EFFECTS OF PCDDs, PCDFs AND COPLANAR PCBs ON IMMUNE RESPONSE AND THYROID HORMONE SYSTEMS IN JAPANESE MOTHERS

Junya Nagayama[1], Hiroshi Tsuji[2], Takao Iida[3], Reiko Nakagawa[3], Takahiko Matsueda[3], Hironori Hirakawa[3] Asuka Shiraha[4], Takashi Yanagawa[4], Jun'ichiro Fukushige[5] and Tadayoshi Watanabe[6]

1 Laboratory of Environmental Molecular Epidemiology, School of Health Sciences, Faculty of Medicine, 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 Department of Mathematical Statistics, Graduate School of Mathematics, Kyushu University, Fukuoka 812-8581, Japan
5 Fukuoka Children's Hospital, Fukuoka 810-0063, Japan
6 Watanabe O.B.G.Y. Clinic, Fukuoka 813-0044, Japan

Introduction

We already have been contaminated with highly toxic polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs), which are so-called dioxins[1,2]. As a consequence, relatively great levels of these chemicals have been determined in Japanese breast milk and total mean concentrations of PCDDs, PCDFs and Co-PCBs in the breast milk were about 1.2 to 1.4 ppt in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalent quantity (TEQ) values on whole weight basis[3,4]. We have reported the effects of perinatal and lactational exposures to these compounds on immune response and thyroid hormone systems in Japanese infants[5,6,7,8,9,10,11,12]. Their effects on these systems were also studied in Japanese mothers[13,14].

In this study, in order to clarify the effects of PCDDs, PCDFs and Co-PCBs on immune response and thyroid hormone systems more in detail, we investigated the changes of the lymphocyte subsets and thyroid related chemicals in the peripheral blood of Japanese mothers in relation to their concentrations of the breast milk.

Materials and Methods

In our studies, 124 mothers (mean age : 29 years old and the range : 21 ~ 38 years old) volunteered to participate in all. 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 2 to 4 months after childbirth, was used to determine 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[15].

TEQ concentrations of PCDDs, PCDFs and Co-PCBs were calculated by using 1998 WHO toxic equivalent factor (TEF) values [16]. The TEQ-sum of all congeners of PCDDs, PCDFs, Co-PCBs and dioxins (PCDDs + PCDFs + Co-PCBs) determined in every breast milk sample was summarized as the total 2,3,7,8-TCDD TEQ concentration. Concentrations of PCDDs, PCDFs, Co-PCBs and dioxins were used as a measure of their contamination levels in Japanese mothers.

Around 1 year after childbirth, 10 ml of peripheral blood samples were individually obtained from 94 mothers (mean age : 28 years old and the range : 21 ~ 37 years old). These blood samples were employed to measure the lymphocyte subpopulations by indirect immunofluorescence using monoclonal mouse anti-human antibodies against CD3, CD4, CD4+8+, CD8, CD16, CD20 and HLA-DR positive lymphocytes, and their relative population densities were calculated [17]. These blood samples were also used to determine serum concentrations of triiodothyronine (T_3), thyroxine (T_4), thyroid stimulating hormone (TSH) and thyroxine binding globulin (TBG) by radioimmunoassay methods using commercially available kits [18].

We are investigating the relative risks of toxic chemicals to these biological systems, but not their causality. For this purpose and in order to conduct reliable and robust analysis, the concentrations of PCDDs, PCDFs, Co-PCBs and dioxins, the percentages of the lymphocyte subsets, as well as CD4+/CD8+ ratios, and the serum levels of thyroid related chemicals were categorized into two groups ; namely, the measurements which were less than the means and equal to or above the 75 percentile points in each year were set by 0 and 1, respectively. Then, Fisher's exact test was applied to the resulted fourfold tables and odds ratios were computed from the tables by logistic regression to evaluate the relative risks. Ninety percent of confidence intervals (C.I.) of odds ratios were also calculated.

Results and Discussion

Respective TEQ-concentrations (mean, min. ~ max.) of PCDDs, PCDFs, Co-PCBs and dioxins on lipid weight basis in 94 breast milk samples were 8.8 pg/g, 2.1 ~ 19 pg/g, 7.0 pg/g, 1.8 ~ 21 pg/g, 9.5 pg/g, 2.1 ~ 31 pg/g and 25 pg/g, 6.3 ~ 51 pg/g. The mean contamination level of Co-PCBs was the highest and that of PCDFs the lowest.

Percentages (mean, min. ~ max.) of lymphocyte subpopulations positive to the monochlonal mouse anti-human antibodies examined and the ratio of CD4+ to CD8+ lymphocytes were as follows : CD3 (72%, 59 ~ 82%), CD4 (41%, 27 ~ 53%), CD4+8+ (1.1%, 0.3 ~ 4.6%), CD8 (29%, 19 ~ 49%), CD16 (11%, 2.6 ~ 22.3%), CD20 (9.7%, 1.3 ~ 18%), HLA-DR (15%, 4.4 ~ 28%) and CD4+/CD8+ (1.46, 0.56 ~ 2.47). Serum levels (mean, min. ~ max.) of T_3, T_4, TSH and TBG in 94 Japanese mothers were 1.4 ng/ml, 1.0 ~ 3.1 ng/ml, 8.2 μg/ml, 5.2 ~ 17 μg/ml, 2.0 μIU/ml, 0.01 ~ 7.6 μIU/ml and 19 μg/ml, 15 ~ 41 μg/ml, respectively.

As shown in Table 1, PCDDs significantly decreased the CD8 positive lymphocytes and increased the CD4 positive / CD8 positive lymphocyte ratios in the blood of Japanese mothers. Co-PCBs also enhanced the CD4 positive / CD8 positive lymphocyte ratios.

We could not find any significant effect of PCDDs, PCDFs, Co-PCBs and dioxins on other lymphocyte subsets in the blood of Japanese mothers. Accordingly, effects of PCDDs on the immune response system in Japanese mothers seemed the strongest.

Table 2 indicates that PCDDs and PCDFs significantly lowered the serum levels of T_3 in the Japanese mothers, exposure to PCDDs, PCDFs and Co-PCBs also decreased the serum levels of T_4, and both PCDDs and PCDFs significantly enhanced the serum levels of TSH. Therefore, effects of PCDDs and PCDFs were considered stronger than that of Co-PCBs on the

thyroid hormone system in the Japanese mothers.

Table 1. Effects of PCDDs, PCDFs and Co-PCBs on the lymphocyte subsets in the blood of Japanese mothers

Dioxins	Odds Ratio	90% C.I.	*p*-Value
CD4 Positive Cells			
PCDDs	0.71	0.16 – 2.88	0.52
PCDFs	1.16	0.30 – 4.33	0.58
Co-PCBs	1.55	0.43 – 5.53	0.42
CD8 Positive Cells			
PCDDs	***0.17***	***0.02 – 0.94***	***0.12***
PCDFs	0.60	0.15 – 2.17	0.41
Co-PCBs	0.54	0.13 – 1.95	0.36
CD4 Positive / CD8 Positive Ratio			
PCDDs	***5.42***	***1.23 – 27.3***	***0.08***
PCDFs	2.56	0.63 – 10.5	0.24
Co-PCBs	***5.00***	***1.22 – 22.5***	***0.07***

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. Nagayama J, Tsuji H, Iida T, Hirakawa H *et al.* (1998) Chemosphere, 37, 1781-1787.
6. Nagayama J, Tsuji H, Iida T, Nakagawa R *et al.* (2000) Organohal Comp 49, 87-90.
7. Nagayama J, Tsuji H, Iida T, Nakagawa R *et al.* (2001) Ibid 53, 121-125.
8. Nagayama J, Tsuji H, Iida T, Hirakawa H *et al.* (2002) Ibid 59, 135-138.
9. Nagayama J, Okamura K, Iida T, Hirakawa H *et al.* (1998) Chemosphere, 37, 1789-1793.
10. Nagayama J, Iida T, Nakagawa R, Matsueda T *et al.* (2000) Organohal Comp 48, 236-239.
11. Nagayama J, Iida T, Nakagawa R, Matsueda T *et al.* (2001) Ibid 53, 140-144.
12. Nagayama J, Iida T, Hirakawa T, Matsueda T *et al.* (2002) Ibid 59, 457-460.
13. Nagayama J, Tsuji H, Iida T, Nakagawa R *et al.* (2002) Ibid 55, 425-428.
14. Nagayama J, Iida T, Nakagawa R, Matsueda T *et al.* (2002) Ibid 56, 115-118.

15. Nakagawa R, Hirakawa H, Iida T and Matsueda T (1999) J AOAC Int 82, 716-724.
16. Van den Berg M, Bimbaum L S, Bosveld ATC, Brunstorm B *et al.* (1998) Environ Health Perspect 106, 775-792.
17. Tsuji H, Murai K, Akagi K and Fujishima M (1990) B J Clin Imunol 10, 38-44.
18. Okamura K, Sato K and Ikenoue H (1988) J Clin Endocrinol Metab 67, 720-726.

Table 2. Effects of PCDDs, PCDFs and Co-PCBs on the thyroid hormone system in the serum of Japanese mothers

Dioxins	Odds Ratio	90% C.I.	*p*-Value
T_3			
PCDDs	***0.32***	***0.09 – 0.95***	***0.08***
PCDFs	***0.11***	***0.01 – 0.50***	***0.01***
Co-PCBs	0.40	0.13 – 1.05	0.11
T_4			
PCDDs	***0.14***	***0.03 – 0.47***	***0.007***
PCDFs	***0.29***	***0.08 – 0.86***	***0.06***
Co-PCBs	***0.28***	***0.09 – 0.74***	***0.03***
TSH			
PCDDs	***2.54***	***0.98 – 6.65***	***0.09***
PCDFs	***3.28***	***1.24 – 8.77***	***0.04***
Co-PCBs	2.06	0.80 – 5.22	0.16