AREAL DIFFRENCES OF CONCENTRATION LEVES OF POLYCHLORINATED DIBENZO-*P*-DIOXINS AND DIBENZOFURANS IN HUMANBREAST MILK FROM VIETNAM AND JAPAN

Tawara K[1], Nishijo M[1], Nakagawa H[1], Kido T[2], Naganuma R[2], Suzuki H[2], Hung TM[3], Thom LTH[3], Dung PT[3], Nhu DD[3]

[1]Department of Epidemiology and Public Health, Kanazawa Medical University, 1-1 Daigaku, Uchinada-machi, Ishikawa Prefecture, Japan; [2]Division of Health Sciences, Graduate School of Medical Science, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa-city, Ishikawa Prefecture, Japan, [3]Division for Mitigation of the Consequences of the Chemicals used during the War on Human Health (10-80 Division), Vietnam Ministry Health, 35 Nguyen Huy Tuong, Thanh Xuan, Ha Noi, Viet Nam

Introduction

A large mount of study over the past three decades has disclosed various concentration levels of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in human breast milk. However few discussions have been progressed so far that some attribute in concentration levels of PCDDs/DFs may be referred to a quality or a feature of exposure sources in the area. Accordingly the studies were conducted to find out areal differences of concentration levels every 2,3,7,8-substituted isomer (7 PCDDs and 10 PCDFs in total) in human breast milk from Viet Nam which had experienced mass pollution by military herbicide operations of the American Armed Force, and Japan in which current typical trends in human exposure to PCDDs/DFs could exist.

Materials and Methods

The study included 162 and 86 healthy mother volunteers from central Viet Nam and Japan respectively. Of 156 Vietnamese volunteers, 87 mothers (26 primiparas and 61 miltiparas; mean age 27.9±5.30 yr) were invited from a polluted commune in Quang Tri Province (QT) where herbicide mixture known as “Agent Orange (AO)” was dispersed between 1962 and 1971. The remaining 75 volunteers (38 primiparas and 37 miltiparas; mean age 25.3 ±3.79 yr) were control mothers who were living in a commune in Ha Tinh Province (HT) which had never experienced military herbicide operation. On the other hand, 86 Japanese mothers consisted of 44 residents (20 primiparas and 24 miltiparas; mean age 29.3±4.00 yr) in Ishikawa Prefecture (IS), and 42 (25 primiparas and 17 miltiparas; mean age 30.4±3.95 yr) residents in Toyama Prefecture (TY). Both prefectures bordering on each other are located in the northern part of Japan, and face to the Sea of Japan.

10 ml of breast milk was collected from each volunteer, and a range of pretreatment procedure for PCDDs/DFs was performed in accordance with Tawara et al[1]. Quantitation was performed by a high resolution mass

spectrometer (HRMS; JEOL MStation-JMS700) equipped with a gas chromatograph (HP-6890).

Data were statistically analyzed using the SPSS version 11.0 series for Windows. To find out isomers with superior concentration levels, first, comparison among groups was examined by two-way analysis of variance (two-way ANOVA). When two-way ANOVA indicated significances, multiple comparison testing followed, using the Tukey's HSD test or the Dunnett test in accordance with the result of the Levene test to examine homogeneity variance.

Logarithmetic transformation of the variables was performed as needed to improve normality, and all values except for the number of samples and volunteer's years in the text and table, are presented as geometric ones.

Results and Discussion

To evaluate the research group difference, two-way ANOVA was conducted with adjustment for parity, because significant differences of PCDDs/DFs levels between primipara and multipara were recognized in every group. Significant main effects of group difference were recognized for all isomers, except for 2,3,7,8-TCDF. With regard to TEQ, $\sum$PCDDs and $\sum$PCDDs/DFs were significantly different among groups, while significant group difference was not recognized from $\sum$PCDFs. Then further multiple comparison testing was conducted, and revealed significant intergroup difference every isomer. Table 1 lists mean values of the PCDDs/DFs every group with adjustment for parity, and demonstrate significances of individual concentration level differences among groups. Significantly highest levels of PCDDs/DFs among the four groups were recognized from either the QT group or the TY group. Furthermore PCDDs with significantly highest levels, except for 1,2,3,4,6,7,8-HpCDD, were focused on the TY group, while such PCDFs, except for 2,3,4,7,8-PCDF and 2,3,4,6,7,8- HxCDF, were focused on the QT group. In contrast significantly lowest levels of PCDDs/DFs among the four groups were recognized from either the HT group or the IS group. Unlike the instance of highest levels, however, most isomers with lowest levels were confined to the HT group, independently of PCDDs and PCDFs. Taking the results into consideration, we discussed the relationship between significances of individual concentration level differences and exposure sources to dioxins.

It has been pointed out hitherto that the largest sources of dioxin emissions in such industrial countries as Japan are municipal and medical waste incinerators. Both QT and HT are located in central Viet Nam which possesses no facilities like incinerators for refuse disposal. Thus, exposure sources to dioxins in both QT and HT are exception to the major source for industrial countries. The most distinct exposure source to dioxins among the four research groups was "AO", dioxin-contaminated herbicide. QT Province borders the demilitarized zone along the 17th parallel which once divided the north from the south in wartime, and represents one of the most heavily AO-exposed areas in the country[2]. It is well known that 2,3,7,8-TCDD was a contaminant of 2,4,5-T portion of AO mixture. Thus, past studies focusing on AO effect to human health tended to distinguish exposure

sources between 2,3,7,8-TCDD and other 2,3,7,8-substituted isomers, and to focus on 2,3,7,8-TCDD level only, when was evaluated dioxin transfer from environment to human tissue including breast milk. On the contrary, as mentioned above, our result showed that isomers significantly superior in the QT group concentrated on PCDFs rather than PCDDs. Additionally, such isomers were not always similar to those in the HT group. That is, 1,2,3,4,6,7,8-HpCDD, 1,2,3,7,8-PCDF, 1,2,3,4,7,8-HxCDF 1,2,3,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF and 1,2,3,4,7,8,9-HpCDF were prominent isomers in the QT group. Accordingly, our studies clearly show that these five isomers are characteristic isomers in QT which determine a quality or a feature of exposure to dioxin.

On the other hand, superior isomers in the TY group were almost similar to those in the IS group, though the concentration levels were significantly different. In other word, unlike the case of Viet Nam, exposure sources to dioxins of the TY group and the IS group are substantially similar. This suggests that characteristic isomers in Japan can be distinguished, when the isomers significantly superior in the TY group were compared with the prominent isomers in the QT group mentioned above. As a result, our study found out 1,2,3,7,8-PCDD, 1,2,3,6,7,8-HxCDD, 2,3,4,7,8-PCDF and 2,3,4,6,7,8-HxCDF, which determine a quality or a feature of Japanese exposure sources to dioxin.

With regard to 2,3,7,8-TCDD, 2,3,7,8-TCDD level in the QT group was not remarkable, in comparison to that in other groups. However the HT level was significantly higher than the IS level. This suggests that 2,3,7,8-TCDD concentration in Viet Nam is comparatively high even at low level. Further discussion point is that 2,3,7,8-TCDF level was highest in the HT group, though significant group difference was not recognized. As mentioned above, distinct exposure sources to dioxin in HT are neither applicable to AO nor refuse incinerators. Accordingly it is suggested that further research should be introduced in HT to evaluate exposure sources. Additionally, although the tendency was observed, that significantly highest levels of most PCDFs were focused on the TY group, significant group difference for $\sum$PCDFs was not recognized. That is, our studies suggest that exposure evaluation for dioxins using TEQ is not necessarily appropriate, because submission of concentration values to I-TEF of WHO-TEQ seem to smooth characteristic PCDDs/DFs levels.

In conclusion, we revealed areal differences of concentration levels of 2,3,7,8-substituted isomers in human breast milk from Viet Nam and Japan, and suggest level differences of the isomers are attributed to a quality or a feature of exposure sources in the area. Additionally, it is suggested that exposure evaluation for dioxins using TEQ is not necessarily appropriate, due to smoothing characteristic PCDDs/DFs levels.

References

1. Tawara k, Honda R, Nishijo M, Nakagawa H. *J. Kanazawa Med. Univ.* 2003;28:17 (in Japanese).
2. PalmerM G. *Social Science and Medicine* 2005;60:1061.

Table 1 Mean concentration of PCDDs/DFs every group with adjustment for parity and results of multiple comparison test

(pg /g-lipid)	Quang Tri Province				Ha Tinh Province				Toyama Prefecture				Ishikawa Prefecture			
Isomers	MEAN	(	S D	)	MEAN	(	S D	)	MEAN	(	S D	)	MEAN	(	S D	)
2,3,7,8-TCDD	0.828	(	2.04	)	0.549	(	1.66	)	0.893	(	2.49	)	0.351	(	1.75	)
				###				** ##				###				***
1,2,3,7,8-PCDD	2.294	(	1.92	)	1.152	(	1.74	)	4.883	(	1.69	)	1.617	(	1.67	)
				*** ###				***				###				*** #
1,2,3,4,7,8-HCDD	1.436	(	2.38	)	0.422	(	1.86	)	1.633	(	2.1	)	0.614	(	1.67	)
				###				***				###				*** #
1,2,3,6,7,8-HxCDD	6.173	(	1.83	)	1.117	(	1.73	)	15.304	(	1.83	)	5.803	(	1.72	)
				*** ###				***				###				*** ###
1,2,3,7,8,9-HxCDD	1.649	(	2.21	)	0.377	(	1.84	)	2.015	(	2.57	)	0.909	(	1.68	)
				###				***				###				*** ###
1,2,3,4,6,7,8-HpCDD	13.398	(	1.81	)	1.328	(	1.74	)	7.675	(	1.96	)	2.894	(	1.6	)
				###				***				*** ###				*** ###
OCDD	44.118	(	1.82	)	5.418	(	1.66	)	61.888	(	1.77	)	25.569	(	1.64	)
				* ###				***				###				*** ###
2,3,7,8-TCDF	0.518	(	1.75	)	1.063	(	1.47	)	0.931	(	1.91	)	0.567	(	1.61	)
				***				###				###				***
1,2,3,7,8-PCDF	0.645	(	2.15	)	0.468	(	1.73	)	0.376	(	3.21	)	0.146	(	1.75	)
				###				* ###				** ###				***
2,3,4,7,8-PCDF	4.396	(	1.88	)	2.759	(	1.54	)	9.273	(	1.74	)	3.335	(	1.71	)
				*** ###				***				###				***
1,2,3,4,7,8-HxCDF	12.894	(	2.09	)	1.383	(	2.29	)	2.41	(	1.69	)	1.008	(	1.87	)
				###				***				*** ###				***
1,2,3,6,7,8-HxCDF	7.577	(	2.12	)	1.137	(	1.84	)	2.578	(	1.72	)	0.946	(	1.76	)
				###				***				*** ###				***
2,3,4,6,7,8-HxCDF	0.987	(	2.26	)	0.355	(	1.87	)	1.681	(	2.21	)	0.604	(	1.84	)
				** ###				***				###				*** ###
1,2,3,7,8,9-HxCDF	0.279	(	2.97	)	0.088	(	2.01	)	0.099	(	2.45	)	0.091	(	2.55	)
				###				***				***				***
1,2,3,4,6,7,8-HpCDF	10.634	(	2.18	)	0.918	(	2.21	)	1.161	(	3.8	)	0.604	(	1.83	)
				###				***				*** ##				***
1,2,3,4,7,8,9-HpCDF	1.465	(	2.77	)	0.104	(	1.91	)	0.105	(	3.12	)	0.117	(	1.78	)
				###				***				***				***
OCDF	0.221	(	3.28	)	0.109	(	1.97	)	0.195	(	1.01	)	0.188	(	2.12	)
				###				***				##				##
TEQ-PCDD	4.347	(	1.79	)	1.955	(	1.61	)	8.032	(	1.72	)	2.76	(	1.66	)
				*** ###				***				###				*** ##
TEQ-PCDF	4.677	(	1.93	)	1.878	(	1.53	)	5.501	(	1.71	)	2.04	(	1.68	)
				###				***				###				***
TEQ-PCDDs/DFs	9.154	(	1.82	)	3.876	(	1.52	)	13.636	(	1.68	)	4.824	(	1.66	)
				*** ###				***				###				***

A double-underlined figure represents highest value of concentration level among groups. Similarly, a single-underlined figure represents lowest value of concentration level among groups. An upper asterisks and a lower sharp in a cell represent significance level acquired by multiple comparison test for highest level (***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$), and lowest level (###: $p < 0.001$, ##: $p < 0.01$, #: $p < 0.05$), respectively.