THE BIOLOGICAL MONITORING PROGRAM OF PERSISTENT ORGANIC POLLUTANTS IN JAPAN: 2. CONCENTRATIONS OF DIOXINS AND POLYCHLORINATED BIPHENYLS IN BREAST MILK, CORD BLOOD AND MATERNAL BLOOD

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Abstract

Persistent organic pollutants (POPs) such as dioxins and polychlorinated biphenyls (PCBs) are bioaccumulative chemical toxins that are resistant to degradation. POPs are thought of as hazardous contaminants. The Ministry of the Environment of Japan (MOE) has been conducting environmental monitoring of POPs since FY2002 on the basis of the Stockholm Convention on POPs. Since we provided some biological samples for the POPs biological monitoring project, we reanalyzed the report from the MOE. In this presentation, we summarize the data on dioxins and PCBs in human pair samples of breast milk, cord blood and maternal blood. We also analyze the associations of the concentrations of these compounds with thyroid-stimulating hormone (TSH) and thyroid hormones in maternal and cord blood, since disruption of the hypothalamus-pituitary-thyroid axis is a hypothetical mechanism for dioxin- and PCB-induced adverse effects. Concentrations of dioxins and PCBs in each biological sample were at levels similar to those in previous reports on Japanese, and high correlations among the three biological samples were observed. Furthermore, single regression analysis showed a statistically significant correlation of dioxins and PCBs with TSH and thyroid hormones such as total thyroxine (T4) and triiodothyromine (T3).

Introduction

POPs such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and PCBs are bioaccumulative chemical toxins that are resistant to degradation. Generally, the main source of exposure to POPs for the general population is thought to be food because the physicochemical attributes of POPs such as lipophilicity and persistence cause bioaccumulation in the food chain, and consequently they can be found in humans at considerable concentrations. Although these concentrations tend to decrease and to be at the background level in industrialized nations, long-term exposure may cause potential risks to human health.

In humans, POPs have been claimed to possess endocrine-disrupting potency. Dioxins, expressed as toxic equivalent (TEQ) levels, were correlated significantly with lower T3 and T4 levels in maternal blood, and with higher blood concentrations in breast-fed infants[1]. There was a significant negative association between dioxin concentrations in breast milk and total T4 in the blood of breast-fed infants[2.] On the other hand, higher environmental background exposure to dioxins significantly increased the T4 concentration in the blood of infants[3]. These findings suggest that exposure to dioxins may affect the hypothalamus-pituitary-thyroid axis. A growing number of reports have demonstrated the association between adverse effects in children and exposure to POPs at low doses over a longer period. In particular, human perinatal exposure to PCBs has been shown to be associated with immunological changes[4], neural and developmental changes[5, 6], lower psychomotor development[7, 8], defects of short-term memory and spatial learning ability[9] and lower cognitive development[10]. Therefore, monitoring and epidemiological verification of exposure to POPs are necessary to assess the health risks to the Japanese population.

In Japan, the MOE has been conducting the POPs monitoring project[11] since FY2002 for the monitoring of chemicals in each of the environmental media and to obtain data that can contribute to effective evaluations in the Stockholm Convention on POPs. Recently the biological monitoring of human samples was added. We have been collaborating with the POPs biological monitoring project of the MOE by providing biological samples

from our prospective birth cohort study, The Tohoku Study of Child Development (TSCD)[12]. We reanalyzed the results and summarized the data of dioxins and PCBs in human pair samples of breast milk, cord blood and maternal blood[13]. We also analyzed the associations of the concentrations of these compounds with TSH and thyroid hormones in maternal and cord blood, since the disruption of the hypothalamus-pituitary-thyroid axis is a hypothetical mechanism for dioxin- and PCB-induced adverse effects.

Materials and Methods

The biological samples analyzed were randomly selected from the participants in the TSCD, and provided anonymously to the MOE. These samples were measured by IDEA Consultants, Inc. (Tokyo, Japan) as part of the MOE project. This study protocol was previously reported[12]. Briefly, the maternal peripheral blood was collected using heparin as the anticoagulant agent in the morning when the pregnancy was at 28 weeks. The cord blood was collected immediately after delivery. These whole blood samples were frozen at –80°C until the chemical analysis. The breast milk was collected one month after delivery, and then frozen similarly. The TSCD was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine, and all mothers provided signed informed consent.

Chemical analysis was conducted following the methods in the environmental monitoring report on persistent organic pollutants (POPs) in Japan 2002-2004[14]. Briefly, the biological samples were spiked with ^{13}C-labeled POPs as internal standards before extraction. The samples were extracted with liquid-liquid extraction and then extracts were purified by multilayer silica gel column chromatography. Active carbon dispersed silica gel column chromatography was further used for purification of PCDD/Fs and dioxin-like PCBs (DL-PCBs). For the other POPs, extracts were purified by Florisil column chromatography except for silica gel column chromatography for toxaphene. Congener-specific determination of the compounds was performed by high resolution gas chromatograph-high resolution mass spectrometry (HRGC-HRMS) or negative ion chemical ionization mass spectrometry (GC-NICIMS) for toxaphene by isotope dilution quantification. Although control samples were analyzed for every 9-sample batch, they did not contain significant amounts of the target compound. TSH, total T4, and total T3 were measured from the plasma of cord and maternal blood by SRL, Inc. (Tokyo, Japan). The statistical analyses were performed using JMP ver. 5.1.2..

Results and Discussion

Concentrations of TEQ and total PCBs in breast milk, cord blood and maternal blood are shown in Table 1. TEQ was calculated by the WHO (1998) toxic equivalency factor[15] (TEF) assuming that the amount of congeners below the determination limit was zero. These data were roughly in agreement with previous studies[16-21]. In these biological samples, TEQ and PCB levels in breast milk were higher than in cord blood and maternal blood. Concentrations of TEQ and total PCBs among the three biological samples showed high correlations (Figure 1). Therefore, to predict the concentrations of dioxins and PCBs for the purpose of biological monitoring, it might be useful to measure the concentrations in breast milk. The homologue pattern of PCBs in breast milk was similar in composition to those of cord blood and maternal blood. The predominant homologues in the biological samples were HxCBs, followed by HpCBs, PeCBs and TeCBs.

The correlations between TEQ and PCBs in breast milk, cord blood and maternal blood were very high (Table 2). It was found that the contribution ratio of DL-PCBs to total TEQ was about 40% and the percentage of the DL-PCB concentration in total PCBs was 10% by the congener-specific analysis of PCBs in these biological samples. Because of the high correlation between TEQ and PCBs, the levels of exposure to dioxins for the population could be estimated from the results of PCB measurements. We may be able to simplify the monitoring method by eliminating the determination of dioxins from the analytical procedures.

The working hypothesis is that dioxins and PCBs cause adverse effects via disruption of thyroid hormone regulation and metabolism. Indeed, as shown in Table 3, there were significant correlations of PCBs with T3, and T4 in maternal blood ($p<0.05$). Similarly, there were correlations of TEQ and PCBs with several thyroid function indicators in breast milk and cord blood ($p<0.05$). Although the exact mechanisms by which dioxins and PCBs affect the levels of TSH and thyroid hormones are not fully understood, these results suggest that exposure to dioxins and PCBs could cause hormonal disturbance of thyroid function.

Table 1. Concentrations of TEQ (pg-TEQ/g-fat) and PCBs (ng/g-fat) in breast milk, cord blood and maternal blood

Compound names	Breast milk Median (Min - Max)	Cord blood Median (Min - Max)	Maternal blood Median (Min - Max)
Dioxins			
PCDD/Fs-TEQ	9.9 (2.0-25)	5.4 (0.28-16)	8.6 (2.8-26)
DL-PCBs-TEQ	6.8 (2.1-21)	2.9 (0.74-7.3)	4.8 (1.4-11)
Total TEQ	17 (4.2-45)	8.3 (1.1-22)	13 (4.8-33)
PCBs			
Total PCBs	102 (31-274)	40 (12-128)	76 (20-163)

n=68 for breast milk and cord blood, n=49 for maternal blood.
TEQ was calculated by the WHO (1998) TEF assuming that the amount of congeners below the determination limit was zero.

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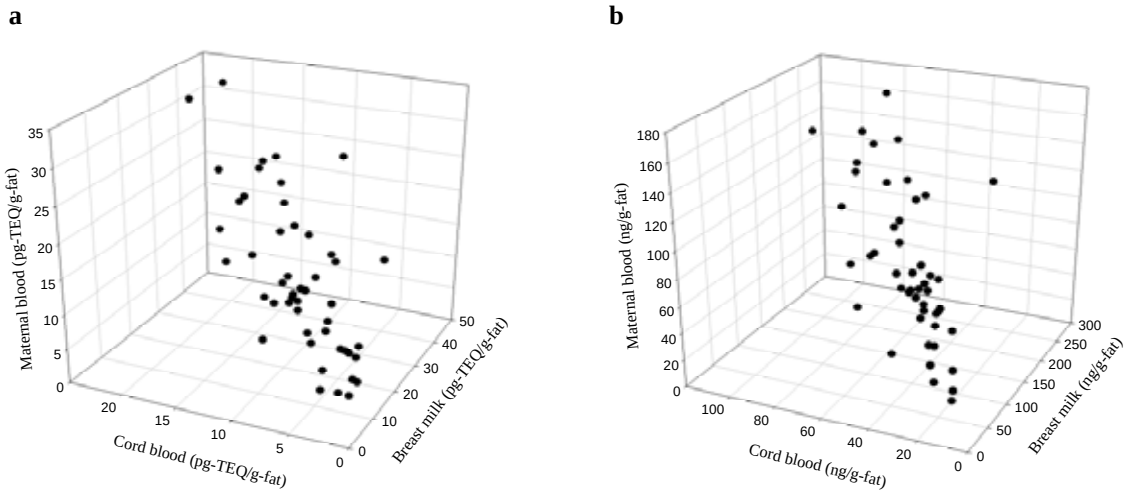

Fig. 1 Relationship of TEQ (a) and PCBs (b) among pair samples of breast milk, cord blood and maternal blood.

Table 2. Pearson correlation coefficients of TEQ and PCBs in breast milk with those of cord blood and maternal blood

	Breast milk		Cord blood		Maternal blood	
	TEQ	PCBs	TEQ	PCBs	TEQ	PCBs
Breast milk						
TEQ	-					
PCBs	0.901	-				
Cord blood						
TEQ	0.763	0.633	-			
PCBs	0.716	0.808	0.826	-		
Maternal blood						
TEQ	0.938	0.854	0.794	0.756	-	
PCBs	0.841	0.927	0.699	0.843	0.892	-

Pearson's r ($p < 0.001$) after log-transformed. n=49 for breast milk, cord blood and maternal blood.

Table 3. Pearson correlation coefficients of TEQ and PCBs with TSH, total T4 and total T3 in breast milk, cord blood and maternal blood

	Maternal blood			Cord blood		
	TSH	T4	T3	TSH	T4	T3
Breast milk (n=68, except for n=67 for maternal TSH/T4/T3)						
TEQ	0.232	0.158	0.225	0.146	-0.224	-0.138
PCBs	0.205	0.231	0.257*	0.137	-0.163	-0.075
Cord blood (n=68, except for n=67 for maternal TSH/T4/T3)						
TEQ	0.203	0.038	0.150	0.071	-0.270*	-0.051
PCBs	0.239	0.131	0.240	0.024	-0.144	0.085
Maternal blood (n=49, except for n=48 for maternal TSH/T4/T3)						
TEQ	0.264	0.271	0.375*	0.145	-0.238	-0.094
PCBs	0.278	0.287*	0.402*	0.185	-0.150	-0.038

n=68 for breast milk and cord blood, n=49 for maternal blood.
Pearson's r after log-transformed. * $p<0.05$