PRENATAL EXPOSURE TO OH-PCBs IN RELATION TO BODY WEIGHT AND NEURODEVELOPMENT

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Introduction

In recent years, certain neurodevelopmental disorders, such as learning disabilities, autism, attention deficit, and hyperactivity, have been increasing in prevalence.[1] Evidence has been accumulating over several decades that industrial chemicals can cause neurodevelopmental damage and that subclinical stages of these disorders might be common.[2] Considerable attention has been directed to fetal exposure to chemicals with possible neurodevelopmental effects, as the developing human brain is much more susceptible than the mature brain to these chemicals.[3] PCB, one of the chemicals of concern, and its metabolite are neurotoxic[2] and are still found in humans at comparatively high concentrations, especially in populations that consume fish, such as the Japanese.

In the present study, the concentration of PCB and its hydroxylated metabolites were measured in preserved umbilical cords from subjects of the prospective cohort study (Tokyo Children's Health, Illness and Development (T-CHILD) ;National Center for Child Health and Development, Tokyo, Japan) to evaluate any associations between prenatal exposure to OH-PCBs and the physical and neural development of infants up to 2 years old.

Materials and methods

Study population. The Seiiku Cohort Study (SCS) recruited about 1,600 women in early pregnancy from 2003 to 2005 at the National Children's Medical Center, Setagaya, Tokyo, Japan. In 2007 and 2008, we asked 910 cohort participants to offer their children's umbilical cords stored at home for analysis of hydroxylated PCBs (OH-PCBs). A total of 126 members, including 10 who had children with neurodevelopmental disorders (cases) agreed to provide their children's umbilical cords.

Analytical procedures. About 1/2 – 1/3 of the umbilical cords (0.0317g – 0.1180g) were used for analysis. The umbilical cords were powdered using a multi-beads shocker, and PCBs were extracted in hexane after alkaline degradation with a KOH/ethanol solution. The alkaline phase containing OH-metabolites was acidified and extracted with hexane. This organic phase was purified with sulfuric acid, and then a hydroxyl group was methoxylated with tetramethylsilyl diazomethane. The clean-up process of the OH-PCB fraction and the PCB fraction was performed using silica gel chromatography and a multilayer silica gel column, respectively.

In this study, 35 selected OH-PCBs and PCBs in the umbilical cords were analyzed using a gas chromatograph (6890 series, Agilent Technologies, USA) and high-resolution mass spectrometry (resolution > 10,000; Autospec- Ultima, Micromass, England). An RH12ms column (INVENTx, USA) and an HT8-PCB

column (SGE Analytical Science, Australia) were used to separate the OH-PCB and PCB congeners, respectively. The results of the PCB analysis were expressed as the sum of the congeners grouped by number of chlorines.

Diagnosis of the neurodevelopmental disorders. Neurodevelopmental disorders, including mental retardation, pervasive developmental disorder (PDD), ADHD, and autism, were diagnosed at the time of the 2-year-old health checkup by pediatricians and clinical developmental psychologists using the Enjoji developmental test[4] and the Japanese version of the Denver Developmental Screening Test (JDDST)[5].

Data analyses. The differences in population characteristics between the case and the control were analyzed with the Mann-Whitney U-test. The differences in the OH-PCB concentrations in umbilical cords between the case and the control were also analyzed with the Mann-Whitney U-test. The differences in the frequency distribution between the case and the control according to the OH-PCB congener levels in the umbilical cords in the quartile were tested with Fisher's exact test, and the trends, with the Cochran-Armitage trend test. Multiple regression models were used to examine the association between physical development (body weight, height, and head circumference) and OH-PCB congener levels in the umbilical cords in the quartile. All statistical analyses were performed using SAS statistical software (version 9.2 for Windows; SAS Institute Inc., Cary, NC, USA). Statistical significance was set at $p < 0.05$.

Results and discussion

The Japanese traditionally keep a small portion of their baby's umbilical cord in a wooden box as a keepsake. This tradition provides a unique opportunity to monitor, after birth, the chemical exposure during fetal life.

Of 126 mother-infant pairs who provided their preserved umbilical cords, one mother-infant pair was excluded from further analysis because of low birth weight (1,540g). The mean maternal age was 33.9 years, and the mean gestational age was 272.9 days. The mean birth weight, birth height, and head circumference were 2,964g, 48.1 cm, and 33.0 cm, respectively. There were no statistical differences in these characteristics between the case and the control.

Relatively higher concentrations were observed for 4'-OH-2,3',4,5,5'-pentachlorobiphenyl (4PM120), 4'-OH-2,2',4,5,5'-pentachlorobiphenyl (4PM101), 4-OH-2,3,3',4,5'-pentachlorobiphenyl (4M107), 3-OH-2,2',4,4',5,5'-hexachlorobiphenyl (3M153), 4-OH-2,2'3,4',5,5'-hexachlorobiphenyl (4M146), 3'-OH-2,2',3,4,4',5'-hexachlorobiphenyl (3PM138), 4'-OH-2,3,3',4,5,5'-hexachlorobiphenyl (4PM159), 4'-OH-2,3,3',4',5,5'-hexachlorobiphenyl (4PM162), 4-OH-2,2',3,4',5,5',6-heptachlorobiphenyl (4M187), and 4'-OH-2,2',3,3',4,5,5'-heptachlorobiphenyl (4PM172). Except 4PM159, these congeners were detected in most of the samples. The median concentration of total OH-PCBs was 375 pg/g dry weight.

There is no data to compare with the present results. Kawashiro et al. measured 9 OH-PCBs in fresh frozen umbilical cords and cord blood from 6 Japanese mother-infant pairs[6]. On the basis of the median concentration

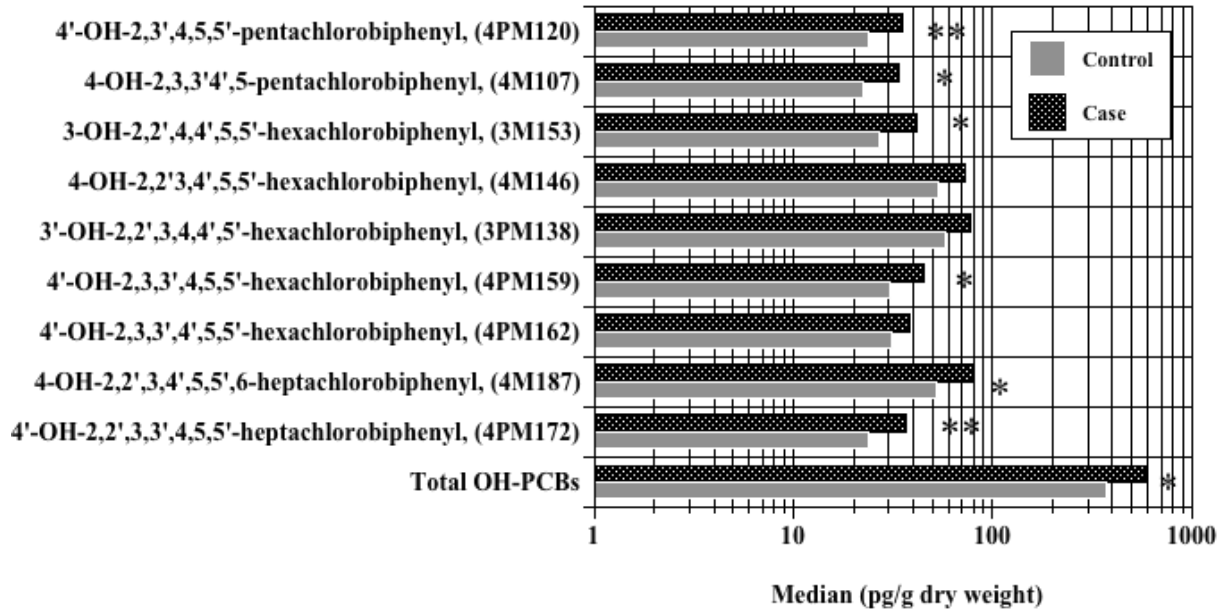

Fig. 1. The levels of major OH-PCBs congeners between the case and the control.

(*,** significantly different from control at $p < 0.05, 0.01$, respectively.)

(pg/g wet weight), the two dominant OH-congeners in the cords were 4M146 (1.6) and 4M187 (1.5), followed by 3PM138 (0.84) and 4M109 (0.76), while 4M187 (11) was the most dominant in cord blood followed by 4M146 (6.3), 3PM138 (3.9), and 4M109 (2.8). The difference in the relative abundance of congeners between blood and cord suggests the tissue-specific distribution profile of congeners. In the present study, on the basis of the median concentration (pg/g dry weight), the order of dominant congeners was 3PM138 (59) > 4M146 (54) > 4M187 (53) > 4PM162 (32) > 4PM159 (31) > 3M153 (28) > 4M107 (25) > 4PM120 (25) > 4PM101 (25) > 4PM172 (24). In the present study, 3PM138 was relatively higher than the results in Kawashiro's study. The reason for this difference is not understood, as the diets of the two populations were most likely similar.

The levels of major OH-PCB congeners and total OH-PCBs were not significantly associated with body weight, height, and head circumference of infants up to 1.5 years old. However, body weight and height at 2 years old for all infants were significantly associated with total OH-PCBs and 4M187. For male infants, body weight at 2 years old was significantly associated with total OH-PCBs, 4M107, and 4M187. Both 4M107 and 4M187 are potent inhibitors of the human estrogen sulfotransferase (hEST)[7]. This inhibition would result in estrogenic effects. It is noteworthy that prenatal exposure to endocrine-disrupting chemicals may increase one's

body weight[8]. Lamb et al. reported that tri-ortho-substituted PCBs, such as 4M187, were marginally associated with increased height in boys[9].

A comparison of the levels of major OH-PCBs congeners between the case and the control was made (Fig. 1). 4PM120, 4M107, 3M153, 4PM159, 4M187, 4M172, and total OH-PCBs were significantly higher in the case than the control (Fig. 1, Mann-Whitney U-test). The frequency distribution between the case and the control according to the OH-PCB congener levels in umbilical cords in the quartile was analyzed. Compared to the control, the cases were more likely to distribute in a higher quartile of 4PM120, 4PM101, 4M107, 3M153, 4M187, 4PM172, and total OH-PCBs (Cochran-Armitage trend test). To our knowledge, this is the first study investigating neurodevelopmental disorders in humans in relation to OH-PCBs in preserved umbilical cords. OH-PCBs are suggested to be active toxins of PCB neurodevelopmental toxicity since OH-PCBs readily cross the placenta and are transported to the brain by strong binding affinity to transthyretin (TTR)[10]. Several mechanisms of the neurotoxicity of OH-PCBs, such as disruption of thyroid hormone homeostasis[11] and estrogenic and anti-estrogenic actions[12], are proposed. Human studies have provided evidence that higher neonatal fT4 is associated with total OH-PCBs and with 4M187 [13], and the lower Mental Development Index (MDI) was associated with cord 4M107 in Eastern Slovakia[14].

Although the number of the case is small, several OH-PCBs might be involved in the etiology of neurodevelopmental disorders. Further epidemiological studies are needed to confirm our findings.

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