FOLLOW-UP SURVEY OF DIOXINS AND RELATED CHEMICALS IN THE BLOOD OF YUSHO PATIENTS IN 2002

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Introduction

In 1968, a mass poisoning, the so-called Yusho incident[1)], occurred in western Japan due to cooking oil contaminated by heat-degraded polychlorinated biphenyls (PCBs). The cause of Yusho disease is thought to be ingested toxic substances, including not only PCBs but also polychlorinated dibezo-*p*-dioxin (PCDDs) and polychlorinated dibenzofuran (PCDFs) in Kanemi rice oil[2)]. The medical aspects of this poisoning have been demonstrated by many researchers[3)]. A follow-up survey of the blood concentrations of PCDDs, PCDFs, and non-*ortho*- coplanar PCBs (Non-Co-PCBs) in Yusho patients is very important when evaluating the health of Yusho patients and for their possible treatment. Since 1998, extensive studies have been performed by the Yusho study group involving follow-up surveys of the human tissues and/or the blood concentrations of the casual compounds in Yusho patients as well as clinical trials for the accelerating the excretion of these compounds in Yusho patients[4),5)].

We have reported that high levels of toxic substances such as PCDFs have persisted in Yusho patients even up through 1998, more than 30 years after the original incident, while at dioxin2003 Meeting, we reported the results of a follow-up survey performed in 2001[6)]. In the present study, we determined the blood concentration of dioxin-like isomers using samples collected in the fiscal year 2002 from Yusho patients as well as from Yusho-suspected people who lived in Japan, using high-resolution gas chromatograph/high-resolution mass spectrometer

(HRGC/HRMS) equipped with a solvent-cut large volume (SCLV) injection system [7].

Materials and Methods

The subjects included in this survey were noted as follows: 279 Yusho patients (authorized by the Yusho medical team) and 92 Yusho-suspected people (those suspected to have ingested Kanemi rice oil, though unauthorized by the medical team according to the diagnostic criteria for Yusho disease). The blood samples were collected from a total of 381 people who had given their informed-consents at their medical checkups in the 2002 fiscal year. 10 mL of blood samples were collected using a vacuum blood collecting pipe containing heparin and stored at -4°C for later analysis. Blood lipid was extracted through the use of an accelerated solvent extractor (ASE-200, Dionex, Sunnyvale, CA). Each blood sample was accurately weighted to 5 g and mixed with 4 g Isolute (International Sorbent Technology Ltd., Hengoed, Mid Glamorgen, UK). After the mixed sample was loaded into the extraction cell, ^{13}C-labeled-PCDDs, ^{13}C-labeled PCDFs, and ^{13}C-labeled PCBs, as internal standards, were added. Acetone and n-hexane (1:4, v/v) were used as the extraction solvents. The lipid obtained was dissolved in n-hexane and treated with concentrated sulfuric acid. The separated hexane layer was applied to a silver nitrate/silica gel column (0.5 g) and eluted with 15 ml of hexane. The eluted solution was loaded into an active carbon column (0.5 g) after being evaporated to 1 ml and separated into two fractions. The first fraction, containing mono-*ortho*-Co-PCBs, was eluted with 10 ml of 10% (v/v) dichloromethane /n-hexane. PCDDs, PCDFs, and Non-Co-PCBs were eluted with 25 ml of toluene as the second fraction. The method employed here requires only a reduced amount of blood collected from Yusho patients compared with the conventional method. The column packing (silver nitrate silica gel, active carbon column, and anhydrous sodium sulfate) used in this experiment was washed in order to reduce blank materials by ASE-200 under the same conditions as the lipid extraction with n-hexane or toluene. Concentrations of the PCDDs, PCDFs, and Non-Co-PCBs were measured using HRGC/HRMS (Autospec Ultima E, MicroMass Ltd., Manchester, UK) equipped with a SCLV (SGE International, Victoria, Australia) injection system. The column used for solvent-cut was a BPX-5 fused silica pre-capillary column (0.25 mm i.d.×6 m, 0.25 µm film thickness), and the analytical column was a BPX-5 fused silica capillary column (0.15 mm i.d.×30 m, 0.15 µm film thickness), (SGE International, Victoria, Australia).

Results and Discussion

Concentration of dioxins in the blood of Yusho patients and of normal subjects: Table 1 shows the concentrations of PCDDs, PCDFs, and Non-Co-PCBs in the blood of Yusho patients, of Yusho-suspected persons, and of normal controls. In typical Yusho patients, the mean TEQ concentrations of PCDDs, PCDFs, and Non-Co-PCBs in blood collected in 2002 were 20, 105, and 12 pg-TEQ/g lipid, respectively. In the case of the Yusho-suspected group, the mean

TEQ concentrations in blood were 14, 19, and 10 pg-TEQ/g lipid, respectively. On the other hand, the levels of normal subjects were 11, 5.8, and 8.0 pg-TEQ/g lipid, respectively. The levels of PCDDs and Non-Co-PCBs were similar in the three groups; however, the level of PCDFs in Yusho patients was 5.5 and 18 times higher than those of Yusho-suspected individuals and normal controls, respectively.

The mean total-TEQ concentration of the blood from Yusho patients was about 4.6 times higher than that of normal subjects, and even after the passage of 34 years, the levels of this substance remains high in victims of this accident. The levels of PCDDs in Yusho-suspected individuals were similar to those reported by Masuda et al.[8)] who surveyed 152 normal persons in Fukuoka in 2000; however, the levels of PCDFs in this group was 3.2 times higher than that of normal persons. In the Yusho-suspected group, 4 people showed more than 200 pg/g lipid of 2,3,4,7,8-PeCDF concentration. In this case, it is suggested that this is a result of exposure to Kanemi rice oil.

Contribution of each isomer to the total TEQ: Figure 1 shows the toxic contribution of each congener to the total TEQ. The toxic contributions of PCDDs, PCDFs, and Non-Co-PCBs in typical Yusho patients, in the Yusho-suspected group, and in normal subjects were 14, 77, and 9%, 33, 44, and 23%, and 44, 24, and 32% of the total TEQ value, respectively. The order of the toxic contribution rate of PCDFs-TEQ which was thought to be the primary cause of Yusho disease was the Yusho patient group, the Yusho-suspected group, and normal subjects, with PCDFs still constituting a high rate in relation to the total TEQ in typical Yusho patients, as mentioned above.

In the Yusho-suspected group, however, the toxic contribution rate was intermediate between typical Yusho patients and normal subjects. However, some individuals in the Yusho-suspected group showed a high toxic contribution rate of PCDFs -TEQ in relation to total TEQ due perhaps to exposure to contaminated Yusho oil at the onset of 1968.

Table 1. Concentrations of dioxins (pg/g lipid) and related compounds in blood of Yusho patients and normal subjects.

Congeners	Yusho patients (N=279)				Yusho-suspected group (N=92)				Normal subject (N=152)*			
	Mean	SD.	Min.	Max.	Mean	SD.	Min.	Max.	Mean	SD.	Min.	Max.
2378-TCDD	1.7	0.81	ND	4.4	1.4	0.9	ND	5.0	1.9	0.82	ND	5.0
12378-PeCDD	11	5.9	ND	47	8	4.0	ND	22	5.7	2.3	1.7	15
123478-HxCDD	2.9	1.8	ND	11	3.1	1.9	ND	11	3.4	1.7	ND	9.3
123678-HxCDD	53	42	6.0	291	29	19	5.9	106	20	9.6	4.4	45
123789-HxCDD	5.1	3.8	ND	41	5.4	3.9	ND	18	3.7	1.9	ND	10
1234678-HpCDD	63	54	11	556	76	55	14	288	20	15	5.1	96
OCDD	877	728	172	9159	909	560	229	2836	371	529	49	4200
2378-TCDF	1.4	0.9	ND	6.3	ND	-	ND	6.2	2.1	1.2	ND	5.7
12378-PeCDF	ND	-	ND	6.3	ND	0.56	0.5	2.9	1.7	1.7	ND	8.8
23478-PeCDF	192	252	3.1	1890	33	52	2.2	263	8.3	4.4	2.2	26
123478-HxCDF	59	99.6	ND	770	9	16	ND	112	5.1	3.0	1.3	29
123678-HxCDF	22	29.1	ND	210	6.9	5.2	ND	26	4.4	2.0	1.9	18
234678-HxCDF	1.4	1.0	ND	10	1.6	1.2	ND	10	ND	-	-	-
123789-HxCDF	ND	-	-	-	ND	-	-	-	2.6	1.5	ND	14
1234678-HpCDF	3.2	4.0	ND	39.8	3.3	4.0	ND	27.4	5.2	5.0	1.4	39
1234789-HpCDF	ND	-	ND	3.5	ND	-	-	-	1.7	0.7	ND	3.5
OCDF	ND	-	ND	9.1	ND	-	ND	7.5	NA	-	NA	NA
3445-TCB(#81)	5.6	3.2	ND	41	5.4	ND	ND	15.5	NA	-	NA	NA
3344'-TCB(#77)	11	7.1	ND	46	10.7	6.7	ND	44.6	5.9	4.4	1.5	22
33445-PenCB(#126)	103	72	ND	561	92	78	ND	387	82	79	10	430
33455'-HxCB(#169)	200	154	13	1131	83	68	5.0	318	45	30	10	160
TotalPCDDs-TEQ	20	10	3.3	79	14	6.9	2.8	39	11	4.2	4.0	26
TotalPCDFs-TEQ	105	138	2.1	1029	19	27.7	1.6	145	5.8	2.8	2.0	21
TotalPCDDs/PCDFs-TEQ	124	147	5.4	1108	32	31	5.6	162	16	6.8	6.4	41
TotalNon-ortho-coplanar PCBs-TEQ	12	7.7	ND	59	10	8.3	ND	42	8.0	7.8	0.28	44
TotalTEQ	136	148	7	1126	42	35	7	178	24	13.6	9.0	81

***:** The data of the normal subjects is cited from Masuda et al. (reference 8).

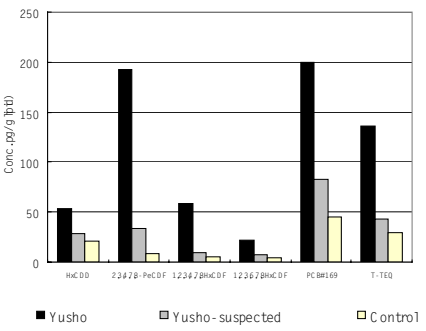

Figure 1. Comparison of concentration levels of Yusho persisted isomers in the blood of Yusho, Yusho-suspected and

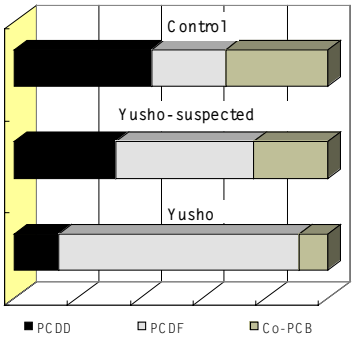

Figure 2. Percent distribution of PCDD, PCDF and non-*ortho*-Co-PCB to the total TEQ in blood of Yusho patient, Yusho-suspected and control.

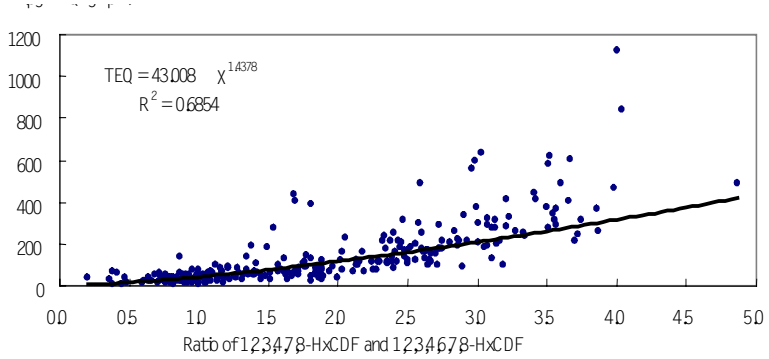

Figure 3. Relationship between TEQ and ratio of 1,2,3,4,7,8-HxCDF, and 1,2,3,6,7,8-HxCDF.

Features of persisting isomers in Yusho patients: The predominant toxic substances present in Yusho patients, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, and 1,2,3,6,7,8-HxCDF, were compared with the levels present in normal subjects. The mean concentration ratio of 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF was 1.1 for normal subjects and Yusho-suspected individuals, while the ratio was 1.9 for typical Yusho patients. This constituted a significant difference of the ratio between Yusho patients and normal subjects ($p<0.05$). Figure 3 shows the relationship between TEQ and the concentration ratio of 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF. A significant relationship between TEQ and the ratio of 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF was observed. The pattern and similarity of concentrations of 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF in Yusho oil and in the blood of Yusho patients are quite similar. These findings indicate that the ratio of 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF is an effective indicator of exposure to Yusho oil and could provide a diagnostic tool for Yusho patients. It is necessary to continue this follow-up investigation in the future, and to accumulate further data which will aid in the health management of Yusho patients.

References

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