

## CYP1A1 expression in breast milk cells of Japanese population

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### Introduction

Dioxins are persistent, lipophilic compounds that are ubiquitous in the environment. Concern over the reproductive and developmental toxicity of dioxins has been growing since they have endocrine-disrupting properties and have adversely affected the health of offspring in experimental and epidemiological studies<sup>1</sup>. Monitoring of maternal body burdens of dioxins and their biological responses to dioxin exposure is needed to estimate the potential health risk to their offspring. Breast milk has been used for monitoring dioxins in humans for decades<sup>2, 3</sup>. Breast milk has some advantages in exposure monitoring. Sampling is non-invasive, and dioxin levels are relatively high because of the high lipid content. It is assumed that mammary glands are exposed to a higher level of dioxins than other tissues since mammary glands synthesize and store milk fat. Breast milk contains leukocytes and exfoliated ductal epithelial cells<sup>4</sup>. If these cells responded to dioxins and expressed CYP enzymes, a sensitive biomarker for dioxin exposure, they would be useful as biomarkers for dioxin exposure.

In the present study, the expression of CYP enzymes in intact milk cells or cells cultured with TCDD was investigated. In addition, breast milk samples were collected from mothers within one week of childbearing, and the expression of CYP1A1 mRNA in milk cells was determined. The relationship between CYP1A1 mRNA expression in milk cells and dioxin levels in the cream layer of breast milk was analyzed.

### Materials and Methods

#### *Expression of AhR and Arnt in intact milk cells and responsiveness of cultured milk cells to TCDD*

Prior to a population study, the expression of AhR and Arnt in intact milk cells and the responsiveness of cultured milk cells to TCDD were determined. Milk cells were prepared by centrifugation from breast milk obtained from volunteers.

Total RNA was isolated using ISOGEN (Wako, Osaka, Japan) and RNeasy (QUIAGEN, Valencia, CA) according to the manufacturers' instructions. The expression of AhR and Arnt mRNA in intact cells was determined by RT-PCR.

Isolated milk cells were cultured in a DMEM/Ham's F12 medium (Gibco BRL) containing 10% FBS. Cells were treated with 1pM, 10pM, 100pM, 1nM, or 10nM TCDD in 0.1% DMSO for 4

hours. Total RNA was isolated as mentioned above, and the expression of CYP1A1 and CYP1B1 mRNA was determined by real-time RT-PCR.

*Study population and breast milk sampling*

Forty-nine pregnant women (age 15-39) who had delivered babies at Uechi Obstetrics and Gynecology Clinic (Utsunomiya, Japan) 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 50ml polypropylene tube with a meshed insert. Milk cells and the cream layer were separated by centrifugation and stored at  $-80^{\circ}\text{C}$  until further analysis.

Information on reproductive history, life style, and dietary habits of the participants was obtained by a questionnaire. The study proposal was approved by the Committee on Medical Ethics of National Institute for Environmental Studies (NIES), and informed consent was obtained from all participants prior to their enrollment.

*Dioxin analysis*

TEF (WHO 1998) assigned 29 PCDD/Fs and dioxin-like PCBs, and PCB#74 in the cream layer was analyzed by HRGC/HRMS with a Solvent Cut Large Volume (SCLV) injection technique<sup>5</sup>. Non-dioxin-like PCBs, PCB#99, #138, #146, #153, #163+#164, #170, #177, #178, #180, #182+#187, #183, #194, and #198+#201, were analyzed by HRGC/HRMS.

**Results and Discussion**

*In vitro study*

Breast milk cells consist mainly of spherical suspension cells, including oil droplets and a few adhesive epithelial keratinized cells. The expression of AhR and Arnt was confirmed in milk cells. The mean values of the constitutive expression of CYP1A1 and CYP1B1 in milk cells from volunteers (n=6) were 51270 and 394 copies/100ng RNA, respectively. The expression of CYP1A1 was much higher than that of CYP1B1 in contrast to the low CYP1A1 expression in peripheral lymphocytes observed in our previous study<sup>6</sup>. As CYP1A1 is sensitive to TCDD exposure, milk cells would be more suitable material than peripheral lymphocytes for monitoring dioxin exposure. In cultured milk cells, CYP1A1 was induced by TCDD at a dose of 100pM or more, and CYP1B1 was not induced at the doses tested here.

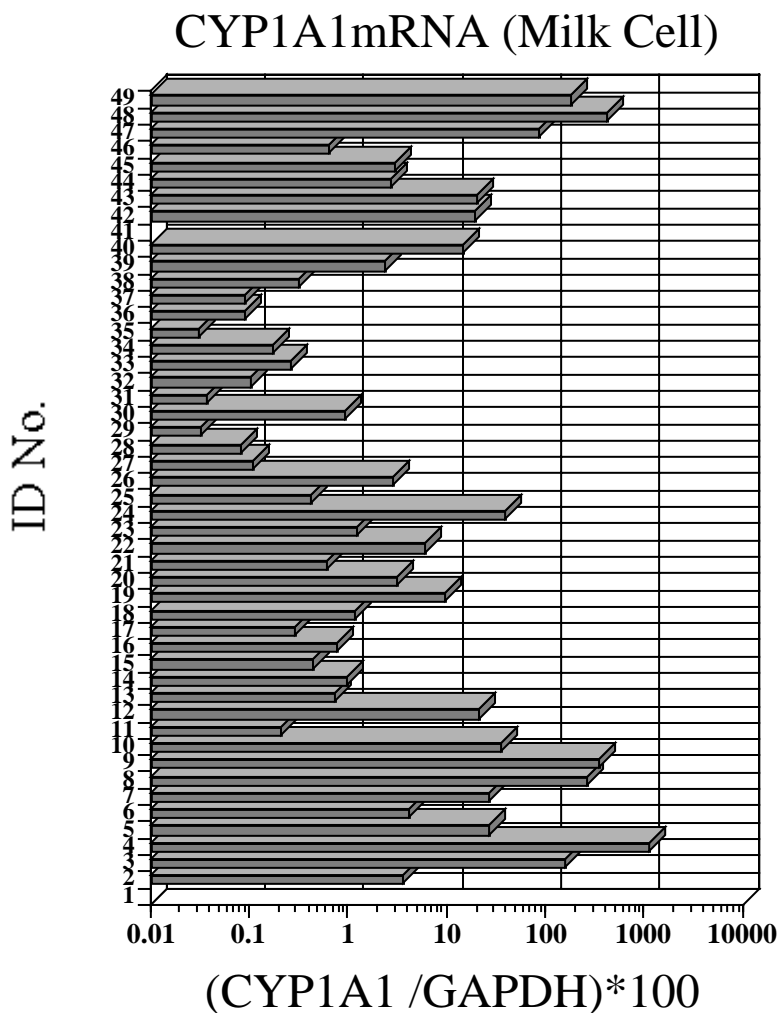


Fig. 1. CYP1A1 mRNA expression in breast milk cells.

#### *Population study*

The expression of CYP1A1 mRNA in milk cells from mothers within one week of childbearing is shown in Fig. 1. The median of  $(\text{CYP1A1}/\text{GAPDH}) \times 100$  was 2.62 (range 0.03 – 1140). Individual variation was very high. Dioxin levels were measured in the cream layer of breast milk. Prior to 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. Dioxin concentration in the cream layer showed a log-normal like distribution. The geometric mean concentrations of PCDDs, PCDFs, Co-PCBs, and their sum were 6.2, 3.4, 3.9, and 13.6 pg TEQ/g fat, respectively. The geometric mean concentration of the sum of non-coplanar PCBs measured in this study was 45,200 pg/g fat. The levels of PCBs #138, #153, and #180

were relatively high among non-coplanar PCBs. A trend of increased levels of dioxins (PCDDs + PCDFs+Co-PCBs) with age was observed ( $r=0.336$ ,  $p < 0.05$ ). A strong correlation was observed between dioxins and age in primiparous mothers ( $r=0.741$ ,  $p<0.001$ ). No significant correlation was observed between  $(\text{CYP1A1}/\text{GAPDH}) \times 100$  and dioxins (PCDDs + PCDFs+Co-PCBs). However, among non-smoking mothers undergoing natural childbirth, a significant correlation was observed between  $\log(\text{CYP1A1}/\text{GAPDH}) \times 100$  and dioxins (PCDDs + PCDFs+Co-PCBs) ( $r=0.458$ ,  $p<0.03$ ) (Fig. 2). Since modulation of CYP1A1 expression in placenta by smoking was reported<sup>7, 8</sup>, smoking would affect the expression of CYP1A1 in the background population. Further study is needed to elucidate the factors responsible for the inter- and intra-individual variability of CYP1A1 expression in milk cells.

In summary, milk cells expressed much higher CYP1A1 mRNA than that found in peripheral lymphocytes. CYP1A1/GAPDH in milk cells correlated with dioxin levels among non-smoking mothers, indicating the possible use of milk cells as biomarkers for dioxin exposure.

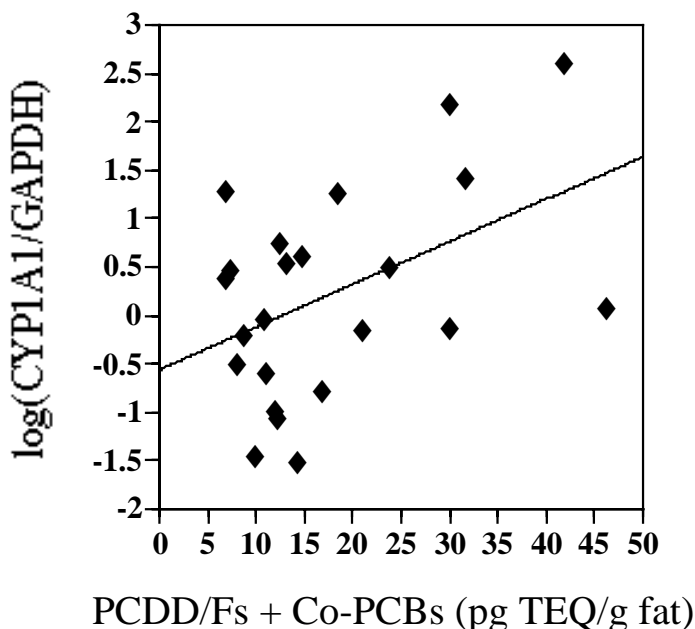


Fig. 2. Relationship between CYP1A1 expression and concentration of dioxins in milk in non-smoking mothers. ( $r=0.458$ ,  $p<0.03$ )

### Acknowledgement

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