ASSOCIATION OF BIRTH WEIGHT WITH DIOXIN LEVELS IN BREAST MILK, LIFESTYLE, AND CYP1A1 POLYMORPHISMS OF MOTHERS IN TOCHIGI, JAPAN

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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 body burdens of dioxins in mothers and their biological responses to dioxin exposure are needed to estimate the potential health risk to their offspring. In the present study, the association of birth weight with dioxin levels in breast milk and lifestyle of mothers was investigated since low birth weight is associated with ill health in later life of infant[2]. The association of CYP1A1 and GSTM1 polymorphisms with dioxin levels in breast milk was also investigated.

Materials and Methods

Sample collection

Ninety-nine women (age 15-40) who had delivered babies at the Uechi Obstetrics and Gynecology Clinic (Utsunomiya, Tochigi, Japan) from June, 2003 to October, 2004 participated in this study. Samples of breast milk were obtained from the women at the clinic within one week of childbearing. Milk samples were collected in a 50-mL polypropylene tube with a meshed insert (Clotspin™, Gentra Systems, Inc., Minneapolis, MN). The milk cells and cream layer were separated by centrifugation (252 ×*g* for 5 min) and stored at –80°C until further analysis. Blood samples were also collected from 49 of the women at the clinic within one week of childbearing.

A questionnaire was used to obtain information on the reproductive history, lifestyle, and dietary habits of the participants. 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

TEF (WHO, 1998) assigned 29 PCDD/Fs and dioxin-like PCBs, and 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 as described previously[4].

Genotyping methods

Genomic DNA was extracted from peripheral blood and adjusted to 10ng/µl. Genotyping of CYP1A1 m1 (rs 4646903) and CYP1A1 m2 (rs 1048943) was performed using the MegaBACE SNuPe Genotyping Kit (GE Healthcare Life Sciences).

For CYP1A1 m1, a 752-bp PCR fragment was generated with the following primers: 5'-TACTGGCACAGAGGTAGTCTC-3' and 5'-CTGAGGTGGGAGAATCGTGTG-3'. The SNuPe reaction primer was 5'- TTTCACTGTAACCTCCACCTCC-3'. For CYP1A1 m2, a 383-bp PCR fragment was generated with the following primers: 5'-CCACTCACTTGACACTTCTGA-3' and 5'-GGTAGACAGAGTCTAGGCCTC-3'. The SNuPe reaction primer was 5'-AGAAAGACCTCCCAGCGGSCAA-3'. The SNuPe products were analyzed on a MegaBACE 1000 DNA Analysis Workstation (GE Healthcare Life Sciences).

For GSTM1, a 273-bp PCR fragment was generated with the following primers: 5'-CTGCCCTACTTGGATTGGATGGG-3' and 5'-TGGATTGTAGCAGATCATGC-3'. The GSTM1 null genotype was assessed by the absence of a 273-bp fragment using agarose electrophoresis.

Statistical analysis

Birth weight was dichotomized to the ≤25 percentile (lower) and >25 percentile or the ≥75 percentile (higher) and <75 percentile. The multivariate logistic regression model was used to identify variables predictive of lower or higher birth weight.

Statistical analyses were performed with MATLAB® 7.01 (The MathWorks, Inc., Natick, MA) and SAS ver. 8.2 (SAS Institute).

Results and Discussion

Dioxin levels were measured in the cream layer of breast milk. The fat-based dioxin levels in the cream layer were almost the same as those in whole milk[5].

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 (DFCo=PCDDs + PCDFs + coplanar-PCBs) was 15.1 pg TEQ/g fat. This level was lower than the level recently reported from Tokyo[6] and developed European countries[7]. The difference might be due to local environmental differences, as reported in a nationwide survey on breast milk dioxins[8].

Genotyping data was obtained in 37 cases. The frequencies of the CYP1A1 m1, CYP1A1 m2, and GSTM1 null genotypes were 27.0%, 13.5%, and 45.9%, respectively.

Lower birth weight (≤25 percentile) was associated with gestational age at delivery (OR=0.785, 95% CI, 0.675-0.912, $p< 0.0015$) and meat intake (OR=1.347, 95% CI, 1.068-1.701, $p< 0.012$). Lower birth weight was not associated with dioxins (DFCo) (OR=1.027, 95% CI, 0.893-1.181, $p< 0.7069$) and total non-coplanar PCBs (OR=0.992, 95% CI, 0.953-1.032, $p< 0.6767$) in breast milk. In 37 cases in which genotyping information was available, multivariate logistic regression analysis was performed by incorporating genotyping information. Again, lower birth weight (≤25 percentile) was associated with gestational age at delivery (OR=0.823, 95% CI, 0.694-0.976, $p< 0.0251$) and meat intake (OR=1.403, 95% CI, 1.038-1.896, $p< 0.0274$). Higher birth weight (≥75 percentile) was associated with total non-coplanar PCBs (OR=1.025, 95% CI, 1.003-1.046, $p< 0.0244$). Polymorphisms of CYP1A1 and GSTM1 were not associated with birth weight.

The CYP1A1 m2 genotype (homo) had lower levels of dioxins in breast milk than other genotypes (hetero or wild). The levels of PCDF and DFCo (PCDD + PCDF + CoPCB) were significantly lower in the CYP1A1 m2 genotype than in others (Fig. 1). The lower dioxins levels in the breast milk of the CYP1A1 m2 genotype might be explained by the increased microsomal enzyme activity[9]. As the combination of the CYP1A1 m2 and GSTM1 null genotype is associated with strong inducibility of CYP1A1 gene transcription by 2,4,7,8-TCDD[10], the CYP1A1 and GSTM1 genotypes should be taken into account when the dioxins levels in breast milk are used as the exposure marker of dioxins.

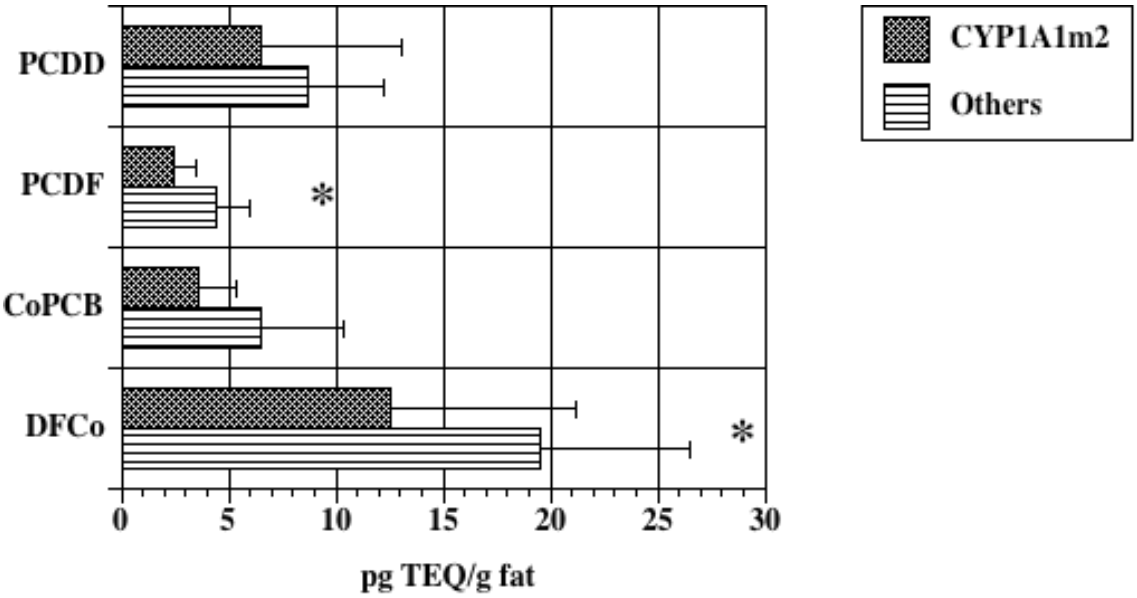

Fig. 1. Comparison of dioxin levels in breast milk between CYP1A1m2 genotype and others.
*Significantly different between two groups ($p<0.05$ by t-test).

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