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DECREASE IN OVALBUMIN-INDUCED PULMONARY ALLERGIC RESPONSE BY BENZALDEHYDE BUT NOT ACETALDEHYDE EXPOSURE IN A GUINEA PIG MODEL.

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LUNG ALLERGIC RESPONSE TO ALDEHYDES

ABSTRACT

The pulmonary effects of two environmentally relevant aldehydes were investigated in non-sensitized or ovalbumin (OA)-sensitized guinea pigs (GPs). Four week-old male Hartley GPs, weighing about 400 g, were intraperitoneally injected with 1 ml of a NaCl solution containing 100 µg OA and 100 mg $Al(OH)_3$. They were then exposed either to acetaldehyde (200 ppb) or benzaldehyde (500 ppb) for 4 weeks (6 hours/day, 5 days/week). At the end of exposure, GPs were challenged with an OA aerosol (0.1% in NaCl) and pulmonary functions were measured. The day after, guinea pigs were anesthetized and several endpoints related to inflammatory and allergic responses were assessed in blood, whole lung histology, and bronchoalveolar lavage (BAL). Sensitized non-exposed GPs showed bronchial hyperresponsiveness to OA, an increased number of eosinophils in blood and BAL, together with a rise in total protein and leukotrienes (LTB_4 and $LTC_4/D_4/E_4$) in BAL. In non-sensitized GPs, exposure to acetaldehyde or benzaldehyde did not induce any change in the tested parameters, with the exception of irritation of the respiratory tract as detected by histology and an increased number of alveolar macrophages in animals exposed to acetaldehyde. In sensitized GPs, exposure to acetaldehyde induced a moderate irritation of the respiratory tract but no change in biological parameters linked to the inflammatory and allergic responses. In contrast, exposure to benzaldehyde induced a decrease both in OA-induced bronchoconstriction and eosinophil and neutrophil number in BAL, an increase in the bronchodilatator mediator PGE_2 and a decrease in the bronchoconstrictor mediators $LTC_4/D_4/E_4$. Further investigations are needed to determine if the attenuated response observed in sensitized GPs exposed to benzaldehyde is due to an alteration of the mechanism of sensitization or to a more direct effect on various mechanisms of the allergic response.

KEY WORDS

Acetaldehyde. Benzaldehyde. Inhalation toxicology. Guinea pigs. Ovalbumin-sensitization. Respiratory functions. Allergy. Inflammation.

INTRODUCTION

Carbonyl compounds, particularly aldehydes, are receiving increasing attention as air pollutants because of their potential as irritants and carcinogens (Grafstrom, 1990; Leikauf, 1992; Morris, 1997). Aldehydes are fairly common primary compounds, which derive from stationary sources (incineration and home wood fire) or mobil sources (diesel, internal combustion and jet engine emissions), but they are more frequently secondary pollutants in so far as they are almost obligatory intermediates in the photo-oxidation of organics compounds in the atmosphere (Carlier et al., 1986).

Aldehydes are present both outdoors and indoors with concentrations inside often higher than outside (Reiss et al., 1995). Approximately 50 percent of the total aldehyde burden is formaldehyde, which can range from 10 to 30 ppb in heavily contaminated air masses (Leikauf, 1992). Estimates for other aldehydes indicate that another 5 to 10 percent of the total aldehyde burden is acrolein, which ranges from 5 to 30 ppb during peaks (Leikauf, 1992). The remaining 40 to 45 percent consists of other aldehydes such as acetaldehyde (Leikauf, 1992; Reiss et al., 1995) and, to a lesser extent, propanal, butanal and benzaldehyde (Reiss et al., 1995).

The toxicity of inhaled formaldehyde (Bardana & Montanaro, 1991; IARC, 1995b; McLaughlin, 1994) and acrolein (Ghilarducci & Tjeerdema, 1995; IARC, 1995a; Kehrer & Biswal, 2000) is well documented, but less data are available on inhaled acetaldehyde and practically no studies on other aldehydes such as benzaldehyde. In humans, acute exposure to acetaldehyde vapors resulted in irritation of the eyes and mucous membranes, reddening of the skin, pulmonary edema and sore throat (IARC, 1999). Repeated exposure causes dermatitis and conjunctivitis (IARC, 1999). Acute, subchronic and chronic exposure to acetaldehyde produces respiratory tract injury in the rat, especially in the nasal cavities (Morris, 1997). Inhaled benzaldehyde also causes damage to the respiratory epithelium lining the nasal septum in rats and impairment of the central nervous system (Laham et al., 1991). All of these studies, however, have been done with quite high levels of aldehydes (above 100 ppm) which are not representative of ambient concentrations.

In parallel, epidemiological studies indicate an increase in the number of asthma cases in urban population, especially children (Beasley et al., 2000). Atmospheric pollutants are thought to be at least responsible for damage to the respiratory function in humans and are believed to increase susceptibility to airborne allergens (Higgins et al., 2000).

Our hypothesis is that acetaldehyde or benzaldehyde exposure induces increased pulmonary allergic responses in laboratory animals. To test it, the effects of exposure to low levels of acetaldehyde (200 ppb) or benzaldehyde (500 ppb) were assessed on the respiratory functions of guinea pigs using a model of ovalbumin-sensitized animals (Lebrec et al., 1996; Ormstad et al., 2000). Concentrations were chosen in order to get close to environmental levels. The concentrations used are about ten times higher than recorded peak levels in heavily polluted areas. Lower levels would have led to technical problems, in particular in terms of exposure concentration variability. These compounds were administered in such a way as to simulate human chronic exposures to low concentrations.

METHODS

Animal model

Four-week male Hartley guinea pigs (GPs) were obtained from Charles River France. They were individually identified with an ear tattoo. Clinical observations were performed at reception and the animals (weighing 414.4 $\pm$ 24.6 g) were randomly assigned to six groups with a method that provided approximately equal initial average body weight for each group.

Pulmonary effects were investigated in ovalbumin (OA)-sensitized or non-sensitized (control) GPs. Half of the animals used in this study were intraperitoneally injected on the first day and 24h later with 0.5 ml of a NaCl solution containing 100 μg OA and 100 mg $Al(OH)_3$. Control GPs were injected only with NaCl solution. Sensitization was checked after 3 weeks by intradermal reaction to 25 μl of a solution containing 200 µg OA/ml. Reaction results were observed 24 h and 48 h later. Animal provocation was performed 4 weeks after the first injection by aerosolized OA inhalation (0.1% in saline for 10 min). Non sensitized GPs were not challenged with OA except half of the control group, in order to check if aerosolized OA had an effect by itself.

Generation of aldehyde-containing air

Acetaldehyde (Rectapur, 98% in purity) and benzaldehyde (99% in purity) were supplied by Merck Eurolab. Acetaldehyde bottles were stored at 4°C. Benzaldehyde was stored in a ventilated room at ambient temperature.

Acetaldehyde atmospheres were generated by injection of acetaldehyde vapor in pure air with a gas syringe (SGE) and a perfusion pump (A99, Razel). To control acetaldehyde concentration in the exposure chambers, acetaldehyde vapor was injected with the proper flow in the main airflow passing through the exposure chamber.

Benzaldehyde vapor was generated by bubbling air in liquid benzaldehyde. The vapor obtained was then mixed with pure air circulating in the chambers. Bubbling airflow rate, height of the liquid benzaldehyde column and main airflow rate through the chambers determined the concentration in the atmosphere.

Exposure conditions

Groups of 8 animals (control or OA-sensitized) were exposed by whole body inhalation in 140 liters Plexiglas® chambers, either to acetaldehyde (200 ppb, 0.36 mg/m^3) or benzaldehyde (500 ppb, 2.2 mg/m^3). The total airflow through the chambers was 3000 L/h for benzaldehyde and 1000 to 2500 L/h for acetaldehyde. The exposure schedule to each aldehyde was six hours/day, five days/week, for four weeks.

Between exposures, four animals were housed per cage in suspended Plexiglas® cages fitted with dust-free bedding. Animals were not fed during exposure but had access to food and water *ad libitum* between each exposure period.

Analytical chemistry

Inhalation chambers atmosphere samples were collected, twice a day, every day (90 min and 270 min after the beginning of exposure), on dinitrophenylhydrazine silicate adsorbent tubes (Sep pack). After desorption with acetonitrile, analyses were performed by HPLC chromatography with UV detection (model 9050, Varian) under the following characteristics: Kromasil column (C18 3.5µ 150 mm), elution solutions: acetonitrile and water, flow: 0.6 l/min, detection: 365 nm.

Experimental design

Six groups of eight guinea pigs were used:

Group 1: air-exposed control

Group 2: air-exposed ovalbumin-sensitized

Group 3: benzaldehyde-exposed

Group 4: benzaldehyde-exposed ovalbumin-sensitized

Group 5: acetaldehyde-exposed

Group 6: acetaldehyde-exposed ovalbumin-sensitized

Animals were acclimated for at least 7 days before the experiment started. At day 0 and day 1, intraperitoneal injections of OA (groups 2, 4 and 6) or NaCl (groups 1, 3, 5) were performed. At day 21, sensitization was determined (intradermal reaction). Aldehyde exposure began the same day as animal sensitization and lasted from day 0 to day 26. Assessment of respiratory function parameters took place both before and after OA provocation, three days after the end of exposure (day 29). The day after (day 30), animals were sacrificed by intraperitoneal injection of pentobarbital sodium in order to evaluate the inflammatory and atopic responses.

Respiratory function parameters

The parameters studied were the breathing rate or respiratory frequency (RF), the tidal volume (TV) which is the volume of gas inhaled per breathing cycle, the minute volume (MV=TV×RF), the inspiratory (PIF) and expiratory (PEF) peak flows, the relaxation time (RT) and the enhanced pause (*Penh*) which represents the resistance of airways and the state of bronchoconstriction (Hamelmann et al., 1997). They were measured by means of whole body plethysmographs connected to a data acquisition software package (BUXCO Electronics Inc., Sharon, CT, USA). Baseline measurements were made during 15 min individually on each animal. They were interrupted for 10 min during which the animals were stimulated with the allergen. Measurements were recorded continuously after that time for one hour.

Hematology and serum chemistry

The day after the assessment of respiratory function parameters, animals were anesthetized with intraperitoneal injection of 6% pentobarbital sodium (0.2 ml/100g). Blood samples were taken from the *vena cava*. The following hematological parameters were determined with a hematology automated instrument (Technicon H1, Bayer): red blood cell (RBC) and white blood cell (WBC) counts, hemoglobin (Hb), hematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin

(MCH), mean corpuscular hemoglobin concentration (MCHC), platelets and leukocyte differential counts.

The following biochemical parameters were determined in plasma, using an automated instrument (Cobas Fara II, Roche): alanine aminotransferase (ALT), albumin (Alb), aspartate aminotransferase (AST), calcium (Ca), cholesterol (Chol), gamma-glutamyl transferase (γ-GT), glucose (Gluc), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), inorganic phosphorus (Phos), total proteins (Prot) and triglycerides (Trig).

Histopathology

At necropsy, a gross pathological examination was conducted and the observations recorded. Trachea, lungs and tracheo-bronchial lymph nods were collected from two animals per group. Nasal tissue was also collected from all animals. Tissues were fixed in 10% formaldehyde, processed and embedded in paraffin. They were sectioned at 5 µm, stained with hematoxylin-eosin-saffron and examined by light microscopy. Nasal tissues were first decalcified for 24 h (Decalcifier II solution, Surgipath) before processing and embedding.

Cellular and biochemical pulmonary parameters

From six animals per groups, lungs were lavaged *in situ* with 3 x 20 ml of a 0.9 % NaCl solution. The collected cells were isolated by centrifugation (350 g, 5 min), counted with an hematology automated analyzer (Technicon H1, Bayer) and cellularity determined microscopically. The remaining cells were resuspended in RPMI medium (Gibco) and seeded onto 6-well plates at a concentration of 3 x 10^6 cells/well. After 3 hours of attachment, the medium was changed and 2 ml of serum free RPMI medium, supplemented with 0.2% bovine serum albumin, was added. After 18 h in a 5% CO_2 humid atmosphere at 37°C, the conditioned culture media were collected and stored frozen at -80°C until used. The remaining bronchoalveolar lavage fluid (BALF) was concentrated about 10-fold using a centrifugal filter device (Ultrafree 15, Millipore) and kept frozen at -80°C for assessment of protein and eicosanoid concentrations. Total protein content in BALF was assessed by the method of Bradford (1976). Prostaglandin E_2, LTB_4 and $LTC_4/D_4/E_4$ were measured in BALF and conditioned culture media using immunoenzymatic systems (PGE_2 EIA system, LTB_4 EIA system, $LTC_4/D_4/E_4$ EIA system, Amersham Pharmacia Biotech).

Statistical analyses

Data from respiratory function parameters were analyzed using a non-parametric test (Kruskal-Wallis ANOVA, SPSS). For cellular and biochemical data, interaction between sensitization and aldehyde exposure was assessed using a two-way analysis of variance (ANOVA). These data were further analyzed using a one-way ANOVA and the Scheffe test (SPSS) to compare:

1) control to sensitized GPs responses for a given treatment (air, acetaldehyde or benzaldehyde),

2) the effect of aldehydes in control versus sensitized GPs.

Significance was considered achieved for $p<0.05$.

RESULTS

Animal model characteristics

Sensitized guinea-pigs presented the following characteristics, related to inflammatory and allergic responses (Table 1):

- Increased *Penh* reflecting enhanced bronchoconstriction.
- Changes in pulmonary histology reflecting an irritation of the respiratory tract.
- Increased number of eosinophils in blood.
- Increased number of inflammatory cells (macrophages, neutrophils and eosinophils) in BAL.
- Increased levels of proteins in BALF reflecting an alteration of vascular and epithelial permeability of the respiratory tract.
- Increased levels of the bronchoconstrictor lipid mediator $LTC_4/D_4/E_4$.

No allergic reaction was noted in non-sensitized GPs challenged with OA. Therefore, all results from control GPs have been pooled.

Exposure concentrations

The concentrations recorded in the chambers are reported in Table 2. The geometric mean was used to estimate the average exposure because atmospheric concentrations tend to be log-normally distributed (Nikas et al., 1991; Rappaport, 1991). In this case, the geometric mean gives a better estimate of the integrated exposure. For acetaldehyde, target values differed slightly from observed

values and a quite high variability was observed. However, we estimated that the animals received approximately the target dose during the 4-week interval.

Mortality, clinical signs and body weights

Exposures to benzaldehyde or acetaldehyde did not induce any mortality. However, two sensitized air-exposed GPs died during the OA provocation. These animals developed acute pulmonary edema due to bronchoconstriction and inflammatory response. No alteration of body weight gain or impairment of state of health was noted during exposures (data not shown).

Respiratory functions

In control GPs, ovalbumin (OA) provocation induced no modifications in respiratory functions. In contrast, sensitized air-exposed GPs showed a typical asthmatic response to OA challenge, *i.e.* bronchoconstriction (increased *Penh*), an increase of the breathing rate and clinical signs of dyspnea, cyanosis of the ears and ocular membranes. Other respiratory parameters evaluated were also modified by OA challenge in control sensitized GPs. A significant increase in MV, PEF, PIF, TV and a significant decrease in RT were observed (data not shown).

In non-sensitized GPs, no difference in bronchial reactivity (*i.e. Penh*) was observed between air-exposed and aldehyde-exposed animals (fig. 1A). Other respiratory parameters evaluated in non-sensitized animals were also not modified by aldehyde exposure.

For *Penh*, sensitized acetaldehyde-exposed GPs show a narrowed peak response to OA stimulation compared to air-exposed animals (fig. 1B). Sensitized benzaldehyde-exposed GPs show no marked response to OA stimulation, except for one animal, for which an intense bronchoconstriction was observed after 50 minutes (bringing the average to a very high value at that time). In benzaldehyde-exposed animals, a similar suppression of allergic response was observed for the other respiratory parameters (data not shown).

Histopathology

For benzaldehyde or acetaldehyde-exposed GPs, histological examination of nasal cavities revealed a slight irritation (metaplasia/hyperplasia) of the respiratory epithelium for non-sensitized GPs and a proliferative subacute allergic rhinitis for sensitized GPs. Rhinitis alterations were more important for benzaldehyde than for acetaldehyde in sensitized GPs.

For the two aldehydes, histological examination of the trachea and lungs showed a slight irritation of respiratory epithelium for non-sensitized GPs and a moderate one for sensitized GPs. An increase of OA-induced typical allergic histological alterations was also noted for aldehyde-exposed sensitized GPs.

Blood parameters

The only modification was a significant increase in eosinophils in air-exposed sensitized GPs compared to air-exposed non-sensitized GPs (Fig. 2A). The other cells (monocytes, lymphocytes, neutrophils and basophils) were not modified by OA-sensitization. Exposure to acetaldehyde or benzaldehyde produced no significant effect on the number of monocytes in non-sensitized GPs compared to the corresponding control (Fig. 2B).

In air-exposed animals, sensitization and challenge induced a significant increase in total proteins but not albumin. Sensitization also induced a significant increase in γ-GT. In non-sensitized and sensitized GPs, benzaldehyde or acetaldehyde exposure induced significant reduction of triglycerides. Acetaldehyde exposure also significantly decreased blood cholesterol in both sensitized and non-sensitized GPs (data not shown).

Bronchoalveolar lavage (BAL) cells

In non-sensitized GPs, acetaldehyde exposure (but not benzaldehyde exposure) induced a significant increase in the number of alveolar cells, reflecting a pulmonary inflammation (Fig. 3A).

OA-sensitization animals significantly increased the number of cells recovered by BAL (Fig. 3A). This difference between sensitized and non-sensitized GPs was not found in benzaldehyde-exposed GPs. In sensitized benzaldehyde-exposed GPs, the increased number of alveolar cells was not significantly different from healthy benzaldehyde-exposed GPs, which in turn was not different from control air-exposed GPs. In control air-exposed GPs, the predominant cells recovered from BAL were alveolar macrophages (AM) (Fig. 3). In air-exposed sensitized animals, an influx of alveolar macrophages (AM), eosinophils (PE) and neutrophils (PN) was observed (Fig. 3C and 3D).

Benzaldehyde exposure did not modify the BAL cytology in non-sensitized GPs. In sensitized animals, benzaldehyde exposure induced a significant decrease in the number of PN in BAL (Fig. 3).

Acetaldehyde exposure in non-sensitized GPs induced a significant increase in the number of AM in BAL, reflecting an inflammatory response (Fig. 3B). In sensitized animals, acetaldehyde exposure did not significantly modify the BAL cytology compared to sensitized air-exposed GPs (Fig. 3).

Bronchoalveolar lavage (BAL) proteins

In non-sensitized GPs, neither benzaldehyde nor acetaldehyde exposure modified total protein concentrations in BALF compared to air-exposed GPs (Fig. 4). OA sensitization significantly enhanced total protein concentration in BALF (Fig. 4). This protein increase was abolished in sensitized GPs exposed to benzaldehyde (*i.e.* not significantly different from non-sensitized air-exposed GPs). In contrast, the sensitization-induced increase in protein concentrations was still observable in acetaldehyde-exposed GPs (Fig. 4).

Eicosanoid levels

In non-sensitized GPs, neither benzaldehyde nor acetaldehyde exposure modified PGE_2, LTB_4 and $LTC_4/D_4/E_4$ concentrations in BALF compared to air-exposed GPs (Fig. 5).

OA sensitization increased $LTC_4/D_4/E_4$ concentrations in BALF. Benzaldehyde exposure of sensitized GPs reversed this pattern and decreased $LTC_4/D_4/E_4$ concentrations in BALF. Acetaldehyde exposure had no significant effect on the lipid mediator concentrations in BALF of sensitized GPs.

The concentration of PGE_2 in alveolar macrophage conditioned media was not significantly modified by either the sensitization or the aldehyde exposure. LTB_4 and $LTC_4/D_4/E_4$ were not detected in alveolar macrophage conditioned media.

DISCUSSION

The OA-induced allergic reaction was associated with a pulmonary inflammatory response, characterized by increased levels of the proinflammatory lipid mediator LTB_4, neutrophil influx and increased levels of proteins in BALF, reflecting an alteration of vascular and epithelial permeability of the respiratory tract. These findings, which have been previously described (Karol et al., 1989; Morley et al., 1995; Santing et al., 1994; Underwood et al., 1995), are relevant to some physiologic features of asthma (Bice et al., 2000). Animal sensitization and challenge also induced an increase in blood total proteins but not albumin, which was probably due to enhanced synthesis of proteins related to inflammation and allergy (complement, orosomucoid, immunoglobulins) (Lebrec et al., 1996; Ormstad

et al., 2000). The increase in blood γ-GT, which is a marker of hepatic damage, may be due to the use of the adjuvant $Al(OH)_3$, a known hepatotoxic compound (Klein et al., 1989; Stein et al., 1987).

Very few studies have explored the toxicity of inhaled benzaldehyde or acetaldehyde. Inhalation exposure of Sprague-Dawley rats to benzaldehyde concentrations ranging from 500 to 1000 ppm for 14 consecutive days produced a mild irritation of the mucosa and severe impairment of the central nervous system as evidenced by abnormal gait, tremors and a positive Straub sign (Laham et al., 1991). These changes also included hypothermia and a reduction of the breathing rate. Histopathologic examination of tissues from exposed rats showed a goblet cell metaplasia that was largely confined to the respiratory epithelium lining the nasal septum in male rats. Hematologic changes were also observed, mostly in female rats which appeared to be more susceptible than males to the toxic effects of benzaldehyde. Acute, subchronic and chronic inhalation exposure to acetaldehyde produces respiratory tract injury with the most severe damage being produced in the nasal cavity (Morris, 1997). The 4-h LC_{50} in the rat is 13,300 ppm (Appelman et al., 1982). Repeated exposures (6h/day, 5 days/week, 4 weeks) of rats to 400, 1 000, 2 200 or 5 000 ppm produced marked injury to the nose. Degeneration of olfactory nasal tissues was noted at all concentrations. Tracheal and laryngeal lesions were observed at the 2 200 and 5 000 ppm concentrations only, and mild injury to the lower respiratory tract was observed only at the highest exposure level (Appelman et al., 1982). Subchronic (13, 26 or 52 weeks) and chronic (28 months) exposure of rats produced injury to the nasal mucosa and larynx without apparent injury to the tracheobronchial tree or lungs (Woutersen et al., 1984; 1986). Chronic exposure of rats to 750 ppm results in nasal adenocarcinomas, whereas exposure to 1 500 ppm produces both adenocarcinomas and squamous cell carcinomas (Woutersen et al., 1984; 1986).

In our experimental conditions, which occurred at considerably lower levels than those used in the above studies, benzaldehyde or acetaldehyde exposure did not markedly modify blood parameters. Decreased hemoglobin and hematocrit have been noted in rats exposed to 1 000 ppm benzaldehyde during 14 days (Laham et al., 1991). At that dose, female rats had also a decrease in number of RBC whereas male showed a significant increase in number of WBC. Blood content was also modified by acetaldehyde or benzaldehyde at doses between 400 and 5 000 ppm during 2 to 4 weeks (Appelman et al., 1982; Laham et al., 1991). Blood biochemical parameters were not modified except for a decrease in triglycerides in aldehyde-exposed animals and a decrease in cholesterol in acetaldehyde-

exposed animals. A decrease in blood cholesterol (but not in triglycerides) has been noted in male rats exposed at 500 and 750 ppm benzaldehyde for 14 days (Laham et al., 1991). However, no biological significance could be attributed to these changes because the values reported fall within the normal range usually found in control rats of that age range.

In non-sensitized animals the two aldehydes caused respiratory tract irritation, which is known for acetaldehyde (Appelman et al., 1982) and suspected for benzaldehyde (Laham et al., 1991). This irritation was accompanied, in acetaldehyde-exposed animals, by pulmonary inflammation, characterized mainly by a macrophage influx in alveolar spaces. Such a response was not observed in benzaldehyde-exposed animals. Acetaldehyde-induced macrophagic response is similar to that obtained after exposure to ozone (Hotchkiss et al., 1989) or coal dust (Oberson et al., 1994). The macrophage increase, observed in our study, may be due to recruitment of blood monocytes as demonstrated for other pollutants such as ozone (Hotchkiss et al., 1989; Zhao et al., 1998). This hypothesis is supported by the fact that the number of blood monocytes decreased in acetaldehyde-exposed GPs. Histological results did not show allergic features, such as eosinophilic infiltration, in aldehyde-exposed non-sensitized GPs.

Numerous studies, including clinical observations, epidemiology and human or animal controlled exposures suggest a connection between air pollutants and an increase in the incidence of asthma and allergy (Devalia et al., 1998; Gilmour, 1995; Koenig, 1999; Sandström et al., 1998). However, a decrease in allergic reaction has been recently observed in OA-sensitized mice exposed during the aerosol challenge phase to 0.7 or 5 ppm NO_2 (Morris et al., 2001b) or a mixture of nasal irritants (3 ppm acrolein + 60 ppm acetaldehyde + 40 ppm acetic acid) (Morris et al., 2001a). Male C57/B6J mice were sensitized by weekly ip OVA injection for 3 weeks followed by daily 1-hour OVA aerosol challenge for 3 or 10 days. Mice were exposed to air or pollutants for 2 hours immediately after each daily OVA exposure. BAL eosinophil levels were significantly lower in OVA-pollutant compared to OVA-air animals. OVA-air exposed mice demonstrated a markedly enhanced airway resistance to aerosolized metacholine, an effect that was absent in the OVA-NO_2 exposed mice. Mechanisms of decreased allergic response in exposed animal are unknown. According to Morris *et al*., the decrease in OVA-induced allergic airway disease may be due to pharmacokinetics alterations (alteration in regional deposition pattern of OVA aerosol) or dynamic alterations (change in cytokine levels for example).

In conclusion, in our experimental conditions, both acetaldehyde and benzaldehyde show irritant properties at concentrations relevant to human exposures. In sensitized animals, exposure to acetaldehyde does not modify the inflammatory and allergic responses induced by sensitization. In contrast, exposure to benzaldehyde modifies several respiratory functional, cellular and biochemical parameters leading to a large decrease in the severity of asthmatic responses. In a mixture of pollutants, benzaldehyde might therefore be able to mitigate the effects of other compounds in the asthmatic response. Further investigations are needed to determine if the attenuated response observed in sensitized GPs exposed to benzaldehyde is due to an alteration of the mechanism of sensitization or to a more direct effect on various mechanisms of the allergic response.

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FIGURE CAPTIONS

FIGURE 1. Bronchoconstriction in air and aldehyde-exposed guinea pigs for non-sensitized (A) and sensitized (B) guinea pigs. Acetaldehyde exposure: 200 ppb for 4 weeks. Benzaldehyde exposure: 500 ppb for 4 weeks. ν: Air-exposed animals, λ: benzaldehyde-exposed animals, σ: acetaldehyde-exposed animals. Data are means $\pm$ SEM for n=8 animals.

FIGURE 2. Number of eosinophils (A) and monocytes (B) in blood of air and aldehyde-exposed guinea pigs. Acetaldehyde exposure: 200 ppb for 4 weeks. Benzaldehyde exposure: 500 ppb for 4 weeks. **a**: significantly different ($p<0.05$) from the corresponding non-sensitized GP group. Data are means $\pm$ SD for n=6 animals.

FIGURE 3. Number of total cells (A), alveolar macrophages (B), eosinophils (C) and neutrophils (D) in BAL of air and aldehyde-exposed guinea pigs. Acetaldehyde exposure: 200 ppb for 4 weeks. Benzaldehyde exposure: 500 ppb for 4 weeks. **a**: significantly different ($p<0.05$) from the corresponding non-sensitized GP group. **b**: significantly different ($p<0.05$) from the control GP group. **c**: significantly different ($p<0.05$) from the air sensitized GP group. Data are means $\pm$ SD for n=6 animals.

FIGURE 4. Total proteins in BALF of air and aldehyde-exposed guinea pigs**.** Acetaldehyde exposure: 200 ppb for 4 weeks. Benzaldehyde exposure: 500 ppb for 4 weeks. **a**: significantly different ($p<0.05$) from the corresponding non-sensitized GP group. **c**: significantly different ($p<0.05$) from the air sensitized GP group. Data are means $\pm$ SD for n=6 animals.

FIGURE 5. Eicosanoids in BALF of air and aldehyde-exposed guinea pigs. A: PGE_2, B: LTB_4, C: $LTC_4/D_4/E_4$. Acetaldehyde exposure: 200 ppb for 4 weeks. Benzaldehyde exposure: 500 ppb for 4 weeks. **a**: significantly different ($p<0.05$) from the corresponding non-sensitized GP group. **c**: significantly different ($p<0.05$) from the air sensitized GP group. Data are means $\pm$ SD for n=6 animals.

FIGURE 6. PGE_2 in alveolar macrophage conditioned media of air and aldehyde-exposed guinea pigs. Acetaldehyde exposure: 200 ppb for 4 weeks. Benzaldehyde exposure: 500 ppb for 4 weeks. Data are means $\pm$ SD for n=6 animals.

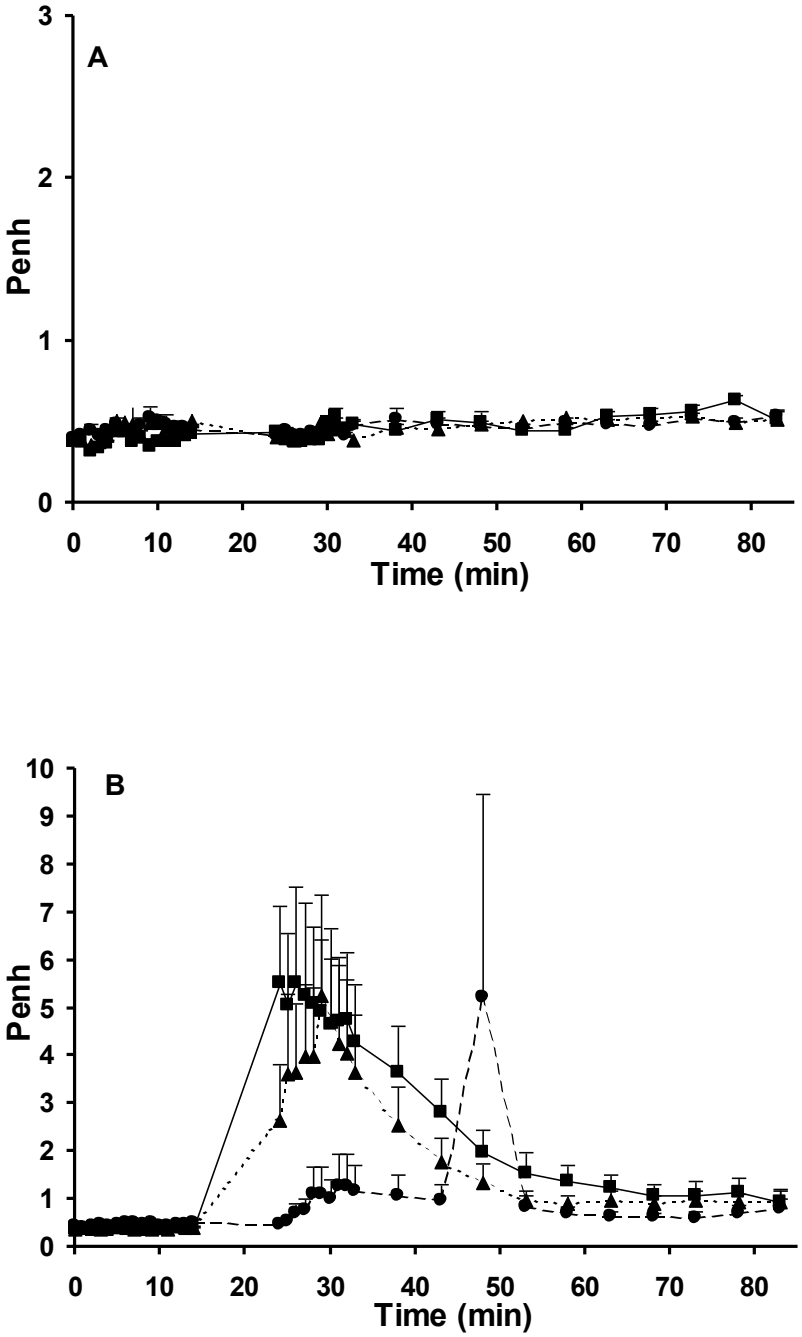
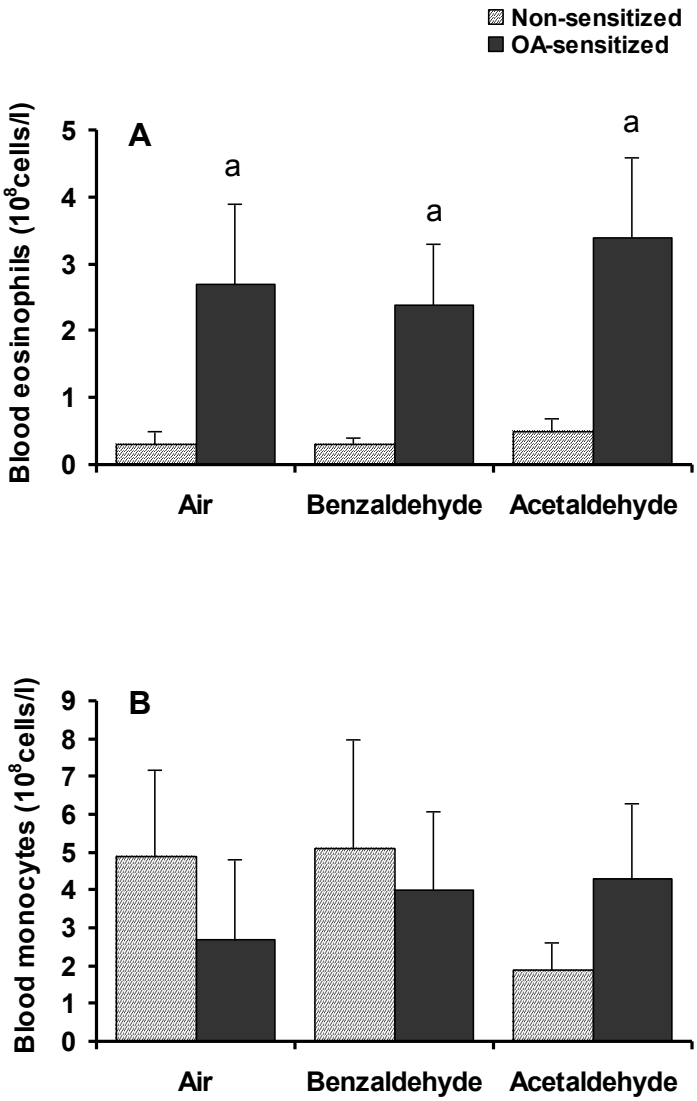


FIGURE 1. Bronchoconstriction in air and aldehyde-exposed guinea pigs for non-sensitized (A) and sensitized (B) guinea pigs. Acetaldehyde exposure: 200 ppb for 4 weeks. Benzaldehyde exposure: 500 ppb for 4 weeks. ν: air-exposed animals, λ: benzaldehyde-exposed animals, σ: acetaldehyde-exposed animals. Data are means $\pm$ SEM for n=8 animals.



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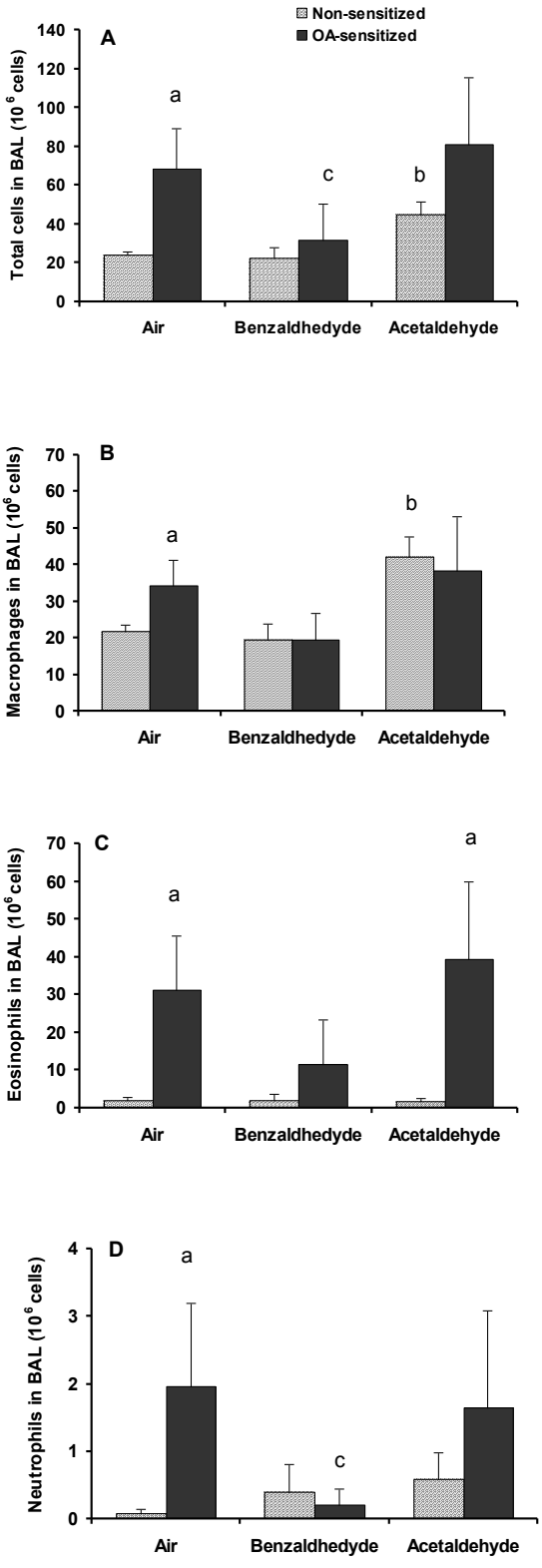
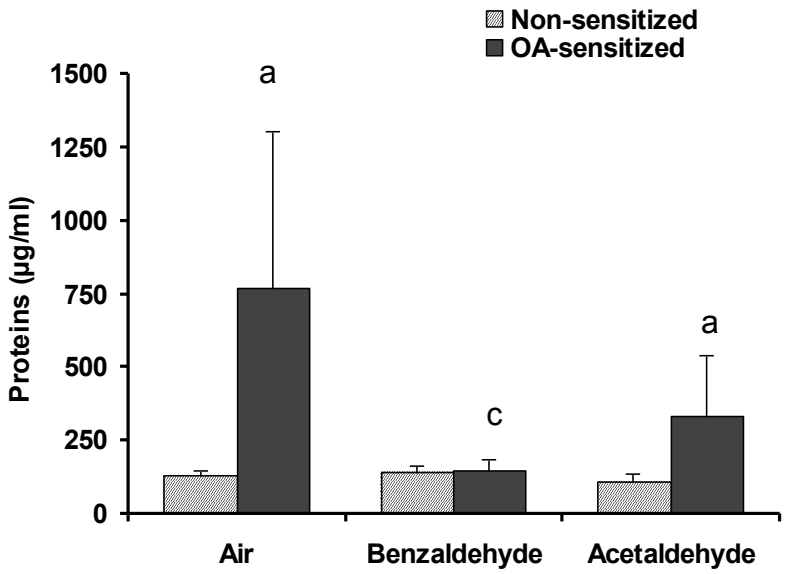


FIGURE 3. Number of total cells (A), alveolar macrophages (B), eosinophils (C) and neutrophils (D) in BAL of air and aldehyde-exposed guinea pigs. Acetaldehyde exposure: 200 ppb for 4 weeks. Benzaldehyde exposure: 500 ppb for 4 weeks. **a**: significantly different ($p<0.05$) from the corresponding non-sensitized GP group. **b**: significantly different ($p<0.05$) from the control GP group. **c**: significantly different ($p<0.05$) from the air sensitized GP group. Data are means ± SD for n=6 animals.



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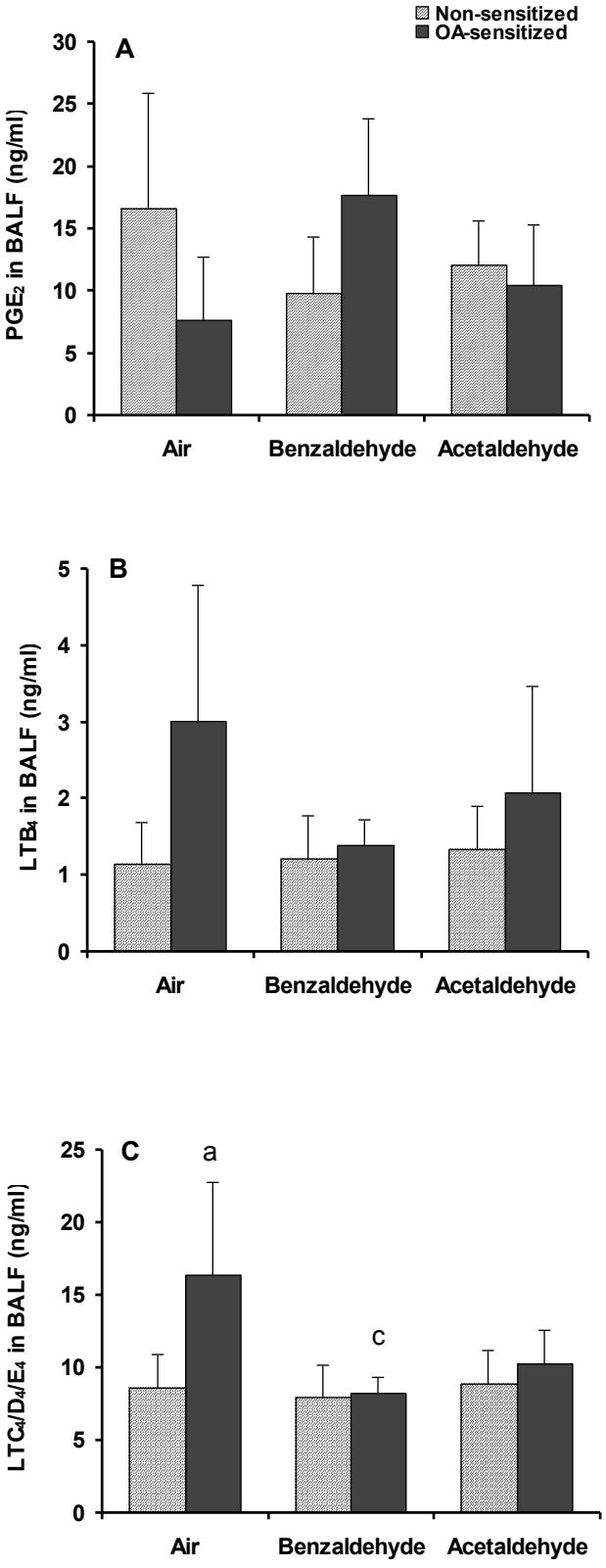


FIGURE 5. Eicosanoids in BALF of air and aldehyde-exposed guinea pigs. A: PGE_2, B: LTB_4, C: $LTC_4/D_4/E_4$. Acetaldehyde exposure: 200 ppb for 4 weeks. Benzaldehyde exposure: 500 ppb for 4 weeks. **a**: significantly different ($p<0.05$) from the corresponding non-sensitized GP group. **c**: significantly different ($p<0.05$) from the air sensitized GP group. Data are means $\pm$ SD for n=6 animals.

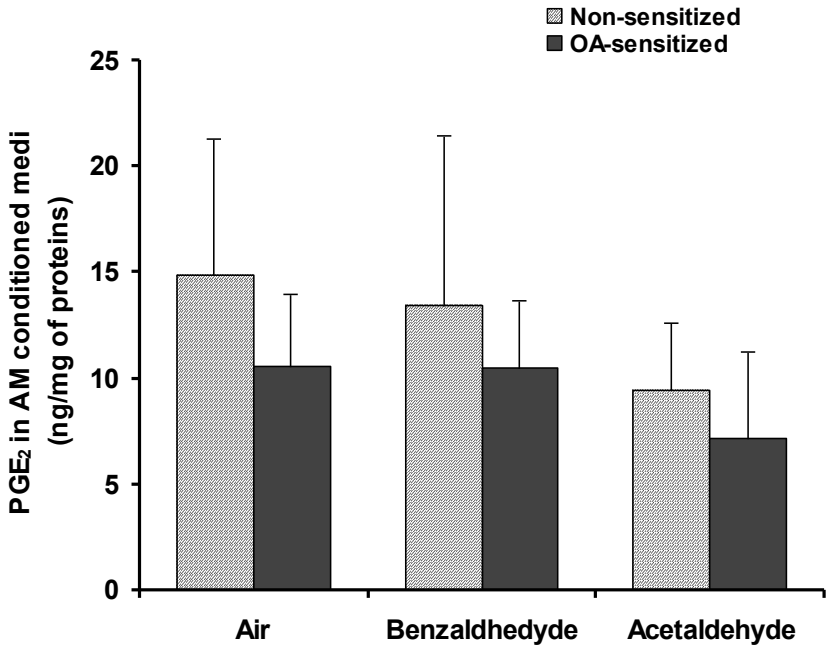


FIGURE 6. PGE_2 in alveolar macrophage conditioned media of air and aldehyde-exposed guinea pigs. Acetaldehyde exposure: 200 ppb for 4 weeks. Benzaldehyde exposure: 500 ppb for 4 weeks. Data are means $\pm$ SD for n=6 animals.

TABLE 1. Baseline characteristics of sensitized and non-sensitized animal groups

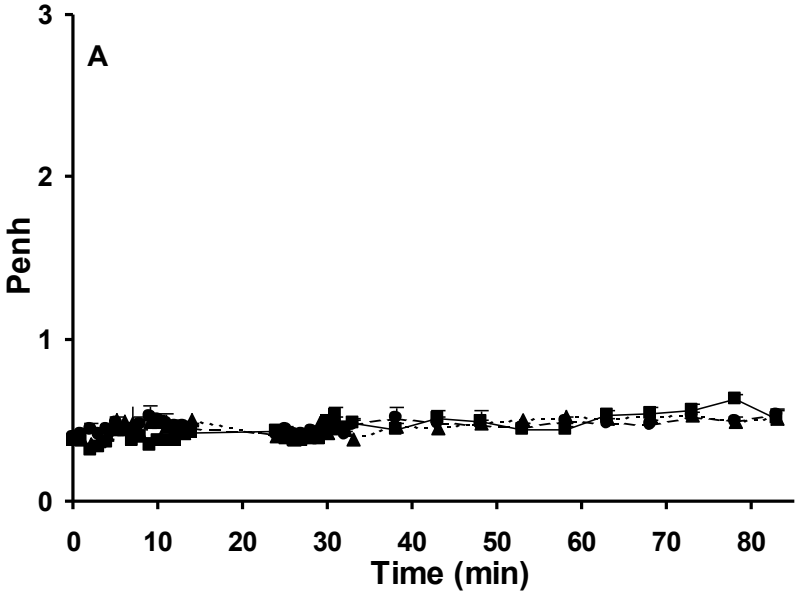
Parameters	Non-sensitized GPs	Sensitized GPs
Penh (bronchoconstriction)	0.5 [a]	* 5.5 [b]
Eosinophils in blood (10^6 / ml)	0.03 ± 0.02	* 0.27 ± 0.12
Histology		*Eosinophilic and lymphocytic chorionic infiltration*
Alveolar cells (x 10^6)	24.3 ± 1.6	* 68.2 ± 21.0
Macrophages	21.9 ± 1.6	* 34.3 ± 6.9
Eosinophils	2.0 ± 0.9	* 31.1 ± 14.4
Neutrophils	0.08 ± 0.06	* 2.0 ± 1.2
Proteins in BALF (µg/ml)	133.3 ± 14.1	* 767.8 ± 538.5
Eicosanoids in BALF (ng/ml)		
PGE_2	16.7 ± 9.3	7.7 ± 5.1
LTB_4	1.1 ± 0.6	3.0 ± 1.8
$LTC_4/D_4/E_4$	8.6 ± 2.4	* 16.4 ± 6.4

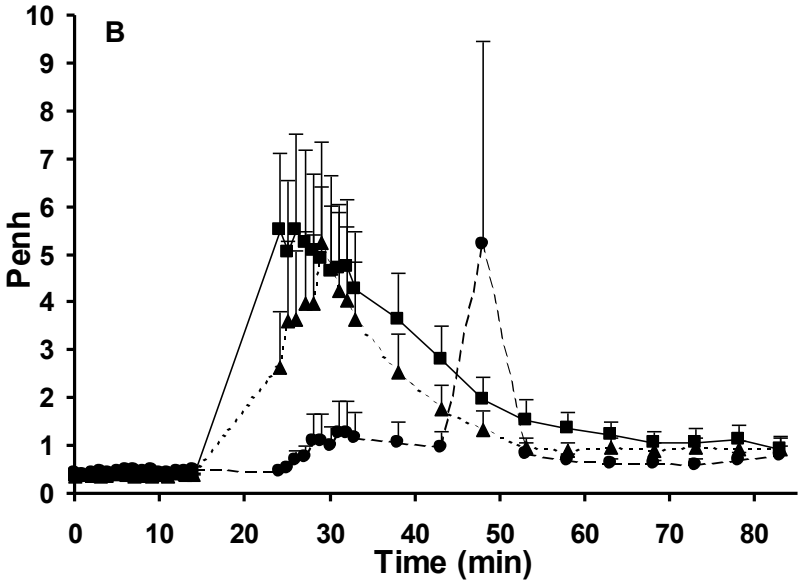
Penh: [a] mean of values recorded before and after OA challenge in air-exposed non-sensitized GPs, [b] mean of the maximal value noted after OA challenge in air-exposed sensitized GPs. Other parameters: data are means ± SD for n=6 animals. *: significantly different from control ($p<0.05$).

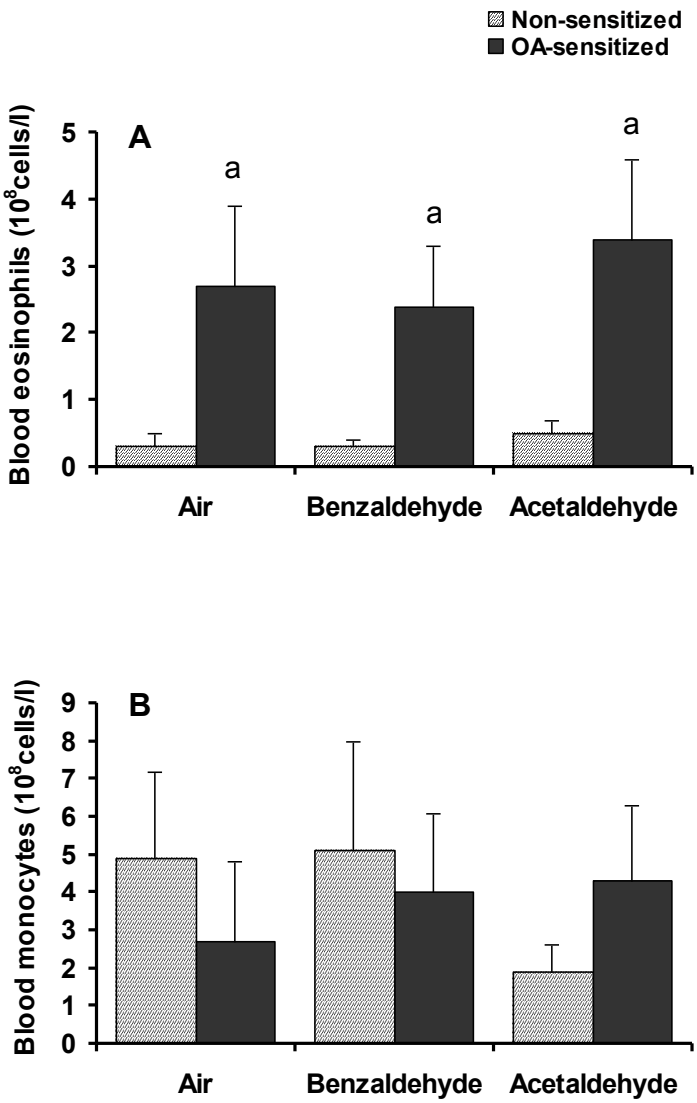
TABLE 2. Aldehyde exposure levels (ppm)

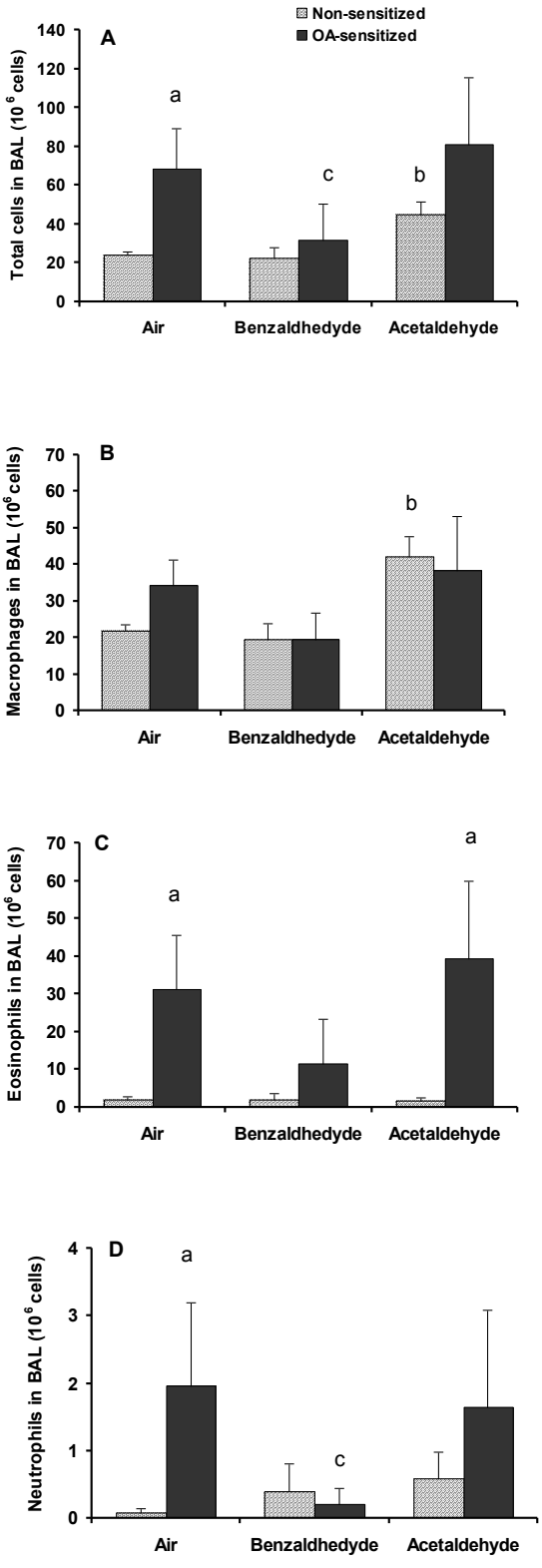
Exposure groups	Target concentrations (ppm)	Exposure concentrations (ppm)	
		Non-sensitized GPs	OA-sensitized GPs
Control (Air)	0	0[a]	0[a]
Benzaldehyde	500	532.1 ×÷ 0.2	497.7 ×÷ 0.2
Acetaldehyde	200	149.9 ×÷ 0.6	221.9 ×÷ 0.6

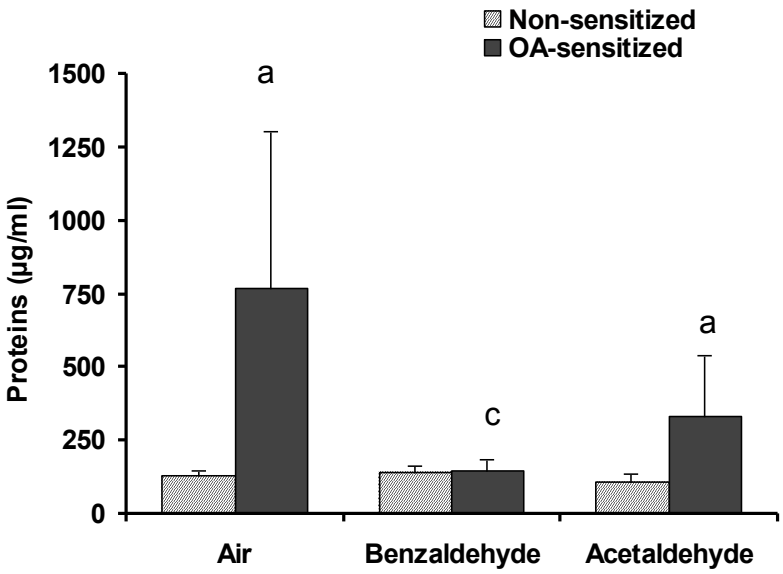
[a] Control values are assumed, not measured. Data are geometric means ×÷ geometric SEM (n = 40) for the duration of exposure (geometric SD or SEM correspond to multiplicative factors).

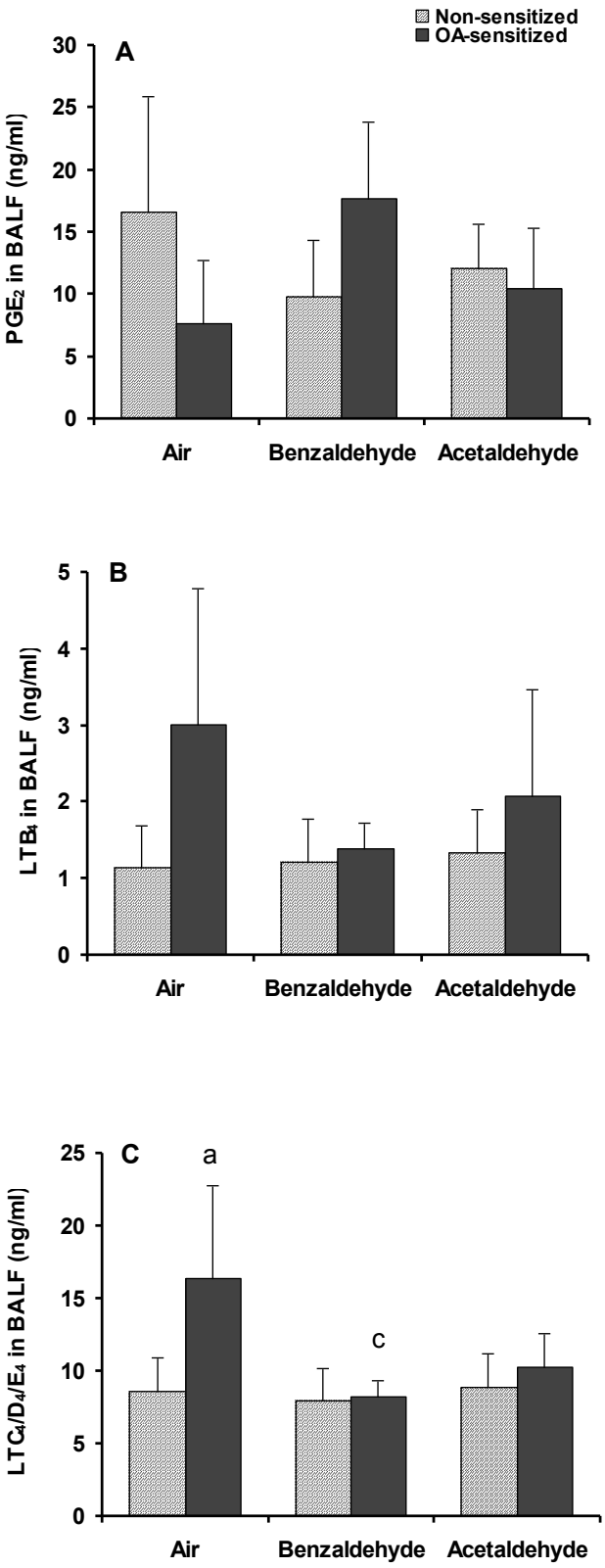

A
Penh
Time (min)
0
1
2
3
0
10
20
30
40
50
60
70
80

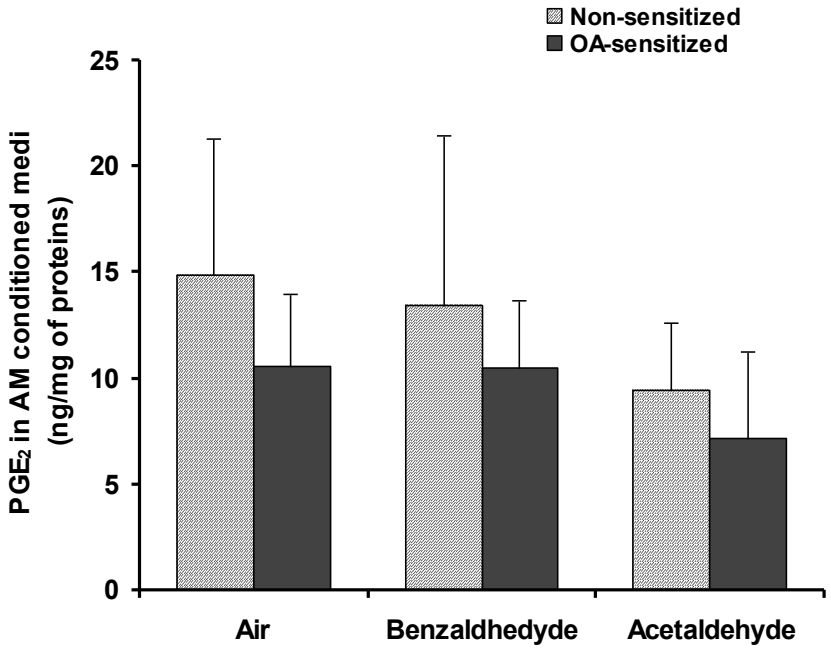

B
Penh
Time (min)
0
1
2
3
4
5
6
7
8
9
10
0
10
20
30
40
50
60
70
80


Non-sensitized
OA-sensitized
A
Blood eosinophils (10^8cells/l)
0
1
2
3
4
5
a
a
a
Air
Benzaldehyde
Acetaldehyde
B
Blood monocytes (10^8cells/l)
0
1
2
3
4
5
6
7
8
9
Air
Benzaldehyde
Acetaldehyde


Non-sensitized
OA-sensitized
A
Total cells in BAL (10 6 cells)
140
120
100
80
60
40
20
0
a
c
b
Air
Benzaldhedyde
Acetaldehyde
B
Macrophages in BAL (10^6 cells)
70
60
50
40
30
20
10
0
b
Air
Benzaldhedyde
Acetaldehyde
C
Eosinophils in BAL (10^6 cells)
70
60
50
40
30
20
10
0
a
a
Air
Benzaldhedyde
Acetaldehyde
D
Neutrophils in BAL (10^6 cells)
4
3
2
1
0
a
c
Air
Benzaldhedyde
Acetaldehyde


Non-sensitized
OA-sensitized
Proteins (µg/ml)
1500
1250
1000
750
500
250
0
a
c
a
Air
Benzaldehyde
Acetaldehyde


Non-sensitized
OA-sensitized
A
PGE2 in BALF (ng/ml)
30
25
20
15
10
5
0
Air
Benzaldehyde
Acetaldehyde
B
LTB4 in BALF (ng/ml)
5
4
3
2
1
0
Air
Benzaldehyde
Acetaldehyde
C
a
c
LTC4/D4/E4 in BALF (ng/ml)
25
20
15
10
5
0
Air
Benzaldehyde
Acetaldehyde


Non-sensitized
OA-sensitized
25
20
15
10
5
0
PGE2 in AM conditioned medi
(ng/mg of proteins)
Air
Benzaldhedyde
Acetaldehyde