FOLLOW-UP SURVEY OF DIOXINS AND RELATED CHEMICALS IN THE BLOOD OF YUSHO PATIENTS IN 2001

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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), polychlorinated dibenzofuran (PCDFs) in Kanemi rice oil. The medical aspects of this poisoning have been demonstrated by many researchers[2)]. We have reported that the levels of toxic substances such as PeCDFs persist in Yusho patients even in 1998, more than 30 years after the original incident[3)- 5)]. Examinations have been carried out for the sake of comparing the victim of the Yusho incident with healthy individuals, since follow-up survey of the blood concentrations of PCDDs, PCDFs, non-*ortho*- coplanar PCBs (non-*ortho*-Co-PCBs), and mono-*ortho*-coplaner PCBs (mono-*ortho*-Co-PCBs) in Yusho patients is very important when evaluating the health of Yusho patients and for their possible treatment. A follow-up survey was conducted during the medical checkups of Yusho patients who lived in Fukuoka prefecture in the 2001 fiscal year. We determined the blood concentration of these dioxin-like isomers in 78 blood samples collected in 2001 using by a high-resolution gas chromatograph/high-resolution mass spectrometer (HRGC/HRMS) equipped with a solvent-cut large volume (SCLV) injection system[6)].

Materials and Methods

The blood samples were collected from seventy eight patients who had given their informed-consents at their medical checkups in the 2001 fiscal year. 10 mL of blood samples were collected using a vacuum blood collecting pipe containing heparin and stored at 4 degrees for later analysis. Blood lipid was extracted by 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-*ortho*-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 Co-PCBs were measured using HRGC/HRMS (Autospec Ultima E, MicroMass Ltd., Manchester, UK) equipped with an 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 for 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), respectively.

Results and Discussion

Concentration of dioxins in blood of Yusho patients and control

Table 1 shows the concentrations of PCDDs, PCDFs, Co-PCBs, and PCBs in the blood of Yusho patients and control. In typical Yusho patients (Group A PCB pattern), the mean TEQ concentrations of PCDDs, PCDFs, mono-*ortho*-Co-PCBs, and non-*ortho*-Co-PCBs in blood collected in 2001 were 36, 260, 9.3, and 64 pg-TEQ/g lipid, respectively. In the case of the Group B PCB pattern, the mean TEQ concentrations of PCDDs, PCDFs, mono-*ortho*-Co-PCBs, and non-*ortho*-Co-PCBs in blood were 27, 169, 8.9, and 32 pg-TEQ/g lipid, respectively. On the other hand, in the group C PCB pattern, the levels were 26, 16, 13, and 11 pg-TEQ/g lipid, respectively, and similar to the levels of control as shown in Table 1. The levels and composition of dioxins were similar to those reported by Masuda et al [7] who surveyed for 152 not exposed subjects in Fukuoka in 2000. Mean total-TEQ concentration of the blood of Yusho patients was about 13 times higher than that of control, and even after the passage of 33 years, the levels of this substance remains high in victims of this accident.

Contribution of each isomer to the total TEQ

Figure 1 shows the toxic contribution of each congener to the total TEQ. The toxic contribution of PCDDs, PCDFs, non- *ortho*-Co-PCBs, and mono-*ortho*-Co-PCBs in typical Yusho patients (Group A PCB pattern) were 10, 70, 3, and 17% of the total TEQ value, respectively. The toxic contribution rate of PCDFs TEQ which was through to be main chemicals of causes of Yusho disease was still at a high rate in relation to total TEQ in typical Yusho patients as mentioned above. The Yusho patients showing B pattern also showed the same contribution rate as the A pattern. In the patients of group C, however, the toxic contribution rate was different from the A and B patterns, rather than similar to those of control. However, some patients in the C 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 in 1968.

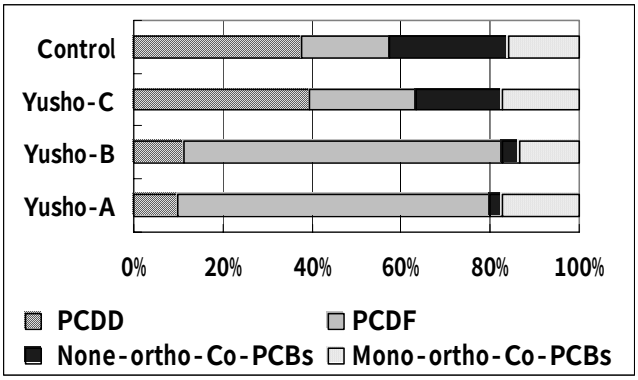

Figure 1
Percent distribution of PCDD, PCDF, Non-*ortho*-Co-PCB and mono-*ortho*-Co-PCB to the total TEQ in blood of Yusho patient and control

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

Congeners	Pattern A (N=20)		Pattern B (N=31)		Pattern C (N=25)		Control(N=152)*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2,3,7,8-TCDD	1.4	0.9	1.5	0.9	2.4	1.1	1.9	0.8
1,2,3,7,8-PeCDD	25	14	18	11	19	8.1	5.7	2.3
1,2,3,4,7,8-HxCDD	1.4	0.9	1.9	1.5	3.5	1.8	3.4	1.7
1,2,3,6,7,8-HxCDD	93	56	57	34	28	11	20	9.6
1,2,3,7,8,9-HxCDD	4.0	1.5	4.5	2.6	5.2	2.5	3.7	1.9
1,2,3,4,6,7,8-HpCDD	19	7.1	22	13	39	30	20	15
OCDD	419	168	484	284	1109	1176	371	529
2,3,7,8-TCDF	2.7	3.2	1.3	1.3	1.7	2.9	2	1.2
1,2,3,7,8-PeCDF	1.1	0.8	0.8	0.5	1.4	1.1	2	1.7
2,3,4,7,8-PeCDF	476	404	312	263	27	22	8.3	4.4
1,2,3,4,7,8-HxCDF	159	148	98	111	8.4	7.9	5.1	3.0
1,2,3,6,7,8-HxCDF	56	44	32	30	7.1	3.0	4.4	2.0
2,3,4,6,7,8-HxCDF	1.4	0.7	1.6	1.0	2.1	1.5	ND	-
1,2,3,7,8,9-HxCDF	1.1	0.3	1.1	0.2	1.1	0.3	2.6	1.5
1,2,3,4,6,7,8-HpCDF	4.4	2.6	3.9	2.5	3.5	1.8	5	5.0
1,2,3,4,7,8,9-HpCDF	ND	-	ND	-	ND	-	2	0.7
OCDF	ND	-	ND	-	ND	-	NA	-
344'5-TCB(#81)	ND	-	ND	-	5.5	2.7	58	4.4
33'4'4'-TCB(#77)	8.6	5.5	7.4	3.3	7.0	3.5	NA	-
33'44'5-PenCB(#126)	56	28	69	35	120	71	82	79
33'44'55'-HxCB(#169)	371	215	204	97	83	38	45	30
2'344'5-PenCB(#123)	160	69	245	134	552	341	431	433
23'44'5-PenCB(#118)	10860	5595	16418	9899	28066	14348	10766	9576
2344'5-PenCB(#114)	4034	3264	3289	2086	2005	981	803	658
233'44'-PenCB(#105)	2036	986	3237	1940	5404	3322	2706	2673
23'44'55'-HexCB(#167)	3498	2145	3680	2133	4082	1828	1771	1390
233'44'5-HexCB(#156)	93807	68790	42495	24890	10857	4999	4452	3112
233'44'5'-HexCB(#157)	26280	19152	12552	7887	2906	1321	1141	833
233'44'55'-HpCB(#189)	9879	6210	5189	2898	1180	661	543	388
Total PCDDs-TEQ	36	18	27	13	26	9.7	11	4.2
Total PCDFs-TEQ	260	219	169	145	16	12	5.8	2.8
Total PCDDs/PCDFs-TEQ	296	231	196	153	42	18	16	6.8
Total None-ortho-coplanar PCBs-TEQ	9.3	3.7	8.9	4.0	13	7.3	8.0	7.9
Total Mono-ortho-coplanar PCBs-TEQ	64	46	32	17	11	4.5	4.6	3.5
Total Co- PCBs-TEQ	74	48	41	19	24	11	13	11
Total TEQ(DDDF/Mono-/Non-Co-PCB)	370	270	237	166	66	25	29	17
Total PCBs(ng/g lipid)	1220	610	1097	933	624	233	-	-

*:The data of control is sited from Masuda et.al. (Refernce No. 7)

Comparison of persisting isomers in the blood of Yusho patients and those inYusho oil

The constituent pattern of Yusho oil and the typical isomers which persisted in blood of Yusho patients were compared. Figure 2 shows the distributive constituent concentrations of the Yusho oil, those in the blood of Yusho patients, and control. The pattern and similarity of concentration in Yusho oil and the blood of Yusho patients are quite similar in regard to 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF. However, the ratio of 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF was not similar in regard to each isomer. Furthermore, the patterns of PCB#118, PCB#156 and PCB#157 do not reflect the pattern of Yusho oil in the blood of Yusho patients. On the other hand, the patterns of these chemicals in control differed from those of Yusho patients.

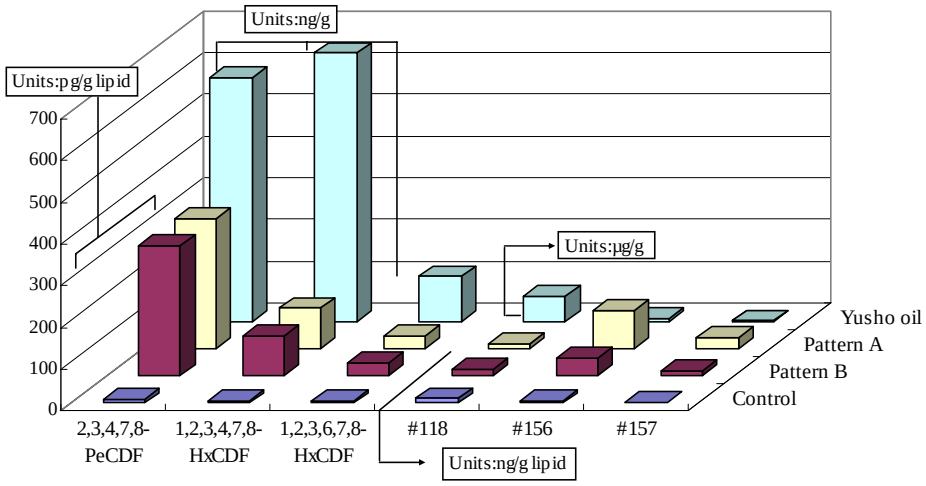

Figure 2 Comparison of persist isomers in blood of Yusho patients and Yusho oil

Conclusions

Thirty-tree years had passed since the Yusho accident, and the total blood concentrations of PCDFs in the blood of Yusho patients were still higher than those of controls. Among the different groups of PCB patterns (A, B and C), the concentrations of 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, and 1,2,3,6,7,8-HxCDF showed significant differences. These findings indicate that these isomers are hard to be metabolized or excreted in the human body since traces of them continued to be detected in these patients dating from when they had been contaminated by the rice oil in the original incident. It is necessary to continue this follow-up investigation in the future, and to accumulate the data which will aid in the health management of Yusho patients.

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