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Does acute exposure to aldehydes impair pulmonary function and structure?

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ABSTRACT

Mixtures of anhydrous ethyl alcohol and gasoline substituted for pure gasoline as a fuel in many Brazilian vehicles. Consequently, the concentrations of volatile organic compounds (VOCs) such as ketones, other organic compounds, and particularly aldehydes increased in many Brazilian cities. The current study aims to investigate whether formaldehyde, acetaldehyde, or mixtures of both impair lung function, morphology, inflammatory and redox responses at environmentally relevant concentrations.

For such purpose, C57BL/6 mice were exposed to either medical compressed air or to 4 different mixtures of formaldehyde and acetaldehyde. Eight hours later animals were anesthetized, paralyzed and lung mechanics and morphology, inflammatory cells and IL-1β, KC, TNF-α, IL-6, CCL2, MCP-1 contents, superoxide dismutase and catalalase activities were determined. The extra pulmonary respiratory tract was also analyzed. No differences could be detected between any exposed and control groups.

In conclusion, no morpho-functional alterations were detected in exposed mice in relation to the control group.

1. Introduction

Atmospheric composition has been undergoing increasing human-related interference, who continuously neglect the consequences or dismiss the putative ambient and health undesired aggressions. According to Anderson and colleagues (1996) one of the main causes of air pollution is the production of contaminants resulting from combustion of fossil fuels. Formaldehydes and acetaldehydes are the main aldehydes present in vehicle emissions and are also generated by industrial processes (Gaffney et al., 1997). Once released into the atmosphere, these aldehydes undergo ozone-initiated oxidation resulting in the subsequent formation of ultra-fine particles and potentially irritating gaseous organic products such as formaldehyde, hydrogen peroxide, hydroxyl radicals,

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and other low volatile oxygenated compounds (Lamorena and Lee, 2008).

The International Agency for Research on Cancer (IARC, 2006) classified formaldehyde in Group 1, which includes substances that are proven to be carcinogenic for humans, and establishes 0.016 ppmv as an exposure limit for an 8-h workday in a Time-Weighted Average (TWA) (2006). In Brazil, the regulatory standard NR-15 from the Consolidation of Labor Laws, establishes a TWA of 1.6 ppmv; while the equivalent US agency (OSHA – Occupational Safety and Health Administration) sets an exposure limit of 0.75 ppmv. Acetaldehyde belongs to Group 2B, in which are included possibly carcinogenic substances for humans, since their carcinogenicity was demonstrated in animals (IARC, 1999). In Brazil, NR-15 sets a limit of tolerance of 78 ppmv, while the ACGIH (American Conference of Governmental Industrial Hygienists) establishes an exposure limit of 25 ppmv, and the OSHA establishes a TWA of 100 ppmv for this compound.

In 1999, the Agency for Toxic Substances and Disease Registry (ATSDR), based on clinical symptoms and on lowest-observed-adverse-effect level (LOAEL) of 0.4 ppmv, established the formaldehyde concentration of 0.04 ppmv as the Minimal Risk Level (MRL) for acute inhalation (14 days or less). The formaldehyde non-observed-adverse-effect level (NOAEL) corresponds to 0.08 ppmv (Nielsen et al., 1999). Increased sneezing, itching, mucosal congestion, transient sensation of burning in the eyes and nasal passages and nasal abnormalities (elevated eosinophil counts and transient increase in protein content in the nasal lavage fluid) are common signs and symptoms in humans (Pazdrak et al., 1993).

Studies on the toxicological profile of acetaldehyde are not conclusive. However, there is evidence suggesting a MRL of 2 ppmv based on a NOAEL of 40 ppmv for humans exposure. In experimental animals the LOAEL ranges from 150 ppmv to 500 ppmv, and the impairments are predominantly observed in the nasal and olfactory epithelia (Appelman et al., 1986; Oyama et al., 2010).In animals, eye and upper airway irritation constitute the early signs of exposure to formaldehyde. According to experimental studies, such effects are usually observed in concentrations from 0.5 to 1 ppmv (Monticello et al., 1996; Monticello et al., 1991; Nielsen et al., 1999). The NOAEL calculated for mice is 0.3 ppmv (Nielsen et al., 1999).

Several studies approached solely the measurement of environmental concentrations of aldehydes, either outdoors or indoors, but their boundaries and health effects are still controversial. Therefore, studies to investigate the various physiological and pathophysiological characteristics are necessary. Some studies on anatomical and clinical changes caused by exposure to formaldehyde and acetaldehyde were conducted in rodents (Jung et al., 2007; Davarian et al., 2005; Kawano et al., 2012; Liteplo, 2002). However, the effects of acute exposure on lung mechanics have not been studied yet.

Hence, the current study aims to investigate the biological impact of formaldehyde, acetaldehyde or mixtures of both (typical environmental or slightly higher concentrations). For such purpose, lung function, respiratory tract morphology and inflammatory and redox responses were addressed.

2. Methods

2.1. Animals

Two hundred and sixteen male and 216 female C57BL/6 mice weighing 25–30 g were obtained from Roberto Giannichi Filho Breeding Unit. The room temperature was kept constant and at (24 ± 2) °C, average relative humidity around 45%, an electronically controlled light/dark cycle of 12 h/12 h, food and water *ad limbitum*.

2.2. Exposure protocol

In each experimental day, a different mixture of aldehydes was evaluated and animals were divided in two groups: (1) control group (C) and Exposed (E). All mice underwent an 8-h exposure period during the active cycle, always between 6:00 pm and 2:00 am of the following day. The group of exposed animals consisted of 24 animals (12 males and 12 females) and the control group consisted of 12 animals (6 males + 6 females).

2.3. Generation, control and measurement of the gaseous aldehydes

A mixture of medical compressed air and the studied gaseous aldehyde was adjusted by means of mass flow controllers (MFC, model MKS 2179, MKS Instruments, Andover, MA, USA for the gaseous aldehydes and model MKS 2579, MKS Instruments, Andover, MA, USA for the medical compressed air). A built-purpose routine that was written in Labview 8.2 (National Instruments, Austin, TX, USA) controlled airflows of both medical compressed air and aldehyde. This program also acquired and exported analogical data through analogical-to-digital and digital-to-analogical converters, respectively (USB 6008, National Instruments, Austin, TX, USA) to the MKS type 247 amplifier (MKS Instruments, Andover, MA, USA). Thus, this computer controlled the flows of desired gaseous aldehyde within the main stream of the medical air sent to the rack of isolated cages where the experimental animals were located. A second computer was used to register in real-time the final concentration of the aldehyde in the air delivered to the animals. Data were stored in *.PRN files throughout the exposure period.

2.4. Exposure groups

Three concentrations of each aldehyde or mixtures of formaldehyde and acetaldehyde were selected to infer the overall behavior of a dose-response curve. The rationale for selecting the concentrations was to establish the lowest ones in the same order of magnitude of the highest environmental concentrations previously described in Brazilian towns, i.e., around 0.1 ppmv (0.151 ppmv of formaldehyde – Martins et al., 2007; and 0.093 ppmv of acetaldehyde – De Andrade et al., 1998). For the formaldehyde we used 0.2 ppmv (same magnitude of the environmental concentration). For acetaldehyde we used 3 ppmv (higher than the maximum environmental concentration), owing to the concentration of acetaldehyde commercially available, limitation in the maximum dilution allowed by the mass controllers, and also the resolution of the FTIR gas analyzer for acetaldehyde. The highest concentration used to infer the overall behavior of the dose-response curve was approximately 10 to 100-fold the lowest one for each aldehyde. The third concentration was set near the geometric mean of the other two extreme doses. For the mixtures, we used the previous formaldehyde concentrations and set the acetaldehyde concentration to simulate ratios of both concentrations (i.e., [formaldehyde]/[acetaldehyde]) observed in the combustion of ethanol (1/20–1/10) and gasohol (1/4–1/2).

On each experimental day one aldehyde mixture was used diluted in medical compressed air: 0.2 ppmv, 1.0 ppmv and 3.0 ppmv of formaldehyde (FA); 3 ppmv, 100 ppmv and 500 ppmv of acetaldehyde (AC); mixture 1: 0.3 ppmv FA + 3,8 ppmv AC (low), 1.3 ppmv FA + 16.3 ppmv AC (medium), 3.0 ppm FA+ 40 ppmv AC (high), and mixture 2: 1 ppmv FA + 3.4 ppm AC (low), 2.3 ppmv FA + 8.5 ppm AC (medium), 3.4 ppm FA + 10.4 ppmv AC (high). Concomitantly, animals were exposed to medical compressed air (control group):