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Research Article

Short-term exposure to formaldehyde promotes oxidative damage and inflammation in the trachea and diaphragm muscle of adult rats

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ABSTRACT

Formaldehyde (FA) is an environmental pollutant widely used in industry. Exposure to FA causes irritation of the respiratory mucosa and is associated with inflammation and oxidative stress in the airways. This study aimed at investigating the oxidative effects on the inflammatory response in the trachea and the diaphragm muscle (DM) of rats exposed to different concentrations of formaldehyde. Twenty-eight Fischer male rats were divided into four groups: control group (CG) exposed to the ambient air; and three groups exposed to the following formaldehyde concentrations of 1% (FA1), 5% (FA5) and 10% (FA10), respectively. The exposure occurred for twenty minutes, three times a day for five days. Oxidative stress analyses were performed by carbonyl protein, lipid peroxidation and catalase activity. The assessment of inflammatory cell influx in both organs and the mucus production in the trachea was carried out. There was an increase of lipid peroxidation in the trachea and the DM of FA1 and FA5 groups compared to the CG and FA10. The oxidation of DM proteins increased in FA10 group compared to CG, FA1 and FA5. The catalase enzyme activity in the DM was reduced in FA1, FA5 and FA10 compared to the CG. Meanwhile, there was a reduction in the enzymatic activity of FA10 compared to the CG in the trachea. The morphometric analysis in the DM demonstrated an influx of inflammatory cells in FA10 compared to the CG. In FA10 group, the tracheal epithelium showed metaplasia and ulceration. In addition, the tracheal epithelium showed more mucus deposits in FA5 compared to CG, FA1 and FA10. The results demonstrated that the exposure to formaldehyde at different concentrations in a short period of time promotes oxidative damage and inflammation in the DM and the trachea and causes metaplasia, ulceration and increased mucus at the latter.

1. Introduction

Formaldehyde (FA) is a volatile substance at room temperature, is soluble in water, is reactive to human tissue (Medinsky and Bond, 2001) and has a common inhalation route by which individuals are exposed to it (Ezratty et al., 2007; Lambert et al., 2003). This product can be formed by a natural consequence of daily activities in most environments as a result of emissions from cars, burning cooking gas, smoke and tobacco (Li et al., 2007). Moreover, it can be used in an aqueous solution, as a disinfectant and preservative. More recently, its application has grown in various industrial areas, including the production of adhesives, wood covers, plastic, textiles, leather, the manufacture of chemical products (Bosetti et al., 2008), building materials (Li et al., 2007) and cosmetics (Flóyret et al., 2001; OSHA, 2011). As a result of this wide distribution presented by FA, the exposure of individuals to this environmental pollutant increases morbidity and mortality from diseases of the respiratory tract such as asthma, emphysema and bronchitis (Horvath et al., 1988). The consequences presented above could affect health professionals such as pharmacists, doctors, nurses, dentists and veterinarians who are exposed to the actions of FA as a result of their activities (Thrasher and Kilburn, 2001). The same can be considered for individuals using techniques in pathological and histological studies. In addition, students and professionals who handle preserved parts, such as those existing in anatomy labs, are considered risk groups (Wantke et al., 2000).

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The FA is a potent trigger of inflammation in the lower airways (Bardana and Montanaro, 1991) by activating inflammatory cells (Horvath et al., 1988; Lino-dos-Santos-Franco et al., 2010). After acute exposure to this chemical agent, local irritation and skin sensitization may occur (Li et al., 2007; Swenberg et al., 1980). Even at small concentrations, FA causes irritation to the eyes and the airways and mild neuropsychological disorders (Ezratty et al., 2007). There are records that reveal carcinogenic action at the site of contact by the epithelial cell proliferation as a result of cytotoxicity and mutation (Li et al., 2007).

In addition, the exposure to a chemical agent with the power to react, such as FA, may increase the production of reactive oxygen species (ROS) which are molecules, responsible for causing damage to the body and normally present in situations of toxicity (Matsuoka et al., 2010). This ROS production mechanism associated with the possibility of the antioxidant system being damaged by excessive exposure to FA is known as redox imbalance, and it may not only cause damage to the respiratory system but also promotes structural and enzymatic changes in other organs (Duong et al., 2011; Lino dos Santos Franco et al., 2006; Barbieri et al., 2010), including those directly associated with ventilation (Bansal et al., 2011).

As an example of structure affected by FA, we can cite the trachea, the main air route, an organ of cartilaginous structure which is lined internally by a pseudostratified columnar ciliated epithelium with goblet cells (Haykal et al., 2014). The mucus produced by the epithelium is a barrier to particles and microorganisms present in inhaled air which are driven by the ciliary movement to the larynx where they are eliminated through the cough reflex. Studies show that the exposure to toxic substances could lead to changes in the epithelium which impairs its function and can even lead to cancer development (Thomassen et al., 1989).

The diaphragm muscle (DM) is one of the structures which is directly related with ventilation that may be affected by FA due to its systemic action. The DM is a thin dome-shaped fibromuscular structure (Heidi, 2009), innervated by the phrenic nerve (Michael and Gail, 2009) and classified, by histological parameters, as a skeletal striated muscle; and its embryonic development begins in the seventh week of gestation and is completed in the tenth (Maish, 2010). It performs two functions, firstly acting as a mechanical barrier between the pleural and abdominal cavity, secondly keeping the pressure gradient between the two cavities. Thus, this muscle is essential in human ventilation (Bardana and Montanaro, 1991), since it is responsible for 50–70% of the inspiration, and helps the expiration occur smoothly and not suddenly, the reason for which it is recognized as the major muscle in the ventilation (Barreiro et al., 2005). Therefore, this study aimed to investigate the oxidative effects on the inflammatory response in the trachea and the DM of rats exposed to different concentrations of formaldehyde.

2. Materials and methods

2.1. Animals

Twenty-eight adult male Fischer rats were obtained from the Laboratory of Experimental Nutrition of the Federal University of Ouro Preto (UFOP). Throughout the period of the experiments, the animals were kept under temperature and humidity control ($21 \pm 2\,^{\circ}C$ and $50 \pm 10\%$, respectively) and received standard feed and water *ad libitum*. The handling of the animals was carried out according to international animal protection standards, approved by the Ethics of the Federal University of Ouro Preto Committee (CEUA-UFOP), protocol n° 2011/01.

2.2. Exposure to Formaldehyde

Using an inhalation chamber of 30 L (25 cm × 30 cm × 40 cm) coupled to an ultrasonic nebulizer (Unique Group, Indaiatuba, São Paulo, Brazil), three groups of 7 animals were exposed to different solutions with formaldehyde at the following concentrations: 1% (FA1), 5% (FA5) and 10% (FA10), adapted from Lino dos Santos Franco's research (Lino dos Santos Franco et al., 2006; Lino-dos-Santos-Franco et al., 2010, 2011a,b). The exposures of 20-min duration were performed three times a day for five consecutive days, totaling 60 min/day. In the control group (CG), the animals were kept in ambient air. After 24 h of the last exposure, the animals were euthanized with an overdose of ketamine (50–75 mg/kg) and xylazine (5–10 mg/kg) intraperitoneally.

2.3. Homogenate tissues

After euthanasia, portions of the trachea and the DM of each animal were placed in the hemolysis tube with 1 mL of buffer (KPE) and homogenized in 10 replicates serum using a tissue homogenizer. Then, the homogenized samples were centrifuged at 7500 rpm for 10 min, the supernatant was collected and stored at $-80\,^{\circ}C$ for biochemical analyses (Steu et al., 2008).

2.4. Histology

The trachea and the DM were removed and immersed in fixative solution containing formaldehyde at 4% for 48 h, then embedded in paraffin. Serial sagittal sections were obtained for histological and morphometric analyses. Five-μm-thick tissue sections were stained with Hematoxylin and Eosin (H & E) for histopathological analyses and periodic acid-Schiff (PAS) for quantifying mucus production.

2.4.1. Histopathological and morphometric analyses

Photomicrographs were obtained by Leica light microscopy using software DM5000 Leica QWin Plus version 3.0. The Inflammatory cells were quantified in the trachea and the DM by counting the number of cell nuclei present in tissue sections using ImageJ software (NIH, USA). The mucus production was indirectly investigated by quantifying the number of cells with glycogen deposits in the trachea by staining with periodic acid-Schiff (PAS). For these morphometric analyses, 20 randomly images performing $1.5 \times 10^6\ \mu m^2$ of area were used. To determine the concentration of the tissue glycogen, random images were transformed into binary images to evaluate the gray-scale intensity of 100 random cells.

2.5. The analysis of oxidative damage

The formation of thiobarbituric acid reactive substances (TBARS) during an acid-heating reaction as previously described by Draper (Draper and Hadley, 1990) was used as an indication of lipid peroxidation. Briefly, the samples from the trachea and the DM homogenates were mixed with 1 mL of 10% trichloroacetic acid and 1 mL of 0.67% thiobarbituric acid; they were subsequently heated in a boiling water bath for 30 min. TBARS levels were determined by the measurement of the absorbance at 532 nm and were expressed as malondialdehyde equivalents (nmol/mg protein).

2.6. The determination of carbonyl protein

The determination of the carbonyl content in oxidatively modified protein was performed according to the method described by Levine (Levine et al., 1994). Each sample was precipitated with 10% (w/v) trichloroacetic acid (TCA). After centrifugation, the precipitate was treated with 10 mmol of 2,4-dinitrophenylhydrazine (DNPH) in 2 N HCl, incubated in the dark for 30 min at room temperature and then treated with 10% TCA. After centrifugation, the precipitate was washed twice with ethanol/ethyl acetate