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Androgen disruption by dioxin exposure in 5-year-old Vietnamese children: Decrease in serum testosterone level

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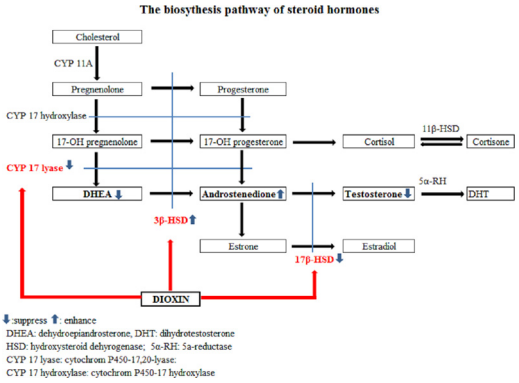
HIGHLIGHTS

- The cohort study has been conducted in one of three dioxin hotspot areas in Vietnam.
- Seven serum steroid hormones were analysed from 5-year-old children in the hotspot.
- DHEA and T levels were significantly lower in the hotspot.
- The A-dione level was significantly higher in the hotspot.
- DHEA, A-dione, and T significantly correlated with dioxin levels in breast milk.

GRAPHICAL ABSTRACT



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ABSTRACT

Dioxins have been suspected to be potential substances causing endocrine disruptions in humans. We are conducting the research in one of three dioxin exposure areas (hotspots) in Vietnam. We previously reported that the salivary dehydroepiandrosterone (DHEA) level decreased in 3-year-old Vietnamese children and that it was significantly inversely correlated with polychlorinated dibenzodioxin/dibenzofuran levels in their mother's breast milk. In this study, we investigated the influence of exposure to dioxin on steroid hormone biosynthesis in the same children when they reached 5 years of age, focusing on androgens. Thirty-five and 50 mother–child pairs from dioxin hotspot and non-sprayed areas, respectively, participated in this study. Maternal

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breast milk was donated at 4 to 16 weeks postpartum in 2008 to measure dioxin levels by gas chromatography/high-resolution mass spectrometry. Serum was collected from 5-year-old children in 2013. Seven steroid hormones were measured by liquid chromatography/mass spectrometry. Most dioxin congeners in breast milk were 2- to 10-fold higher in the hotspot than in the non-sprayed area. DHEA and testosterone (T) were significantly lower in the hotspot and showed negative correlations with most dioxin congeners. Similar results were observed for the activities of cytochrome P450-17, 20 lyase (CYP17 lyase), and 17β-hydroxysteroid dehydrogenase (HSD). Conversely, the elevated androstenedione (A-dione) level and 3β-HSD activity in children from the hotspot were positively correlated with dioxin levels. Moreover, a positive correlation was shown between T and 17β-HSD. It is possible that dioxin inhibits 17β-HSD activity, leading to a decrease in the T level. Multiple regression analysis indicated that dioxin had a strong association with the DHEA, A-dione, and T levels. In conclusion, the present study suggests that dioxin is associated with low levels of DHEA and T and inhibition of the activity of steroidogenic enzymes such as CYP17 lyase and 17β-HSD in 5-year-old children.

1. Introduction

Endocrine disrupting chemicals (EDCs) have been described as human-made substances that alter hormone regulation in humans and wildlife. EDCs include many synthetic chemicals such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polycyclic aromatic hydrocarbon, polychlorinated biphenyls, pesticides, phthalates, perfluorooctanoate, and bisphenol A. Humans were exposed to dioxin through contamination from Agent Orange during the Vietnam War and through the industrial explosion in the Seveso disaster in Italy. Both incidents resulted in high and long-term dioxin exposure in humans. Dioxins are lipophilic and highly stable, have half-lives ranging from 7 to 11 years in humans, and are excreted in breast milk (Kreuzer et al., 1997; Schecter and Gasiewicz, 2003). The main source of human exposure to dioxin is consumption of contaminated food. During pregnancy, some dioxins may pass from the mother to the foetus via the placenta and umbilical cord (Schecter et al., 1990). After birth, infants can receive dioxin from their mother by drinking breast milk (Schecter, 1991; Suzuki et al., 2005; Wang et al., 2004). Moreover, the dioxin level in breast milk is 50% to 70% of the starting level after breastfeeding for 12 to 23 months (Abraham et al., 1996; Fürst et al., 1989; Schecter et al., 1998). In one study, the total toxic equivalent (TEQ) of dioxin levels in the blood of breast-fed infants was 10 times higher than that of formula-fed infants at 11 months of age (Abraham et al., 1996). Patandin et al. (1999) reported that breastfeeding for 6 months can contribute 12% and 14% to the body burdens of dioxin in men and women, respectively, at the age of 25 years.

Early-life exposure to dioxin (in utero and via breastfeeding) can cause various adverse health effects in children. Numerous published literatures have stated that perinatal exposure to dioxin and/or PCBs correlated with intrauterine and postnatal growth retardation, immunotoxicity (a greater susceptibility to infectious diseases), and neurotoxicity (neurodevelopmental deficit, modified behavioural sexual dimorphism, and subtle cognitive and motor development delays) in subsequent generations (Brouwer et al., 1999; Nishijo et al., 2012; Tran et al., 2016; Vreugdenhil et al., 2002; Weisglas-Kuperus et al., 2000; Winneke et al., 2005; Winneke et al., 2014). Recently, perinatal exposure to EDCs has been linked to hormone alterations and abnormal reproductive outcome at birth (Cao et al., 2008; Goudarzi et al., 2017; Rennert et al., 2012; Sathyanarayana et al., 2017). Nevertheless, few human epidemiological studies have focused on whether lactational or food-chain exposure to dioxin can disrupt steroid hormones in children.

Since 2008, we have conducted cohort epidemiological studies in the dioxin hotspot areas in Vietnam to determine the levels of PCDD and PCDF congeners in maternal breast milk and steroid hormones in mothers and their children. Our previous reports presented significant differences in dioxin congener levels in breast milk and steroid hormone concentrations in mothers between the hotspots and the non-sprayed area (Kido et al., 2014; Nhu et al., 2010). The frequency of low birth weight was significantly higher in the hotspot than in the non-sprayed area, and this difference was associated with the dioxin congener levels and high glucocorticoid levels in mothers (Van Tung et al., 2016). Moreover, disturbances of dehydroepiandrosterone (DHEA) synthesis were also observed in children from the hotspot area. The salivary DHEA level increased at 1 year of age and decreased at 3 years of age. This DHEA disruption was associated with dioxin congener levels in maternal breast milk (Anh et al., 2017; Kido et al., 2016). These results suggest that exposure to dioxin during breastfeeding continuously affects growth and steroidogenesis in children.

In the present study, to elucidate hormone disruption by dioxin in 5-year-old children (the third generation from the time of herbicide/dioxin spraying), we collected serum instead of saliva for hormone analysis. We first measured seven steroid hormone concentrations in serum and calculated from serum hormone levels the activity of enzymes involved in androgen hormone biosynthesis. From these results, we attempted to elucidate the target enzymes of dioxin and the mechanism of androgen disruption in children.

2. Subjects and methods

2.1. Study areas

The study area was Phu Cat district, located in Binh Dinh province. It was formerly a United States Airbase and was used for herbicide storage, supply facilities, and washing of aircrafts during the Vietnam War (1965–1971). Phu Cat was one of three areas characterised by elevated levels of dioxin contamination, known as “hotspots” in Vietnam (Dwernychuk, 2005).

Kim Bang district, located in Ha Nam province, was chosen as the non-sprayed area in this study because this area was not exposed to herbicides in the war.

The hotspot and non-sprayed areas are agricultural areas; therefore, they have been not contaminated by industrial pollution.

2.2. Study participants and sample collection

Lactating mothers (60 from Phu Cat and 63 from Kim Bang) participated in this study beginning in September 2008. These mothers were breastfeeding their 4- to 16-week-old infants and had resided in the area for >5 years. After the medical staff had explained the purpose of the study, all participants agreed to donate 10 to 20 mL of milk samples. They also underwent measurement of anthropometric indicators (height, weight) and were interviewed about their age, family income, residence period, occupation, education, disease, smoking, drinking, and any therapy related to hormone metabolism. The detailed demographics of the mothers were reported in a previous study (Kido et al., 2014).

Their children were followed up until reaching the age of 5 years in 2013. Thirty-six of 60 original children in the hotspot and 51 of 63