POLYCHLORINATED BIPHENYLS CONCENTRATIONS IN BLOOD OF YUSHO PATIENTS DURING MEDICAL CHECK-UPS PERFORMED IN 2012

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Introduction

In 1968, Yusho incident occurred in western Japan. Yusho patients had ingested rice oil contaminated with large amounts of polychlorinated biphenyl (PCBs) that were used as a heat-transfer medium in rice oil production. Several studies on Yusho incident have reported that the cause of the disease was thought to be ingested toxic substances, including not only PCBs but polychlorinated dibenzofuran (PCDFs), polychlorinated quarterphenyls (PCQs), and polychlorinated terphenyls (PCTs)[1)]. However, we showed that Yusho patients still have higher concentrations of PCBs after over 40 years[2)]. Furthermore, we found two characteristic groups of PCB isomers in the blood sample. One group is consisted with the major isomers (hexaCB-153, hexaCB-138, heptaCB-180, and heptaCB-182/ heptaCB-187) that were generally detected in the human blood, another group is the specific isomers (hexaCB-156, hexaCB-157, heptaCB-181, and heptaCB-189) that were highly detected in that of Yusho patients. The data from the isomer profiles may provide us with newly important information related to exposure evaluation of Yusho patients and with valuable information for future epidemiologic studies. In this study, we analyzed PCBs in the blood collected from Yusho patients during medical check-ups in 2012. We focused the major and specific PCB isomers in the blood sample, and compared with the concentrations between this study and the results obtained from Yusho patients and normal controls during medical check-ups performed in 2005.

Materials and methods

Sampling

Medical check-ups for Yusho patients have been conducted annually to determine the health status of patients since Yusho incident. The blood samples examined in this study were collected from 139 participants who received a medical check-up in 2012. Informed consent was obtained from all participants. The mean age of Yusho patients in 2012 was 62.0 years old. Additionally, that of Yosho patients and nomal controls in 2005 was 67.3 and 68.1 years old, respectively.

Ten ml of the blood sample was collected using a vacuum blood collecting tube containing heparin, and were stored at 4°C until the start of the PCB analyses.

Analysis

The extraction and purification of PCB congeners was used with the method described previously[3)-4)]. Briefly, the extraction of lipid from 5 g, a portion of each blood samples was performed with an accelerated solvent extractor (ASE) system, and the extract was refined with concentrated sulfuric acid, a silver nitrate silica gel column, an activated carbon dispersed silica gel column and sulfoxide cartridge column as a further clean-up method.

PCBs analysis was performed using a high resolution gas chromatography / high resolution mass spectrometry (HRGC/HRMS). The measurement conditions were as follows: the gas chromatograph was an HP-6890 (Agilent Technologies, USA) equipped with an Autospec Ultima NT (Waters, USA); the column used was a HT8-PCB fused silica precapillary column, 0.25 mm i.d.×60 m (SGE Ltd.); The column oven temperature of the HT8-PCB was programmed at a rate of 20°C min^{-1} from an initial temperature of 130°C (1 min hold) to a temperature of 220°C, then at a rate of 3°C min^{-1} to a temperature of 280°C, then at a rate of 20°C min^{-1} to a final temperature of 300°C (3.5 min hold). The carrier gas of helium flow rate (constant flow) was 1.3 ml/min. The injection temperature was maintained at 280°C and each sample (2 μl) was injected in the splitless mode. The ionizing energy, accelerating voltage, and trap current were 38 eV, 8.0 kV and 650 μA, respectively. Analysis was performed using EI ionization and selected ion monitoring mode. The resolution was maintained at 10000 at 5% valley.

Results and discussion

The concentrations of PCB isomers in the blood of Yusho patients in 2005 and 2012 were shown in Table 1, including the data of the normal controls previously reported.

Table 1 Comparison of PCB concentrations in the blood sample collected from medical check-ups.

	Concentration (pg g^{-1} lipid)			Ratio	
	Yosho Patients (YP)		Normal Controls (NC)	YP/NC*	YP/NC**
	2005 (n=237)	2012 (n=139)	2005 (n=127)	2005/2005	2012/2005
IUPAC#	Mean	Mean	Mean		
245-TrCB (#29)	32	16	25	1.3	0.65
244'-TrCB (#28)	1371	1428	2571	0.53	0.56
344'-TrCB (#37)	19	426	-	-	-
22'55'-TeCB (#52)	977	753	1290	0.76	0.58
22'45'-TeCB (#49)	235	179	303	0.78	0.59
22'44'-TeCB (#47)	438	342	605	0.72	0.57
22'35'-TeCB (#44)	312	170	458	0.68	0.37
23'4'6-TeCB (#71)	87	27	192	0.45	0.14
234'5-TeCB (#63)	84	79	146	0.57	0.54
244'5-TeCB (#74)	9835	9209	19472	0.51	0.47
23'4'5-TeCB (#70)	197	135	259	0.76	0.52
23'44'-TeCB (#66)	1514	1582	2338	0.65	0.68
233'4'-/2344'TeCBs (#56/60)	489	282	884	0.55	0.32
22'35'6-PeCB (#95)	667	527	833	0.80	0.63
22'355'-PeCB (#92)	601	530	860	0.70	0.62
22'455'-PeCB (#101)	1680	1480	1898	0.88	0.78
22'44'5-PeCB (#99)	17682	12882	12505	1.4	1.0
234'56-PeCB (#117)	1401	1076	927	1.5	1.2
22'345'-PeCB (#87)	646	459	692	0.93	0.66
22'344'-PeCB (#85)	142	118	218	0.65	0.54
233'4'6-PeCB (#110)	365	231	411	0.89	0.56
233'4'5-PeCB (#107)	693	765	987	0.70	0.77
2'344'5-PeCB (#123)	276	194	466	0.59	0.42
23'44'5-PeCB (#118)	16343	17117	24353	0.67	0.70
2344'5-PeCB (#114)	1895	1722	1697	1.1	1.0
233'44'-PeCB (#105)	3473	3484	5061	0.69	0.69
22'355'6-HxCB (#151)	1254	1027	1255	1.0	0.82
22'33'56'-HxCB (#135)	470	312	475	0.99	0.66
22'34'56-HxCB (#147)	612	524	484	1.3	1.1
22'344'6-HxCB (#139)	742	151	822	0.90	0.18
22'33'56-HxCB (#134)	25	10	27	0.92	0.36
233'55'6-HxCB (#165)	1412	-	-	-	-
22'34'55'-HxCB (#146)	21873	22521	13899	1.6	1.6
22'33'46'-HxCB (#132)	188	128	282	0.67	0.45
22'44'55'-HxCB (#153)	134448	128123	89821	1.5	1.4
22'3455'-HxCB (#141)	303	161	324	0.94	0.50
22'344'5-HxCB (#137)	6019	5497	2968	2.0	1.9
22'33'45'-HxCB (#130)	4466	3773	2620	1.7	1.4
233'4'5'6-HxCB (#164)	27083	28766	19350	1.4	1.5
22'344'5'-HxCB (#138)	66117	57218	40872	1.6	1.4
22'33'44'-HxCB (#128)	865	673	876	0.99	0.77
23'44'55'-HxCB (#167)	3927	3788	3649	1.1	1.0
233'44'5-HxCB (#156)	30958	25632	7982	3.9	3.2
233'44'5'-HxCB (#157)	8418	6488	2024	4.2	3.2
22'33'566'-HpCB (#179)	219	137	208	1.1	0.66
22'33'55'6-HpCB (#178)	9511	10297	6248	1.5	1.6
22'344'56-HpCB (#182/187)	43375	42047	28083	1.5	1.5
22'344'5'6-HpCB (#183)	10460	8087	6145	1.7	1.3
22'344'56-HpCB (#181)	315	159	71	4.5	2.2
22'33'4'56-HpCB (#177)	8533	8023	5753	1.5	1.4
22'33'455'-HpCB (#172)	5948	5909	2974	2.0	2.0
22'344'55'-HpCB (#180)	110380	113585	59481	1.9	1.9
233'44'5'6-HpCB (#191)	1813	1467	766	2.4	1.9
22'33'44'5-HpCB (#170)	39700	34771	17268	2.3	2.0
233'44'55'-HpCB (#189)	4542	4050	1052	4.3	3.8
22'33'55'66'-OcCB (#202)	4547	3870	2812	1.6	1.4
22'33'45'66'-OcCB (#200)	663	351	648	1.0	0.54
22'33'4566'-OcCB (#201/198)	25584	9644	10093	2.5	0.96
22'344'55'6-OcCB (#203)	19357	9078	7820	2.5	1.2
22'33'44'56-OcCB (#195)	4389	3477	1820	2.4	1.9
22'33'44'55'-OcCB (#194)	19132	19268	8595	2.2	2.2
233'44'55'6-OcCB (#205)	873	463	309	2.8	1.5
22'33'455'66'-NoCB (#208)	948	1048	775	1.2	1.4
22'33'44'566'-NoCB (#207)	414	323	339	1.2	0.95
22'33'44'55'6-NoCB (#206)	3088	2995	1960	1.6	1.5
22'33'44'55'66'-DeCB (#209)	1153	760	1361	0.85	0.56
Total TrCBs	1407	1869	2596	0.54	0.72
Total TeCBs	14169	12758	25961	0.55	0.49
Total PeCBs	42514	40586	51021	0.83	0.80
Total HxCBs	308929	284796	187798	1.6	1.5
Total HpCBs	234795	228534	128048	1.8	1.8
Total OcCBs	74546	46151	32096	2.3	1.4
Total NoCBs	4450	4366	3075	1.4	1.4
Total PCBs	652325	619820	431955	1.5	1.4

YP/NC* : Ratio = Blood PCB concentrations from Yusho patitents in 2005 / those from normal controls in 2005.
YP/NC** : Ratio = Blood PCB concentrations from Yusho patitents in 2012 / those from normal controls in 2005.

As the result, 8 isomers of mono-*ortho* PCBs and 57 isomers of non-dioxin-like PCBs in the 209 PCB isomers were identified in the blood of Yusho patients in 2012. The total concentrations of PCB isomers in the blood of Yusho patients in 2005 and 2012 were 652 and 620 ng g^{-1} lipid, respectively. These results indicated that PCB concentrations in the blood of Yusho patients seem mostly unchanged for 7 years. Their total concentrations were 1.4-1.5 times higher than those of the normal controls in 2005.

In this study, we focused the major and specific PCB isomers detected in the blood sample. The concentrations of major isomers, such as hexaCB-153, hexaCB-138, heptaCB-180, and heptaCB-182/heptaCB-187 in the blood of Yusho patients, were 134, 66, 110, and 43 ng g^{-1} lipid in 2005, additionally 128, 57, 114, and 42 ng^{-1} lipid in 2012, respectively (Table 1). We confirmed that hexaCB-153, hexaCB-138, heptaCB-180, and heptaCB-182/heptaCB-187 highly contributed in the total PCB concentrations detected in the blood of Yusho patients. In addition, these major isomers were also found in high concentration in that of the normal controls, as shown in Table 1. Thus, the ratios relative to the normal controls were in the range of 1.5-1.9, there were no significant differences between Yusho patients and the normal controls. Previous studies have reported that major isomers are metabolized to hydroxylated PCBs in human body[5)]. It is a possibility that these compounds in Yosho patients have gradually decreased by the metabolic effects for a long time. Furthermore, it was considered that daily consumed fish and seafood might affect the residue accumulation in this study.

On the other hand, the concentrations of specific isomers such as hexaCB-156, hexaCB-157, heptaCB-181, and heptaCB-189 in the blood of Yusho patients were 31, 8.4, 0.32, and 4.5 ng g^{-1} lipid in 2005, additionally 26, 6.5, 0.16, and 4.1 ng g^{-1} lipid in 2012, respectively. The concentrations of the specific isomers were lower than those of major isomers, whereas the ratios of the specific isomers were higher than those. The ratios of hexaCB-156, hexaCB-157, heptaCB-181, and heptaCB-189 in Yusho patients were 3.2-3.9, 3.2-4.2, 2.2-4.5, and 3.8-4.3, respectively, higher than those of the normal controls in 2005. These results indicated that Yusho patients still have higher concentrations of hexaCB-156, hexaCB-157, heptaCB-181, and heptaCB-189 in their blood than do unaffected people, even though over 40 years have passed since the outbreak of Yusho. These four isomers can therefore be considered to be the most important compounds for evaluating the PCBs exposure of Yusho patients. Further study is required to elucidate the accumulating property of specific isomers in Yusho patients.

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