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Alteration of syncytiotrophoblast mitochondria function and endothelial nitric oxide synthase expression in the placenta of rural residents

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ABSTRACT

The impact of environmental organophosphate (OP) pesticide exposure on respiratory complexes, enzymatic antioxidant defense activities, and oxidative damage markers in the syncytiotrophoblast and cytotrophoblast mitochondria was evaluated. Placental progesterone (PG) levels and endothelial nitric oxide synthase (eNOS) expression were studied. Samples from women non-exposed (control group-CG) and women living in a rural area (rural group-RG) were collected during pesticide spraying season (RG-SS) and non-spraying season (RG-NSS).

In RG-SS, the exposure biomarker placental carboxylesterase decreased and syncytiotrophoblast cytochrome *c* oxidase activity increased, while 4-hydroxynonenal levels decreased. PG levels decreased in RG-SS and in the RG. Nitric oxide synthase expression decreased in RG, RG-SS and RG-NSS. No significant changes in mitochondrial antioxidant enzyme activities were found. These results suggest that the alteration of syncytiotrophoblast mitochondrial complex IV activity and steroidogenic function may be associated to pesticide exposure. Reduction in placental PG and eNOS expression may account for low newborn weight in RG.

1. Introduction

Among modern non-persistent pesticides, the organophosphate pesticides (OP) are the most commonly used worldwide. As a result of their widespread use, OP poisoning is a major cause of morbidity and mortality, especially in developing countries [1]. Pesticides follow a dynamic fate in the environment, which includes gaining access not only to pest-specific targets but also to non-target organisms, such as human beings. Agricultural pesticide application represents a major source of human pesticide exposure. Populations residing next to crops may be exposed to pesticides via various exposure routes simultaneously [2]. In this sense, Bradman et al. (2011) [3] reported that, dietary intake as well as temporal and spatial proximity to agricultural use represent the most relevant routes among the multiple determinants of OP exposure. The OP oxon metabolites are the actual powerful inhibitors of type B-esterases [4], which include carboxylesterase (CE) [5], a sensitive indicator of environmental OP exposure [6].

Potential health effects associated with pesticide exposure during pregnancy have become a major public health concern due to high maternal and fetal sensitivity to xenobiotics [7]. Several toxic effects of prenatal OP exposure have been documented at birth, such as intrauterine growth restriction (IUGR) increased risk [8], negative correlation between OP exposure and birth weight and length [9], and gestational duration [10]. Long-term effects have been registered at later life stages, including neurobehavioral [11],

Abbreviations: α-NA, alpha-naphthyl acetate; CAT, catalase; CE, carboxylesterase; CG, control group; CT, cytotrophoblast; eNOS, endothelial nitric oxide synthase; GST, glutathione S transferase; HNE, 4-hydroxynonenal; IUGR, intrauterine growth restriction; Mn-SOD, Mn-superoxide dismutase; NO, nitric oxide; OP, organophosphate; PG, progesterone; RG, rural group; RG-NSS, non-spraying season rural group; RG-SS, spraying season rural group.

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social functioning deficits [12] as well as adverse cardiovascular risk profiles in susceptible children [13].

Placental dysfunction is the underlying mechanism of many pregnancy complications [14]. Even though it has been proposed that the placenta acts as a temporary depot of OP [15], few studies have addressed the possible OP placental toxicity. Women exposed to the OP parathion presented changes in the placental morphology, with microcalcifications, microinfarcts, and atypical characteristics of tertiary villi [16]. More recently, we have shown that environmental OP exposure modulates cytokine, arginase and ornithine decarboxylase expression in human placenta [17].

The placenta is a metabolically active organ that fulfills several physiological functions such as fetal nutrition, gas exchange, protein and hormone synthesis [18]. Placental defects in the energy provision system may impact seriously on fetal developmental processes. In fact, several lines of evidence indicate that placental mitochondrial dysfunction is associated to some pathological conditions such as IUGR [19] and placental insufficiency, probably leading fetal programming effects [20,21].

Two mitochondria types have been described in the placenta, the syncytiotrophoblast (SCT) and cytotrophoblast (CT) mitochondria [22]. It has been reported that large rounded mitochondria are observed in CT cells. In contrast, the SCT contains smaller irregular mitochondria with a condensed matrix [22,23]. Both display different morphological features and dissimilar activity levels of cytochrome P450 enzymes, that participate in progesterone (PG) synthesis, with higher activity occurring in SCT mitochondria [24].

Many experimental studies have shown that pesticide exposure exerts deleterious effects in mitochondria by disturbing oxidative balance [25,26]. Mitochondrial dysfunction in pregnant women exposed to low doses of complex pesticide mixtures was suggested by Bonvallot et al. (2013) by investigating different urinary metabolites in early pregnancy [27]. We have also reported changes in SCT mitochondrial phospholipid profile associated to OP environmental exposure in women residing next to an agricultural area [28].

Taking into consideration that the mitochondria may represent a target for OP toxicity in the placenta, in this work we analyzed several mitochondrial hallmarks in placentas from pregnant women environmentally exposed to pesticides. Alterations in mitochondrial respiratory complex activities and PG levels were addressed. Also, enzymatic antioxidant defense activities and oxidative damage marker were studied. In addition, endothelial nitric oxide synthase (eNOS) expression was assessed considering that nitric oxide (NO) modifies mitochondrial respiration [29,30] and is involved in OP toxicity [31,32]. Finally, relationships between biochemical variables and fetal and placental growth indicators were evaluated.

2. Materials and methods

2.1. Chemicals

Analytical grade reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Merck Laboratories (Darmstadt, Germany), and BioRad (Hercules, CA, USA).

2.2. Antibodies

Rabbit polyclonal NOS (C-20), rabbit polyclonal β-actin (I–19), and goat polyclonal 4-HNE (P-16) antibodies purchased from Santa Cruz Biotechnology (Dallas, Texas USA). Horseradish peroxidase-conjugated goat anti-rabbit IgG and rabbit anti-goat IgG antibodies were from Sigma (St. Louis, MO, USA).

2.3. Studied population

The study included sixty-three healthy pregnant women (17–35 years old). From 2010 to 2013, forty-three women were recruited at the Sanatorio del Personal de Industrias Químicas at Cinco Saltos City, Rio Negro, a hospital located in northern Patagonia, Argentina. They were included in the rural group (RG) because they belonged to a small population living in an area surrounding fruit cultivation areas where pesticides are applied by ground-based spraying equipment during the dry season (september to december). The distance from the houses to the crops was between 50 m and 10 km. The strongest wind from west and south-west occurs during pesticide intensive application period. In the farms, aerial drift from the target area is frequent, increasing the potential environmental exposure of the rural population. In addition, they may be exposed to pesticides related to the commonly used irrigation technique of periodic flooding, as Loewy et al. (2011) [33] have reported that pesticide residues, mainly chlorpyrifos, azinphosmethyl and carbaryl, are found both in water (surface and subsurface) and soil, indicating off-site migration. Water source in the rural population was 5% from groundwater and 95% drinking water. The control group (CG) consisted of pregnant women with no history of pesticide exposure who attended the San Lucas prenatal clinic in Neuquén City (n = 20), Argentina.

Women in the third trimester of pregnancy were asked to participate in this study. They were included if they had medium income level, belonged to the same ethnic group – Hispanic – and were undergoing planned caesarean section due to previous caesarean section or fetal breech presentation. Placentas from caesarean deliveries were chosen because maternal oxidative stress is lower in a time-scheduled procedure than in women undergoing vaginal delivery [34]. Women were excluded if they smoked, suffered from a serious chronic disease or were medicated (except those included in Group A according to U. S. Food and Drug Administration), or developed a pregnancy complication (i.e., gestational diabetes, hypertension, preeclampsia). Groups were matched for reported smoking status and alcohol consumption.

At the time of recruitment, women were asked to complete a guided questionnaire including place of residence, physical characteristics, level of education, and lifestyle. Written informed consent was obtained from each participant. Information about pregnancy complications, placenta weight, the newborn morphometric parameters at birth (weight, height, head circumference), and gestational age were collected from medical records.

This study was approved by the ethical committee of the local Advisory Committee of Biomedical Research in Humans.

2.4. Sample collection

The spraying season group (RG-SS, n = 22) included tissue samples from placentas of rural residents collected from September to December, while the non-spraying season group (RG-NSS, n = 21) included those collected from April to August. Villous placental samples were collected immediately following caesarean delivery. Suitable amount of the sample was obtained from the central area of the maternal side of the placenta because the expression of various components may vary according to the location [35]. For subcellular fractioning, samples were collected in ice-cold Hepes buffer with NaCl 0.85%, pH 7.0, containing 0.11% buthylated hydroxytoluene as an antioxidant and then processed immediately. Subsamples were frozen at −20 °C for carboxylesterase (CE) activity and PG determinations.