SEXUAL DIFFERENCE IN EFFECTS OF DIOXINS ON IMMUNE RESPONSE SYSTEM IN JAPANESE INFANTS

Junya Nagayama[1], Hiroshi Tsuji[2], Takao Iida[3], Hironori Hirakawa[3], Takahiko Matsueda[3], Jun'ichiro Fukushige[4] and Tadayoshi Watanabe[5]

[1]Laboratory of Environmental Health Sciences, School of Health Sciences, Kyushu University, Fukuoka 812-8582, Japan
[2]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan
[3]Department of Environmental Sciences, Fukuoka Institute of Health and Environmental Sciences, Fukuoka 818-0135, Japan
[4]Fukuoka Children's Hospital, Fukuoka 810-0063, Japan
[5]Watanabe O.B.G.Y. Clinic, Fukuoka 813-0044, Japan

Introduction

We already have been contaminated with highly toxic organochlorine compounds such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) [1, 2]. Consequently, levels of these compounds have been determined in Japanese breast milk and total concentrations of PCDDs, PCDFs and Co-PCBs in the breast milk were about 1.2 to 1.4 ppt in the mean toxic equivalent quantity (TEQ) values on whole weight basis [3, 4]. According to their levels in the breast milk, breast-fed Japanese infants are considered to take relatively a large amount of these compounds, namely, about 100 to 200 TEQ-pg/kg body weight[3]. Infants seem more sensitive to the toxic chemicals than adults, so we should give due attention to their possible health effects in infants.

In this study, in order to clarify the sexual differences in effects of perinatal exposure to PCDDs, PCDFs and Co-PCBs, which are so-called dioxins, on the immune response system, we investigated the lymphocyte subsets in the peripheral blood of Japanese male and female infants in relation to their concentrations of the breast milk.

Materials and Methods

Ninety-three healthy mothers (mean age : 29 years old and the range : 24~38 years old) 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 (50~100 ml), sampled about 3 months after the childbirth, was used to determine the concentrations of PCDDs, PCDFs and Co-PCBs by gas chromatography/mass spectrometry using a Finnigan MAT-90 mass spectrometer (Finnigan MAT, Germany) directly interfaced with a Varian Model 3400 gas chromatograph [3].

TEQ concentrations of PCDDs, PCDFs and Co-PCBs were calculated by using 1998 WHO toxic equivalent factor (TEF) values [5]. By multiplying the concentration (pg/g) and the TEF value, the concentration in a TEQ value of each congener was calculated (TEQ-pg/g). The TEQ-sum of all congeners of PCDDs, PCDFs and Co-PCBs determined in the breast milk was summarized as the total TEQ concentration.

RISK ASSESSMENT

About 1 year after birth, 5 to 10 ml of peripheral blood samples were individually obtained from 93 infants (54 males and 39 females). These blood samples were employed to measure lymphocyte subsets 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, CD4 and CD8 double positive (CD4+8+) cells, CD16 for natural killer T cells, CD20 for B cells and HLA-DR for activated T cells (Ortho Pharmaceutical Corp., Raritan, NJ and Becton-Dickinson, Mountain View, CA) [6]. The relative population densities of the lymphocyte subsets were also calculated.

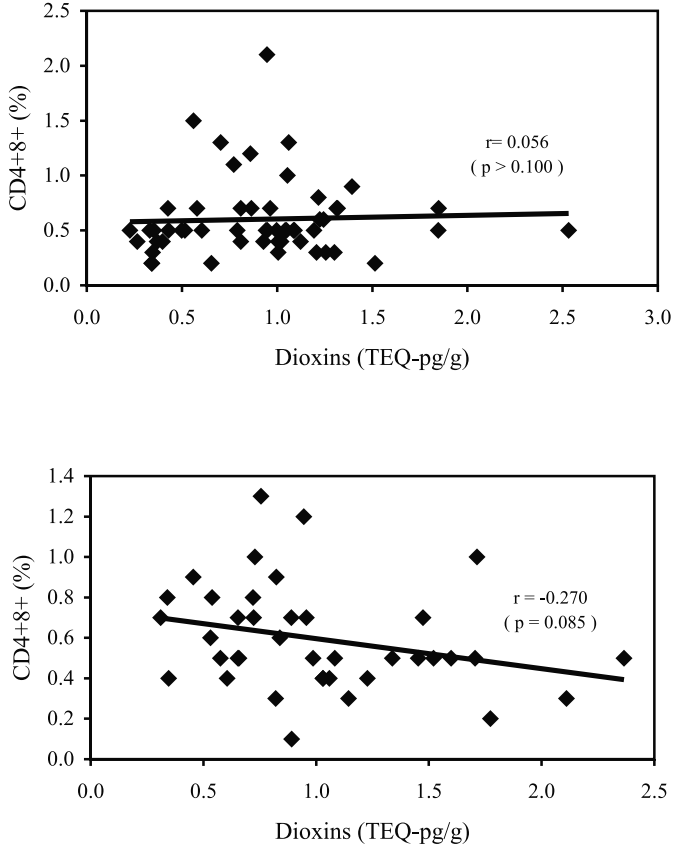

Figure 1. Correlation of the dioxin levels in the breast milk with the percentages of CD4 and CD8 double positive lymphocytes in the blood of the male (upper) and female (lower) infants

Analysis of variance (ANOVA) was applied to examine the relationship of perinatal exposure to dioxins to each variable of interest and statistical significance was evaluated by Student's *t*-test.

Results and Discussion

Median contamination level of dioxins in the breast milk for the male infants was 0.96 TEQ-pg/g on whole weight basis with the range of 0.23 to 2.92 TEQ-pg/g and that for the female infants 0.89 TEQ-pg/g with 0.15 to 2.37 TEQ-pg/g. Like this, large personal variation was observed in

concentrations of dioxins in Japanese breast milk and these were used as the exposure variables of the male and female infants.

Percentages of the lymphocyte subsets positive to the monoclonal mouse anti-human antibodies examined in the blood of the male infants (median, min.~ max.) were as follows : CD3 (62.2 %, 37.1~ 75.4 %), CD4 (39.1 %, 22.4~61.7 %), CD8 (19.3 %, 12.3~41.2 %), CD4+8+ (0.5 %, 0.2~2.1 %), CD16 (9.4 %, 2.7~25.4 %), CD20 (20.2 %, 5.5~38.7 %) and HLA-DR (24.2 %, 8.2~44.6 %). Similarly, those in the blood of the female infants : CD3 (58.0 %, 37.2~76.6 %), CD4 (41.3 %, 15.7~56.4 %), CD8 (18.3 %, 10.6~33.8 %), CD4+8+ (0.5 %, 0.1~1.3 %), CD16 (6.8 %, 1.7~19.9 %), CD20 (23.6 %, 12.3~56.2 %) and HLA-DR (26.6 %, 11.2~62.1 %). Those were used as the response variables of the infants.

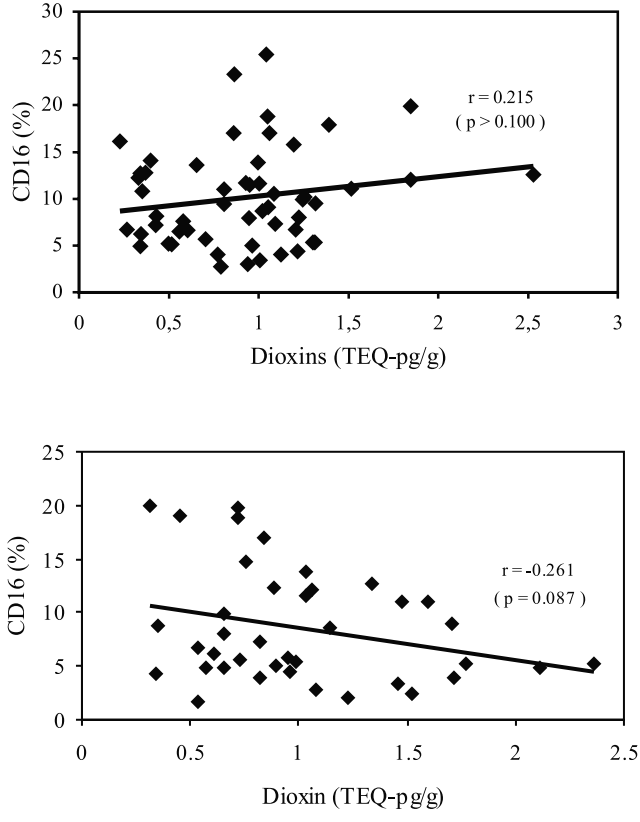

Figure 2. Correlation of the dioxin levels in the breast milk and the percentage of CD16 positive lymphocytes in the blood of the male infants (upper) and of the female infants (lower)

Some significant relationships between the exposure variables and response variables were observed only in the female infants and not in the male infants. Those were the correlations between the dioxin level and the percentage of CD4 and CD8 double positive lymphocytes, CD8 positive lymphocytes, or CD16 positive lymphocytes. Figs 1 and 2 show the relationships between dioxin levels and percentages of CD4 and CD8 double positive cells, or those of CD16 positive cells.

RISK ASSESSMENT

On the thyroid hormone system of Japanese infants, dioxins seem to give more effects in males than in females [7]. These results, however, of this study may indicate that effects of dioxins on the immune response system in the rapidly growing stage of human life seem stronger in the females than in the males.

References

1. Hirakawa H, Iida T, Matsueda T, Tokiwa H, Nagata T and Nagayama J (1992) Organohal Comp 10, 93-96.
2. Hirakawa H, Matsueda T, Iida T, Nakamura M, Nagata T and Nagayama J (1994) Ibid 21, 419-422.
3. Matsueda T, Iida T, Hirakawa H, Fukamachi K, Tokiwa H and Nagayama J (1993) Chemosphere 27, 187-194.
4. Hirakawa H, Iida T, Matsueda T , Nakagawa R, Hori T and Nagayama J (1995) Organohal Comp 26, 197-200.
5. Van den Berg M, Birnbaum LS, Bosveld ATC, Brunstorm B *et al.* (1998) Environ Health Perspect 106, 775-792.
6. Tsuji H, Murai K, Akagi K and Fujishima M (1990) B J Clin Immunol 10, 38-44.
7. Nagayama J, Iida T, Hirakawa H, Matsueda T, Tsuji H, Fukushige J and Watanabe T (2002) Organohal Comp, in press.