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Environmental exposure to organophosphate pesticides: Assessment of endocrine disruption and hepatotoxicity in pregnant women

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ABSTRACT

In utero exposure is the first point of contact with environmental xenobiotics that may affect the maternal-placental-fetal balance. Considering that maternal pathophysiological changes affect intrauterine development, this pilot study was conducted to address how environmental exposure to organophosphate pesticides (OPs) during pregnancy may contribute to maternal endocrine disruption and disturbed hepatic function. A prospective study was carried out with pregnant women ($n=97$) living in a rural area of the Rio Negro province where OPs are intensively applied throughout 6 months of the year. Blood samples were obtained and biomarkers of OPs exposure (cholinesterases and β-glucuronidase), cortisol (CT) and progesterone (PG) levels, as well as glycemia, were determined. Parameters of liver injury were assayed by measuring aspartate aminotransferase (AST) and alanine aminotransferase (ALT); liver function was assayed by measuring albumin. Biomonitoring carried out during the pre-spraying period (PreS) and spraying period (SP) showed that the population studied was exposed to OPs, proven by the fact that plasma (PCh) and erythrocyte cholinesterase (AChE) decreased very significantly ($p<0.01$) during SP. CT values increased very significantly ($p<0.01$) in the first trimester of pregnancy during SP with respect to PreS. Individual values above the upper limit of the CT and PG reference range were found both in PreS and SP. This finding could be associated with changes in hormone metabolism pathways produced by OPs exposure. During the second trimester of pregnancy there were increases in ALT values and the AST/ALT ratio in SP, suggesting subclinical hepatotoxicity. In SP, glycemia was unchanged while albuminemia increased. Although anthropometric newborn parameters and pregnancy alterations were within normal values for the general population, the increase in CT in the maternal compartment may lead to impaired newborn health later in life.

1. Introduction

Organophosphate pesticides (OPs) have been the most widely used group of insecticides for many decades. Although they are essential tools in crops protection and public health they constitute a large source of potential hazard to humans. Exposure may occur in three different scenarios: occupational exposure, environmental exposure for communities living in areas with intensive agricultural production, and dietary exposure of the general population (Mantovani et al., 2008).

The long-term health effects among workers after an acute or chronic low-level exposure to OPs have been studied extensively (Roldán-Tapia and Sánchez-Santed, 2004; Edwards and Tchounwou, 2005). Alterations in hormone levels (Curtis, 2001; Recio et al., 2005; Blanco-Muñoz et al., 2010; Kitamura et al., 2011), biomarkers of liver (Hernández et al., 2006) and kidney function (Khan et al., 2008), as well as hematological parameters (Del Prado-Lu, 2007; Rastogi et al., 2008) have also been reported. Furthermore, according to epidemiological data (Rezg et al., 2010; Slotkin, 2011), there may be an increased risk of type 2 diabetes induction.

Women and children of farm resident families and those living close to application and/or disposal sites have an increased risk of exposure through skin contact and contaminated water, plants, food (Beamer et al., 2009; Gbaruko et al., 2009; Satoh and Gupta, 2010) and house dust (Lu et al., 2000; Coronado et al., 2006). Several authors have focused on children's health from the time of their conception in association to household and agricultural

Abbreviations: AChE, acetylcholinesterase; ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; BG, β-glucuronidase; CT, cortisol; GCs, glucocorticoids; OPs, organophosphates; PCh, plasma cholinesterase; PG, progesterone; SP, spraying period; PreS, pre-spraying period

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exposure to OPs during pregnancy. They found an increased risk of growth restriction (Levario-Carrillo et al., 2004), miscarriage and congenital defects resulting in fetal death (Thomas et al., 1992), as well as altered fetal growth and length of gestation (Souza et al., 2005; Whyatt et al., 2005; Harley et al., 2011).

Although it is well established that maternal health during pregnancy helps ensure a positive outcome in pregnancy, information about the biochemical effects of environmental exposure to pesticides during pregnancy is scarce. Pregnancy is regulated by a complex interplay of signals between the fetus and mother (Mendelson, 2009), and progesterone (PG) and glucocorticoids (GCs) play an important role. PG provides trophic support for placental, and consequently, fetal growth (Mark et al., 2006), and maintains uterine quiescence (Kennedy et al., 2009). GCs are potent inhibitors of fetoplacental growth and yet play a role in the late maturation of fetal organs. In fact, impaired GCs production and HPA axis regulation activity have been associated with altered gestation length and timing of birth (Odermatt and Gumy, 2008). Moreover, the role of typical stress hormones such as GCs may determine the extent of embryonic, placental and maternal responses to PG and prolactin early in pregnancy (Douglas, 2011).

During pregnancy there are profound changes in maternal metabolism. There is a marked drop in insulin sensitivity late in pregnancy, seen by all investigators, and this drop is comparable to that found in type 2 diabetes and obesity. In contrast, the changes seen in insulin sensitivity early in pregnancy are not consistent. Hepatic glucose output in the fasted state increases despite increased fasting insulin concentrations, suggesting that decreased hepatic sensitivity occurs (Lindsay, 2009). There is evidence to suggest that maternal hyperglycemia less severe than that used to define overt diabetes is related to clinically important perinatal disorders (HAPO, 2008) and is associated to fetal hyperglycemia, producing abnormal stimuli for fetal growth (Osorio et al., 1996).

Owing to the immaturity of the fetal liver, the maternal liver and to a lesser extent, the maternal kidney are in charge of biliary compound biotransformation and elimination (Marin et al., 2008). In addition, the maternal liver is involved in xenobiotic detoxification, thus influencing their transfer to the placenta and fetus.

In view of the above information, this study was designed to evaluate the relationship between environmental exposure to OPs during pregnancy and the mothers' blood biochemical and endocrine parameters. Serum cortisol (CT), PG, glycemia and biomarkers of hepatotoxicity and liver function were analyzed in pregnant women living in an area of agricultural exploitation. Moreover, alterations in pregnancy and newborn morphological parameters were recorded. Exposure to OPs was assessed by the determination of blood cholinesterases and β-glucuronidase (BG). Blood cholinesterases comprise AChE and PCh. The activities of both cholinesterases are widely recognized as reference biomarkers to evaluate acute and chronic occupational exposure to OPs and carbamate pesticides (Gil and Pla, 2001; Quandt et al., 2010) as well as environmental exposure to these toxicants (Souza et al., 2005). BG activity is considered a novel biomarker of OPs and carbamate exposure (Satoh and Hosokawa, 2006), which seems to be a very sensitive biomarker at high (Soltaninejad et al., 2007) and low-level occupational OPs exposure (Inayat-Hussain et al., 2007; Ueyama et al., 2010). However, there is no information available about BG activity in studies concerning OPs environmental exposure during pregnancy.

2. Methods

2.1. Subjects and study design

We performed a prospective study of 97 healthy pregnant women, between the ages of 15 and 36, entering prenatal care at the Allen Public Hospital, province of Rio Negro, Argentina, from November 2007 to August 2008. They belonged to a population living in small towns near farms located in the Upper Valley of the Negro River, the major site of pear and apple production in Argentina, situated in the province of Rio Negro in the northern part of Argentine Patagonia. In this fruit cultivation area, insecticides, mainly OPs such as azinphos methyl, phosmet, chlorpyrifos and dimethoate, as well as carbamates like carbaryl and dithiocarbamates such as ziram, captan and mancozeb, are applied for six months a year, during the dry seasons: spring and summer (September to February). Pesticides are usually finely dispersed as droplets or particles at the time of pulverization and aerial drift from the target area is frequent, increasing the potential environmental exposure of the population.

This study was carried out with the full ethical approval of the local Advisory Committee of Biomedical Research in Humans. Consent was obtained at the time of routine blood testing.

A questionnaire was administered to record physical characteristics, reproductive history, educational level and lifestyle habits. Health status was checked by the medical staff. Women with arterial hypertension, gestational diabetes, cholestasis of pregnancy, those on medication (except those included in Group A according to the FDA), consuming alcohol or drugs and those with serious pregnancy complications such as eclampsia, preeclampsia or any other chronic condition were excluded. Information about pregnancy complications and the status of the newborn at birth was collected (weight, length, head circumference and gestational age).

Blood samples collected between November 2007 and February 2008 were considered samples of the spraying period (SP), and those collected between April 2008 and August 2008 were considered samples of the pre-spraying period (PreS).

2.2. Confounding factors and standardization

Given that parameters such as CT (Allolio et al., 1990; Jensen et al., 2002), PG (Vause and Saroya, 2005), albumin (Maher et al., 1993), transaminases (Bacq et al., 1996) and BG (Isaksson et al., 1984) vary throughout gestation, pregnant women with similar weeks of gestation (first trimester PreS: 10.2 ± 2.3, SP: 9.4 ± 2.4; second trimester PreS: 19.5 ± 4.5, SP: 20.3 ± 5.5) were selected during sampling.

CT secretion is affected by circadian rhythms, physical activity, food consumption, smoking, caffeine, alcohol and steroid medication (Nepomnaschy et al., 2006). To reduce the influence of these confounding variables, samples were obtained after an overnight fasting period, early in the morning before participants performed any major physical activity. As 13 percent of women reported that they were current smokers, they were asked not to smoke overnight prior to the sampling. Furthermore, women from both groups (PreS and SP) were matched for smoking habit and alcohol consumption in each trimester of pregnancy (first and second).

2.3. Exposure measures and biochemical determinations

Maternal blood samples from the first and second trimester of pregnancy were obtained by venipuncture at the Allen Public Hospital's laboratory. They were collected in heparinized and EDTA-treated tubes and without an anticoagulant. Heparinized blood samples were analyzed for cholinesterase activity. EDTA-treated samples were analyzed for red cell count in a Cell-Dyn 1400 hematology analyzer. Although AChE and PCh can be used as sensitive biomarkers to detect exposure to OPs pesticides, a key limitation of using PCh for the assessment of exposure is the high inter-individual and even intra-individual variations in enzyme activity (Hernández et al., 2006). Therefore, we measured both AChE and PCh activities at 30 °C following the method by Voss and Sachsse (1970). A blank was added to every subject's blood. AChE activity was normalized by red blood cell count and expressed as nmoles of hydrolyzed substrate $\times$ min^{-1} $\times$ millions^{-1} of erythrocytes. ChP activity was expressed as nmoles of hydrolyzed substrate $\times$ min^{-1} $\times$ μL^{-1} of whole blood. Peyster et al. (1994) showed that in control pregnant women blood cholinesterases did not change in the first and second trimester but diminished in the third trimester, therefore we compared PreS and SP of first and second trimester taken together.

Serum was separated immediately by low-speed centrifugation. Aliquots were stored at −20 °C until analysis for BG activity, which was performed within one week as it remains unchanged in this period (as was previously verified in our laboratory). Aliquots for hormones assays were also stored at −20 °C and analyzed within one week.

CT and PG were determined by electrochemiluminescence and expressed as μg/dL and μg/L, respectively. Transaminase activity, aspartate amine transferase (AST) and alanine amino transferase (ALT), as well as albumin, were determined using Wiener Laboratory, S.A. kits (Rosario, Argentina) and were expressed as U/L. Serum AST and ALT were determined by kinetic assays and albumin, glucose and β-glucuronidase (BG) were measured by colorimetric methods. Results were expressed as g/L and U/L, respectively.

2.4. Data analysis

Categorical variables (education, smoking status, alcohol consumption, groundwater consumption, indoors pesticides use) were compared using the Pearson's chi-