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The exposure to formaldehyde causes renal dysfunction, inflammation and redox imbalance in rats

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ABSTRACT

Twenty-eight Fischer male rats were divided into four groups: control group (CG), exposed to the ambient air, and groups exposed to formaldehyde (FA) at concentrations of 1% (FA1%), 5% (FA5%) and 10% (FA10%). Kidney function was assessed by dosage of uric acid, creatinine and urea. Morphometry was performed on the thickness of the lumen of Bowman's capsule and diameter of the lumen of the renal tubules. We evaluated the redox imbalance through the catalase and superoxide dismutase activity as well as oxidative damage by lipid peroxidation. Inflammatory chemokines CCL2, CCL3 and CCL5 were analyzed by enzyme immunoassays. There was an increase in the concentration of urea in FA10% compared with CG and FA1%. The levels of creatinine, renal lumen and lipid peroxidation increased in all FA-treated groups compared with CG. The concentration of uric acid in FA10% was lower compared with all other groups. There was an increase in the space of Bowman's capsule in FA5% and FA10% compared with CG and FA1%. However, the superoxide dismutase activity was higher in FA5% compared with other groups while CCL5 was higher in FA1% compared with CG. The exposure to formaldehyde in a short period of time leads to changes in the kidney function, inflammation and morphology, as well as promoted the increase of superoxide dismutase activity and oxidative damage.

1. Introduction

Formaldehyde (FA) is a colorless gas, highly soluble in water and irritates in its pure form. FA is a contaminating compound commonly found in the environment due to its wide use in industries such as the production of building materials, textiles, sterilization of products, plastics and cosmetics (Bakar et al., 2015; Checkoway et al., 2015; Ciftci et al., 2015). FA can also be found in cigarette smoke, in car emissions, fuel oil and natural gas, contributing to increased air pollution (Zararsiz et al., 2007b). The exposure to FA is increasingly common, either by environmental or laboratory conditions, where professionals and/or students in the medical field are constantly exposed (Schroeter et al., 2014; Zararsiz et al., 2007b). FA is also endogenously produced by the L-methionine metabolism, histamine, methanol and methyl alanine, being a key intermediate for the biosynthesis of purines and other amino acids (Checkoway et al., 2015; Gulec et al., 2006).

In 2006, the International Agency for Research on Cancer (IARC) described FA as a carcinogen (IARC, 2006). In addition, several studies have shown that chronic exposure to FA may also result in sensory irritation, salivation, dyspnea, headache, insomnia, seizures and neurodegenerative disorders (Bakar et al., 2015; Gulec et al., 2006). The toxicity caused by the exposure to FA by aerobic metabolism and by inflammation can lead to the production and the release of reactive oxygen species (ROS) (Birben et al., 2012; Gulec et al., 2006; Saito et al., 2005). At low concentrations, ROS have physiological functions in cellular processes, but in high amounts, they can cause adverse changes in the cell components, including proteins, lipids and deoxyribonucleic acid (DNA) (Birben

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et al., 2012; Saito et al., 2005). A change in the balance between oxidant/antioxidant in favor of oxidants is named oxidative stress (Birben et al., 2012). To protect against the deleterious effects of ROS, the cells present a complex enzymatic antioxidant defense system including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Gulec et al., 2006; Matsuoka et al., 2010).

Studies have shown that prolonged exposure to FA can result in degeneration and necrosis of proximal tubule kidney and consequently impaired urinary system (IARC, 2006; Kum et al., 2007; Zararsiz et al., 2007b). Furthermore, the exposure to FA induces a number of pathophysiological conditions, including inflammatory diseases by interfering in the concentration of T $CD3^+$ cells, natural killer (NK) cells, TNF, IL-6 and IL1–β (Lino-dos-Santos-Franco et al., 2011; Moro et al., 2016; Seow et al., 2015). The kidney is one of the most sensitive organs to the inflammation and is an important source of chemokines and cytokines in the tubular epithelium due its close contact with high blood flow (Grunz-Borgmann et al., 2015). Thus, the aim of the study was to analyze the oxidative effects on renal inflammatory response in Fischer rats exposed to different concentrations of FA.

2. Materials and methods

2.1. Animals

Twenty-eight male Fischer rats, between 10 and 12 weeks of age and body mass 180–200 g from the Experimental Nutrition Laboratory of the Federal University of Ouro Preto, were used in this study. The animals were kept in boxes with environment temperature, controlled light and humidity (21 ± 2 °C, 12-h cycles of light/dark, 50 ± 10%, respectively) receiving commercial diet for rat and water, both *ad libitum*. This study was performed in accordance with standards of animal protection and the ethical principles of the Brazilian Society of Science in Laboratory Animals and approved by the Ethics Committee on Animal Use of this university (Protocol 2011/01).

2.2. Exposure to formaldehyde (FA)

Animals were exposed to FA by an ultrasonic nebulizer (Unique Group, Indaiatuba, São Paulo, Brazil) coupled to an inhalation chamber of 30 L (25 cm × 30 cm × 40 cm). Three groups of 7 animals were exposed to different concentrations of FA (1% 5% and 10%) and a control group exposed to ambient air. The exposure proceeded for 20 min, 3 times a day (morning, afternoon, evening) for 5 consecutive days (Maiellaro et al., 2014; Murta et al., 2016). After 24 h of the experimental protocol, the animals were euthanatized with an overdose of Ketamine (50–75 mg/kg) and Xylazine (5–10 mg/kg) intraperitoneally.

2.3. Homogenized tissue

After euthanasia, the right kidney was removed and homogenized in 1 mL of pH 7.5 potassium phosphate buffer and centrifuged at 10.000 × *g* for 10 min at 4 °C. The supernatant was collected and stored in a freezer at −80 °C for biochemical analyses.

2.4. Renal function

Blood was obtained by cardiac puncture and collected into tubes. After, the samples were centrifuged at 10.000 rpm for 10 min. Serum was collected for analyses of uric acid, creatinine and urea. Analyses were spectrophotometrically performed using commercial kits (Bioclin®, Belo Horizonte, Brazil).

2.5. Histology and morphometric analyses

The left kidney was removed and immersed in fixative solution containing 4% formaldehyde for 48 h. Serial sections were performed to four micrometers thick, which were stain with hematoxylin-eosin (HE) for histopathological analyses. The variables analyzed were: Bowman's space and the lumen of the renal tubules. Bowman's space was calculated by the area of the Bowman's capsule and the glomerular tuft and subtracted from the second of the first area. Glomeruli were evaluated in the afferent artery measuring hence the equatorial portion of the glomerulus. The lumen of the renal tubules was calculated by tracing the boundaries thereof by ImageJ® software (National Institutes of Health, Bethesda, Maryland, USA)

2.6. The activity of catalase (CAT)

CAT activity was measured as the decreased rate of hydrogen peroxide to an absorbance of 240 nm represented by U/mg of the protein (Aebi, 1984). Protein content was performed on samples of tissue homogenate by the method of Bradford (Bradford, 1976).

2.7. The activity of superoxide dismutase (SOD)

SOD activity was measured in the tissue according to the Marklund method (Marklund and Marklund, 1974) which is based on the enzyme's ability to inhibit the autoxidation of pyrogallol. The absorbance was read in the ELISA reader at 570 nm. Protein content was performed on samples of the homogenate tissue by the method of Bradford (Bradford, 1976).

2.8. Analysis of oxidative damage

Lipid peroxidation was determined by testing reactive substances thiobarbituric acid (TBARS) described by Buege (Buege and Aust, 1978). The homogenized tissue was centrifuged for 10 min at 13.000 rpm and the supernatant was read in a spectrophotometer at 535 nm. The concentration was represented in nmols per milligram of the protein (nmol/mg protein).

2.9. Immunoassays

The renal parenchyma was used for the evaluation of the inflammatory chemokines CCL2, CCL3 and CCL5. The immunoassays were performed in 96-well plates by the addition of the 100 μL of a monoclonal antibody to protein (or peptide) of interest, diluted in PBS containing 0.1% bovine serum albumin-BSA (SIGMA). After 12 h at room temperature incubation, the plates were blocked with 300 μL/well of a PBS/1% BSA solution for 1 h at 37 °C. Samples were applied in a volume of 100 μL to each well. The avidin-HRP (1:2000) and the substrate ABTS liquid were used at the end of the reaction before the reading an ELISA reader at 490 nm. All chemokine ELISA kits were purchased from Peprotech (Ribeirão Preto, Brazil) and performed according to the manufacturer recommendations.

2.10. Statistical analysis

The normal distribution of each variable was assessed using the Kolmogorov-Sminorv test and presented as mean ± standard error of mean (SEM). For comparison among groups, a one-way ANOVA followed by Tukey's post-test was used. We used the Kruskal-Wallis test followed by Dunn's post-test for discrete data and expressed them as median, minimum and maximum values. In both cases, the difference was considered significant when p value was <0.05. The statistical analyses were performed using