Science of the Total Environment 595 (2017) 842–848

Contents lists available at ScienceDirect

Science of the Total Environment

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

The relationship between dioxins exposure and risk of prostate cancer with steroid hormone and age in Vietnamese men

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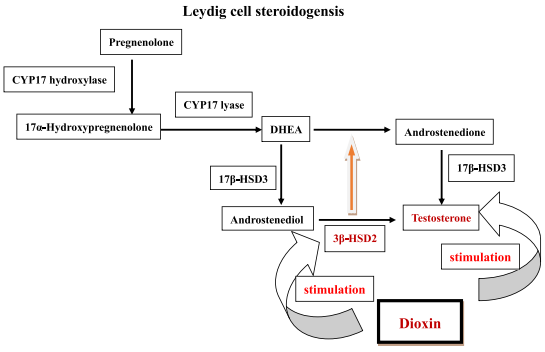
HIGHLIGHTS

- In the hotspot group, sex hormone levels were significantly increased with age.
- Mean 3β-HSD activity levels were higher in the hotspot than the non-sprayed group.
- In the hotspot group, 3β-HSD activity levels were significantly increased with age.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 November 2016
Received in revised form 1 April 2017
Accepted 2 April 2017
Available online xxxx

Editor: H. Hollert

Keywords:
Polychlorinated dibenzodioxins
Polychlorinated dibenzofurans

ABSTRACT

Although Vietnam's massive herbicide exposure in 1960s and 1970s was clearly injurious to health, not all causal relationships have been clarified. We therefore explored associations among dioxins, steroid hormones, age and prostate cancer risk in men. We compared serum levels of dioxin, steroid hormones and prostate specific antigen (PSA) in men aged 56–81 years from herbicide-exposed hotspots ($n = 50$) with those from non-sprayed regions ($n = 48$). Mean serum levels of dioxin congeners in the hotspot group were 1.5–11.3 times higher than the non-sprayed group depending on specific compound. Levels of testosterone, estradiol and 3β-hydroxysteroid dehydrogenase (3β-HSD) activity in the hotspot group were also significantly higher than in non-sprayed group. Estradiol levels were significantly related to levels of several specific dioxin derivatives in both group. Significant positive correlations were also found between DHT and 1234678-HpCDD or 1234678-HpCDF; and between 3β-HSD activity and 123678-HxCDD, 123478-HxCDF, 123678-HxCDF, or HxCB#169. After adjusting for age,

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http://dx.doi.org/10.1016/j.scitotenv.2017.04.013

Testosterone
3β-Hydroxysteroid dehydrogenase activity
Hotspot

body mass index, and tobacco use, multiple linear regressions showed levels of dihydrotestosterone (DHT), estradiol, testosterone and 3β-HSD activity were not associated with dioxins in the two groups; however, levels of DHT, testosterone and 3β-HSD activity increased significantly with age in the hotspot group. The hotspot and non-sprayed groups did not significantly differ in PSA levels. But six of the hotspot subjects had PSA levels >3 ng/mL, 3 of whom were suspected to have prostate cancer (PC) after digital rectal examination. Our findings suggest that dioxin exposure can lead to increased levels of several sex steroid hormones with age. The correlation of dioxin with steroid hormone levels and prostate cancer risk should be studied further.

1. Introduction

Dioxin and dioxin-like compounds comprise the chemical class of polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs). As environmental pollutants, these highly toxic compounds have well-established harmful effects in animals and humans.

During Operation Ranch Hand (1962–1971), the United States Air Force sprayed approximately 107 million lbs. of herbicides in the south of the former Demilitarized Zone at the 17th parallel for defoliation and crop destruction. The best-known herbicide was Agent Orange (AO), which is 50:50 mixture of n-butyl esters of 2,4-dichlorophenoxyacetic acid (2,4D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5T); the 2,4,5T was also contaminated with varying levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Stellman et al., 2003). Dioxins can cause reproductive and developmental problems, damage the immune system, interfere with hormone signaling and cause cancer (WHO, 2010). In 1998, the U.S. National Academy of Sciences concluded that "limited/suggestive evidence" indicated an association between AO and prostate cancer (PC) (Institute of Medicine, 1998). In the largest study to date ($N = 13,144$), United States Vietnam veterans who reported exposure, AO was found to double the risk of developing PC, particularly its most aggressive form, compared with those who were not exposed (Chamie et al., 2008). Several other recent studies of Vietnam veterans also support the association of AO exposure to increased risk of PC (Ansbaugh et al., 2013; Yi and Ohrr, 2014). Li and Wang found that the PCB congener PCB-126 induced differential changes in androgen, cortisol, and aldosterone biosynthesis in human adrenocortical H295R cells (Li and Wang, 2005). As androgens are fundamental for the development and maintenance of the prostate gland and play a key role in PC biology, a long-standing hypothesis has been that circulating hormone levels affect PC risk (Lindstrom et al., 2010).

During the war, many Vietnamese people and Vietnam veterans were heavily exposed to dioxins, which is now manifesting as health problems, including skin disorders, liver damage and adverse reproductive effects (Constable and Hatch, 1985; Tamburro, 1992; Wolfe et al., 1995; Institute of Medicine, 2008). We had found breast milk and serum dioxins levels were significantly higher in residents of hotspot regions than in those from non-sprayed regions (Kido et al., 2014; Manh et al., 2014; Manh et al., 2015; Kido et al., 2016); and that salivary and serum adrenal steroid hormone levels in Vietnamese women and their children were associated with breast-milk dioxins levels (Kido et al., 2014; Kido et al., 2016). However, few studies have addressed effects of dioxins exposure on steroid hormone or PC in Vietnamese men (Sun et al., 2014; Sun et al., 2016). Here, we explored associations among dioxins, steroid hormones and PC risk with age in Vietnamese men.

2. Subjects and methods

2.1. Study areas

Phu Cat airbase, a dioxins hotspot located in Binh Dinh Province, was a former United States airbase during the Vietnam War (Manh et al., 2014).

The control region is a non-sprayed region in the Kim Bang district in Ha Nam Province, in the northern part of Vietnam. The control region was not sprayed with chemical herbicides during the war. Both the hotspot and the control region are rural and there is no industrial pollution nearby (Manh et al., 2014).

2.2. Study population

The study subjects included 50 and 48 men, from the dioxins hotspot and the non-sprayed region, respectively. All men aged over 50 residing in three areas located in and around the airbase were recruited by local hospital staff in 2009 and 2011. Ninety-seven men consented to participate. Among them, 50 subjects were selected at random for analysis of steroid hormone levels. In the non-sprayed region, all male residents over 50 were recruited similarly, and 85 men agreed to participate. Of those, 48 men were selected randomly. In August 2009 and August 2011, blood (10 mL each) was collected from the men at 8:00–10:30 a.m. Serum samples were obtained by centrifuge, then stored in a cooling box and frozen in dry ice for several days. All samples were transported to Japan for analysis. The serum samples were stored at −70 °C until analysis. Levels of PSA and steroid hormones were determined for all subjects; levels of dioxins congeners were determined for 48 men from the dioxins hotspot and 36 men from the non-sprayed region.

This study was approved by the Medical Ethics Committee of Kanazawa University.

2.3. Measurement of serum hormone estimation by LC-MS/MS

Serum steroid analysis was carried out by LC-MS/MS (API-4000 Applied Biosystems, MDS SciEx, Toronto, ON, Canada) as described previously (Kido et al., 2014). Briefly, serum (200 μL) was diluted with purified water to a volume of 1.0 mL, and cortisol-$^{2}H_4$ (1 ng), dihydroepiandrosterone (DHEA)-$^{2}H_4$ (100 pg), testosterone-$2H_3$ (100 pg), progesterone-$^{13}C_3$ (100 pg), estrone-$^{13}C_4$ (100 pg), and estradiol-$^{13}C_4$ (100 pg) were added as internal standards. After extraction with ethyl acetate, the extract was applied onto a cartridge column (C18) to removed impurities. The obtained purified fractions were derivatized with picolinic acid according to the procedure described by Yamashita et al. (Yamashita et al., 2009). The reaction mixture was then applied onto an interSept pharm cartridge column to remove excess reagents. Levels of nine hormones were simultaneously determined by LC-MS/MS. The lowest estimation levels per assay were 50 pg for cortisol; 50 pg for cortisone; 5 pg for DHEA; 1 pg for testosterone; 1 pg for dihydrotestosterone (DHT); 10 pg for progesterone; 10 pg for androstenedione (A-dione); 1.0 pg for estrone, and 0.5 pg for estradiol. Both the accuracy and precision in inter- and intra-day assays were within ±20% of the lowest level and within ±15% for concentrations other than the lowest concentration.

Enzyme activities were calculated from serum steroid levels using the formulas below:

$$5\alpha\text{−reductase activity } (\%) = \text{DHT/testosterone}$$