RISK II

Effects of Lactational Exposure to Chlorinated Dioxins and Related Chemicals on Lymphocyte Subpopulations and Thyroid Functions in Japanese Babies

Junya Nagayama : Laboratory of Environmental Health Sciences, School of Health Sciences, Kyushu University, Higashi-ku, Fukuoka 812, Japan
Takao Iida, Hironori Hirakawa and Takahiko Matsueda : Department of Environmental Chemistry, Fukuoka Institute of Health and Environmental Sciences, Dazaifu, Fukuoka 818-01, Japan
Hiroshi Tsuji : Second Department of Internal Medicine, Faculty of Medicine, Kyushu University, Higashi-ku, Fukuoka 812, Japan
Ken Okamura : Laboratory of Clinical Chemistry, School of Health Sciences, Kyushu University, Higashi-ku, Fukuoka 812, Japan
Mayumi Hasegawa and Kayo Sato : Department of Midwifery, School of Health Sciences, Kyushu University, Higashi-ku, Fukuoka 812, Japan
Etsuko Kitahara : Department of Nursing, School of Health Sciences, Kyushu University, Higashi-ku, Fukuoka 812, Japan
Hsing-Yi Ma and Takashi Yanagawa : Department of Mathematical Statistics, Faculty of Science, Kyushu University, Higashi-ku, Fukuoka 812, Japan
Hisaji Igarashi and Jun'ichiro Fukushige : Department of Pediatrics, Faculty of Medicine, Kyushu University, Higashi-ku, Fukuoka 812, Japan
Tadayoshi Watanabe : Watanabe O.B.G.Y. Clinic, Higashi-ku, Fukuoka 813, Japan

Introduction

We have reported the concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) in breast milk[1), 2), 3)]. According to levels of these compounds in breast milk, it is estimated breast-fed babies in Japan consume from about 100 to 200 pg/kg/day as the Toxic Equivalents (TEQ) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD)[2)]. These TEQ values are much greater than the Acceptable Daily Intake (ADI) of 2,3,7,8-TCDD, namely, 1 - 10 pg/kg/day for adults. Babies are considered more sensitive to such kinds of chemicals. Therefore, we should give due attention to their possible health consequences to sucklings.

In order to clarify biological and/or biochemical effects of PCDDs, PCDFs and Co-PCBs, we

investigated the lymphocyte subpopulations and thyroid functions in the blood of 37 healthy breast-fed babies in relation to their concentrations of the breast milk.

Methods

Forty healthy women volunteered to participate in this study. They had a normal pregnancy without use of medicines. Breast milk (50 - 100 mℓ), sampled about 3 months after childbirth, was used to determine concentrations of PCDDs, PCDFs and Co-PCBs by GC-MS and the amounts of TEQ of 2,3,7,8- TCDD were calculated with Toxic Equivalency Factor (TEF) values proposed by NATO Committee on Challenge to Modern Society (1988) for PCDDs and PCDFs, and those by WHO-ECEH and IPCS (1994) for Co-PCBs[1), 2), 3)].

About 1 year after birth, 5 to 10 mℓ of peripheral blood samples were individually obtained from 37 breast-fed babies. The blood samples were used to measure the lymphocyte subpopulations by indirect immunofluorescence using monoclonal mouse anti-human antibodies against CD3, CD4, CD8, CD16, CD20 and HLA-DR, and the relative population densities of the lymphocyte subpopulations were calculated[4)]. They were also employed to determine serum concentrations of triiodothyronine (T_3), thyroxine (T_4), thyroid stimulating hormone (TSH) and thyroxine binding globulin (TBG) by radio-immunoassay methods using commercially available kits[5)].

Analysis of variance (ANOVA) was applied to fit the concentrations of PCDDs, PCDFs and Co-PCBs in TEQ of 2,3,7,8-TCDD as a function of variables of interest and statistical significance was evaluated by Student's *t* -test.

Results

1) Concentrations of PCDDs, PCDFs and Co-PCBs in breast milk

Medians and ranges in total concentration of PCDDs, PCDFs and Co-PCBs as TEQ of 2,3,7,8-TCDD are given in Table 1. Median concentrations on the whole and fat weight bases were 1.00 and 25.7ppt, respectively. Range was 0.34 to 2.37ppt on the whole weight basis and 15.2 to 48.5ppt on the fat weight basis.

2) Percentages of peripheral lymhocyte subpopulations in breast-fed babies

As shown in Table 2, median percentages of peripheral lymphocyte subpopulation in 37 breast-fed babies were as follows. Mature T-cell expressing CD3 was the highest (54.6%), helper/inducer T-cell (CD4) : 34.5%, human leucocyte antigen expressing DR domain (HLA-DR) : 31.0%, B-cell (CD20) : 27.1%, suppressor/cytotoxic T-cell (CD8) : 19.3%, Natural killer T-cell (CD16) : 7.9% and T-cell reactive to both CD4 and CD8 the lowest (0.5%). T-cell subpopulation expressing CD4 had a tendency to show higher percentage than that for CD8 in the peripheral blood of 37 babies and their ratio (CD4/CD8) was 1.8 in median.

Table 1. Medians and ranges in total concentration of PCDDs, PCDFs and Co-PCBs as TEQ of 2,3,7,8-TCDD in the breast milk of 40 mothers

Compound	Median (min.~ max.) in TEQ of 2,3,7,8-TCDD (ppt)	
	Whole Basis	Fat Basis
PCDDs PCDFs Co-PCBs	1.00 (0.34~2.37)	25.7 (15.2~48.5)

Table 2. Percentages of lymphocyte subpopulations in the peripheral blood of 37 breast-fed babies

Lymphocyte Subpopulation (Positive Cells)	Median (min.~ max.) Percentage
CD3	54.6 (31.2~69.8)
CD4	34.5 (15.7~51.4)
CD8	19.3 (10.6~38.4)
CD4+CD8	0.5 (0.2~1.3)
CD16	7.9 (2.1~23.3)
CD20	27.1 (12.8~56.2)
HLA-DR	31.0 (15.5~62.1)
CD4/CD8	1.8 (0.8~3.3)

3) Thyroid function tests in breast-fed babies

Results of the examination in thyroid functions in the serum of 37 breast-fed babies are summarized in Table 3. We could not find any apparent abnormal figure in all the tests.

4) Correlation between the concentrations of PCDDs, PCDFs and Co-PCBs in breast milk and percentages of peripheral lymphocyte subpopulations in breast-fed babies

We observed positive correlation between the total levels of TEQ as 2,3,7,8-TCDD in the breast milk and the percentages of CD4-positive cells in the blood of the babies (p=0.086) and also negative

correlation between the 2,3,7,8-TCDD TEQ levels and the percentages of CD8-positive cells (p=0.070). Consequently, as indicated in Fig. 1, the ratios of CD4 to CD8 showed a significant increasing tendency with the total TEQ levels in the breast milk (p=0.015).

Table 3. Results of thyroid function tests in the serum of 37 breast-fed babies

	Median (min. ~ max.)
Triiodothyronine (T_3, ng/mℓ)	2.00 (1.00~2.50)
Thyroxine (T_4, μg/dℓ)	11.4 (7.7~16.4)
Thyroid stimulating hormone (TSH, μU/mℓ)	2.69 (0.92~5.86)
Thyroxine binding globulin (TBG, μg/mℓ)	25.1 (17.8~31.8)
T_4/TBG	0.45 (0.30~0.61)

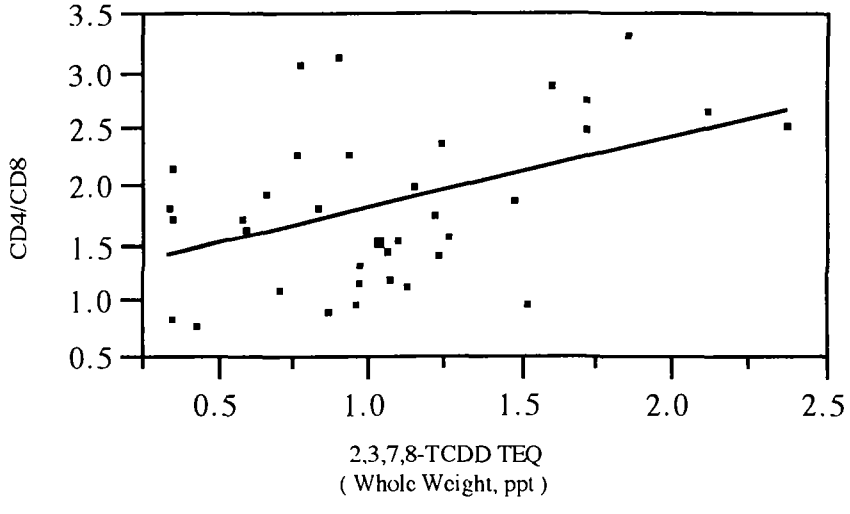

Fig. 1. Correlation between 2,3,7,8-TCDD TEQ levels in the breast milk and the ratios of percentages of CD4-positive to CD8-positive cells in the blood of breast-fed babies (p=0.015)

5) Correration between the concentrations of PCDDs, PCDFs and Co-PCBs in breast milk and thyroid functions in breast-fed babies

Significant negative correlation was observed between the total levels of TEQ as 2,3,7,8-TCDD in the

breast milk and the levels of T_4 in the serum of the babies (p=0.035). The result is shown in Fig. 2. Other thyroid functions did not indicate any significant correlation with the total TEQ levels.

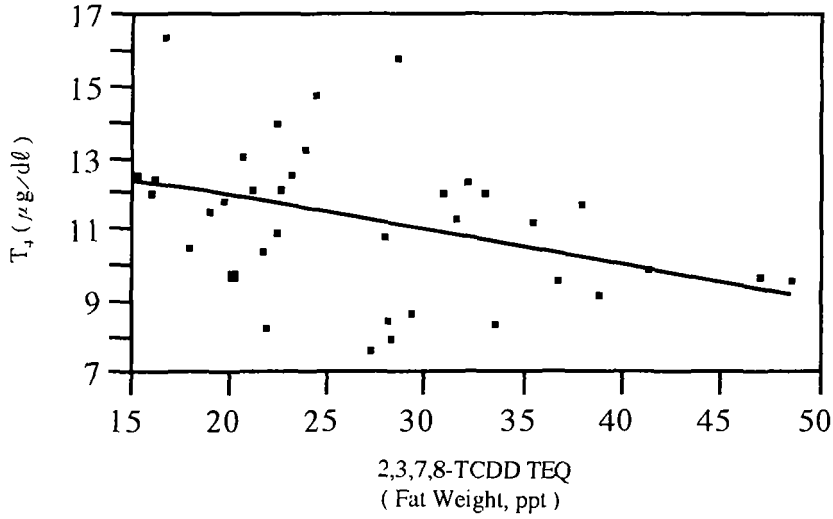

Fig. 2. Correlation between 2,3,7,8-TCDD TEQ levels in the breast milk and the levels of T_4 in the serum of breast-fed babies (p=0.035)

Discussion and Conclusions

The presence of PCDDs, PCDFs and Co-PCBs in breast milk results in daily intakes from 40 to 280 pg/kg body weight as TEQ of 2,3,7,8-TCDD with the median figure of 120pg/kg for breast-fed babies (Table. 1). These intakes far exceed the ADI of various countries.

Possibly due to such kinds of great intakes of Dioxin-like chemicals from breast milk, significant correlations were observed between peripheral lymphocyte subpopulations in the breast-fed babies and 2,3,7,8-TCDD TEQ levels of the breast milk (Fig. 1), and also between serum T_4 levels of the babies and the 2,3,7,8-TCDD TEQ levels (Fig. 2). Recently, no relationship between pre- and postnatal PCB/Dioxin exposure and upper or lower respiratory tract symptoms or humoral antibody production was reported[6]. However, at 11 weeks of age, calculated cumulative intakes of Dioxins by breast milk consumption were significantly correlated with aminotransferase activities in plasma and negatively with platelet counts in newborns[7]. Concerning effects on thyroid hormone regulation, contrary to our results, T_4 and the ratio of T_4/TBG were significantly higher in high-exposed babies than in low-exposed ones at 7 days and 11 weeks of age[8].

The results of studies mentioned above and our present investigation suggest that exposure to background levels of Dioxins and related chemicals via breast milk may have some effects in breast-fed babies.

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