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Prenatal pyrethroid insecticide exposure and thyroid hormone levels and birth sizes of neonates

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HIGHLIGHTS

- We examined relationship between neonatal fT4 and TSH and maternal pyrethroid exposure.
- The relationship between pyrethroid exposure and neonatal body size was also exploired.
- Urinary metabolite (3-PBA) level was used as a biomarker of pyrethroid exposure.
- Multiple regression analysis did not extract urinary 3-PBA as significant for neonatal fT4 and TSH.
- Maternal urinary 3-PBA was a significant predictor of birth size and head circumference.

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ABSTRACT

Pyrethroid insecticides have been shown to possess thyroid hormone disrupting properties in previous animal studies. In this study, the relationship between maternal exposure to pyrethroid insecticides during pregnancy and neonatal thyroid hormone status (free thyroxine (fT4) and thyroid stimulating hormone (TSH) in whole blood) and birth sizes were explored in 147 mother–neonate pairs in Tokyo. The concentration of 3-phenoxybenzoic acid (3-PBA) in maternal urine, sampled in the first trimester of gestation, was used for pyrethroid exposure assessment. Neonatal fT4 and TSH were within the normal range except for one elevated TSH (but normal fT4) in a neonate. Multiple regression analyses with stepwise variable selection did not extract maternal 3-PBA as significant for neonatal fT4 and TSH, indicating that maternal pyrethroid exposure had no apparent effect on the neonatal thyroid hormone status of the neonate subjects. For birth weight and head circumference, maternal 3-PBA was selected as significant with a positive partial regression coefficient along with other factors known to increase birth sizes of neonates (gestational weeks or maternal BMI). It was not clear if this was causal because no biological mechanism was apparent.

1. Introduction

The health effects of prenatal pesticide exposure have been of serious concern to health authorities for some years. Epidemiologic studies of the health effects on neonates and infants of prenatal exposure to organochlorine (OC) and organophosphorus (OP) pesticides are many (Burns et al., 2013); however, those on pyrethroid insecticides (PYR) are still few (Berkowitz et al., 2004; Xue et al., 2013). Pyrethroid insecticides are a major subclass of insecticides used in residential settings and in agriculture, forestry, and horticulture worldwide because they are considered less toxic than OC and OP pesticides. In many areas of application PYR are currently replacing the OP pesticides that replaced OC pesticides in the past. Biomonitoring studies revealed that metabolites of PYR have been frequently detected in urine samples from occupationally non-exposed general populations (Becker et al., 2006; Ueyama et al., 2008; Kimata et al., 2009; Barr et al., 2010; Wielgomas et al., 2013) demonstrating that human exposure to PYR is widespread within and across populations. One of the most frequently measured metabolites of PYR is 3-phenoxybenzoic acid (3-PBA) and it

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has been frequently detected in urine samples from pregnant women (Qi et al., 2012; Zhang et al., 2013).

Reproductive effects in adult males (Han et al., 2008; Xia et al., 2008; Meeker et al., 2008, 2009; Ji et al., 2011; Toshima et al., 2012) have been a major concern when the chronic toxicity of PYR at environmental levels was considered. The structural similarities of PYR to triiodothyronine (T3) and thyroxine (T4), have also suggested that they may act as environmental thyroid hormone disruptors (THD). The thyroid hormone effects of PYR and 3-PBA have been demonstrated by in vitro receptor assays (Du et al., 2010), and altered thyroid hormone levels were found in experimental animals exposed to PYR (Akhtar et al., 1996; Kaul et al., 1996; Giray et al., 2010).

We have measured urinary concentrations of 3-PBA in pregnant Japanese women and possible associations between serum levels of free T4 (fT4), thyroid stimulating hormone (TSH) and thyroxin binding globulin (TBG) were investigated but no significant relationships were revealed (Zhang et al., 2013). This absence of associations was consistent with the finding for adult male subjects recruited in an infertility clinic in the USA (Meeker et al., 2009). However, since the fetus is generally more vulnerable to the toxic actions of chemicals than are adults, and trans-placental transfer of PYR or 3-PBA has been demonstrated in rodents (Nassr et al., 2010) and also indicated in humans (Pérez et al., 2010), it is essential to assess possible thyroid effects of PYR in the fetus. The purpose of the present study was to determine if prenatal exposure to PYR has any effects on the thyroid hormone status of neonates. Assessment of a possible effect of prenatal PYR exposure on birth size was a further purpose of this study as birth size is a routine measure in reproductive and developmental studies.

2. Subjects and methods

2.1. Subjects

The subjects of this study were 147 pairs each consisting of a mother and her newborn baby. The mothers, in their first trimester of gestation, were recruited at Showa University Hospital located in Tokyo, during 2009–2011. Inclusion criteria were that each mother was 1) living in the Tokyo metropolitan area, 2) of reproductive age (20–50 years old), and 3) free of any known disease affecting normal thyroid functions (Hisada et al., 2013). The women voluntarily participated in our study after giving informed consent. The ethics committees of the University Hospital and of the University of Tokyo approved the study.

The original cohort for this study consisted of 315 women in the first trimester of gestation who agreed to participate (424 women were approached) (Zhang et al., 2013). Blood and urine samples were available from 231 women. Of these 231, 37 left the cohort by the time of delivery for the following reasons: spontaneous abortion (7), change of hospital (12), multiple pregnancy (8), and refusal for other reasons (10). The other 47 pairs of mother and baby were not included because of a failure to sample blood from the baby.

2.2. Sampling

Blood and spot urine samples were collected from subject mothers at regular maternal health checks during the 10th–12th gestational weeks. Urine samples were taken by the subjects themselves into paper cups and aliquots of each sample were then dispensed into several polypropylene (PP) tubes by hospital staff. Maternal blood was collected by hospital staff on the same day as urine sampling; no anticoagulant was added to the blood samples. Immediately after sampling, serum was recovered from each sample by centrifugation and was transferred into a PP tube. All PP tubes for urine and serum collection were rinsed with ultrapure water and methanol (HPLC Grade, Kanto Chemical Co. Ltd., Japan) prior to use.

Neonatal blood was sampled on to filter paper from the heel of each baby. The filter papers with blood spots for this study were obtained in addition to those used for routine screening for congenital metabolic diseases before discharge (typically on the 5th day postpartum).

Maternal serum and urine and filter papers with neonatal blood spots were stored at −20 °C until analyzed.

2.3. Thyroid hormone analysis

Concentrations of TSH and fT4 in neonatal whole blood spots on filter papers were determined by enzyme linked immunosorbent assay (ELISA) with a commercial kit (ENZAPLATE, Siemens, Japan) at the Japan Public Health Association, Tokyo, Japan. This was the analytical method used for routine mass screening for cretinism in Japan (Naruse et al., 1986). Reproducibility of the measurements of fT4 and TSH in this laboratory was 3.8% and 7.2%, respectively.

Concentrations of fT4, TSH and TBG in maternal serum were determined by electrochemiluminescence immunoassay (ECLIA) with a commercial kit (ECLusys TSH, fT4, Roche, Tokyo, Japan) and radioimmunoassay (RIA) with a TBG commercial kit (RIA-gnost TBG, CIS bio international, Gif-sur-Yvette, France) at a commercial laboratory (SRL Co. Inc. Tokyo, Japan). Reproducibilities of the measurement of fT4, TSH and TBG in this laboratory were 3.5%, 2.0% and 4.4% respectively (Hisada et al., 2013).

2.4. 3-PBA and iodine in maternal urine

Urinary 3-PBA analysis was based on a previous method (Baker et al., 2004) with some modifications and was described in our previous paper (Zhang et al., 2013). Briefly, urine was spiked with stable isotope labeled 3-PBA, followed by acidic deconjugation, solid phase extraction and free 3-PBA was determined by high performance liquid chromatography (Agilent 1100, Agilent technologies, CA, USA) tandem mass spectrometry (Micromass Quattro Ultima, Manchester, UK) (HPLC–MS/MS). The detection limit of 3-PBA was 0.02 ng/mL in urine according to the S/N = 3 definition. When the concentration was below the detection limit, one-half of the detection limit concentration was substituted in the statistical analyses. Our urinary 3-PBA analysis followed a strict internal analytical quality assurance protocol by measuring procedural blanks and an in-house quality control urine sample in each batch of measurements (Zhang et al., 2013).

Iodine concentrations in maternal urine were measured by inductively coupled plasma mass spectrometry (Agilent 7500ce, Agilent Technologies Japan, Tokyo, Japan) according to Caldwell et al. (2005). Iodine was measured because it is an essential constituent of thyroid hormones. The detection limit of urinary iodine was 3 ng/mL. External quality control was performed by measuring urine reference material (RM) (Urine Blank, Sero, Norway); the mean and standard deviation (SD) of triplicate measurements were 144 ± 3 ng/mL, which was consistent with the assigned range of the RM (131–147 ng/mL).

To compensate for urine dilution effects, specific gravity (SG) was measured by a hand-held refractometer (Clinical Refractometer ERMA Inc. Japan) and urine 3-PBA and iodine concentrations were adjusted to SG = 1.020.

2.5. Anthropometry

Birth weight, length, chest circumference and head circumference of neonates were measured immediately after birth by following the standard protocol.

2.6. Food frequency questionnaire

We asked the subject mothers about the frequency of consumption of the following food categories: rice, bread, meat, fish, shellfish, eggs, milk and dairy products, vegetable and fruit, seaweeds, soy products and oil. These categories were selected because they are commonly consumed in Japan. Frequency was to be classified into 6 categories;