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Inverse association of highly chlorinated dioxin congeners in maternal breast milk with dehydroepiandrosterone levels in three-year-old Vietnamese children

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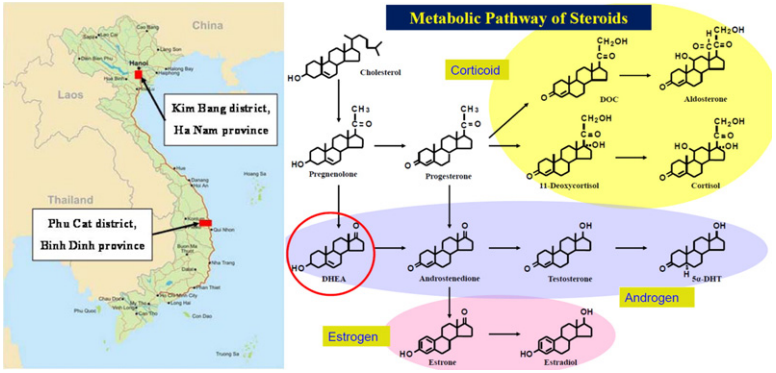
HIGHLIGHTS

- Dioxin levels in breast milk were higher in the hotspot than the non-exposed region.
- Salivary steroid hormones were analyzed from 3-year-old children of these mothers.
- DHEA levels were significantly lower in the hotspot than in the non-exposed region.
- DHEA levels were inversely correlated with highly chlorinated dioxin congeners.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aims to evaluate the endocrine-disrupting effect of dioxin congeners on adrenal steroid hormones in mother–child pairs. In our previous study, we found that cortisol and cortisone levels were higher in the blood and the saliva of mothers living in a dioxin hotspot area than in mothers from a non-exposed region in Vietnam. In this follow-up study, we determined the salivary steroid hormone levels in 49 and 55 three-year-old children of these mothers in the hotspot and non-exposed region, respectively. Steroid hormones were determined by liquid chromatography–tandem mass spectrometry, and dioxin in the maternal breast milk was determined by gas chromatography–mass spectrometry. Dioxin levels in the breast milk of mothers from the hotspot (median total toxic equivalents polychlorinated dibenzodioxins/polychlorinated dibenzofurans; (TEQ PCDD/Fs) of 11 pg/g lipid) were three to four times higher than those of mothers in the non-exposed region (median TEQ PCDD/Fs of 3.07 pg/g lipid). Salivary dehydroepiandrosterone (DHEA) levels in children were found

Abbreviations: DHEA, dehydroepiandrosterone; F, cortisol; E, cortisone; A-dione, androstenedione; OCDD, octachlorodibenzodioxin; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; CYP17, cytochrome P450C17; LC–MS/MS, liquid chromatography–tandem mass spectrometry; GC–MS, gas chromatography–mass spectrometry.

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to be significantly lower in the hotspot than in the non-exposed region, while cortisol and cortisone levels were not different between the two regions. Highly chlorinated dioxin congeners, such as octacholorodibenzodioxin (OCDD), 1,2,3,4,6,7,8-heptacholorodibenzodioxin (HpCDD) and 1,2,3,4 (or 6), 7,8-hexachlorodibenzodioxin Hx(CDD), showed stronger inverse associations with the children's salivary DHEA than other lowly chlorinated dioxin congeners. Glucocorticoid levels in the mothers exhibited a significantly positive correlation with OCDD and HpCDD/F (polychlorinated dibenzofurans). In conclusion, highly chlorinated dioxin congeners are more strongly correlated with endocrine-disrupting effects on adrenal hormones, resulting in high cortisol levels in the mothers and low DHEA levels in their three-year-old children.

1. Introduction

Dioxin (polychlorinated dibenzodioxins, polychlorinated dibenzofurans) is one of the most toxic chemical substances known and is a persistent environmental contaminant. It can be released into the environment as a by-product of various chemical manufacturing and combustion processes.

Dioxin involves a number of isomers and congeners with a dibenzo-*p*-dioxin, dibenzofuran or biphenyl skeleton, and different numbers of chloride atoms, with the toxic potency differing markedly from one isomer to the next. As such, and to allow for a simple evaluation of their hazards to health, the toxic equivalency factor (TEF) was established and has been widely used for some time (Berg et al., 2006). Although dioxin was suspected to cause endocrine disruption for a long time, very few epidemiological studies were carried out on its effects on the steroid hormone biosynthesis in humans (Nhu et al., 2011; Manh et al., 2013; Kido et al., 2014; Sun et al., 2014). In our previous research on women from a dioxin hotspot region in Vietnam, the salivary and serum levels of six steroid hormones, including sex hormones, were simultaneously determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Kido et al., 2014). The results of that study demonstrated that the levels of cortisol (F) and cortisone (E) were higher in the hotspot than in a non-exposed region, and these hormone levels were positively associated with dioxin concentrations in breast milk. Furthermore, we found saliva to be a useful matrix for hormone assays in epidemiological studies.

There are two main contaminated regions in the world as a result of dioxin exposures with one in Southern Vietnam and the other at Seveso in Italy (Stellman et al., 2003; Warmer et al., 2011). Although many Vietnamese were exposed to herbicide/dioxin to a greater extent, most studies concerning adverse health effects have been carried out on American veterans (Giri et al., 2004). Large numbers of residents in Southern Vietnam have been known to suffer from adverse health effects as a result of herbicide/dioxin exposure. Similarly, dioxin levels in human milk were found to be higher than 950 pg/g lipid at the end of the war in 1970 (Schecter et al., 1995). Current levels in the sprayed region of Vietnam are much lower (0.2–0.5%) due to the wash-off by tropical rain and chemical breakdown over the 40 years since spraying ceased (Schecter et al., 1991; Manh et al., 2014). However, levels are still three to five times higher in breast milk and serum from residents in and around the three former US air bases (Bien Hoa, Da Nang and Phu Cat) than in non-exposed regions (Manh et al., 2014; Hue et al., 2014; Thuong et al., 2014; Pham et al., 2015). In addition to direct exposure from soil, indirect exposure is known to occur as a result of apparent food-chain transfer of dioxins to humans. This is a particularly important source of exposure for the health of babies fed with maternal milk on a daily basis. Like other endocrine-disrupting chemicals, dioxin is suspected to have an effect on human hormones at low doses (Vandenberg et al., 2012). Indeed, the adverse effects such as cancer, diabetes, immunosuppression and neurotoxicity associated with dioxin exposure may be considerably mediated by alterations to endocrine function (Huisman et al., 1995; Diamanti-Kandarakis et al., 2009; Miyashita et al., 2011).

Recent human studies have shown that high circulating levels of maternal cortisol during pregnancy correlate negatively with birth weight, thereby suggesting that excess glucocorticoids can cross the placental barrier (Braun et al., 2013; Reynolds, 2013). Similarly, an increase in the frequency of low birth weights was found to be associated with high dioxin concentrations in the milk and blood of mothers from Japan (Tawara et al., 2009; Konishi et al., 2009). It is also very important to monitor the development from child to adult as intrauterine growth retardation or a low birth weight have been linked to a late onset of diseases such as cardiovascular disease and type 2 diabetes in adulthood (Pinney et al., 2011). These concepts have led to the developmental origin of health and disease (DOHaD) hypothesis (Pinney and Simmons, 2009). As such, endocrine-disrupting chemicals may affect both exposed individuals and their children and subsequent generations.

In this study, we focused on the adrenal hormone levels of mother–child pairs and elucidated the dioxin effects on the steroid biosynthesis pathway. As it is difficult to obtain blood samples from infants in epidemiological studies, we have developed a simple technique for collecting saliva from children and determining the hormone levels by LC–MS/MS.

The first aim of this study was to determine the adrenal hormone levels in three-year-old children and to compare the results for their mothers in the previous report (Kido et al., 2014). Then, any hormone relations among these mother–child pairs will be identified.

The second aim was to identify which dioxin congeners were associated with adrenal hormone variations in the mothers and their children. In the previous report, we only reported the total TEQ of PCDD/Fs; therefore in this report, we further describe the relation of each dioxin congener to the hormone levels. Cytochrome P450C17 (CYP17) has two catalytic actions, 17a-hydroxylase and 17,20-lyase, on the steroid (pregnane) and plays a role in the turning point into androgen and corticoid biosynthesis (Li and Wang, 2005). We therefore note that the ratio of androgen (C19 steroid)/corticoid (C21steroid) can reflect the two enzymatic activities.

2. Subjects and methods

2.1. Study region

2.1.1. Agent orange/dioxin hot-spot

The dioxin hot-spot selected was Phu Cat air base, where chemical herbicides were stored during the Vietnam War and the aircraft used to spray

Agent Orange/dioxin were washed (Manh et al., 2014). The Phu Cat district is located in Binh Dinh province and is one of the three representative dioxin hotspots in South Vietnam (Manh et al., 2014; Hue et al., 2014; Pham et al., 2015). Records show that approximately 17,000 drums of Agent Orange, 9000 drums of Agent White and 2900 drums of Agent Blue were stored at Phu Cat (Young, 2008).

2.1.2. Control region

The non-exposed region selected as the control region was the Kim Bang district in Ha Nam province in the north of Vietnam, which was not exposed to chemical herbicides during the war and has not been affected by industrial pollution (Manh et al., 2014).