EFFECTS OF DIOXINS, PCBs AND ORGANOCHLORINE PESTICIDES ON WET WEIGHT BASIS ON IMMUNE RESPONSE AND THYROID HORMONE SYSTEMS IN JAPANESE MOTHERS

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Introduction

Foods in Japan have been polluted with some organochlorine compounds such as pesticides, polychlorinated biphenyls (PCBs) and dioxins [1] and Japanese people have also been contaminated with these compounds [2, 3, 4]. Consequently, some pesticides such as hexachlorocyclohexans (HCHs), 1,1,1-trichloro-2,2-bis-(4-chlorophenyl)-ethane (DDT), dieldrin and heptachlor epoxide (HCE), and PCBs have been determined in Japanese breast milk and their mean or median concentrations on lipid weight basis were about 420, 330, 3, 4 and 110 ppb, respectively [4, 5, 6]. Their levels, however, were still 100 to 10,000 times higher than those of dioxins, that is, polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalent (TEQ) values as a whole in the breast milk of Japan [5]. We have reported the effects of perinatal and lactational exposure to these compounds on immune response and thyroid hormone systems in Japanese infants [7, 8, 9, 10, 11, 12, 13, 14]. Their effects on these systems were also studied in Japanese mothers [15, 16].

In this study, in order to clarify the effects of dioxins, PCBs and organochlorine pesticides on immune response and thyroid hormone systems more in detail, we investigated the changes of the lymphocyte subsets and thyroid hormones and the related chemicals in the peripheral blood of Japanese mothers in relation to their contamination levels of the breast milk on wet weight basis.

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 [17], and also employed to determine those of PCBs and organochlorine pesticides by ECD gas chromatographic method [5, 18].

TEQ concentrations of PCDDs, PCDFs and Co-PCBs were calculated by using 1998 WHO toxic equivalent factor (TEF) values [19]. The TEQ-sum of all congeners of 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 dioxins, PCBs and organochlorine pesticides 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 used to measure the lymphocyte subsets 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 [20]. These blood samples were also employed 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 [21].

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 dioxins, PCBs and organochlorine pesticides, the percentages of the lymphocyte subsets, and the serum levels of thyroid hormones and the 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

Concentrations of HCH and DDT were about 100 times higher than those of dieldrin and HCE. Mean contamination levels of chlordane and PCBs were around 4 ng/g and 3 to 5 times less than those of HCH and DDT. Mean concentration of dioxins was 0.96 pg-TEQ/g, which was around 180 times lower than those of dieldrin and HCE.

Percentages (mean, min. ~ max.) of lymphocyte subsets 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 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, PCBs significantly decreased the percentage of HLA-DR+ lymphocyte subset and HCE increased the percentage of CD4+8+ lymphocyte subset. HCE also lowered the percentage of CD20+ lymphocyte subset.

We could not find any significant effect of dioxins, PCBs and organochlorine pesticides on other lymphocyte subsets. Accordingly, effects of HCEs on the immune response system in Japanese mothers seemed the strongest.

Table 2 indicates that chlordane and PCBs significantly lowered the serum levels of T_3, exposure to dioxins also decreased the serum levels of T_4, and PCBs significantly enhanced the serum levels of TSH. In addition, dioxins and chlordane lowered the serum level of TBG. Therefore, effects of dioxins were considered stronger on thyroid hormone system than on immune response system in Japanese mothers. More detail effects of dioxins on thyroid hormone system is seen in Table 3.

References

1. Nakagawa R, Hirakawa H, Hori T. *J.AOAC Int* 1995;78:921-929.
2. Kashimoto T, Takayama K, Mimura M *et al*. *Chemosphere* 1989; 19:921-926.
3. Hirakawa H, Iida T, Matsueda T, Nagayama J. *Organohal Comp* 1996;30:127-130.
4. Iida T, Hirakawa H, Matsueda T, Nakagawa R, Hori T, Nagayama J. Ibid. 1999;44:123-127.
5. Nakagawa R, Hirakawa H, Iida T, Matsueda T, Nagayama J. *J. AOAC Int* 1999;82:716-724.
6. Nagayama J, Tsuji H, Okamura K, Iida T *et al*. *Organohal Comp* 1998;37:163-167.
7. Nagayama J, Tsuji H, Iida T, Hirakawa H *et al*. *Chemosphere* 1998;37:1781-1787.
8. Nagayama J, Tsuji H, Iida T, Nakagawa R *et al*. *Organohal Comp* 2000;49:87-90.

Table 1. Effects of Dioxins, PCBs and organochlorine pesticides on lymphocyte subsets in the blood of Japanese mothers (p-value < 0.20)

Compound	Odds Ratio	90% C.I.	p-Value
HLA-DR + Cells			
DDT	0.16	0.01 – 0.89	0.11
PCBs	***0.15***	***0.01 – 0.80***	***0.08***
CD4+8+ Cells			
DDT	***0.00***	***–***	***0.07***
HCE	***6.61***	***1.76 – 28.9***	***0.02***
CD8+ Cells			
PCBs	3.75	0.99 – 15.2	0.11
CD4+/CD8+			
Dieldrin	0.23	0.02 – 1.25	0.19
CD20+ Cells			
HCE	***0.00***	***–***	***0.05***

Table 2. Effects of Dioxins, PCBs and Organochlorine Pesticides on thyroid hormone system in the serum of Japanese mothers (p-value < 0.20)

Compound	Odds Ratio	90% C.I.	p-Value
T_3			
Dioxins	0.44	0.14 – 1.20	0.15
Chlordane	***0.36***	***0.12 – 0.94***	***0.07***
PCBs	***0.29***	***0.06 – 0.97***	***0.10***
T_4			
Dioxins	***0.14***	***0.03 – 0.48***	***0.007***
TSH			
DDT	2.18	0.77 – 6.02	0.17
PCBs	***2.31***	***0.94 – 5.68***	***0.10***
TBG			
Dioxins	***0.34***	***0.09 – 1.01***	***0.10***
Chlordane	***0.38***	***0.12 – 1.00***	***0.09***

Table 3. Effects of Dioxins, PCDDs, PCDFs and Co-PCBs on thyroid hormone system in the serum of Japanese mothers

Compound	Odds Ratio	90% C.I.	*p*-Value
T_3			
Dioxins	0.44	0.14 – 1.20	0.15
PCDDs	***0.23***	***0.05 – 0.76***	***0.05***
PCDFs	0.58	0.19 – 1.59	0.30
Co-PCBs	***0.30***	***0.08 – 0.85***	***0.06***
T_4			
Dioxins	***0.14***	***0.05 – 0.48***	***0.008***
PCDDs	***0.08***	***0.01 – 0.35***	***0.004***
PCDFs	***0.26***	***0.07 – 0.77***	***0.04***
Co-PCBs	***0.13***	***0.03 – 0.44***	***0.005***
TSH			
Dioxins	1.70	0.60 – 4.66	0.29
PCDDs	1.29	0.46 – 3.46	0.45
PCDFs	***2.62***	***0.99 – 6.93***	***0.09***
Co-PCBs	1.75	0.66 – 4.54	0.25
TBG			
Dioxins	***0.34***	***0.09 – 1.01***	***0.10***
PCDDs	0.43	0.12 – 1.31	0.19
PCDFs	0.44	0.12 – 1.30	0.19
Co-PCBs	***0.29***	***0.08 – 0.83***	***0.05***

9. Nagayama J, Tsuji H, Iida T, Nakagawa R *et al*. *Ibid* 2001;53:121-125.
10. Nagayama J, Tsuji H, Iida T, Hirakawa H *et al*. *Ibid* 2002;59:135-138.
11. Nagayama J, Okamura K, Iida T, Hirakawa H *et al*. *Chemosphere* 1998;37:1789-1793.
12. Nagayama J, Iida T, Nakagawa R, Matsueda T *et al*. *Organohal Comp* 2000;48:236-239.
13. Nagayama J, Iida T, Nakagawa R, Matsueda T *et al*. *Ibid* 2001;53:140-144.
14. Nagayama J, Iida T, Hirakawa T, Matsueda T *et al*. *Ibid* 2002;59:457-460.
15. Nagayama J, Tsuji H, Iida T, Nakagawa R *et al*. *Ibid* 2002;55:425-428.
16. Nagayama J, Iida T, Nakagawa R, Matsueda T *et al*. *Ibid* 2002;56:115-118.
17. Nakagawa R, Hirakawa H, Iida T, Matsueda T. *J AOAC Int* 1999;82:716-724.
18. Hirakawa H, Iida T, Matsueda T, Nakagawa R, Hori T, Nagayama J. *Organohal Comp* 1995;26:197-200.
19. Van den Berg M, Birnbaum LS, Bosveld ATC, Brunstorm B *et al*. *Environ Health Perspect* 1998;106:775-792.
20. Tsuji H, Murai K, Akagi K, Fujishima M. *B J Clin Imunol* 1990;10:38-44.
21. Okamura K, Sato K, Ikenoue H. *J Clin Endocrinol Metab* 1988;67:720-726.