STATUS OF HELPER AND SUPPRESSOR T LYMPHOCYTE SUBPOPULATIONS IN JAPANESE INFANTS LACTATIONALLY EXPOSED TO ORGANOCHLORINE PESTICIDES, PCBs AND DIOXINS

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

[1]Laboratory of Environmental Health Sciences, School of Health Sciences, Kyushu University, Fukuoka 812-8582, Japan; [2]Third Department of Internal Medicine, School of Medicine, Fukuoka University, Fukuoka 814-0180, 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

Our environments including food have been polluted with some organochlorine compounds such as dioxins which are polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs), polychlorinated biphenyls (PCBs) and pesticides [1, 2]. Accordingly, Japanese people have also been contaminated with these chemicals [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 [5, 6, 7] and their mean or median concentrations on fat weight basis were about 420, 330, 3, 4 and 110 ppb, respectively [6, 7]. Their levels were considered more than 100 to 10,000 times higher than that of dioxins in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) toxic equivalent (TEQ) value [6]. Therefore, we should give due attention to possible health consequences of these organochlorine pesticides and PCBs as well as dioxins in Japanese infants.

We have already reported effects of the lactational exposure to these compounds on lymphocyte subpopulations in the peripheral blood of Japanese infants [8]. In this study, however, numbers of infants and samples of human milk are increased, and their effects on them were investigated more in detail.

Materials and Methods

In this study, 124 mothers 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 organochlorine pesticides and PCBs by ECD gas chromatographic method [6, 9] and dioxins by high resolution GC/MS method [6].

TEQ concentrations of PCDDs, PCDFs and Co-PCBs were calculated by using 1998 WHO toxic equivalent factor (TEF) values [10]. The TEQ-sum of all congeners of PCDDs, PCDFs and Co-PCBs determined in every breast milk sample was summarized as the total 2,3,7,8-TCDD TEQ

concentration or level.

About 1 year after birth, 5 to 10 ml of peripheral blood samples were individually obtained from 93 infants. These blood samples were employed to measure the lymphocyte subpopulations by indirect immunofluorescence using monoclonal mouse anti-human antibodies against helper (CD4 positive) and suppressor (CD8 positive) T cells, and their relative population densities were calculated [11].

Lactational exposure to dioxins, PCBs or organochlorine pesticides was estimated as a product of their respective daily intake, which was calculated with their respective level in the milk times an expected intake of breast milk in Japanese infants, that is, 120 g/kg body weight, multiplied by individual duration (days) of breast feeding.

In order to conduct more robust and reproducible statistical analysis, data were categorized into two groups. According to estimated intakes of the compounds, which were adjusted for years, and the levels of immune response variables such as percentages of the lymphocyte subpopulations and CD4/CD8 ratios, donated by 0 (less than the mean including minimum value) and 1 (the last quartile including maximum value). Then, we examined the relationship between the immune response variables and the intakes of the organochlorine pesticides, PCBs and dioxins by simple logistic regression analysis, and calculated odds ratios. In addition, multiple logistic regression analysis was done to compute the joint effect of every two compounds, each of which showed less than 0.300 of *p*-value in simple logistic regression analysis, on the immune response system.

Table 1. Estimated total intakes of organochlorine pesticides, PCBs and dioxins from the breast milk in 10-month-old infants

Compound	Estimated Total Intake	
	Mean	Std Err Mean
Organochlorine pesticide (μg/kg body weight)		
ß-HCH	563	51
Dieldrin	5.5	0.6
DDT*	442	37
HCE	5.8	0.6
Chlordane**	103	8.6
PCBs (μg/kg body weight)	150	9.5
Dioxins*** (ng-TEQ/kg body weight)	30	1.6

* : Sum of *p, p'*-DDE and *p, p'*-DDT

** : Sum of oxychlordane, *trans*-nonaclor and *cis*-nonaclor

*** : Sum of PCDDs, PCDFs and Co-PCBs

Results and Discussion

Estimated total intakes of the organochlorine compounds from the breast milk are indicated in Table 1. The mean intake of ß-HCH or DDT was about 100 times higher than that of dieldrin or HCE. The intakes of chlordane and PCBs were 100 and 150 μg/kg body weight, respectively and 3 to 6 times less than those of ß-HCH and DDT. In dioxins, the mean intake was 30 ng-TEQ/kg body weight, which was two orders of magnitude lower than those of dieldrin and HCE.

Table 2. Percentages of helper (CD4+) and suppressor (CD8+) lymphocyte subpopulations in peripheral blood of Japanese infants

Lymphocyte Subpopulation (Positive Cells)	Percent	
	Median (min. ~ max.)	Normal Range*
CD4	39.6 (15.7 ~ 61.7)	25 ~ 56
CD8	19.1 (10.6 ~ 41.2)	17 ~ 44
CD4/CD8	2.08 (0.62 ~ 4 .52)	0.6 ~ 2.9

* : Determined by the biggest center of clinical examinations in Japan, SRL Corp., Tokyo, Japan for adults.

Table 3. Relationship between percentages of CD4 and CD8 positive lymphocytes and their ratio (CD4/CD8) in the peripheral blood of Japanese infants and lactational exposure to organochlorine pesticides, PCBs and dioxins by simple logistic regression analysis (p-value<0.250)

Response Variable	Exposure Variable (Odds Ratio, p-value)
	Organochlorine pesticides, PCBs and Dioxins
CD4	**HCE (1.66, 0.074)**, Chlordane (1.52, 0.149)
CD8	**HCE (1.80, 0.035)**
CD4/CD8	—

Boldface shows statistically significant exposure variable (p-value<0.100).

As shown in Table 2, observed range of the percentages of helper (CD4+) T cells was greater than the normal range, which was determined for Japanese adults. Lower levels of the percentages of suppressor (CD8+) T lymphocytes were less than the lower limit and higher levels of CD4/CD8 ratios larger than the upper one.

Results of simple and multiple logistic regression analyses concerning effects of lactational exposure to the organochlorine compounds on the immune response system are indicated in Tables 3 and 4, respectively.

In simple logistic regression analysis, HCE significantly increased the percentages of helper and suppressor T lymphocytes in peripheral blood of Japanese infants. In our previous results[8], total intakes of dioxins showed significant positive correlation with the percentages of helper T cells and CD4/CD8 ratios and negative correlation with those of suppressor T cells. Effect, however, of other compounds on helper (CD4+) and suppressor (CD8+) T lymphocyte subpopulations, and CD4/CD8 ratios was not observed in this study.

In multiple logistic regression analysis, HCE and chlordane jointly affected the percentages of CD4 positive T cells and significantly increased the odds ratio more than each of them did. We, however, do not know the clinical significance of all the results mentioned above at present.

Table 4. Joint effects of two compounds exposed through breast milk on the lymphocyte subsets (CD4 and CD8) and their ratio in the peripheral blood of Japanese infants by multiple logistic regression analysis

	Exposure Variable (Organochlorine pesticides, PCBs and Dioxins)			
Response Variable	X_1	X_2	Odds Ratio	*p*-value
CD4	**HCE (1.85)**	**Chlordane (1.31)**	**2.42**	**0.069**
CD8	Dieldrin (1.40)	HCE (1.67)	2.35	0.120
CD4/CD8	—	—	—	—

Number in parenthesis is odds ratio of the single compound.
Boldface indicates statistically significant joint effect of the two compounds (X_1, X_2) at *p*-value less than 0.100.

References

1. Swedish Environmental Protection Agency (1998) Persistent Organic Pollutants, pp. 9-129, ISBN 91-620-1189-8, ISSN 1100-231X.
2. Nakagawa R, Hirakawa H and Hori T (1995) J AOAC Int 78, 921-929.
3. Kashimoto T, Takayama K, Mimura M, Miyata H, Murakami Y and Matsumoto H (1989) Chemosphere 19, 921-926.
4. Hirakawa H, Iida T, Matsueda T and Nagayama J (1996) Organohal Comp 30, 127-130.
5. Iida T, Hirakawa H, Matsueda T, Nakagawa R, Hori T and Nagayama J (1999) Organohal Comp 44, 123-127.
6. Nakagawa R, Hirakawa H, Iida T, Matsueda T and Nagayama J (1999) J AOAC Int 82, 716-724.
7. Nagayama J, Tsuji H, Okamura K, Iida T, Hirakawa H, Matsueda T, Hasegawa M, Sato K, Tomita A, Yanagawa T, Igarashi H, Fukushige J and Watanabe T (1998) Organohal Comp 37, 163-167.
8. Nagayama J, Tsuji H, Iida T, Hirakawa H, Matsueda T, Okamura K, Hasegawa M, Sato K, Ma H-Y, Yanagawa T, Igarashi H, Fukushige J and Watanabe T (1998) Chemosphere 37, 1781-1787.
9. Hirakawa H, Iida T, Matsueda T, Nakagawa R, Hori T and Nagayama J (1995) Organohal Comp 26, 197-200.
10. Van den Berg M, Birnbaum LS, Bosveld ATC, Brunstorm B *et al.* (1998) Environ Health Perspect 106, 775-792.
11. Tsuji H, Murai K, Akagi K and Fujishima M (1990) B J Clin Immunol 10, 38-44.

12. Gillesby, B. E., Stanostefano, M., Porter, W., Safe, S., Wu, Z. F., and Zacharewski, T. R. (1997) *Biochemistry* **36**, 6080-6089.
13. Pohl, R. J., and Fouts, J. R. (1980) *Anal. Biochem.* **107**, 150-155.
14. Vickers, P. J., Dufresne, M. J., and Cowan, K. H. (1989) *Mol. Endocrinol.* **3**, 157-164.
15. Thomsen, J. S., Wang, X., Hines, R. N., and Safe, S. (1994) *Carcinogenesis* **15**, 933-937.
16. Wang, W. L., Thomsen, J. S., Porter, W., Moore, M., and Safe, S. (1996) *Br. J. Cancer*, **73**, 316-322.
17. Veldscholte, J., Ris-Stealpers, C., Kuiper, G G., Jenster, G., Berrevoets, C., Claassen, E., Van Rooij, H. C., Trapman, J., Brinkmann, A. O., and Mulder, E. (1990) *Biochem. Biophys. Res. Commun.* **173**, 534-540.
18. Wong, C., Kelce, W. R., Sar, M., and Wilson, E. M. (1995) *J. Biol. Chem.* **270**, 19998-20003.
19. Ricci, M. S., Toscano, D. G., Mattingly, C. J., Toscano, W. A. Jr. (1999) *J. Biol. Chem.* **274**, 3430-3438.