Effects of Dioxins and Polychlorinated Biphenyls (PCBs) on Thyroid Function in Infants Born in Japan: Report from Research on Environmental Health

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Abstract. Dioxins (polychlorinated-dibenzo-p-dioxin (PCDD) + polychlorinated-dibenzofuran (PCDF)) and polychlorinated biphenyls (PCBs) are potentially hazardous compounds which have structural similarity to thyroid hormones. Animal studies have demonstrated that PCDDs, PCDFs and PCBs can alter thyroid hormone homeostasis. Both dioxins and PCBs are highly lipophilic and are present in large amounts in human milk samples in highly industrialized, densely populated countries such as Japan and European countries. We collected breast milk from 80 mothers (group A) living in Tokyo, Saitama, Ishikawa, and Osaka prefectures at 5, 30, 150, 300 postpartum days and measured PCDDs, PCDFs and PCBs. The mothers were limited to those aged from 25 to 34 years, and all were primiparous. Blood was taken from infants who were breast-fed at the age of one year. For evaluation of thyroid and immune function, blood was also taken from thirty infants who were bottle-fed (group B) as a control. Serum T4, T3 and FT4 levels were 10.6 ± 1.6 ug/dl, 1.6 ± 0.2 ng/ml, 1.42 ± 1.25 ng/dl in Groups A and were 11.1 ± 2.0 ug/dl, 1.7 ± 0.3 ng/ml, 1.43 ± 0.20 ng/dl in Group B respectively, and were not significantly different between the two groups. TSH levels were 2.3 ± 1.0 μ U/ml and 1.8 ± 0.8 μ U/ml, respectively and were within the normal range, however that of group A was significantly higher than that of group B ($p=0.027$). There was no correlation between serum levels of TSH, T3, T4 and FT4 and intake of dioxins. We concluded that intake of dioxins from breast milk in Japanese infants does not alter thyroid function seriously, however, the long-term effects remain to be evaluated.

Key words: dioxins, PCBs, breast milk, thyroid function, TEQ

Introduction

Received: September 27, 2000

Accepted: October 14, 2000

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Polychlorinated-dibenzo-p-dioxin (PCDD) and polychlorinated-dibenzofuran (PCDF), summarized as dioxins, are tricyclic aromatic compounds (Fig. 1). Because the number of

Fig. 1 Chemical structures of dioxins and T4

chloride atoms can vary between one and eight, there are potentially 75 different PCDD and 135 PCDF congeners. They are mainly formed as by-products during synthesis of organochlorine chemicals and during the combustion of municipal and hazardous waste. Polychlorinated biphenyl (PCB) is a compilation of 207 possible congeners with different chlorine substitutions. In the late 1970s, the production and the use of these compounds were banned because their adverse health effects had become evident. However, there are still significant quantities of PCB in use in old transformers and capacitors (1, 2).

Both dioxins and PCB are highly lipophilic and chemically stable compounds that accumulate in the food chain. For human beings, the main sources are dairy products, meals and particularly fish in Japan. They are stored mainly in the human adipose tissue, with elimination half-lives of 6 to 10 years. Small amounts of dioxins and PCB can reach the fetus by means of transplacental transport, whereas much higher levels reach breast-fed infants. Dioxins and PCB levels can be

especially high in human milk samples in highly industrialized, densely populated countries such as Japan and European countries. Dioxin and PCB have structural similarity to thyroid hormones (Fig. 1), and it has been suggested that PCBs block conversion of T₄ to T₃ and binding of T₃ to thyroid hormone receptor (TR), as well as inhibiting the binding of T₃-TR complexes to thyroid hormone elements (TREs) (1-3).

The effects of pre- and postnatal exposure to dioxins and PCBs on thyroid function in pregnant women and their infants have been reported in European countries (1, 4-9), however, there are few reports from Japan (10). The contents of dioxins and PCBs in human milk and their intake by infants through human milk have hitherto not been studied on a large scale in Japan, therefore, in this study we have made a nationwide study from Japan.

Subjects and Methods

We collected breast milk from 80 mothers (group A) living in Tokyo, Saitama, Ishikawa, and Osaka prefectures at 5, 30, 150, 300 postpartum days and measured fourteen isomers for PCDDs, 15 for PCDFs and 12 for coplanar PCBs (co-PCBs). To express the toxic potency of the mixture of dioxin and PCB in breast milk samples, the toxic equivalent factor (TEF) approach was used. That is, TEF values were assigned to the dioxin and co-PCB to present their relative toxic potencies toward 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most toxic dioxin congener, which has a TEF value of one. By multiplying the concentration (pg/g milk fat) and TEF value, the toxic equivalent quantity (TEQ) of each congener was calculated (pg TEQ/g milk fat).

Mothers were limited to those from 25 to 34 years of age, and all the mothers were primiparous and resident for more than 5 years in same area. Intake of dioxin was estimated in two ways: (1) (Dioxin concentration in mother's milk) × (average intake of mother's milk) (ml/day) × (days

until discontinuation of breast-feeding). (2) (Dioxins contents of first mother's milk - dioxin contents of last mother's milk) $\times$ (BMI)

At the age of 1 year, blood was taken from 55 of 80 infants of group A for evaluation of thyroid and immune functions. Blood was also taken from 30 infants who were bottle-fed at the age of 1 year as a control (group B).

Dried blood on filter paper was also taken to examine thyroid function and thyroid autoantibodies and was compared with those taken in the newborn period at 5 days. Thyroid and immunologic functions were examined together with growth and developmental assessment in each infant at the age of 1 year.

PCDDs, PCDFs and PCBs in human milk were determined by GC/MS at the Japan Food Research Laboratory. Serum TSH, T3, T4 and FT4 levels were determined by RIA methods at the Specific Reference Laboratory, Tokyo (SPL) using standard assay kits. Dried blood on filter paper was used to determine levels of TSH, FT4 and anti-thyroid antibodies by methods previously reported (11).

The medical ethical committee of the Kitasato University Institutional Review Board (IRB B98-05) approved the study protocol as did each other institute's IRB. The parents gave written informed consent.

Results

Thyroid function in Groups A and B

Serum T4, T3 and FT4 levels in group A were $10.6 \pm 1.6 \mu\text{g/dl}$, $1.6 \pm 0.2 \text{ ng/ml}$, and $1.42 \pm 1.25 \text{ ng/dl}$, and in Group B were $11.1 \pm 2.0 \mu\text{g/dl}$, $1.7 \pm 0.3 \text{ ng/ml}$, and $1.43 \pm 0.20 \text{ ng/dl}$, respectively, and were not significantly different between the two groups. TSH levels were $2.3 \pm 1.0 \mu\text{U/ml}$ and $1.8 \pm 0.8 \mu\text{U/ml}$, respectively, and were within the normal range, however, that of group A was significantly higher than that of group B ($p=0.027$) (Table 1).

Thyroid function and intake of dioxins TEQ

There was no correlation between serum levels of T3, T4, FT4 and estimated intake of dioxins calculated by equation (2) in the method. There was no correlation between the serum level of TSH and estimated intake of the dioxin TEQ in group A (Fig. 1) ($r=0.081$).

Thyroid autoantibodies

None of the infants in Groups A and B were positive for anti-thyroglobulin or anti-microsomal antibodies.

Table 1 Thyroid function in infants with breast feeding (Group A) and bottle feeding (Group B) at the age of 1 year

		TSH ($\mu\text{U/ml}$)	T3 (ng/ml)	T4 ($\mu\text{g/dl}$)	FT4 (ng/dl)
Breast feeding	Mean	2.3	1.6	10.6	1.42
	S.D.	1.0	0.2	1.6	0.26
	n	55	55	55	55
Bottle feeding	Mean	1.8	1.7	11.1	1.43
	S.D.	0.8	0.3	2.0	0.20
	n	32	32	32	32
p		0.027	0.18	0.271	0.828

Table 2 Thyroid function analyzed using dried blood on filter paper for infants aged 1 year and newborn infants

		Breast feeding	Bottle feeding
		TSH (μ U/ml)	TSH (μ U/ml)
5 days of age	Mean	3.4	1.8
	S.D.	1.9	1.2
	n	48	15
1 year of age	Mean	2.7	2.1
	S.D.	1.1	1.0
	n	48	15
p		0.159	0.551

Fig. 2 Correlation between serum TSH level and estimated intake of dioxin TEQ

Correlation between thyroid function at 1 year of age and that of newborn infants at 5 days of age

The results of thyroid function analyzed using dried blood on filter paper for infants at 1 year of age and 5 days of age are shown in Table 2. The TSH was within normal range in all cases and there were no significant differences between 5 days and 1 year of age or between Groups A and B. There was no significant correlation in dried blood on filter paper of TSH levels at 5 days and at 1 year of age and in none of the samples from dried blood on filter paper was the TSH level more than 10 μ U/ml.

Discussion

Although there were significant differences in serum TSH levels between breast-fed and bottle-fed infants, the levels of TSH were within the normal range. Serum T3, T4 and FT4 levels were also within normal ranges and there was no difference between the two groups. It is not clear at present whether intake of dioxins from mother's milk affected serum TSH in the two groups. Pluim et al. reported that mean TSH levels at birth and 1 week after birth were similar in a high-exposure group and low-exposure group, but at the age of 11 weeks, the mean TSH concentration was significantly higher in the high-exposure group (1.81 ± 0.19 vs 2.90 ± 0.12 μ U/dl) (4). A correlation between dioxin content in mother's milk and thyroid function in mothers as well as in their infants was reported from The Netherlands (1). Higher dioxin levels in human milk correlated with lower levels of maternal thyroid hormone, and with higher plasma levels of TSH in the infants in the 2nd week and 3rd month after birth. The median TSH levels in the high dioxin-exposed breast-fed infants were 2.3 μ U/ml at the second week and 2.0 μ U/ml at three months of age, which was almost the same level as in our breast-fed infants at the age of one year. Nagayama et al. reported a significant positive correlation between serum TSH and

estimated total intakes of dioxins and PCB from breast milk and a negative correlation with T4 (10). The levels of serum TSH and T4 were within normal ranges in their report. Their estimated intake of dioxins and PCBs from the breast milk was calculated by multiplying their TEQ levels in breast milk at 2 to 3 months after birth by the expected intake of breast milk in the Japanese baby, namely 120 ml/Kg body weight, by the individual duration of breast feeding (days). Since as we reported recently (12), the concentrations of dioxins and PCBs decrease and the intake of milk per Kg of body weight also decreases with the number of days after birth, the intake of dioxins estimated by this method is rather rough and accordingly the data should be treated with great caution.

Effects of lactational exposure to dioxins and related chemicals on immune function (5, 6), lymphocyte subpopulations (5), cognitive abilities (8), and growth and development (9) has been reported in human children as well as experimental animals (13, 14). Research on the above subjects is now progressing in Japan and will be reported in the near future.

Conclusion

It was concluded that, although the infants were exposed to fairly large amounts of dioxins and PCBs in breast milk in Japan, the thyroid function at 1 year of age was not impaired seriously. However long term effects remain to be evaluated.

Acknowledgments

We thank all the parents involved in this study and those persons in the local governments concerned. We also thank Mrs. Michiyo Kanda for preparing the manuscript. This study was supported by the Research on Environmental Health and Research on Children and Families, the Ministry of Health and Welfare, Japan.

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