EFFECTS OF LIPID EXTRACTION FROM BREAST MILK ON RISK ASSESMENT OF DIOXINS AND OTHER POPs IN JAPANESE INFANTS

Nagayama J [1], Matsueda T [2], Hirakawa H [2], Kunisue T [3], Tanabe S [3], Tsuji H [4], Kohno H [5] , Shimomura H [6], Yanagawa T [7]

[1] Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan; [2] Department of Environmental Sciences, Fukuoka Institute of Health and Environmental Sciences, Fukuoka 818-0135, Japan; [3] Center for Marine Environmental Studies, Ehime University, Matsuyama 790-8577, Japan; [4] Kitakyushu-Tsuyazaki Hospital, Fukuoka 811-3307, Japan; [5] Fukuoka Children's Hospital, Fukuoka 810-0063, Japan; [6] Shimomura OBGY Clinic, Fukuoka 811-1254, Japan; [7] Biostatistical Center, Kurume University, Fukuoka 830-0011, Japan

Abstract

We examined the perinatal exposure to dioxins, PCBs and organochlorine pesticides on immune response and thyroid hormone systems in Japanese infants, and prenatal exposure to these organochlorine compounds on the incidence of congenital hypothyroidism (cretinism) by their concentrations on both wet and lipid weight bases. Due to the difficulties of quantitative lipid extraction from the breast milk, their effects on these two biological systems and the incidence of cretinism were great difference in the concentrations on wet or lipid weight basis.

At present, concentrations on wet weight basis seem more precise than those on lipid weight basis, so we had better use them for the evaluation of toxic effects or risks of chemical compounds. For more precise assessments of their toxic effects and risks, we have to establish the international standard method for the quantitative extraction of lipid from biological samples.

Introduction

In general, effects and risk evaluation of toxic compounds such as dioxins, PCBs and other persistent organic pollutants (POPs) for human beings have been done based upon their concentrations on lipid weight basis. Lipid extraction, however, from objects to be examined is seemed quite difficult and such an example was reported in Dioxin 2006 in Oslo, Norway [1]. At that time, we showed the examples of lipid extraction from the blood in three individual men and the effects of quantitative lipid extraction on the concentrations of several organochlorine pesticides. We also reported the different effects of prenatal exposure to dioxins, PCBs and organochlorine pesticides on the incidence of congenital hypothyroidism by lipid and wet weight bases [1]. Accordingly, we specially emphasized the effects of lipid extraction from the objects to be examined on the risk evaluation of such toxic compounds in human beings.

In this study, we compared the concentrations of dioxins, PCBs and organochlorine pesticides on wet and lipid weight bases in Japanese breast milk in two different methods of lipid extraction, and also stressed the importance of quantitative lipid extraction on the risk evaluation of these toxic compounds in human beings.

Materials and Methods

Study I : 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 June~October, 1994~1996. Pregnancy and delivery were completed without overt signs of serious illness or complications. Only babies born at term (37~42 weeks of gestation) without congenital anomalies or diseases were included

Breast milk sample (15g) was weighted into a 200ml separatory funnel (A), and then 2 ml 10% potassium

oxalate solution and 20 ml ethanol were added. The mixture was shaken vigorously for 1 min. Then, 20 ml diethylether-petroleum ether (1 + 1, V/V) was added, and the mixture was shaken for 5 min. The ether phase was transferred into another separatory funnel (B), containing 20 ml 5% NaCl. The aqueous phase was extracted twice with 10 ml diethyl ether-petroleum ether (1 + 1, V/V). The ether phase was transferred into the second funnel (B) and shaken for 5 min. The ether phase was separated from the aqueous phase, dried, concentrated and weighed as lipid.

Organochlorine pesticides and PCBs in these samples of the breast milk were determined by ECD gas chromatographic method [2, 3], and dioxins by HRGC-HRMS technique using a Finnigan MAT-900 mass spectrometer directly interfaced with Hewlett-Packard 5890 Series II gas chromatograph [2, 4].

About 1 year after birth, 5 to 10 ml of peripheral blood samples were individually obtained from 100 infants. These blood samples were employed to measure lymphocyte subsets [5] and also determine the serum concentrations of T_3, T_4 and TSH [6].

Study II : Positive cases of the mass-screening for congenital hypothyroidism (cretinism) in 2001~2004 in Fukuoka visited Fukuoka Children's Hospital, Fukuoka, Japan for the minute examination of cretinism, and finally 22 neonates were diagnosed as cretinism. One hundred and one normal neonates, namely, negative cases of the mass-screening for the cretinism, were born in Shimomura OBGY Clinic, Fukuoka, Japan. Fifty to 100 ml of breast milk of both the positive and negative cases was obtained within 4 weeks after childbirth.

Approximately 10g of human breast milk sample was added to 10g diatomaceous earth (Extrelunt NT; Merck, Germany) packed in a glass column and extracted with diethyl ether. After the Kuderne-Danish (K-D) concentration of the extract, 2 ml of the aliquot was dried at 80°C to determine the lipid content.

Chemicals analyzed in this study were dioxins, PCBs, DDT, HCH, chlordane and HCB. Extraction was conducted using the method reported by Hirai *et al* [7]. Clean-up and separation processes were followed by the method previously described [8, 9]. Identification and quantification of dioxins were performed using HRGC (Agilent 6890)-HRMS (JEOL JMS-700D and GC mate). Quantification of PCBs and organochlorine pesticides was done using GC-ECD (Hewlett-Packard 6890).

In case of dioxins, their TEQ concentrations were computed by using 1998 WHO toxic equivalency factor (TEF) values [10].

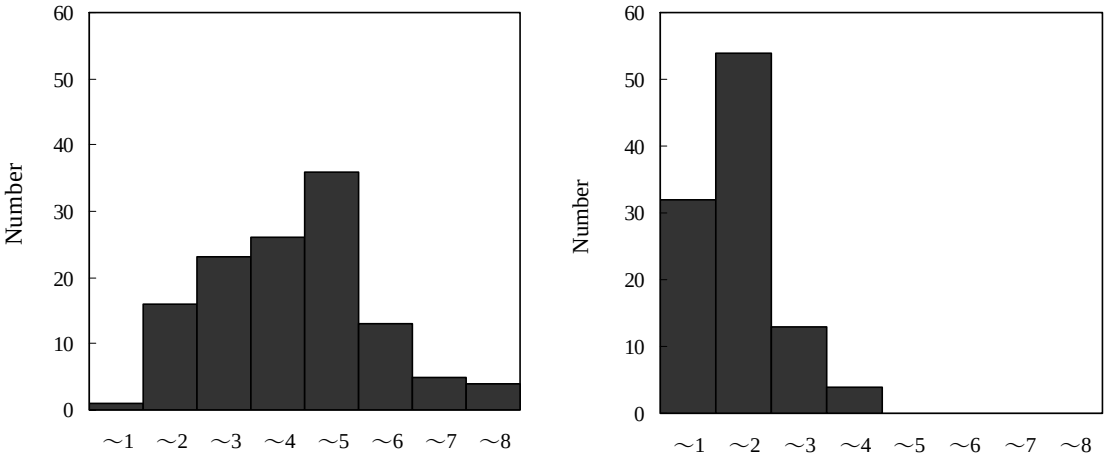
We are studying the relative risks of toxic compounds to these biological systems and to the incidence of cretinism, but not their causality. For this purpose and in order to conduct reliable and robust analysis, exposure and response variables were categorized into two groups; namely, the measurements which were less than the median and equal to or over the median in each year were set by 0 and 1, respectively, except healthy neonates and neonates with cretinism, which were set 0 and 1, respectively, in the case-control study. 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

As indicated in Fig. 1, the mean lipid content of breast milk of Study I was about 2.6 times higher than that of Study II, and both the min. and max., respectively, lipid contents of Study I were also around 2 times greater than those of Study II.

Tables 1 and 2 shows the concentrations of PCDDs, PCDFs, Co-PCBs and dioxins on both wet and lipid weight bases in Studies I and II, respectively.

In the wet weight basis, their mean concentrations of Study I were 3 to 4 times higher than those of Study II. In the lipid weight basis, however, due to the lower lipid contents of Study II than those of Study I, their mean concentrations of Study I were less than 1.5 times greater than those of Study II. Same kinds of results were also seen in PCBs and organochlorine pesticides, as shown in Tables 3 and 4.



Study I (N=124)
Mean : 3.82
Median : 3.85
Min : 0.98
Max : 7.19
S.D. : 1.46

Study II (N=103)
Mean : 1.47
Median : 1.4
Min : 0.42
Max : 3.7
S.D. : 0.68

Fig. 1. Distribution of lipid content in samples of Japanese breast milk

Table 1. Comparison of contamination levels on wet weight basis of PCDDs, PCDFs, Co-PCBs and Dioxins in the breast milk of Japanese mothers

	Concentration (Mean ± S.D., TEQ-pg/g)			
	PCDDs	PCDFs	Co-PCBs	Dioxins
Study I	0.330 ± 0.175	0.261 ± 0.141	0.345 ± 0.202	0.936 ± 0.472
Study II	0.105 ± 0.063	0.062 ± 0.033	0.110 ± 0.061	0.277 ± 0.149

Table 2. Comparison of contamination levels on lipid weight basis of PCDDs, PCDFs, Co-PCBs and Dioxins in the breast milk of Japanese mothers

	Concentration (Mean ± S.D., TEQ-pg/g lipid)			
	PCDDs	PCDFs	Co-PCBs	Dioxins
Study I	8.92 ± 3.30	7.12 ± 3.10	8.90 ± 4.07	24.9 ± 8.72
Study II	7.92 ± 4.62	4.78 ± 2.52	8.23 ± 4.49	20.9 ± 11.0

Table 3. Comparison of contamination levels on wet weight basis of PCBs and organochlorine pesticides in the breast milk of Japanese mothers

	Concentration (Mean ± S.D., ng/g)			
	PCBs	DDT	HCH	Chlordane
Study I	4.78 ± 2.79	13.9 ± 11.5	17.1 ± 14.9	3.22 ± 2.52
Study II	1.51 ± 1.27	3.67 ± 3.68	1.25 ± 1.24	0.76 ± 0.68

Table 4. Comparison of contamination levels on lipid weight basis of PCBs and organochlorine pesticides in the breast milk of Japanese mothers

	Concentration (Mean ± S.D., ng/g lipid)			
	PCBs	DDT	HCH	Chlordane
Study I	136 ± 92	347 ± 255	419 ± 298	82 ± 60
Study II	113 ± 82	266 ± 228	90 ± 74	57 ± 59

In Studies I and II, effects of these organochlorine compounds on both wet and lipid weight bases on the immune response and thyroid hormone systems and the incidence of cretinism, respectively, were investigated in Japanese infants and we observed their effects were great difference in their concentrations on wet or lipid weight basis. Quantitative extraction, however, of lipid from biological samples such as blood and breast milk and so forth is considered much more difficult than that of chemical compounds and their concentrations on wet weight basis more precise than those on lipid weight basis.

Based upon our findings, at present, we had better use the concentrations on wet weight basis for the evaluation of toxic effects or risks of chemical compounds. Anyway, for more precise evaluation of their toxic effects and risks, we have to establish the international standard method for the quantitative extraction of lipid from biological samples, as soon as possible.

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