SEXUAL DISTINCTION IN EFFECTS OF PERINATAL EXPOSURE TO ORGANOCHLORINE PESTICIDES, PCBs AND DIOXINS ON FREQUENCY OF SISTER CHROMATID EXCHANGES IN JAPANESE INFANTS

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Introduction

Foods in Japan have been polluted with some organochlorine compounds such as pesticides, polychlorinated biphenyls (PCBs) and dioxins, which are polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) [1]. So, Japanese people have also been contaminated with these compounds [2, 3, 4]. Consequently, some pesticides such as hexachlorocyclohexanes (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 fat 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 that of dioxins in 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (2,3,7,8-TCDD) toxic equivalent (TEQ) value as a whole [5]. Therefore, we should give due attention to possible health consequences of these organochlorine pesticides and PCBs as well as dioxins in Japanese infants.

Formation and induction of sister chromatid exchanges (SCEs) have been considered as a good index to the synthetic and sharp genotoxic potency for several mutagenic and carcinogenic chemicals [7, 8]. We have already reported the effects of perinatal exposure to dioxins on the frequency of SCEs in the lymphocytes of Japanese infants [9]. In this study, in order to clarify the sexual distinction in their effects on the SCE frequency, we investigated the changes of SCE frequency in Japanese male and female infants in relation to their contamination levels of the breast milk.

Materials and Methods

Fifty to 100 ml of breast milk at postpartum period of 2 ~ 4 month were collected from 124 healthy mothers, mean age : 29 years old and the range : 22 ~ 41 years old, in July ~ October,

1994, June ~ October, 1995 and June ~ October, 1996. 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. These samples of the breast milk were analyzed for organochlorine pesticides and PCBs by ECD gas chromatographic method [5, 10], and also for dioxins by HRGC-HRMS technique using a Finnigan MAT-95 mass spectrometer (Germany) directly interfaced with Varian Model 3400 gas chromatograph [5, 11]. TEQ concentrations of the dioxins were calculated by using 1998 WHO TEF values [12]. TEQ-sum of all congeners of the dioxins determined in the milk samples was summarized as the total 2,3,7,8-TCDD TEQ concentration or level.

Five to 10 ml of the peripheral blood of 105 infants born of these mothers were individually obtained by venipuncture in January ~ March of 1995, 1996 and 1997. Among them, SCE frequency of the lymphocytes was measured in 66 infants (40 males and 26 females). Lymphocytes in the whole blood were stimulated with phytohemagglutinin and cultured for two replicative cycles in the presence of bromodeoxyuridine (100 μM) as detailed elsewhere [13, 14]. Differential staining of sister chromatids was obtained by a fluorochrome plus Giemsa technique and the frequencies of SCEs which were the solvent (DMSO) treated SCEs ($SCE_{control}$), 7,8-benzoflavone (ANF) treated SCEs (SCE_{ANF}) and ΔSCEs ($SCE_{ANF} - SCE_{control}$) were evaluated.

We are examining the relative risks of toxic chemicals to this genotoxic index, but not their causality. For this purpose and in order to conduct reliable and robust analysis, the concentrations of the organochlorine compounds and the frequencies of SCEs were categorized into two groups ; namely, the measurements which were less than the means and equal to or above the mean 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 confidence intervals (C.I.) of odds ratios were also reckoned.

Results and Discussion

Mean concentrations (male and female infant groups) of HCH, dieldrin, DDT, HCE, Chlordane, PCB and dioxins on lipid weight basis were 368 and 440 ng/g, 2.9 and 5.0 ng/g, 277 and 355 ng/g, 4.5 and 4.7 ng/g, 76 and 81 ng/g, 128 and 114 ng/g, and 23.1 and 23.3 pg/g, respectively, as indicated in Table 1. Contamination levels of HCH, dieldrin and DDT were somewhat higher in the female infant group than in the male one. Respective mean values (male and female infants) of $SCE_{control}$, SCE_{ANF} and ΔSCEs were 8.33 and 8.14/cell, 11.8 and 11.9/cell, and 3.46 and 3.75/cell, as shown in Table 2. There were no significant difference between the male and female infants in the frequencies of $SCE_{control}$, SCE_{ANF} and ΔSCEs.

As indicated in Table 3, perinatal exposure to dioxins significantly increased the frequency of $SCE_{control}$ in the lymphocytes of male infants, but not in those of female infants. HCE significantly enhanced the frequencies of SCE_{ANF} and ΔSCEs only in the lymphocytes of female infants.

Changes in the frequency of ΔSCEs seemed more sensitive biomarker than those in $SCE_{control}$ and SCE_{ANF} in the evaluation of genotoxic potency of chemicals [15, 16]. The results mentioned above were considered to support the idea of sexual distinction in the effects of HCE and/or dioxins on the induction of SCEs in the lymphocytes of Japanese infants. However, this study was done with rather small number of infants. Therefore, further large-scale investigations are needed to get more conclusive results.

Table 1. Concentrations of organochlorine pesticides, PCBs and dioxins in the mother's milk of male and female infants

Compound	Concentration on Lipid Weight Basis*	
	Male Infants	Female Infants
Organochlorine Pesticides (ng/g)		
HCH	368 ± 267	440 ± 298
Dieldrin	2.9 ± 2.5	5.0 ± 5.8
DDT	277 ± 147	355 ± 251
HCE	4.5 ± 4.6	4.7 ± 4.1
Chlordane	76 ± 45	81 ± 69
PCB (ng/g)	128 ± 69	114 ± 58
Dioxins (TEQ-pg/g)	23.1 ± 8.5	23.3 ± 8.2

* : Mean ± Standard Deviation

Table 2. Frequencies of $SCE_{control}$, SCE_{ANF} and ΔSCEs in the lymphocytes of male and female infants

SCEs	Frequency per Cell*	
	Male Infants	Female Infants
$SCE_{control}$	8.33 ± 1.26	8.14 ± 0.71
SCE_{ANF}	11.8 ± 1.6	11.9 ± 1.3
ΔSCEs	3.46 ± 1.52	3.75 ± 1.36

* : Mean ± Standard Deviation

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Table 3. Effects of perinatal exposure to organochlorine pesticides, PCBs and dioxins on the frequencies of $SCE_{control}$, SCE_{ANF} and ΔSCEs in the lymphocytes of Japanese male and female infants

	Male Infant		Female Infant	
Compound	Odds Ratio	90% C.I.	Odds Ratio	90% C.I.
$SCE_{control}$				
Dioxins	***3.67***	***1.22 - 11.9***	0.68	0.15 - 2.92
HCE	0.35	0.09 - 1.17	0.39	0.09 - 1.57
SCE_{ANF}				
HCE	0.86	0.27 – 2.63	***6.00***	***1.41 – 29.7***
ΔSCEs				
HCE	0.86	0.28 – 2.67	***24.0***	***4.17 - 280***