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Thyroid hormone status of newborns in relation to *in utero* exposure to PCBs and hydroxylated PCB metabolites ☆

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Abstract

The associations between *in utero* exposure to polychlorinated biphenyls (PCBs) or hydroxylated PCB metabolites (OH-PCBs), and free thyroxin (fT4) or thyroid-stimulating hormone (TSH) status in the newborn were investigated as a pilot study of a large-scale epidemiologic study on *in utero* PCB or OH-PCB exposure and thyroid function of the newborns. Umbilical cord tissue was used as the media for the biological monitoring of PCBs/OH-PCBs exposure *in utero*. For the measurement of fT4 and TSH, a heel-prick blood sample spotted on filter paper, which is called Guthrie card, is collected from each neonate at day 4–6 postpartum for this study when the mass screening sampling was performed.

We showed that the concentration of total OH-PCBs and one of the OH-PCB congeners (OH-PCB 187) was related significantly to higher fT4 level of newborns. On the other hand, the concentration of total PCBs and PCB congeners (PCB 118, 138, 153, and 180) showed no relationship with fT4 and TSH level of the newborns. The results obtained in this pilot study indicated the possibility that *in utero* OH-PCBs exposure affects thyroid hormone status of newborns.

Keywords: Thyroid hormone; Thyroid-stimulating hormone; Polychlorinated biphenyl (PCB); Hydroxylated PCB metabolites (OH-PCB); *in utero* exposure

1. Introduction

Thyroid hormones play critical roles in the development of central nervous system and brain function, which are absolutely imperative for the maturation of brain in perinatal period (Yen, 2001; Colborn, 2004; de Escobar et al., 2004). Deficiency of thyroid hormones can be one of the etiologies of neurologic developmental defects in children (Haddow et al., 1999; Brosvic et al., 2002; Builee and Hatherill, 2004).

In recent years, it has become evident that exposure to polychlorinated biphenyl (PCBs) and hydroxylated PCB metabolites (OH-PCBs) can impair thyroid hormone functions in laboratory animals (Collins and Capen, 1980; van den Berg et al., 1988; Darnerud et al., 1996; Kato et al., 2004a). The fetus may be especially vulnerable to PCBs and OH-PCBs exposure, and decreased levels of circulating plasma thyroxin (T4) following PCBs and OH-PCBs exposure of pregnant animals has been seen in the offspring (Ness et al., 1993; Goldey et al., 1995; Morse et al., 1995; Roth-Härer et al., 2001; Meerts et al., 2004). Reduction of thyroid hormone levels can lead to an alteration of the

☆This study was conducted in accordance with national and institutional guidelines for the protection of human subjects. The medical ethics committee of the Board of Physicians approved the study.

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normal thyroid feedback loop, resulting in release of thyroid-stimulating hormone (TSH; Yen, 2001). There are three postulated mechanisms how xenobiotic compounds such as PCBs decrease plasma thyroid hormone levels. Firstly, Collins and Capen (1980) reported in rat experiment that PCBs *in utero* and by the milk exposure directly affect the thyroid follicular cells and reduce thyroid hormone levels in the neonatal. Secondly, PCBs facilitate biliary excretion of T4 by inducing UDP-glucuronyl transferase and reduce thyroid hormone level (Beetstra et al., 1991; Morse et al., 1993; Barter and Klaassen, 1994; Kato et al., 2004a). Third, competitive interactions of PCBs and OH-PCBs with the thyroid hormone transport protein, transthyretin (TTR), are indicated, thereby displacing the natural ligand T4 (Brouwer et al., 1990; Lans et al., 1993; Chauhan et al., 2000).

For OH-PCBs, thyroid hormone activity was assessed using a yeast two-hybrid assay and was found to have 5% of the activity of T4 by 2′,4,5′,6-tetrachlorobiphenyl-2-ol (Shiraishi et al., 2003). Further, PCBs and OH-PCBs at very low level suppressed thyroid hormone-induced transactivation in transfection-based reporter assays (Iwasaki et al., 2002). These in vitro findings indicate an additional possibility how PCBs and OH-PCB affect thyroid hormone status.

When compared with the number of publications based on in vitro and in vivo experiments, that of human study is still limited (Koopman-Esseboom et al., 1994; Osius et al., 1999; Longnecker et al., 2000; Persky et al., 2001; Ribas-Fitó et al., 2003; Takser et al., 2005). For this reason, we have launched a study to investigate whether altered thyroid hormone status in newborns is associated with *in utero* exposure to PCBs and OH-PCBs in Japanese. The authors are planning a large-scale epidemiologic study on *in utero* exposure to environmental pollutants, e.g., PCBs and OH-PCBs, and thyroid hormone status of newborns.

We focus on *in utero* PCBs/OH-PCBs exposure because fetuses have the exquisite sensitivity that can interfere with the thyroid. Umbilical cord tissue, which is considered to represent the levels of the chemicals that have passed through the blood–placenta barrier and the quantities of the chemicals that have been delivered to the fetus (Fukata et al., 2005; Grandjean et al., 2005), is used as media for the biological monitoring of PCBs/OH-PCBs exposure *in utero*.

In this paper, the result of a small-scale pilot survey carried out in 2005 is reported.

2. Materials and methods

2.1. Study population

We approached 39 healthy women recruited between January 2005 and December 2005, living in Tokyo Metropolitan Area. Cord tissue and cord blood samples were collected from the subjects who gave their written informed consent after being explained the purpose and the procedure of the present study by the physicians. The medical ethics committee of the Board of Physicians approved the study.

Umbilical cord tissue samples were collected for PCBs and OH-PCBs analysis and cord blood samples were for selenium and other trace element analysis (Iijima et al., to be submitted). A heel-prick blood sample from neonates was used for measurement of TSH and free thyroxin (fT4).

A complete set of the samples (umbilical cord tissue, cord blood, and heel-prick blood) was obtained from 23 subjects. One additional subject supplied only cord blood sample and two additional subjects supplied only cord tissue sample.

2.2. Collection of umbilical cord tissue samples and determination of PCBs and OH-PCBs

Umbilical cord tissue (10–20 cm) was sampled at the time of delivery by the gynecologist and was stored at −20 °C in a glassware that had been vigorously cleaned with hexane and acetone prior to use.

Umbilical cord tissue sample was cut into fine pieces with stainless-steel scissors and homogenized in isopropanol, and extracted in dichloromethane/hexane (1:1) under acidic condition. Lipid content of the umbilical cord tissue was gravimetrically measured from this extract. As internal standard, ^{13}C-labelled PCBs and ^{13}C-labelled OH-PCB were added. The crude extract was then purified with sulfuric acid which was followed by fractionation process using a column packed with silica containing 5% water; PCBs fraction was eluted by hexane and OH-PCBs fraction was eluted by dichloromethane/hexane (1:3). The PCBs fraction was further purified using a column packed with activated alumina eluted by dichloromethane/hexane (1:1). The OH-PCBs fraction, after the methylation by diazomethane, was purified using a column packed with silica containing 44% H_2SO_4.

In this study, all of 209 PCB congeners and six selected OH-PCBs (4-OH-2,3,3′,4′,5-PentaCB (4OH-PCB 107), 3′-OH-2,2′,3,4,4′,5′-HexaCB (3′OH-PCB 138), 4-OH-2,2′,3,4′,5,5′-HexaCB (4OH-PCB 146), 3-OH-2,2′,4,4′,5,5′-HexaCB (3OH-PCB 153), 4′-OH-2,2′,3,3′,4,5,5′-HeptaCB (4′OH-PCB 172), and 4-OH-2,2′,3,4′,5,5′,6-HeptaCB (4OH-PCB 187)) in the umbilical cord tissues were analyzed. Congener-specific PCB analysis was carried out using a gas chromatograph (6890 series, Agilent Technologies, USA) and high-resolution mass spectrometry (resolution >10,000; JMS-700D, JEOL, Japan). An HT8-PCB column (SGE Analytical Science, Australia) and DB-5MS column (Agilent Technologies, USA) were used to separate PCBs and OH-PCBs congener, respectively. For the external quality assurance of our PCBs analysis, a certified reference material (CRM) from the National Research Council Canada CARP-2 was analyzed.

2.3. Collection of neonatal blood and determination of fT4 and TSH

A heel-prick blood sample was obtained as spots on a filter paper for the Guthrie card from each neonate at day 4–6 for this study. The sampling was carried out at the time of blood sampling for the mass screening for inborn errors of metabolism, etc. (phenylketonuria, maple syrup urine disease, homocystinuria, galactosemia, congenital adrenal hyperplasia syndrome and hypothyroidism), which is a routine procedure for virtually all of the neonates in Japan (Naruse and Yamaguchi, 2004). Additional blood spots were sampled on another filter paper for this study.

Dried blood on the filter papers with neonatal blood spots were stored at −20 °C until fT4 and TSH measurement. The fT4 and TSH in the blood spots on the filter paper were determined by enzyme-linked immunosorbent assay (ELISA) with ELISA kit (Eiken Chemical, Japan) as the same analytical method as the routine screening.

2.4. Statistical analyses

PC software SPSS version 12.0J was employed for statistical analyses. Kolmogorov–Smirnov (KS) test was used for normality test of the distribution of PCBs concentration, OH-PCBs concentration, fT4 level,