Toxicology and Applied Pharmacology 300 (2016) 77–81

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Short Communication

Beneficial effects of vitamin C treatment on pregnant rats exposed to formaldehyde: Reversal of immunosuppression in the offspring

Beatriz Silva Ibrahim [a], Éric Diego Barioni [a], Cíntia Heluany [a], Tárcio Teodoro Braga [b], Carine Cristiane Drewes [a], Silvia Goes Costa [c], Niels Olsen Saraiva Câmara [b], Sandra Helena Poliselli Farsky [a], Adriana Lino-dos-Santos-Franco [c,*]

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

ARTICLE INFO

Article history:
Received 10 December 2015
Revised 25 February 2016
Accepted 21 March 2016
Available online 26 March 2016

Keywords:
Oxidative stress
Acute lung inflammation
LPS
Bone marrow
NF-kappa B
Toll-like receptor

ABSTRACT

Inhalation of formaldehyde (FA) during the pregnancy induces oxidative stress in the uterus, and here we hypothesized that this mechanism may be responsible for the impaired immune response detected in the offspring. In order to investigate the protective effects of Vitamin C on the oxidative stress induced by FA in the uterine microenvironment, pregnant Wistar rats were treated with vitamin C (150 mg/kg, gavage) or vehicle (distilled water, gavage) 1 h before FA exposure (0.92 mg/m^3, 1 h/day, 5 days/week), for 21 days, and the 30 days old offspring were submitted to LPS injection (*Salmonella abortus equi*, 5 mg/kg, i.p.). The enhanced gene expression of iNOS, COX-1 and COX-2 and decreased gene expression of SOD-2 in the uterus of FA exposed mothers was rescued by Vit C treatment. Moreover, vitamin C rescued the impaired immune response elicited by LPS in the offspring from FA exposed mothers, by increasing the number of blood and bone marrow leukocytes, and augmenting gene expression of IL-6 and reducing mRNA levels of IL-10 and IFN in the lungs. Vitamin C treatment did not rescue the impaired TLR4-NF-kB pathway in the lung of the offspring, suggesting that FA-induced uterine oxidative stress affects other inflammatory pathways activated by LPS in the offspring. Together, data obtained here confirm our hypothesis that FA-induced oxidative stress in the uterine microenvironment modifies the programming mechanisms of the immune defenses of offspring, leading to an impaired host defense.

1. Introduction

Formaldehyde (FA) is an occupational and indoor pollutant, with potential carcinogenic effects in the airways. It is widely employed in industries and also emitted from furniture, building materials and chipboards (Salthammer, 2013). Moreover, it has been shown that FA air concentrations in the anatomy and in pathology laboratories, as well as in hairdresser salons, are at upper limits than those allowed by the regulatory agencies, leading to the toxicity of biomedical students and workers (Klein et al., 2014; Fox et al., 2013). Taking into account that women are a significant part of the population in these stated activities, FA toxicity in this gender has been recently investigated (Amiri et al., 2015; Haffner et al., 2015). Moreover, our group has previously shown that FA exposure during pregnancy induces programming mechanisms in the immune defenses of the offspring, leading to impaired defenses against infectious or allergic stimulus (Maiellaro et al., 2014; Silva Ibrahim et al., 2015). It is noteworthy that FA-exposed mothers do not present any toxic effects, unless elevated oxidative stress in the uterine tissue, measured by enhanced expression of cyclooxygenase-1 (COX-1) and by reduced expressions of constitutive nitric oxide synthase (cNOS) and superoxide dismutase (SOD-2) (Maiellaro et al., 2014). As it has been fully described that the imbalance of anti and pro oxidant enzymes is a hallmark of oxidative stress (Griffiths et al., 2014), we show that alterations on expressions of these enzymes in the uterus represent an important pathway of in vivo FA exposure on mothers, would implicate in changes in the immune system of offspring.

Many pollutants, such as FA, particulate matter, polycyclic aromatic hydrocarbons, induce reactive oxygen and nitrogen species (ROS and RNS) generation, which all lead to oxidative stress in the lung (Lino-dos-Santos-Franco et al., 2009, 2011; Lim et al., 2010; Jeng, 2010). ROS and RNS are important mediators of the inflammatory process, and directly react with DNA base pairs, causing genetic and epigenetic effects (Thompson and Al-Hasan, 2012). In the latter context, it has been proposed that adverse effects in the uterus, such as enhanced ROS and RNS production, induces fetal reprogramming mechanisms which reflect on the quality of life and health of the offspring.

* Corresponding author at: Post Graduate Program in Biophotonics Applied to Health Sciences, University Nove de Julho (UNINOVE), Rua Vergueiro, 239/245, São Paulo, SP CEP 01504-000, Brazil.
E-mail address: alsantosfranco@gmail.com (A. Lino-dos-Santos-Franco).

http://dx.doi.org/10.1016/j.taap.2016.03.010
0041-008X/

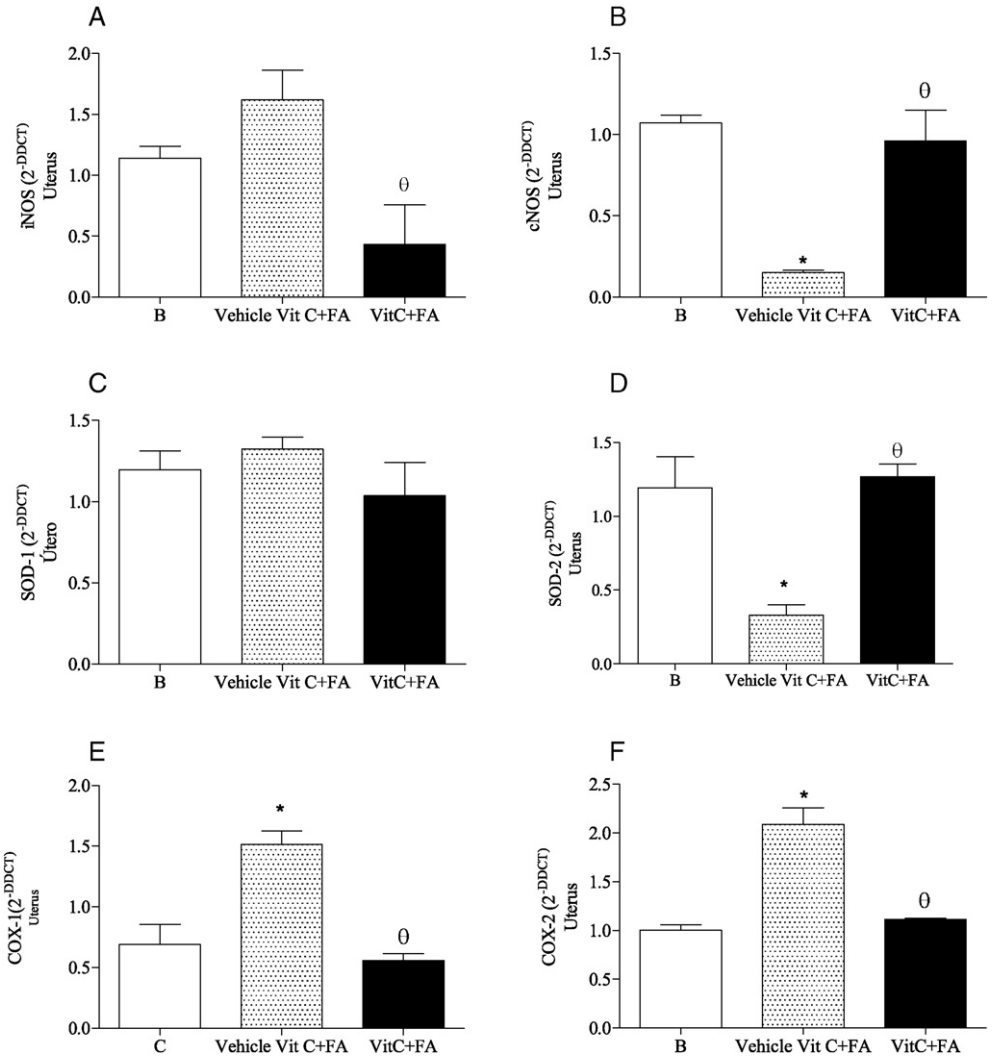

Fig. 1. Treatment with vitamin C during pregnancy protects the uterus against the deleterious effects of FA exposure. Pregnant rats were exposed to FA for 21 days and treated with vitamin C (150 mg/kg, gavage) or vehicle 1 h before each FA exposure. Non-manipulated pregnant rats were used as control. After 20 days of gestation, the gene expressions of iNOS (Panel A), cNOS (Panel B), SOD-1 (Panel C), SOD-2 (Panel D), COX-1 (Panel E), and COX-2 (Panel F), were evaluated in the uterine tissue. Data is mean ± SEM of 5 mothers per group. $^{*}P < 0.05$ in relation to the B group; $^{\theta}P < 0.05$ in relation to the Vehicle + FA group.

The participation of oxidative stress in FA-induced lung toxicity was corroborated by abolishing the deleterious effects of FA in the lung of animals previously treated with Vitamin C (Lino-dos-Santos-Franco et al., 2011; Lino-dos-Santos-Franco et al., 2010; Gulec et al., 2006). It has been fully shown the employment of vitamin C as antioxidant compound, as it prevents membrane lipoperoxidation and acts as a potent reactive species scavenger (Ozdil et al., 2004).

Taking into account that xenobiotic induced oxidative stress is associated with fetal reprogramming mechanisms, and that our previous data show higher levels of oxidative stress in the uterine microenvironment of pregnant rats exposed to FA, we here investigated if the treatment with an antioxidant agent during pregnancy could rescue the immune system defenses in the offspring from mothers exposed to FA.

2. Materials and methods

The experimental protocols were approved by the Ethics Committee on the use of animals (CEUA), the Faculty of Pharmaceutical Sciences (FCF), University of São Paulo (USP). Pregnant rats were treated with vitamin C (150 mg/kg) or vehicle (distilled water) by gavage for 1 h before each FA exposure (0.92 mg/m^3, 1 h/day, 5 days/week) during 21 days of gestation according to Maiellaro et al. (2014). Mating was verified by plug formation. The dose of vitamin C chosen was based on our earlier study (Lino-dos-Santos-Franco et al., 2010), which inhibited the oxidative stress in the lung of FA exposed animals and showed no toxic effects. Previous data showed that exposure to FA vehicle or isolated treatment with Vitamin C did not induce alterations in the mothers as well as in the offspring. Values obtained were similar to those found in the non-exposed mother (basal) (Maiellaro et al., 2014) and in order to reduce the number of animals, only the basal group was employed as control for the experiments. Thirty days after birth, male and female pups were mixed and randomly assigned to three experimental groups: offspring from non-manipulated mothers (Basal), offspring from mothers treated with vehicle of Vitamin C and exposed to FA (Vehicle Vit C + FA), and offspring from mothers treated with vitamin C and exposed to FA (VitC + FA). These procedures were adopted in order to exclude the possibility of the results being influenced by individual susceptibility of offsperimg born from one unique mother. Moreover, we mixed the results obtained from both sex, because no differences were observed between males and females responses. The offspring received an i.p. injection of lipopolysaccharide (LPS, *Salmonella abortus equi*, 5 mg/kg, i.p.) or saline, in order to challenge the innate immune system (Silva Ibrahim et al. (2015). Systemic Lipopolysaccharide (LPS) administration is an approach used experimentally to induce sepsis-related lung injury. Bronchoalveolar and femural lavages, lung myeloperoxidase activity (MPO), and blood analyses, were all performed according to Silva Ibrahim et al. (2015). Lung and