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Impacts of prenatal triclosan exposure on fetal reproductive hormones and its potential mechanism

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ABSTRACT

Background: Triclosan (TCS) has been widely detected in pregnant women. The reproductive endocrine-disrupting effects of TCS have been observed in humans and animals. Little is known about the potential impact of prenatal TCS exposure on fetal reproductive development as well as its potential mechanism.
Objectives: We investigated the potential effect of prenatal TCS exposure on fetal reproductive hormones in cord blood and its potential mechanism in relation to placental steroidogenic enzymes.
Methods: Urinary TCS was detected among 537 healthy pregnant women from a prospective cohort in China. Four reproductive hormones in cord blood, namely E_2 (n = 430), T (n = 424), LH (n = 428) and FSH (n = 373), and three steroidogenic enzymes in placenta, namely *P450arom* (n = 233), *3β-HSD* (n = 227) and *17β-HSD* (n = 222), were measured.
Results: Prenatal TCS exposure was associated with increased testosterone concentrations in cord blood in a dose-dependent manner. Infants with prenatal TCS levels > 0.6 μg/L had, on average, a 0.23 ng/mL (95% CI: 0.05, 0.45, p = 0.02) higher testosterone concentrations in cord blood compared to those with prenatal TCS levels < 0.1 μg/L. Of note, prenatal TCS exposure was associated with increased testosterone and decreased E_2 concentrations in cord blood among male infants. Adverse associations were found between the prenatal TCS exposure and concentrations of three placental steroidogenic enzymes. *3β-HSD* and *P450arom* demonstrated mediating effects in the association between prenatal TCS exposure and testosterone concentrations in cord blood.
Conclusions: Our findings suggested potential impacts of prenatal TCS exposure on reproductive hormones in cord blood mediated by steroidogenic enzymes, and male infants were more vulnerable.

1. Introduction

Triclosan (TCS), a broad-spectrum phenol used in personal care products (PCPs), has been widely detected in the natural environment and human body (U.S. CDC, 2017). The compound has already been listed as an endocrine disrupting chemical (EDC) (WHO-UNEP, 2012) and reproductive endocrine-disruption is among the effects of most concern (Wang and Tian, 2015).

Fetuses are especially vulnerable to EDCs. Up to now, limited and inconsistent evidence is available regarding the potential effects of prenatal TCS exposure on the fetus. The Odense Child Cohort from Denmark reported an association between the prenatal TCS exposure and reduced anogenital distance (AGD) in boys at three months of age, indicating potential reproductive disruption in male fetuses (Lassen et al., 2016). Two in vivo studies reported that prenatal TCS exposure disrupted normal fertility and/or viability of early stage embryos (Crawford and Catanzaro, 2012; Geiß et al., 2016). Given the increasing reports on the wide detection of TCS in the urine of pregnant women as well as in human amniotic fluid (Meeker et al., 2013; Mortensen et al., 2014; Philippat et al., 2013), the potential health effect of prenatal TCS exposure on fetuses has become a matter of growing concern.

Reproductive hormone concentrations are among the most popular endpoints for assessing the developmental and reproductive toxicity of EDCs (Johnson et al., 2016). Two in vivo studies reported that TCS exposure disrupted the levels of reproductive hormones (Kumar et al., 2009; Zorrilla et al., 2009). However, no study has evaluated the

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potential effects of prenatal TCS exposure on fetal reproductive development by measuring reproductive hormones and determining the potential mechanisms.

An increasing number of studies indicate that the placenta is the potential target of prenatal exposure of EDCs (Cantón et al., 2008). Recently, an in vivo study reported obvious bioaccumulation of TCS in the placenta as well as a dramatic decrease in the reproductive hormones among pregnant rats exposed to high doses of TCS (Feng et al., 2016). During pregnancy, reproductive hormones essential for fetal reproductive development are produced in the placenta, where a series of steroidogenic enzymes are involved (Costa, 2016). It is well known that fetal reproductive hormone synthesis is catalyzed by placental steroidogenic enzymes, which have been proved to be closely related to the fetal reproductive hormone levels (Hill et al., 2010). Disruption of the process of steroidogenesis, especially steroidogenic enzyme activities and expressions, has been regarded as one of the key modes of action of TCS as an EDC (Wang and Tian, 2015; WHO-UNEP, 2012). However, little is known about the effect of prenatal TCS exposure on placental steroidogenesis in humans as well as its role in fetal endocrine disruption. The aim of the present study was to investigate the potential effect of prenatal TCS exposure on fetal reproductive development in view of the reproductive hormones in cord blood and its potential mechanism in relation to placental steroidogenic enzymes in a birth cohort from northern China.

2. Methods

2.1. Participants and study design

The study population was from a birth cohort, named the Laizhou Wan Birth Cohort (LWBC), in the southern coastal area of Laizhou Wan (Bay) of the Bohai Sea, Shandong Province, China, as previously described (Chen et al., 2015). Healthy pregnant women were recruited when they were admitted to the local hospital for delivery. Eligibility criteria were described elsewhere (Chen et al., 2015). In total, 773 pregnant women participated in the study from September 2010 to December 2013. After excluding 236 cases without sufficient urine sample for TCS analysis, 537 women were regarded as the study population in the present study. No substantial differences were found in sociodemographic characteristics between the baseline population (n = 773) and the study population (n = 537) (Supplementary Table 1). The research was approved by the Medical Ethics Committee of Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine.

2.2. Urinary TCS measurement

Spot urine from pregnant women was collected during their hospital admission for delivery and was aliquoted and stored at − 80 °C until further analysis. The total (free and conjugated) urinary TCS concentrations were simultaneously measured (Zhao et al., 2015). Four milliliters of urine was incubated (37 °C) in a 1 M ammonium acetate buffer solution (pH = 5.0) with β-glucuronidase/sulfatase (type H-1 from *Helix pomatia*, 20,000 units/mL, Sigma-Aldrich, St. Louis MO, USA) overnight for hydrolysis. The hydrolyzed compounds were extracted and pre-concentrated with solid phase extraction (SPE, 500 mg/3 mL, Supelclean, Sigma-Aldrich). After drying, the residue was redissolved in methanol and further analyzed by UPLC-MS/MS (Agilent 1290-6490, Agilent Technologies Inc., USA). The limit of detection (LOD) was 0.1 μg/mL and solid phase extraction (SPE) recovery was 76.9%, which was higher than those reported by previous studies (62%–66%) (Ye et al., 2008, 2005; Zhou et al., 2012) and indicated adequate sensitivity. The TCS concentration was linear over the range of 0.1–50 ng/mL (r^2 = 0.999). Each analytical batch consisted of standard samples, blank controls, and quality controls. All relative standards of intra and inter batch precisions were < 15%. Urinary creatinine concentrations were analyzed with an automated chemistry analyzer (7100 Hitachi Medical Systems, Tokyo, Japan).

2.3. Reproductive hormone analysis

Cord blood samples were collected from an umbilical vein immediately following baby delivery using a syringe and two 10-mL tubes, allowed to clot, and centrifuged at 1500 rpm for 20 min to collect serum, which was decanted into pre-cleaned glass vials and stored at − 80 °C until analysis. Serum concentrations of four hormones, estradiol (E_2), testosterone (T), luteinizing hormone (LH) and follicle stimulating hormone (FSH) were separately measured via a radioimmunoassay (RIA) using RIA kits (Beijing North Institute of Biotechnology, Beijing, China). The sensitivities of the assays were 2 pg/mL, 0.02 ng/mL,1.0 mIU/mL and 1.0 mIU/mL for E_2, T, LH and FSH respectively. The intra-assay and inter-assay coefficients of variation of the four kits were < 10% and 15% for all kits. The cord blood samples did not provide enough material to measure all four hormones, therefore, assays were performed in the following order of priority, E_2, T, LH and FSH according to the importance of the hormones in TCS exposure (Feng et al., 2016; Kumar et al., 2009; Zorrilla et al., 2009). Besides, six samples were further excluded in the assay for T due to readings < the lower limit of quantification (LLOQ). Finally, there were 430, 424, 428, and 373 cases with measurements of E_2, T, LH, and FSH levels respectively (Supplementary Fig. 1).

2.4. Steroidogenic enzymes analysis

Placenta tissue samples were collected within 30 min after delivery. The placenta was divided into four quadrants with the umbilical cord as the core. Tissue samples were cut with sterile scissors from the center of each quadrant. Each tissue sample, with a general dimension of each sample of approximately 1.0 ∗ 1.0 ∗ 3.0 cm, was deposited into an EP tube and stored at − 80 °C until analysis.

We selected three steroidogenic enzymes based on the literature (Adibi et al., 2010; Kumar et al., 2009; Wu et al., 2012), namely human cytochrome P450-family19-subfamily A-polypeptide 1 (*CYP19A1* or *P450arom*), human 3 beta hydroxysteroid dehydrogenase type 1 (*3β-HSD*), and human 17 beta hydroxysteroid dehydrogenase type 1 (*17β-HSD*), and confirmed that all of the three enzymes were detectable in placenta with ELISA kits (Bluegene, China) in a pilot study (data not shown).

A piece of maternal-side placenta tissue of approximately 0.5 g was cut for analysis. After the samples were rinsed in ice-cold PBS (0.02 mol/L, pH 7.2) and weighted, the tissues were homogenized and further centrifugated (15 min, 3000 rpm) for analysis with ELISA Kits (Bluegene, China). The standard curve ranges were 0–50 ng/mL, 0–100 ng/mL, and 0–100 ng/mL for *P450arom*, *3β-HSD* and *17β-HSD* respectively. The intra-assay and inter-assay coefficients of variation of the three kits were < 10% and 15% for all kits. We measured the placental steroidogenic enzyme concentrations for the 1st and 4th quartile samples according to maternal urinary TCS levels (n = 253). Finally, after excluding readings below LLOQ, there were 233, 227, and 222 cases with measurements of *P450arom*, *3β-HSD*, and *17β-HSD*, respectively (Supplementary Fig. 1).

2.5. Statistical analysis

Because the distributions of the urinary TCS levels (both non-creatinine-adjusted and creatinine-adjusted), reproductive hormone concentrations in cord blood and placental steroidogenic enzyme concentrations were skewed, the median and quartiles were calculated.

The associations between the prenatal TCS levels and reproductive hormone concentrations in cord blood were analyzed by generalized linear models (GLM). The prenatal TCS levels were transferred to tertile groups with levels < LOD assigned to the reference group, and all