PCB and hydroxy PCB congeners in the blood of patients with Yusho PCB poisoning

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Introduction

Rice oil polychlorinated biphenyl (PCB) poisoning called Yusho occurred in Fukuoka and Nagasaki Prefectures, Japan, in 1968 Patients have been suffering from various hormone affected symptoms retaining some persistent PCB and polychlorinated dibenzofuran (PCDF) congeners in the body for more than 35 years[1]. In the body of Yusho patients, highly accumulated PCBs and PCDFs have gradually decreased to or less than 2.3 ppm and 0.6 ppb in lipid of blood, respectively, during more than 30 years. Present levels of PCBs and PCDFs in the blood are still several times higher than those of control persons[2]. The particular patterns of concentrations of PCB congeners, namely relatively high concentrations of 2,3,3'4,4',5-hexaCB (PCB156) and low concentrations of 2,3',4,4',5-pentaCB (PCB118), have kept in the blood of patients for the most part of lifetime. PCB metabolites, hydroxy (HO-) PCBs and methylsulfone ($MeSO_2$-) PCBs have been identified in the human blood and tissues[3]. HO-PCBs were identified in the blood at levels of 2 to 10 times less than those of PCBs[4,5]. Sandau et al[6] identified HO-PCBs and pentachlorophenol (PCP) in umbilical cord plasma of neonate in Quebec, Canada and suggested that PCP and HO-PCBs are possibly altering thyroid hormone status in newborn. We analyzed PCBs, HO-PCBs and $MeSO_2$-PCBs in the blood of Yusho patients for investigating the possible relations to health effects in Yusho patients..

Materials and methods

Blood sample: Blood samples from Yusho patients were collected at annual health examination in Fukuoka from 1999 to 2003 and kept frozen until analysis. Control bloods were sampled from students and instructors at Daiichi college of pharmaceutical sciences in 1993-1997.

Analysis of blood for PCBs, HO-PCBs and $MeSO_2$-PCBs : Blood sample 5-10 g was added with internal standards of 6 ^{13}C labeled PCBs, namely 3,3',4,4'-tetraCB (PCB77), 2,2',4,5,5'-pentaCB (PCB101), PCB118, 3,3',4,4',5-pentaCB (PCB126), 2,2',4,4',5,5'-hexaCB (PCB153) and PCB156 5 ng, ^{13}C labeled 4'-HO-2,2',3,3',4,5,5'-heptaCB (4'HO-PCB172), 4-HO-2,2',3,4',5,5',6-heptaCB (4HO-PCB187) and PCP 5 ng and 4'-Me-5'-$MeSO_2$-2,3,3',4,5-pentaCB 25 ng, ethanol 10 ml and 2M HCl 10 ml. The mixture was extracted with hexane +

diethyl ether (7 : 3) 10 ml three times. The hexane layers were combined and washed with water and evaporated to dryness, remaining fatty residue being weighed. The residue was dissolved in hexane 10 ml and extracted with 0.5 M KOH/ethanol + water (1 : 1) 5 ml twice (HO-PCB fraction). The hexane solution was chromatographed on silica gel (1 g) column, eluting with hexane 30 ml (PCB fraction) and successively eluting with dichloromethane 30 ml ($MeSO_2$-PCB fraction). The PCB fraction was concentrated to 0.1 ml and injected to gas chromatograph/mass spectrometry (GC/MS, Shimadzu QP5000) for analysis of PCB congeners, hexachlorobenzene (HCB) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE). The HO-PCB fraction was acidified with 2M HCl and extracted with hexane + diethyl ether (4 : 1) 5 ml three times. The hexane layers were combined, concentrated to 1 ml and methylated with diazomethane. The methoxy-PCB solution was fractionated on a multi layer column of sulfuric acid/silica gel (1 g) and silica gel (0.5 g), eluting with dichloromethane 10 ml. The dichloromethane solution was evaporated to dryness and dissolved in hexane 2 ml. The hexane solution was fractionated on silica gel (1g) column, eluting with hexane 30 ml.. The hexane eluate was concentrated to 0.1 ml and injected to GC/MS for HO-PCBs and PCP. The $MeSO_2$-PCB fraction was evaporated and dissolved in hexane, which was partitioned with concentrated sulfuric acid 1 g twice. The sulfuric acid layer was diluted with the same amount of water and back extracted with hexane. The hexane extract was fractionated on silica gel (1 g) column eluting with dichloromethane + hexane (1 :1). The eluate was concentrated to 0.1 ml and analyzed for $MeSO_2$-PCBs.

Results and discussion

Concentrations of PCBs, HCB, DDE HO-PCBs and PCP in whole blood of Yusho patients and control persons are shown in Table 1. Their ages, fat contents of blood samples and their concentration ratios of Yusho/control are also displayed in Table 1. HO-PCBs and PCP were first quantified in the blood of Yusho patients and the concentrations were about 1/7 and 1/5, respectively, of the total PCB concentrations in the blood. The concentration ratios of HO-PCBs/total PCBs are comparable to the ratios in the blood of Canadians[4], Latvians and Swedish[5], which reported to be in the range from 1/10 to 1/2. The concentration ratios of PCP/total PCBs were more variable from 1/9 to 2/1 in these persons. The ratio of Yusho/Control concentrations in PCB156 is 8.35 and the highest among the congeners in Table 1. The concentrations of PCB118, 2,3,3',4,4'-pentaCB (PCB105) and PCP in Yusho patients were however lower than those of control persons. These characteristic differences by the PCB congeners have been observed in Yusho patients for more than 30 years and used as one of the characteristic signs for diagnosing Yusho. HCB was probably metabolized to PCP in the human body, and the PCP concentrations were however 5 to 10 times higher than the HCB concentrations. PCP is probably more retainable in the human blood than HCB. In Yusho patients, aromatic hydrocarbon hydroxylase (AHH) might have been strongly induced by the toxic congeners such as 2,3,4,7,8-pentaCDF, PCB156 and others, and the induced AHH has been possibly accelerating the metabolism of some retainable and slowly labile PCBs such as PCB118 and PCB105 as well as PCP. Their concentrations are eventually lowered in the body of Yusho patients and their levels of thyroxin and estradiol may have been simultaneously disturbed.

Table 2 lists the correlation coefficients between the pairs of concentrations of the PCBs, HO-PCBs and pesticides in the blood of Yusho patients (n = 36). Very high correlation coefficients are observed between the concentrations of PCB153, PCB138, PCB 156 and 4HO-CB187. DDE has high correlation coefficients with PCB138 or PCB156 but low correlation coefficients with 4HO-CB146 or 4HO-CB187. HCB and PCP however do not have significant correlations with any other congeners in the Table 2. Interestingly, age shows significant positive correlations ($p < 0.05$) with the concentrations of PCB153, PCB138, DDE and 4HO-CB146, suggesting their accumulative retention in the body during long human life.

Table 1: Concentrations of PCBs, pesticides and HO-PCBs in the blood of Yusho patients and control persons and their ratios

		Number detected	Concentrations, ppb in whole blood					Ratio Yusho/Cont
			Mean	SD	Median	Min	Max	
Age	**Yusho**		**66.4**	**9.9**	**68.0**	**40.5**	**84.5**	3.08
	Control		39.0	21.2	22.1	20.7	62.0	
Lipid (%)	**Yusho**	**36**	**0.229**	**0.069**	**0.213**	**0.129**	**0.362**	1.81
	Control	9	0.130	0.068	0.118	0.033	0.279	
PCB 118	**Yusho**	**36**	**0.078**	**0.046**	**0.063**	**0.0009**	**0.208**	0.77
	Control	9	0.109	0.063	0.081	0.0370	0.197	
PCB 153	**Yusho**	**36**	**0.659**	**0.330**	**0.583**	**0.0249**	**1.921**	2.62
	Control	9	0.340	0.226	0.223	0.1047	0.765	
PCB 105	**Yusho**	**34**	**0.032**	**0.035**	**0.019**	**0.0049**	**0.158**	0.47
	Control	9	0.041	0.025	0.040	0.0109	0.084	
PCB 138	**Yusho**	**36**	**0.496**	**0.352**	**0.373**	**0.0080**	**1.973**	3.75
	Control	9	0.174	0.124	0.100	0.0519	0.405	
PCB 182	**Yusho**	**36**	**0.101**	**0.051**	**0.094**	**0.1654**	**0.245**	1.46
	Control	8	0.073	0.052	0.064	0.0174	0.159	

PCB 156	**Yusho**	**36**	**0.278**	**0.201**	**0.225**	**0.0282**	**1.120**	8.35
	Control	9	0.029	0.018	0.027	0.0084	0.051	
PCB 180	**Yusho**	**36**	**0.433**	**0.224**	**0.406**	**0.0001**	**1.293**	4.49
	Control	9	0.163	0.143	0.090	0.0460	0.422	
Total PCBs	**Yusho**	**36**	**2.793**	**1.474**	**2.460**	**0.634**	**8.740**	3.19
	Control	9	1.203	0.750	0.771	0.418	2.510	
HCB	**Yusho**	**36**	**0.105**	**0.057**	**0.101**	**0.0075**	**0.227**	1.09
	Control	9	0.100	0.052	0.093	0.0389	0.187	
DDE	**Yusho**	**36**	**2.998**	**1.657**	**2.663**	**0.0595**	**6.731**	1.84
	Control	9	2.339	1.772	1.447	0.3856	5.728	
4HO-PCB 146	**Yusho**	**35**	**0.197**	**0.130**	**0.165**	**0.061**	**0.778**	2.12
	Control	8	0.089	0.048	0.078	0.036	0.155	
4HO-PCB 187	**Yusho**	**35**	**0.206**	**0.126**	**0.177**	**0.057**	**0.660**	1.51
	Control	9	0.145	0.072	0.117	0.045	0.251	
PCP	**Yusho**	**34**	**0.562**	**0.385**	**0.444**	**0.186**	**2.314**	0.64
	Control	8	0.979	0.701	0.690	0.335	2.061	
$MeSO_2$-PCB	**Yusho**	**0**	**Not detected (< 0.5)**					
	Control	0	Not detected (< 0.2)					

Boldface : Yusho patients,
PCB138: 2,2',3,4,4',5'-hexaCB, PCB182: 2,2',3,4,4',5',6-heptaCB,
PCB180: 2,2',3,4,4',5,5'-heptaCB, 4HO-PCB146: 4-HO-2,2',3,4',5,5'-hexaCB.

Table 2: Correlation coefficients between the concentrations of PCBs, pesticides and HO-PCBs in the blood of Yusho patients (n = 36) and their ages

	Age	PCB 118	PCB 153	PCB 138	PCB 156	HCB	DDE	4OH-PCB 146	4OH-PCB 187	PCP
Age	1									
PCB118	0.268	1								
PCB153	0.400	0.497	1							
PCB138	0.361	0.481	**0.949**	1						
PCB156	0.270	0.251	**0.742**	**0.760**	1					
HCB	0.211	-0.019	0.053	0.186	0.277	1				
DDE	0.473	0.426	**0.715**	**0.770**	0.280	0.143	1			
4HO-CB146	0.370	0.086	**0.562**	0.430	**0.591**	-0.013	0.093	1		
4HO-CB187	0.269	0.349	0.410	0.249	0.334	-0.233	0.016	**0.555**	1	
PCP	-0.003	0.135	-0.048	0.043	0.085	0.319	-0.035	-0.177	0.138	1

Boldface > 0.525 : p <0.001, Lightface > 0.329 : p < 0.05, Underline < 0.329 : not significant.(p > 0.05)

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