Effects of lactational exposure to organochlorine pesticides, PCBs and dioxins on immune response and thyroid hormone systems in Japanese male and female infants

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Introduction

Our environments including food have been polluted with some organochlorine compounds such as dioxins, polychlorinated biphenyls (PCBs) and pesticides [1, 2]. 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 those of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs), so-called dioxins, in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalent (TEQ) value as a whole [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 perinatal exposure to these compounds on lymphocyte subsets [7, 8, 9, 10] and thyroid hormone statuses [11, 12, 13, 14] in the peripheral blood of Japanese infants. In this study, in order to clarify the sexual distinction in their effects on the immune response and thyroid hormone systems, we investigated the lymphocyte subsets and thyroid related chemicals in the blood of Japanese male and female infants in relation to their concentrations of the breast milk.

Materials and Methods

In this study, ninety-three mothers (mean age : 29 years old and the range : 24 ~ 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 organochlorine pesticides and PCBs by ECD gas chromatographic method [6, 15] and dioxins by high resolution GC/MS method [6]. Lactational exposure in whole breast-feeding periods to the organochlorine compounds were evaluated with individual intakes through the breast milk, which were estimated with multiplying their daily intakes by individual duration of breast-feeding (days).

About 1 year after birth, 5 to 10 ml of peripheral blood samples were individually obtained from 100 infants (57 males and 43 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) [16]. Then the relative population densities of the lymphocyte subsets were calculated. These blood samples were also used to determine the serum concentrations of T_3, T_4, TSH and TBG by radioimmunoassay methods using commercially available kits [17].

We are studying 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 the organochlorine compounds, the percentages of the lymphocyte subsets, as well as CD4+/CD8+ ratio, and the serum levels of thyroid related chemicals were categorized into two groups ; namely, the measurements which were less than the mean and equal to or over the mean in each year 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. In this study, less than 10 percent of *p*-value was considered as statistically significant.

Results and Discussion

Median intake in whole breast-feeding periods of HCH or DDT was about 100 times higher than that of dieldrin or HCE. Median intakes of chlordane and PCBs were around 100 μg/kg body weight and 3 to 5 times less than those of HCH and DDT. In case of dioxins, their TEQ levels were computed by using 1998 WHO toxic equivalency factor (TEF) values [18] and the median intake was about 29 ng-TEQ/kg body weight, which was around 150 times lower than that of dieldrin or HCE.

Table 1. Sexual distinction in effects of lactational exposure to the organochlorine pesticides, PCBs and dioxins on whole weight basis on the subsets of CD16+, HLA-DR+, CD4+8+, CD3+ and CD20+ lymphocytes in the peripheral blood of Japanese male and female infants

Compound	Male Infant		Female Infant	
	Odds Ratio	*p*-value	Odds Ratio	*p*-value
< CD16+ Cells >				
Dioxins	***2.42***	***0.09***	0.60	0.33
HCH	2.14	0.15	2.82	0.12
Dieldrin	***3.33***	***0.05***	1.27	0.50
DDT	***2.55***	***0.09***	0.89	0.56
Chlordane	1.50	0.34	2.32	0.17
PCBs	***3.26***	***0.03***	0.64	0.36
< CD4+8+ Cells >				
Dioxins	2.47	0.10	***0.08***	***0.002***
DDT	1.35	0.41	***0.12***	***0.009***
HCE	2.18	0.15	1.60	0.37
Chlordane	0.96	0.60	***0.19***	***0.02***
PCBs	1.12	0.54	***0.31***	***0.09***
< CD3+ Cells >				
Dioxins	***2.91***	***0.05***	0.83	0.52
DDT	2.09	0.16	0.54	0.28
HCE	***2.52***	***0.09***	2.33	0.20
< CD20+ Cells >				
HCH	2.31	0.12	1.08	0.59
DDT	1.95	0.19	1.33	0.47
HCE	0.87	0.52	0.52	0.28

Boldface shows statistically significant compound ($p<0.10$)

Higher levels of T_3, T_4 and TSH in the serum of Japanese infants were greater than their upper limits of Japanese adults. These hormones play vital roles in an early stage of human life and therefore obviously they are required more in fetuses and sucklings than in adults.

Table 1 summarizes the effects of lactational exposure to the organochlorine compounds on the subsets of CD16+, CD4+8+, CD3+ and CD20+ lymphocytes. Dioxins, dieldrin, DDT and PCBs increased the CD16+ lymphocyte subsets in the blood of male infants, but not in the female infants.

Dioxins, DDT, chlordane and PCBs significantly decreased the CD4+CD8+ lymphocyte subsets only in the female infants. CD3+ Lymphocyte subset was significantly increased by dioxins and HCE.

Table 2. Sexual distinction in effects of lactational exposure to the organochlorine pesticides, PCBs and dioxins on whole weight basis on the subsets of CD4+ and CD8+ lymphocytes and CD4+/CD8+ ratio in the peripheral blood of Japanese male and female infants

Compound	Male Infant		Female Infant	
	Odds Ratio	*p*-value	Odds Ratio	*p*-value
< CD4+ Cells >				
Dioxins	1.16	0.50	1.86	0.27
Dieldrin	0.54	0.24	2.10	0.24
HCE	2.18	0.15	1.60	0.37
PCBs	0.46	0.14	1.22	0.51
< CD8+ Cells >				
Dioxins	1.51	0.32	***0.34***	***0.10***
HCH	1.11	0.54	***0.25***	***0.05***
Dieldrin	2.27	0.15	0.48	0.24
HCE	1.11	0.54	2.33	0.20
PCBs	1.07	0.56	0.53	0.26
< CD4+/CD8+ >				
Dioxins	***0.40***	***0.08***	***2.92***	***0.10***
Dieldrin	***0.36***	***0.09***	***3.56***	***0.08***
HCE	0.45	0.14	1.91	0.28
PCBs	0.72	0.38	***2.92***	***0.10***

Boldface shows statistically significant compound ($p<0.10$)

The effects of lactational exposure to the organochlorine chemicals on the subsets of CD4+ and CD8+ lymphocytes and CD4+/CD8+ ratio are shown in Table 2. Dioxins and HCH decreased the CD8+ lymphocyte subset only in the blood of female infants. Dioxins, dieldrin and PCBs significantly enhanced the CD4+/CD8+ ratio only in the female infants. Dioxins and dieldrin, however, significantly lowered the CD4+/CD8+ ratio in the blood of male infants.

Table 3 indicates the effects of lactational exposure to the organochlorine compounds on the serum levels of T_3, T_4, TSH and TBG. Dieldrin and chlordane significantly decreased the serum levels of T_3 only in the female infants. DDT, however, lowered the serum T_3 levels in both male and female infants. DDT decreased the serum levels of T_4 only in the male infants and HCE enhanced them only in the female infants. DDT increased and HCE lowered the serum TSH levels only in female

infants. HCH increased and PCBs decreased the serum TBG levels only in female infants. The Japanese female infants seemed more sensitive to the organochlorine compounds than the Japanese male infants in their effects on the serum levels of T_3, T_4, TSH and TBG.

Table 3. Sexual distinction in effects of lactational exposure to the organochlorine pesticides, PCBs and dioxins on whole weight basis on the serum levels of T_3, T_4, TSH and TBG in the peripheral blood of Japanese male and female infants

Compound	Male Infant		Female Infant	
	Odds Ratio	*p*-value	Odds Ratio	*p*-value
< T_3 >				
Dioxins	1.14	0.51	0.38	0.12
Dieldrin	0.59	0.28	***0.15***	***0.01***
DDT	*0.26*	*0.02*	*0.32*	***0.09***
HCE	0.67	0.33	0.75	0.46
Chlordane	0.65	0.32	***0.28***	***0.05***
< T_4 >				
Dioxins	0.86	0.49	1.40	0.41
DDT	***0.31***	***0.03***	0.55	0.27
HCE	0.75	0.40	***3.60***	***0.06***
< TSH >				
Dioxins	1.91	0.18	1.30	0.46
DDT	2.28	0.11	***4.40***	***0.03***
HCE	1.18	0.49	***0.29***	***0.08***
< TBG >				
HCH	1.10	0.54	***3.10***	***0.09***
DDT	0.61	0.26	0.36	0.13
HCE	1.10	0.54	2.38	0.17
PCBs	1.02	0.59	***0.33***	***0.09***

Boldface shows statistically significant compound ($p<0.10$)

The results mentioned above seem to support the idea of sexual distinction in the effects of organochlorine pesticides, PCBs and dioxins on the immune response and thyroid hormone systems of Japanese infants. However, this study was done with rather small number of infants. Therefore, further large-scale investigations are required to get more conclusive findings.

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