not through altering the release of chemical mediators.

We examined the effect of repeated transnasal administration of FA on BAL cell component 30 min after antigen challenge for the purpose of assessing its influence on allergic airway inflammation. BAL cell differentiation was not significantly influenced by FA exposure in passively or actively sensitized guinea-pigs. Riedel et al. reported that there was no sign of inflammation of the lower airways in FA-exposed (0.25 p.p.m.) guinea-pigs other than mucosal edema, which was discovered by morphometry [11]. Swiecichowski et al. [27] found no evidence of inflammation after exposure to 0.3 p.p.m. FA for 8 h in guinea-pigs. Bhalla et al. [39] reported that the exposure to 10 p.p.m. FA for a period of 4 h increased goblet cell volume, cell sloughing, and damaged cilia in the rat nasal cavity using scanning electron microscopy. These histopathologic findings suggest that FA not only irritates the upper respiratory tract but also damages the epithelium of upper airways. On the other hand, it is unclear whether FA is a lower respiratory tract irritant. The above-mentioned authors reported that FA exposure did not cause inflammation of the lower respiratory tract [11, 27, 39].

We do not know whether 1.0% FA (100 µL) administered transnasally three times a week for 6 weeks in our guinea-pig model is equal to the concentrations frequently encountered in the indoor environment in daily life. Moreover, the mechanism for enhanced sensitization to inhaled allergens due to FA exposure was not investigated in this study. Mucosal permeability might be increased in the upper airways, where inflammatory reactions have been demonstrated previously in guinea-pigs [40]. Swiecichowski et al. [27] reported that *in vivo* bronchial responsiveness following FA exposure increased with time after exposure and became maximal 2–6 h post exposure, and this effect was still evident 24 h after the exposure. Thus, it is likely that if methacholine responsiveness is measured in sensitized guinea-pigs within 24 h after the last exposure to FA, the FA exposure could enhance the bronchial responsiveness. However, bronchial responsiveness was not altered 48 h after the last exposure of FA in non-sensitized guinea-pigs in the present study, indicating no direct influence of FA on bronchial responsiveness 48 h after the exposure. Thus, our results clearly indicate that transnasal administration of FA enhances antigen-induced bronchoconstriction in sensitized guinea-pigs, with-

out change of bronchial responsiveness. These evidences suggest that nasal FA enhanced sensitizing process to antigen. It is unlikely that sensitization augments FA-induced bronchial hyperresponsiveness or FA-induced bronchoconstriction 48 h after the exposure. Thus, we hypothesize that environmental FA may be a factor for increasing rating of allergic diseases.

In conclusion, our results indicate that repeated transnasal administration of FA enhances allergic bronchoconstriction and potentiates anti-OA homocytotropic antibody (IgG) production, but does not alter non-specific bronchial responsiveness or the release of chemical mediators. These results suggest that FA is not an inducer of asthma but a factor of worsening asthma by enhancement of sensitization in this asthmatic animal study. We conclude that a long-term (6 weeks) exposure to FA can significantly potentiate allergic bronchoconstriction via enhancement of sensitization to antigens.

References

- 1 Sly RM. Changing prevalence of allergic rhinitis and asthma. *Ann Allergy Asthma Immunol* 1999; 82:233–52.
- 2 Salvaggio JE. Over view of occupational immunologic lung disease. *J Allergy Clin Immunol* 1982; 70:5–10.
- 3 Hendrick DJ, Lane DJ. Occupational formalin asthma. *Br J Ind Med* 1977; 34:11–8.
- 4 Godish T. Formaldehyde and building related illness. *J Environ health* 1981; 44:116–21.
- 5 Samet JM, Marbury MC, Spengler JD. Health effects and sources of indoor air pollution. Part II. *Am Rev Respir Dis* 1988; 137:221–42.
- 6 Sauder LR, Chatham MD, Green DJ, Kulle TJ. Acute pulmonary response to formaldehyde exposure in healthy nonsmokers. *J Occup Med* 1986; 28:420–4.
- 7 Kilburn KH, Warshaw R, Thornton JC. Pulmonary function in histology technicians compared with women from Michigan: effects of chronic low dose formaldehyde on a national sample of women. *Br J Ind Med* 1989; 46:468–72.
- 8 Levine RJ, DalCorso RD, Blunden PB, Battigelli MC. The effects of occupational exposure on the respiratory health of West Virginia mortician. *J Occup Med* 1984; 26:91–8.
- 9 Malaka T, Kodama AM. Respiratory health of plywood workers occupationally exposed formaldehyde. *Arch Environ Health* 1990; 45:288–94.
- 10 Tarkowski M, Gorski P. Increased IgE antiovalbumin level in mice expected to formaldehyde. *Int Arch Allergy Immunol* 1995; 106:422–4.
- 11 Riedel F, Hasenauer E, Barth PJ, Kozirowski A, Riegar CHL. Formaldehyde exposure enhances inhalative allergic sensitization in the guinea-pig. *Allergy* 1996; 51:94–9.
- 12 Konzett H, Rössler R. Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. *Arch Exp Path Pharmacol* 1940; 195:71–4.
- 13 Jones T, Masson P, Hamel R et al. Biological activity of leukotriene sulfones of respiratory tissues. *Prostaglandins* 1982; 24:279–89.
- 14 Fujimura M. Inhibitory effects of steroids on slow reacting substance of anaphylaxis (SRS-A) mediated bronchoconstriction in the guinea pig *in vivo*. *Jpn J Allergol* 1983; 32:365–75.
- 15 Songur N, Fujimura M, Mizuhashi K, Saito M, Xiu QY, Matsuda T. Involvement of thromboxane A2 in propranolol-induced

- bronchoconstriction after allergic bronchoconstriction in guinea pigs. *Am J Resp Crit Care* 1994; 149:1488–93.
- 16 Minami S, Okafuji K, Saga T et al. A new quantitative inhalation apparatus for small animals. *Jpn J Chest Dis* 1982; 21:252–8.
 - 17 Kurashima K, Fujimura M, Tsujiura M, Matsuda T. Effect of surfactant inhalation on allergic bronchoconstriction in guinea pigs. *Clin Exp Allergy* 1997; 27:337–42.
 - 18 Santives T, Roska AK, Hensley GT, Moore VL, Fink JN, Abromoff P. Immunologically induced lung disease in guinea pigs. *J Allergy Clin Immunol* 1976; 57:582–94.
 - 19 Yuji Tohda. An experimental study of bronchial asthma: a dual phase response model. *Med J Kinki Univ* 1989; 14:133–49.
 - 20 Watanabe N, Ovary Z. Antigen and antibody detection by *in vivo* methods: a reevaluation of passive cutaneous anaphylactic reactions. *J Immunol Meth* 1997; 14:381–90.
 - 21 Salvaggio JE. Overview of occupational immunologic lung disease. *J Allergy Clin Immunol* 1982; 70:5–10.
 - 22 Gough M, Hart R, Karrh BW et al. Report on the consensus workshop on formaldehyde. *Environ Health Perspect* 1984; 58:323–81.
 - 23 Schachter EN, Witek TJ, Tosun T, Leaderer BP, Beck GJ. A study of respiratory effects from exposure to 2 p.p.m. formaldehyde in healthy subjects. *Arch Environ Health* 1986; 41:229–39.
 - 24 Witek TJ, Schachter EN, Tosun T, Beck GJ, Leaderer BP. An evaluation of respiratory effects following exposure to 2.0 ppm formaldehyde in asthmatics: Lung function, symptoms, and airway reactivity. *Arch Environ Health* 1987; 42:230–7.
 - 25 Green DJ, Sauder LR, Kulle TJ, Bascom R. Acute response to 3.0 ppm formaldehyde in exercising healthy nonsmokers and asthmatics. *Am Rev Respir Dis* 1987; 135:1261–6.
 - 26 Harving H, Korsgaard J, Dahl R. Low concentrations of formaldehyde in bronchial asthma: a study of exposure under controlled conditions. *Br Med J* 1986; 293:310.
 - 27 Swiecichowski AL, Long KL, Miller ML, Leikauf GD. Formaldehyde-induced airway hyperreactivity *in vivo* and *ex vivo* guinea pigs. *Environ Res* 1993; 61:185–99.
 - 28 Dykewicz MS, Patterson R, Cugell DW, Harris KE, Wu AF. Serum IgE and IgG to formaldehyde-human serum albumin: lack relation to gaseous formaldehyde exposure and symptoms. *J Allergy Clin Immunol* 1991; 87:48–57.
 - 29 Grammer LC, Harris KE, Cugell DW, Patterson R. Evaluation of a worker with possible formaldehyde-induced asthma. *J Allergy Clin Immunol* 1993; 92:29–33.
 - 30 Maurice F, Rivory JP, Larsson PH, Johansson SG, Bousquet J. Anaphylactic shock caused by formaldehyde in a patient undergoing long-term hemodialysis. *J Allergy Clin Immunol* 1986; 77:594–7.
 - 31 Kramp JA, Peltenburg LTC, Kerklaan PRM, Spieksma FTM, Valentijn RM, Dijkman JH. Measurement of specific antibodies in individuals exposed to formaldehyde. *Clin Exp Allergy* 1989; 19:509–14.
 - 32 Matsumura Y. The effects of ozone, nitrogen dioxide, and sulfur dioxide on the experimental induced allergic respiratory disorder in guinea pigs. 1. The effects on sensitization with albumin through the airway. *Am Rev Respir Dis* 1970; 102:430–7.
 - 33 Colizzo F, Krantz MJ, Fish JE, Hastie AT. Ciliated respiratory epithelial surface changes after formaldehyde exposure. *J Toxicol Environ Health* 1992; 35:221–34.
 - 34 Krzyzanowski M, Quackenboss JJ, Lebowitz M. Chronic respiratory effects of indoor formaldehyde exposure. *Environ Res* 1990; 52:117–25.
 - 35 Kuper CF, Koornstra PJ, Hameleers DMH et al. The role of nasopharyngeal lymphoid tissue. *Immunol Today* 1992; 13:219–24.
 - 36 Mannix RC, Phalen RF, Walters RB, Kurosaki TT. Effects of sulfur dioxide and formaldehyde on particle clearance in the rat. *J Toxicol Environ Health* 1983; 12:429–40.
 - 37 Casanova-Schmitz M, Starr TB, d'A Heck H. Differentiation between metabolic incorporation and covalent binding in the labeling of macromolecules in the rat nasal mucosa and bone marrow by inhaled [¹⁴C] and [³H] formaldehyde. *Toxicol Appl Pharmacol* 1992; 76:26–44.
 - 38 Swenberg JA, Gross EA, Randall HW, Barrow CS. The effect of formaldehyde exposure on cytotoxicity and cell proliferation. In: Clary JJ, Gibson JE, Waritz RS, eds, *Formaldehyde Toxicology, Epidemiology and Mechanisms*, 1st Edn. New York: Dekker, 1983, pp 225–36.
 - 39 Bhalla DK, Mahavni V, Nguyen T, McClure T. Effects of acute exposure of formaldehyde on surface morphology of nasal epithelia in rats. *J Toxicol Environ Health* 1991; 33:171–88.
 - 40 Pazdrak K, Gorski P, Krakowiak A, Ruta U. Changes in nasal lavage fluid due to formaldehyde inhalation. *Int Arch Occup Environ Health* 1993; 64:419–515.