PCDD/Fs, coplanar PCBs and PCBs in breast milk of Japanese mothers

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Introduction

Dioxins are persistent, lipophilic compounds that are ubiquitous in the environment. Concern about the reproductive and developmental toxicity of dioxins has been growing because dioxins have endocrine-disrupting properties and have adversely affected the health of offspring in experimental and epidemiological studies[1]. Monitoring the effects of dioxins in mothers and their biological responses to dioxin exposure is necessary to estimate the potential health risk to their offspring. In a previous study, we investigated the applicability of CYP1A1 expression in cells in breast milk (milk cells) as a biomarker for dioxin exposure and showed that CYP1A1/GAPDH in milk cells correlated with dioxin levels among non-smoking mothers[2]. As a part of this study, the levels of dioxins (PCDD/Fs and co-planar PCBs) and PCBs in breast milk were determined, and factors affecting these levels were analyzed.

Materials and Methods

Sample collection

Ninety-nine women (age 15–40) who had delivered babies at Uechi Obstetrics and Gynecology Clinic (Utsunomiya, Japan) (2003.6- 2004.10) participated in this study. Samples of breast milk were obtained from the mothers at the clinic within one week of childbearing. The milk samples were collected in a 50-mL polypropylene tube with a meshed insert (Clotspin™, Gentra Systems, Inc., Minneapolis, MN). Milk cells and the cream layer were separated by centrifugation (252 ´*g* for 5 min) and stored at –80°C until further analysis.

Information on the reproductive history, lifestyle, and dietary habits of the participants was obtained with the use of a questionnaire. The study proposal was approved by the Committee on Medical Ethics of the National Institute for Environmental Studies, and informed consent was obtained from all participants before their enrolment.

Dioxin analysis

Sample extraction and cleanup

The cream layer from approximately 10 mL of breast milk was weighed and spiked with a mixture of ^{13}C-labeled PCDD, PCDF, and PCB internal standards. The lipid in the cream layer was extracted by liquid-liquid organic solvent extraction. The extraction was performed by the addition of distilled water, sodium oxalate, and ethanol to the cream layer, followed by subsequent extraction with diethyl ether and *n*-hexane. The *n*-hexane layer was cleaned up and concentrated to dryness after treatment with anhydrous sodium sulphate; the lipid content was determined gravimetrically. The sample was dissolved again into *n*-hexane and further cleaned up by multilayer, silica gel column chromatography.

The eluate was divided into 9:1 for dioxin analysis (PCDD/Fs and non-*ortho* and mono-*ortho* PCBs) and the analysis of other PCBs, respectively. The dioxin fraction was further cleaned up and subfractioned into (1) mono-*ortho* PCBs and (2) PCDD/Fs + non-*ortho* PCBs by active carbon-dispersed silica gel column chromatography.

Quantification

TEF (WHO, 1998) assigned 29 PCDD/Fs and dioxin-like PCBs. PCB 74 in the cream layer was analyzed by HRGC (6890, Agilent, Palo Alto, CA) and high-resolution mass spectrometry (AutoSpec-Ultima, Micromass, Manchester, UK) equipped with a solvent cut large-volume injection system[3] (SGE Japan, Inc., Yokohama, Japan). Non-dioxin-like PCBs (i.e., PCB 99, 138, 146, 153, 163 + 164, 170, 177, 178, 180, 182 + 187, 183, 194, and 198 + 201) were analyzed by HRGC/HRMS.

Statistical analysis

All statistical analyses were performed with MATLAB® 7.01 (The MathWorks, Inc., Natick, MA).

Results and Discussion

Dioxin levels were measured in the cream layer of breast milk. Before the measurement, dioxin levels were compared between the cream layer and whole milk of commercial cow's milk to check whether the dioxin levels in the cream layer were representative of those in whole milk. The fat-based dioxin levels in the cream layer were almost the same as those in whole milk[2].

Descriptive statistical data on the levels of PCDDs, PCDFs, coplanar-PCBs, and non-coplanar PCB (PCBs #138, #153, and #180) in the cream layer of breast milk are given in Table 1. The geometric mean concentrations of PCDDs, PCDFs, coplanar-PCBs, and their sum were 6.7, 3.6, 4.5, and 15.1 pg TEQ/g fat, respectively. The geometric mean concentrations of PCB#138, PCB#153, and PCB#180 were 8344, 21028, and 7201 pg/g fat, respectively. The geometric mean concentration of dioxins (PCDDs + PCDFs + coplanar-PCBs) was 15.1 pg TEQ/g fat. This level was lower than the level reported recently from Tokyo[4] and developed European countries[5]. The difference might be due to local environmental differences, as reported in a nation-wide survey on breast milk dioxins[6].

A trend of increased levels of dioxins (PCDDs + PCDFs + coplanar-PCBs) with age was observed ($r = 0.268$, $p < 0.02$). A strong correlation was observed between dioxins and age in primiparous mothers ($r = 0.655$, $p < 0.0001$). This strong correlation observed between dioxin levels of breast milk and age in primiparous mothers was reasonably understood, as dioxin concentration in adipose tissue has been shown to be significantly correlated with age[7] and the level of dioxins in breast milk decreases as a mother breast-feeds more children[8]. Because age and parity were correlated with dioxin levels in breast milk, as mentioned above, these two factors were controlled in the analysis of relationships between dietary habits and levels of dioxins or PCBs in breast milk by partial correlation coefficient analysis. There was a weak trend of correlation that was not statistically significant between fish consumption and levels of dioxins or PCBs in breast milk. There was a trend of negative correlation between meat consumption and levels of dioxins or PCBs in breast milk. In the case of PCBs #153 and #180, this correlation was statistically significant (partial correlation coefficient (PCC) and the *p* values for PCB#153 and PCB#180 (Fig. 1) were PCC=-0.204, $p < 0.0423$ and PCC=-0.225, $p < 0.0285$, respectively). In general, meat consumption positively correlated with dioxin levels in breast milk. Other factors, such as lifestyle and socio-economic status, might be involved in this phenomenon.

Table 1. Concentrations of pollutants in breast milk (pg TEQ/g fat)

Pollutant	Min.	Max	Median	Geometric Mean
?PCDDs	1.56	21.3	6.38	6.74
?PCDFs	0.91	14.1	3.62	3.57
?Co-PCBs	1.14	23.1	4.52	4.50
?PCDD/Fs + Co-PCBs	3.75	46.4	15.1	15.1
PCB#138 (pg/g fat)	2110	27400	8600	8340
PCB#153 (pg/g fat)	3340	53200	17900	21000
PCB#180 (pg/g fat)	1310	22700	7530	7200

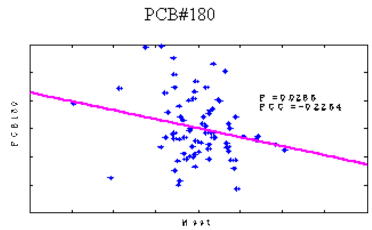

Fig.1 Meat consumption was negatively correlated with PCB#180 by partial coefficient analysis.

Acknowledgments

The authors express their appreciation to the volunteers who participated in this study. This work was partly supported by Grant-in Aid for scientific Research (C) (No. 17510053) from Japan Society for the Promotion of Science.

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