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Acute airway effects of airborne formaldehyde in sensitized and non-sensitized mice housed in a dry or humid environment

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ABSTRACT

We investigated the role of air humidity and allergic sensitization on the acute airway response to inhaled formaldehyde (FA) vapor. Mice were sensitized to the immunogen ovalbumin (OVA) by three intraperitoneal injections followed by two aerosol challenges, giving rise to allergic airway inflammation. Control mice were sham sensitized by saline injections and challenged by saline aerosols. Once sensitized, the mice were housed at high (85–89%) or low (<10%) relative humidity, respectively for 48 h prior to a 60-min exposure to either 0.4, 1.8 or about 5 ppm FA. Before, during and after exposure, breathing parameters were monitored. These included the specific markers of nose and lung irritations as well as the expiratory flow rate, the latter being a marker of airflow limitation.

The sensory irritation response in the upper airways was not affected by allergic inflammation or changes in humidity. At high relative humidity, the OVA-sensitized mice had a decreased expiratory airflow rate compared to the saline control mice after exposure to approximately 5 ppm FA. This is in accordance with the observations that asthmatics are more sensitive than non-asthmatics to higher concentrations of airway irritants including FA. In the dry environment, the opposite trend was seen; here, the saline control mice had a significantly decreased expiratory airflow rate compared to OVA-sensitized mice when exposed to 1.8 and 4 ppm FA. We speculate that increased mucus production in the OVA-sensitized mice has increased the "scrubber effect" in the nose, consequently protecting the conducting and lower airways.

Introduction

High indoor air humidity and mold growth have been associated with upper and lower airway symptoms and with development and exacerbation of asthma (Mendell et al., 2011). However, little is known about the interplay between airway irritants and humidity. Dry eyes and dry airways are commonly reported symptoms in office environments and clinical studies indicate that low relative humidity (RH) has a strong influence by desiccation of eyes and possibly also the upper airways (Wolkoff and Kjærgaard, 2007). Two hypotheses have been suggested; the first proposes that exposure to low RH causes desiccation of eyes and mucous membranes, directly causing the irritation. The second proposes that exposure to low RH has no effects per se, but symptoms are caused by exacerbated sensitivity to sensory irritants due to a compromised mucus barrier. Thus, trigeminal stimulation by volatile compounds should be preceded by a destabilized eye tear film which increases penetration of irritants and causes eye symptoms (Wolkoff et al., 2012). Whatever the mechanism, it may be speculated that a similar mechanism would apply for the airways.

Mice showed increased trigeminal stimulation in the upper airways by 45 min exposure to ammonia under dry (0% RH) compared to humid conditions (95% RH). The difference in responsiveness was apparent from the concentration of ammonia necessary to reduce the respiratory rate by 50% (RD_{50}), which was 582 and 732 mg/m^3 in the dry and humid environments, respectively (Li and Pauluhn, 2010). Rats did not show a similar difference by exposure to dry versus humid ammonia; the RD_{50} values were 972 and 905 mg/m^3, respectively. Mice exposed to a reaction mixture of ozone and limonene showed statistically less sensory irritation at 32% RH than at 2% RH (Wilkins et al., 2003). A similar trend was observed by exposing male subjects to a similar reaction mixture under dry and humid conditions; the decrease in eye blink frequency, a proxy for trigeminal stimulation, was less pronounced at elevated RH, indicating an alleviating effect under humid conditions (Nøjgaard et al., 2005). Exposure of subjects to dry air showed detrimental effects on the upper airways by reduced saccharin clearance from the nose, especially among elderly (Sunwoo et al., 2006); the authors speculated that long-term dry air exposure deteriorates the function of cilia, important for clearance of the

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airways. Thus, exposure to dry air may condition the airways in becoming more susceptible to trigeminal stimulation by sensory irritants like formaldehyde (FA).

The exposure of asthmatic subjects to low (<1 ppm) FA at ambient RH did not show exacerbation of lung functions (Golden, 2011; Wolkoff and Nielsen, 2010). However, increased water loss by dry conditions has been shown to influence lung functions, including decreased forced expiratory volume in 1 s (FEV_1) (McFadden et al., 1999) indicating an increased airway resistance.

Our objective was to determine the respiratory tract effects of exposure to airborne particle-free FA in dry and humid atmospheres and to study the role of allergic sensitization on airway responsiveness to FA exposure using a mouse inhalation model. Effects of FA were studied at all three levels of the airways i.e. the upper, conducting and pulmonary levels. FA was considered to be an appropriate model compound since at low concentrations the effect is primarily at the eyes and upper airways (Nielsen et al., 1999; World Health Organisation, 2010), but at high concentrations sufficient FA levels can penetrate beyond the upper airways and affect the lungs (Nielsen et al., 1999). To our knowledge, the influence of humidity on FA exposure has not been investigated previously.

Method/materials

Chicken egg ovalbumin (CAS 9006-59-1) (OVA) was grade V (purity ≥ 98%) from Sigma-Aldrich, St. Louis, MO, USA. Aluminum hydroxide ($Al(OH)_3$) adjuvant was from Alhydrogel, Brenntag Biosector, Denmark. Formaldehyde was supplied by Kin-Tek (TX, USA) as a certified Trace Source™ permeation tube with paraformaldehyde.

Animals. Inbred BALB/cA male mice were purchased from Taconic, Denmark, and were housed in polypropylene cages (380 × 220 × 150 mm) with pinewood sawdust bedding (Lignocel S8, Brogaarden, Denmark). The photoperiod was from 6 a.m. to 6 p.m., and the temperature and relative humidity (RH) in the animal room were 22 ± 2 °C and 30–50%, respectively. The cages were sanitized twice weekly. Food (Altromin no. 1324, Altromin, Lage, Germany) and tap water were available ad libitum. Treatment of the animals adhered to procedures approved by the Animal Experiment Inspectorate, Denmark with permission numbers 2006/561-1123 and 2011/561-1990.

Sensitization. Mice (n = 30) were immunized to OVA by intraperitoneal (i.p.) injections of 1 μg OVA in combination with 270 μg $Al(OH)_3$ in 100 μL 0.9% saline on day 0. Mice were boosted i.p. on days 14 and 21 with 0.1 μg OVA in 100 μL 0.9% saline. Finally, the animals were exposed 20 min to an aerosol of 0.2% OVA on days 28 and 29 using a Pari Star nebulizer (PARI GmbH, Starnberg, Germany), which mainly delivers respirable particles as specified (Hansen et al., 2007b). For the non-sensitized control mice (n = 30), saline (0.9%) was used for the three i.p. injections and the aerosol exposures. Afterwards, on days 29 and 30, the sensitized and non-sensitized mice were housed at either low (<10) or high (85–89) % RH. On day 31, the mice were exposed to FA.

Generation and monitoring of formaldehyde concentration and ultrafine particles. Formaldehyde was generated from a Kin-Tek (TX, USA) gas standard generator (Model 491MB) by use of a permeation tube and dry air, and led to a 24 L exposure chamber (Larsen and Nielsen, 2012). The airflow rates in the chamber were set between 18.8 and 23.2 L/min. The chamber exposure concentrations of FA were monitored pre- and post-FA exposures and every 10 min during exposures by 10 min air sampling of 4.4 L on dinitrophenylhydrazine (DNPH) sampling cartridges (LpDNPH S10, Supelco, Bellefonte, PA). Humidification of the sampling air to 50% relative humidity by a sparger with clean water was placed in front the cartridge under the dry conditions. The cartridges were eluted within 1 h after sampling and analyzed immediately thereafter by HPLC using a diode array detector using a standard mix ((Supelco, Bellefonte, PA) Carbonyl-DNPH Mix 1) for six-point calibration ($r^2 > 0.999$). The FA concentrations are reported as the mean of five samples. The chamber background air was <0.1 and <0.2 ppm at dry and humid conditions, respectively. Target concentrations were 0.4, 1.8 and 7 ppm, respectively. The FA concentrations measured in the animal exposure chamber were similar under dry and humid environments both at the low (0.42 ± 0.01 ppm) and medium (1.8 ± 0.09 ppm) FA levels. At the highest FA level, the concentrations were 4.0 ppm and 5.7 ppm in the dry and humid environments, respectively.

For the low RH experiments, medicinal grade air (2% RH) was used upstream to dilute the FA, while air passed through a heated sparger (30 °C) containing clean water in experiments with elevated RH (85–89%) at 22.5–23.3 °C measured in the chamber. Humidity and temperature were measured with a calibrated Testo 650 humidity/temperature instrument (Testo GmbH & Co., D-79849 Lenzkirch).

Measurement of ultrafine particles in the center of the exposure chamber was carried out without animals at 4.8 ppm FA and 80% RH with a particle condensation counter from TSI (MN, USA) Model 3007 (particle size: 0.01 to >1.0 μm) for 160 min. The measurement showed that the background level of less than 40 particles/cm^3 remained constant after the FA gas generator was turned on (data not presented). This shows that the generator delivered gaseous FA.

Bioassay. The respiratory effects were studied in a mouse bioassay (Larsen et al., 2000). The bioassay allows the detection of respiratory effects on the upper airway (sensory) irritation as well as effects in the conducting airways and at the alveolar level (Alarie, 1998).

Respiratory parameters. Different types of effects from the respiratory system can be studied simultaneously via continuous computerized monitoring of the breathing pattern of unanesthetized mice (for details, see Boylestein et al., 1995, 1996; Vijayaraghavan et al., 1993, 1994). Brief descriptions of parameters of interest for the present study are given below. For a thorough description of all respiratory parameters, cf. Nielsen et al. (2005).

Sensory irritation pattern. In humans, chemicals stimulating the trigeminal nerve endings of the upper respiratory tract cause irritation that may increase to burning and painful sensations, termed 'sensory irritation' (Alarie, 1973). Sensory irritants decrease the respiratory rate (f, breaths per min) in mice due to a reflex causing a break at the end of the inspiratory phase. The increase in the time of break (TB, s) and the decrease in f, both show concentration–effect relationships and both can be used to quantify sensory irritation effects, although TB is the more specific of the two parameters (Alarie, 1998).

Airflow limitation. Airflow limitation, also known as bronchoconstriction, may be a result of constriction or inflammation of the conducting airways. Narrowing of the bronchi causes increased resistance that reduces the expiratory flow (VD, mL/s).

Pulmonary irritation. Pulmonary irritation is due to stimulation of vagal nerve endings at the alveolar level. Pulmonary irritation may be quantified by "time of a pause" (TP, s), which is characterized by a short break at the end of expiration. The duration of the TP increases with increasing exposure concentration and thus TP is the specific parameter to quantify this effect (Boylestein et al., 1995, 1996; Vijayaraghavan et al., 1993, 1994).

Exposure conditions and data acquisition. Five OVA- and five saline-sensitized mice, were simultaneously exposed head-only at each exposure concentration. Briefly, mice were inserted into body plethysmographs that were connected to the exposure chamber. The respiratory parameters were obtained for each mouse from a Fleisch