Perinatal Exposure to Chlorinated Dioxins and Related Chemicals on Lymphocyte Subpopulations in Japanese Breast-Fed Infants

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Introduction

We already have been contaminated with highly toxic organochlorine chemicals such as PCDDs, PCDFs and Co-PCBs[1) 2)]. Consequently, these compounds have also been determined in Japanese breast milk and mean total concentrations of PCDDs, PCDFs and Co-PCBs in the breast milk were about 1.2 to 1.4ppt in 2,3,7,8-TCDD TEQ values on whole weight basis[3) 4)]. According to their mean levels in the breast milk, breast-fed Japanese infants are considered to have relatively large amounts of these chemicals, namely, about 100 to 200 TEQ pg/kg body weight[3)]. Babies seem more sensitive to the toxic compounds than adults, so we should give due attention to their possible health consequences in breast-fed infants.

In this study, in order to clarify the effects of perinatal exposure to PCDDs, PCDFs and Co-

PCBs on the immune system, we investigated the lymphocyte subsets in the peripheral blood of 93 breast-fed infants in relation to their total TEQ concentrations.

Material and Methods

One hundred and twenty four mothers volunteered to participate in this study. Pregnancy and delivery were completed without overt signs of serious illness or complications. Only babies born at term (37 to 42 weeks of gestation) without congenital anomalies or diseases were included.

Breast milk (50~100mℓ), sampled around 3 months after the childbirth, was used to determine the concentrations of PCDDs, PCDFs and Co-PCBs by high resolution GC/MS method[3]. The international toxic equivalency factor approach of NATO/CCMS was employed for PCDDs and PCDFs[5] and WHO-ECEH and IPCS approach for Co-PCBs[6]. By multiplying the concentration (pg/g) and the toxic equivalency factor value, the concentration in 2,3,7,8-TCDD TEQ value of each congener was calculated (TEQ pg/g). The TEQ-sum of all congeners of PCDDs, PCDFs and Co-PCBs detected in the breast milk was summarized as the total TEQ concentration or level.

About 1 year after birth, 5 to 10mℓ of peripheral blood samples were individually obtained from 93 breast-fed infants. These blood samples were used to measure lymphocyte subpopulations by indirect immunofluorescence using monoclonal mouse anti-human antibodies against CD3 for mature T cells, CD4 for helper/inducer T cells, CD8 for suppressor/cytotoxic T cells, CD16 for natural killer T cells, CD20 for B cells and HLA-DR for activated T cells and their relative population densities were calculated[7].

In order to get more reliable results, the Spearman rank correlation coefficients were computed instead of the Pearson correlation and their statistical significances were evaluated.

Results

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

Respective distributions in total 2,3,7,8-TCDD TEQ concentrations of PCDDs, PCDFs and Co-PCBs on the whole and fat weight bases are indicated in Fig. 1. Median total concentrations on the whole and fat weight bases were 0.94 and 22.6ppt, respectively. The range of total concentrations on the whole weight basis was 0.15 to 2.92ppt and that on the fat weight basis 3.4 to 48.5 ppt.

2) Percentages of lymphocyte subpopulations in the peripheral blood of breast-fed infants

As shown in Table 1, median percentages of lymphocyte subpopulations in the blood of 93 breast-fed infants were as follows. Mature T-lymphocytes (CD3) was the highest, 60.4%, helper/

inducer T-lymphocytes (CD4) : 39.6%, activated T-lymphocytes (HLA-DR) : 25.6%, B-lymphocytes (CD20) : 21.7%, suppressor/cytotoxic T-lymphocytes (CD8) : 19.1%, natural killer T-lymphocytes (CD16) : 8.6% and T-lymphocytes possitive to both CD4 and CD8 the lowest, 0.5%. The median ratio of CD4/CD8 was 2.08. Distributions in percentages of CD8-positive lymphocytes and in the ratios of CD4/CD8 in percentages were shown in Fig. 2.

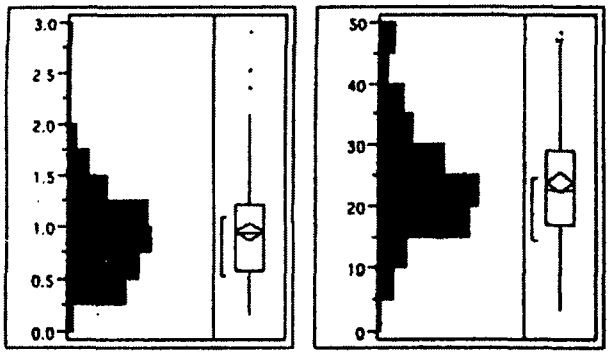

Fig. 1. Distributions in total 2,3,7,8-TCDD TEQ concentrations (ppt) of PCDDs, PCDFs and Co-PCBs on the whole (left) and fat (right) weight bases in the breast milk of 124 mothers

Table 1. Percentages of lymphocyte subpopulations in the peripheral blood of 93 breast-fed infants

Lymphocyte Subpopulation (Positive Cells)	Median (min. ~ max.) Percentage
CD3	60.4 (31.2 ~ 76.6)
CD4	39.6 (15.7 ~ 61.7)
CD8	19.1 (10.6 ~ 41.2)
CD4 + CD8	0.5 (0.1 ~ 2.1)
CD16	8.6 (1.7 ~ 25.4)
CD20	21.7 (5.5 ~ 56.2)
HLA-DR	25.6 (8.2 ~ 62.1)
CD4/CD8	2.08 (0.62 ~ 4.52)

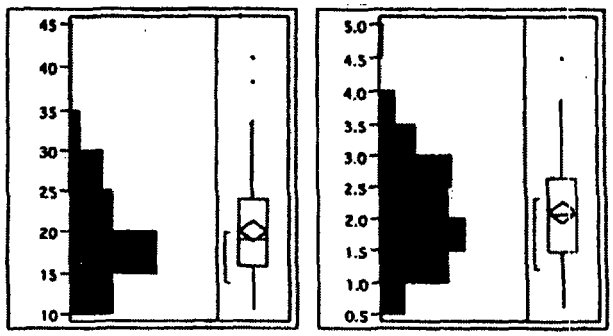

Fig. 2. Distributions in percentages of CD8-positive lymphocytes (left) and in the ratios of CD4/CD8 in percentages (right) in the peripheral blood of 93 breast-fed infants

3) Correlation between the total TEQ concentrations of PCDDs, PCDFs and Co-PCBs of the breast milk and the peripheral lymphocyte subpopulations in breast-fed infants.

The results of this study are based upon the Spearman rank correlations instead of using the Pearson correlations to get more robust findings. Therefore, no significant correlation between the total TEQ concentrations in the breast milk and the peripheral lymphocyte subpopulations was observed.

Discussion

The presence of PCDDs, PCDFs and Co-PCBs in the breast milk results in daily intakes of about 18 to 350 TEQ pg/kg body weight with the median figure of 113 TEQ pg/kg body weight, which was about 10 times higher than the tolerable daily intake in Japan, namely, 10 TEQ pg/kg body weight.

In our formar paper[8], positively significant correlation between the total TEQ concentrations in the breast milk and the ratios of CD4 to CD8 positive lymphocytes was observed. In that paper, the Pearson correlation coefficient was used for statistical evaluation, so some extreme values of data may cause such kinds of correlations. In case of the Pearson correlation of these 93 breast-fed infant and mother pairs, the percentage of CD8-positive lymphocytes and the ratio of CD4/CD8 were also negatively and positively correlated with the total 2,3,7,8-TCDD TEQ concentration, namely, $p=0.09$ and $p=0.06$, respectively. However, in order to avoid such fallible effects, we employed the Spearman rank correlation method. Therefore, the results of this method seemed more reliable. Recently, no relationship between pre- and postnatal PCB/Dioxin exposure and upper or lower rispiratory tract symptoms or humoral antibody production was reported[9].

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