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Formaldehyde inhalation during pregnancy abolishes the development of acute innate inflammation in offspring

Beatriz Silva Ibrahim [a], Cristiane Miranda da Silva [b], Éric Diego Barioni [a], Matheus Correa-Costa [c], Carine Cristiane Drewes [a], Niels Olsen Saraiva Câmara [c], Wothan Tavares-de-Lima [d], Sandra Helena Poliselli Farsky [a], Adriana Lino-dos-Santos-Franco [b,*]

[a] Department of Clinical and Toxicological Analyses, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil
[b] Post Graduate Program in Biophotonics Applied to Health Sciences, University Nove de Julho (UNINOVE), São Paulo, Brazil
[c] Department of Immunology, University of São Paulo, São Paulo, Brazil
[d] Department of Pharmacology, University of São Paulo, São Paulo, Brazil

HIGHLIGHTS

- Exposure to formaldehyde during pre-natal period suppressed the lung acute injury in the offspring.
- Exposure to formaldehyde during pre-natal period reduced the oxidative burst in the offspring.
- Exposure to formaldehyde during pre-natal period decreased the TLR4 expression in the lung tissue in the offspring.
- Exposure to formaldehyde during pre-natal period reduced NF-kB translocation into the nucleus in the offspring.

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ABSTRACT

Formaldehyde (FA) is an environmental and occupational pollutant that induces programming mechanisms on the acquired immune host defense in offspring when exposed during the prenatal period. Hence, here we investigated whether the exposure of FA on pregnant rats could affect the development of an innate acute lung injury in offspring induced by lipopolissacaride (LPS) injection. Pregnant Wistar rats were exposed to FA (0.92 mg/m^3) or vehicle (distillated water), both 1 h/day, 5 days/week, from 1 to 21 days of pregnancy. Non-manipulated rats were used as control. After 30 days of birth, the offspring was submitted to injection of LPS (*Salmonella abortus* equi, 5 mg/kg, i.p.). Systemic and lung inflammatory parameters were evaluated 24 h later. Exposure to FA during gestation abolished the development of acute lung injury in offspring, as observed by reduced number of leukocytes in the bronchoalveolar fluid (BAL), in the blood and in the bone marrow, and decreased myeloperoxidase activity in the lung. Moreover, phagocytes from BAL presented normal phagocytosis, but reduced oxidative burst. Alterations on the profile of inflammatory cytokines were evidenced by reduced mRNA levels of IL-6 and elevated levels of IL-10 and IFN gamma in the lung tissue. Indeed, mRNA levels of toll-likereceptor-4 and nuclear factor-kappa B translocation into the nucleus were also reduced. Additionally, hyperresponsiveness to methacholine was blunted in the trachea of offspring of FA exposed mothers. Together, our data clearly show that FA exposure in the prenatal period modifies the programming mechanisms of the innate defense in the offspring leading to impaired defense against infections.

1. Introduction

Formaldehyde (FA) is an ubiquitous occupational pollutant that is widely used in a variety of industries, including in the construction, paper product, resin, insulating material, wood composite, textile, paint, plastic, adhesive, and cosmetic (Carlson

* Corresponding author at: Post Graduate Program in Biophotonics Applied to Health Sciences, University Nove de Julho (UNINOVE), Brazil Rua Vergueiro, 239/245, São Paulo, SP CEP 01504-000, Brazil. Tel.: +55 36821275.

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et al., 2004). FA is also an indoor air pollutant emitted from furniture, building materials and chipboards (Salthammer, 2013).

The relationship between pollution and lung disease is well established, and pregnant women and children are particularly susceptible to the toxic effects of pollution (Gordon et al., 2014). In this context, some studies show the effects of pollution during pregnancy and its repercussion in the lung disease in the offspring; however, the mechanisms involved are unclear. Experimental studies showed that xenobiotic exposure can increase the susceptibility to development of infections due to the reduction of activity of neutrophils and macrophages. Additionally, prenatal exposure to pollutants leads to important effects in the birth weight, risk of birth defects and infant mortality, growth retardation and deleterious effects on the respiratory system (Dugandzic et al., 2006; Jedrychowski et al., 2005; Gilboa et al., 2005; Lipfert et al., 2000; Djemek et al., 1999).

In earlier studies, the toxic effects of FA on the immune system have been shown. We showed that low doses of FA exposure during pregnancy induce reduced birth weight and interfere with the course of Th2 immune response. The development of allergic lung inflammation and tracheal hyperresponsiveness in the offspring was suppressed by mechanisms mediated by reduced anaphylactic antibody synthesis, and reduced IL-6 and TNF-α secretion. In addition, toxic systemic effects were not detected in pregnant rats exposed to FA, although the oxidative stress in the uterine microenvironment increased (Maiellaro et al., 2014). Thus, we showed the putative programming mechanisms induced by FA exposure on the acquired immune system of offspring.

The immune response is developed through several innate and adaptive mechanisms in order to protect the body against pathogens including bacteria, viruses, fungi and protozoa. The early innate response detects and eliminates pathogenic microorganisms by discrimination between themselves (self) and foreign (non-self) substances of the body, especially by actions of phagocytes, such as, neutrophils, macrophages and dendritic cells (Takeda et al., 2003). The initial response may progress to adaptive immune reaction, involving the humoral and cytotoxic responses mediated by lymphocytes. Lung inflammation caused by environmental agents, including endotoxin or its component identified as lipopolysaccharide (LPS), plays an important role in the progression of chronic respiratory diseases (Liu, 2004). Systemic administration of LPS in animals of laboratory induces neutrophil recruitment into the lung with local TNF-α production and injury to alveolar epithelium and endothelium (Arbour et al., 2000). In addition, LPS also accounts for bronchoconstriction and hyperresponsiveness to methacholine (Lefort et al., 2001; Schnyder-Candrian et al., 2005).

Toll-like receptor 4 (TLR4) plays a critical role in the lung response to systemic LPS administration (Andonegui et al., 2003). Indeed, after the activation of TLR4 by LPS, a wide spectrum of cytokines is synthesized by activation of NF-kB transcription factor. NF-kB plays an important role on the inflammatory diseases including ARDS. In this context, NF-kB appears to be responsible for the expression of pro-inflammatory cytokines such as, IL-1β, IL-6, IL-12, TNF-α, the enzyme COX-2 (cyclooxygenase-2), adhesion molecules including ICAM-1 (intercellular adhesion molecule-1), VCAM-1 (vascular cell adhesion molecule-1), E-selectin and, enzymes involved in microbicidal activity as iNOS (inducible nitric oxide synthase) (Kawai et al., 1999).

In this study, we hypothesized that FA exposure during the pregnancy may alter the profile of innate immune response of offspring caused by LPS. To this purpose, we have evaluated the magnitude of lung inflammation, functional activity of lung phagocytes and ex-vivo tracheal responsiveness to methacholine. Finally, gene expression of cytokines and TLR4, and NF-kB translocation were also assessed.

2. Materials and methods

2.1. Animals

Female and male 2-month-old Wistar rats were obtained from the Institute of Biomedical Sciences, University of São Paulo, and maintained in a light and temperature-controlled room (12/12-h light–dark cycle, 21 ± 2 °C), with free access to food and water. The virgin females were caged overnight with a male, and vaginal smears were taken the following morning. Pregnancy was confirmed by vaginal smear. The experiments were approved by the Institutional Animal Care Committee.

2.2. Exposure to formaldehyde (FA) inhalation

The pregnant rats (5/chamber) were exposed to FA inhalation (0.92 mg/m^3, 1 h/day, 5 days/week) during 21 days of gestation. The dose of FA used in the present study was based on that which does not cause effects on human health (OSHA), but on the other hand, causes immunosuppression in the offspring as observed in earlier studies (Maiellaro et al., 2014). For this purpose, we used a standard glass chamber (20 L) coupled to an ultrasonic nebuliser device (Icel®, Brazil) that produces an aerosol with particles of between 0.5 and 1 μ. to generate a constant airstream in an aqueous solution of formalin (Maiellaro et al., 2014).

The pregnant females rats were divided into 3 experimental groups: B (basal group, $n = 5$), non-manipulated rats; C (control group, $n = 5$), rats exposed to vehicle of FA (distillated water) and P (pollutant group, $n = 5$), rats exposed to FA.

2.3. Experimental design with offspring

After birth, pups were left with their mothers for 21 days (weaning period). The animals were maintained in a light- and temperature-controlled room (12/12-h light–dark cycle, 21 ± 2 °C) with free access to food and water.

Thirty days after birth, male and female pups from each experimental exposure group, were mixed and randomly assigned to experimental groups of 10 animals for each experimental group. These procedures were adopted in order to eliminate the formation of groups containing pups with an over representation from a single mother, excluding the possibility of the results being influenced by individual susceptibility. Indeed, we mixed the results obtained from both sex, because no differences were observed between males and females responses (data not shown).

Pups were submitted to systemic inflammation by injection of lipopolissacaride (LPS, *Salmonella abortus* equi, 5 mg/kg, i.p.) in order to challenge the innate immune system. The dose chosen was based in the sub-septic effects of LPS accordingly Clark et al. (2014). The offspring was assigned into 3 groups: (1) B group, identified as offspring from non-manipulated mothers and without LPS or vehicle treatment ($n = 10$); (2) LPS group, identified as offspring submitted to LPS from mothers exposed to vehicle of FA ($n = 10$); (3) P + LPS group, identified as offspring submitted to LPS from mothers exposed to FA ($n = 10$). The animals were anesthetized with ketamine and xylazine (100 and 20 mg/kg, i.p.) and the analyses were performed 24 h after the injection of LPS.

2.4. Evaluation of acute lung injury by quantification of cells recruited in the bronchoalveolar lavage (BAL) and myeloperoxidase activity (MPO) in the lung tissue

BAL fluids were extracted according to de Lima et al. (1992). Tracheae of rats were cannulated with polyethylene tubing, and the lungs were flushed twice with PBS (10 ml total volume). The collected BAL was centrifuged (1500 rpm for 15 min at 20 °C), and