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Oxidative effects on lung inflammatory response in rats exposed to different concentrations of formaldehyde☆

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ABSTRACT

The formaldehyde (FA) is a crosslinking agent that reacts with cellular macromolecules such as proteins, nucleic acids and molecules with low molecular weight such as amino acids, and it has been linked to inflammatory processes and oxidative stress. This study aimed to analyze the oxidative effects on pulmonary inflammatory response in Fischer rats exposed to different concentrations of FA. Twenty-eight Fischer rats were divided into 4 groups (N = 7). The control group (CG) was exposed to ambient air and three groups were exposed to different concentrations of FA: 1% (FA1%), 5% (FA5%) and 10% (FA10%). In the Bronchoalveolar Lavage Fluid (BALF), the exposure to a concentration of 10% promoted the increase of inflammatory cells compared to CG. There was also an increase of macrophages and lymphocytes in FA10% and lymphocytes in FA5% compared to CG. The activity of NADPH oxidase in the blood had been higher in FA5% and FA10% compared to CG. The activity of superoxide dismutase enzyme (SOD) had an increase in FA5% and the activity of the catalase enzyme (CAT) showed an increase in FA1% compared to CG. As for the glutathione system, there was an increase in total glutathione (tGSH), reduced glutathione (GSH) and oxidized glutathione (GSSG) in FA5% compared to CG. The reduced/oxidized glutathione ratio (GSH/GSSG) had a decrease in FA5% compared to CG. There was an increase in lipid peroxidation compared to all groups and the protein carbonyl formation in FA10% compared to CG. We also observed an increase in CCL2 and CCL5 chemokines in the treatment groups compared to CG and in serum there was an increase in CCL2, CCL3 and CCL5 compared to CG. Our results point out to the potential of formaldehyde in promoting airway injury by increasing the inflammatory process as well as by the redox imbalance.

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1. Introduction

The increased morbidity and mortality of the respiratory inflammatory diseases such as asthma, emphysema and bronchitis have been correlated with the exposure of individuals to the environmental pollutants including particulate matter and chemical substances such as formaldehyde (FA) (Lambert et al., 2003; Fujimaki et al., 2004; Green-McKenzie and Hudes, 2005; Ezratty et al., 2007). Pollutants may cause injury to the airway epithelium, inducing changes in the local immune response, favoring the induction and maintenance of inflammation as in asthma, and even causing lung cancer and cardiovascular disease (Dales and Raizenne, 2004; Güleç et al., 2006; Sul et al., 2007). In particular health professionals as well as all individuals that leads with

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methodologies applied in anatomy, pathology and histology field are in potential risk to the damage actions of the FA due to their labor activities (Wantke et al., 2000).

FA is one of the most studied chemicals nowadays (NAS, 2007) and occurs as a natural product in most living systems and the environment and it has been used in aqueous solution as a disinfectant and preservative and more recently in various industrial applications, including the production of adhesives, timber covers, plastic, textiles, leather, chemical manufacturing (Bosetti et al., 2008), building materials and cosmetics (Li et al., 2007). It is also found in emissions from cars due to the use of methanol as an alternative to fossil fuels because its enzymatic biotransformation generates FA, the kitchen gas burning (Lino dos Santos Franco et al., 2009) and tobacco smoke (Li et al., 2007). FA causes local irritation; skin sensitization after acute and sub-acute exposure in animal experiments and in humans (Swenberg et al., 1980); irritation to the eyes, nose, throat and airway; mild neuropsychological disorders (Ezratty et al., 2007) and is a potent trigger of inflammation of the lower airways (Lima et al., 2015; Bardana and Montanaro, 1991), in particular mediated by eosinophils, neutrophils and phagocytic cells that amplify this inflammatory process through inflammatory cytokines and chemokines (Persoz et al., 2010, 2012).

FA is a well-known crosslinking agent that reacts with cellular macromolecules, such as proteins, nucleic acids and amino acids with cytotoxic, haematotoxic, immunotoxic and genotoxic effects (Cheng et al., 2003; Liteplo and Meek, 2003; Metz et al., 2004). It is also able to induce DNA damage, growth inhibition and DNA repair delay after UV irradiation in various types of human cells (Grafstrom et al., 1983, 1984; Cosma and Marchok, 1988; Emri et al., 2004). In 2006, the International Agency for Research on Cancer (IARC) classified inhaled FA as a chemical able to cause nasopharyngeal cancer in humans. Recently, the IARC highlights that FA can cause leukaemia in humans, expanding the last classification from 2006 (IARC, 2006, 2012).

Exposure to chemical and physical agents as well as cellular aerobic metabolism and inflammation are sources of reactive oxygen species (ROS), including singlet oxygen, hydrogen peroxide, superoxide anion and hydroxyl radical (Lima et al., 2015). These reactive species are important mediators of cell damage, play an important role in the redox imbalance and may contribute to a variety of disorders (Halliwell, 1997). Polyunsaturated fatty acids associated with the membrane are likely to react with ROS, resulting in lipid peroxidation (Campos et al., 2013). This process can interfere with the fluidity of the membrane and cellular compartments, which can lead to cell lysis (Datta and Namasivayam, 2003). In this context, our study aimed to analyze the oxidative effects on pulmonary inflammatory response in Fischer rats exposed to different concentrations of FA.

2. Methods

2.1. Experimental design

Eight-week-old male Fischer rats were housed under controlled conditions in standard laboratory cages (Laboratory of Experimental Nutrition, Department of Food, School of Nutrition, Federal University of Ouro Preto – UFOP) and given free access to water and food. All *in vivo* experimental protocols were approved by the ethics committee (#2011/01) from UFOP. Rats (N = 28) were evenly divided into 4 groups: control group exposed to ambient air (CG), group exposed to 1% formaldehyde (FA1%), group exposed to 5% formaldehyde (FA5%) and group exposed to 10% formaldehyde (FA10%) using an inhalation chamber with 30 L (25 cm × 30 cm × 40 cm) coupled to an ultrasonic nebulizer (Unique Group, Indaiatuba, São Paulo, Brazil). The exposures of 20-min were performed during three times a day for five consecutive days, totaling 60 min/day (Lima et al., 2015; Maiellaro et al., 2014; Oliveira et al., 2015).

2.2. Assessment and analysis of Bronchoalveolar Lavage Fluid (BALF)

Immediately after euthanasia, the chest of each animal was opened to collect BALF. The left lung was clamped, and the right lung and the trachea were cannulated and perfused with 3 ml of saline solution (3 × 1.000 μl). The samples were kept on ice until the end of the procedure to avoid cell lysis. Total mononuclear and polymorphonuclear cell numbers were previously stained with trypan blue and determined in a Neubauer chamber. A differential cell count was performed on cytospin preparations (Shandon, Waltham, MA, USA) stained with fast panoptic coloration kit (Laborclin, Pinhais, Paraná) (Bezerra et al., 2011).

2.3. Assessment of hematological paramenters

For the complete blood count, the blood was diluted with saline (1:2) and erythrocyte hematological parameters, hematocrit and hemoglobin were evaluated using electronic counting device (ABX diagnostics, micro 60) of the Pilot Clinical Analysis Laboratory (LAPAC-UFOP).

2.4. Processing and homogenizing tissue

After BALF collection, the right ventricle of each rat was perfused with saline to remove blood from the lungs. The right lung was clamped so that just the left lung could be perfused with 4% buffered formalin (pH 7.2) at a pressure of 25 cm H_2O for 2 min via the trachea. The left lung was removed and immersed in a fixative solution for 48 h. The material was then processed as follows: tap water bath for 30 min, 2 baths in 70% and 90% alcohol each for 1 h, 2 baths in 100% ethanol for 1 h each, and embedding in paraffin. Serial 5-μm sagittal sections stained with hematoxylin and eosin were obtained from the left lung for histological analyses. After the removal of the left lung for histology, the right lung was immediately removed and stored in crushed ice in labeled tubes. The lungs were subsequently homogenized in 1 ml potassium phosphate buffer, pH 7.5 and centrifuged at 1500 × *g* for 10 min. The supernatant was collected, and the final volume of all samples was adjusted to 1.5 ml with phosphate buffer. The samples were stored in a freezer (−80 °C) for biochemical analyses.

2.5. Immunoassays for inflammatory markers

Plasma and lung tissue homogenates were used for evaluation of the inflammatory chemokines CCL2, CCL3 and CCL5. The immunoassays were performed in 96-well plates which were added 100 of monoclonal antibody to the protein (or peptide) of interest, diluted in PBS containing 0.1% bovine serum albumin - BSA (SIGMA). After incubation for 12 h at room temperature, the unabsorbed antibodies were discarded and the plates blocked with 300 uL/well of a PBS solution containing 1% BSA for 1 h at 37 °C. The plasma samples/or the supernatant were applied in a volume of 100 μl to each well. The reading of the marking intensity was performed on ELISA reader using a wavelength of 490 nm (Martins et al., 2013). All the chemokine ELISA kits were purchased from Peprotech (Ribeirão Preto, Brazil) and particularities in this methodology performed according to the manufacturer.

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