HYDROXYLATED POLYCHLORINATED BIPHENYLS (OH-PCBs) IN THE BRAIN OF MARINE MAMMALS STRANDED ALONG THE JAPANESE COAST

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Introduction

Polychlorinated biphenyls (PCBs) are still detected in a wide range of wild animals; especially accumulation of PCBs in high-trophic species such as marine mammals remains pronounced [1]. In epidemiological and experimental studies, it is noted that PCBs disturb thyroid hormone (TH) homeostasis and cerebral nervous system in human and rodents [2, 3]. Although the competitive binding between PCBs and thyroxine (T4) to transthyretin (TTR) in blood could be included as a mechanism involved in disturbing TH homeostasis, it has been demonstrated that the binding affinity to TTR was much stronger for hydroxylated PCBs (OH-PCBs), which are formed by oxidative metabolism of PCBs by the cytochrome P450 monooxygenases, than the parent compounds [2, 4]. Hence, investigations on residue levels of OH-PCBs in human blood are increasing [5]. OH-PCBs have been also detected in blood of wildlife [6-8], but the observed residue levels and patterns vary by species, possibly because of species-specific metabolic capacity by phase I CYP and/or phase II conjugation enzymes and binding affinity to TTR.

The mechanism of disturbance on the cerebral nervous system by PCBs is not well clarified. Recently, in a study using reporter gene assays, it was shown that extremely low doses of OH-PCBs (10^{-10} M) suppressed TH-induced transcriptional activation of TH receptor (TR), implying that this mechanism may be involved in disturbance of cerebral nervous system by PCBs [9]. Furthermore, it was indicated that OH-PCBs might suppress TH/TR-mediated transcription directly through partial dissociation of TR/Retinoid X receptor (RXR) from the thyroid hormone-response element (TRE) [10]. Thus OH-PCBs may adversely affect gene expression in the brain at low level, but to our knowledge, only one study on residue levels of OH-PCBs in the brain is available in polar bears [8].

The present study attempted to elucidate the residue levels of OH-PCBs in the brain of marine mammals stranded along the Japanese coast.

Materials and Methods

The brain (cerebrum) samples were collected from melon-headed whale (*Peponocephala electra*) (*n* = 5) and striped dolphin (*Stenella coeruleoalba*) (*n* = 3) stranded along the Japanese coast during 2002-2003. Samples were stored in Environmental Specimen Bank (*es*-BANK) for Global Monitoring of Ehime University at –20 °C until analysis.

The brain sample was denatured with HCl and $^{13}C_{12}$-labeled 4'OH-P_5CB120, 4'OH-H_6CB159, 4'OH-H_7CB172, and 4OH-H_7CB187 were spiked as internal standards. 2-propanol was added, and then OH-PCBs were extracted thrice with 50% methyl *t*-butyl ether (MTBE)/hexane. After centrifugation, the organic phases were combined, evaporated and dissolved in hexane. 1 M KOH in 50 % ethanol/H_2O was added and shaken. The partition process was repeated and the alkaline phases were combined. The remaining organic phase was concentrated and lipid was removed by gel permeation chromatography, and then passed through activated silica-gel packed in a glass column. PCBs were eluted with hexane and concentrated for GC-MS analysis. The combined alkaline phase was acidified with sulfuric acid, and then OH-PCBs were extracted twice with 50% MTBE/hexane. The organic phases were combined, evaporated, and dissolved in hexane. After sulfuric acid treatment, the organic phase was concentrated, and OH-PCBs in the organic phase were then methylated by reaction with diazomethane. The derivatized solution was concentrated and passed through activated silica-gel packed in a glass column. CH_3O-PCBs were eluted with 10% dichloromethane/hexane and this fraction was concentrated nearly to dryness. $^{13}C_{12}$-labeled H_6CB157 were then added as syringe spikes. Identification and quantification were performed using a gas chromatograph (Agilent 6890 series) and a high-resolution mass spectrometer (JEOL JMS-800D) with a resolving power of more than 10000. CH_3O-PCBs were monitored by selective ion monitoring (SIM) mode at two most intensive ions ($[M+2]^+$ and $[M+4]^+$). Recoveries for the $^{13}C_{12}$-labeled OH-PCB isomers were within 50-80 %. Identification and quantification of PCB congeners were performed using the analytical condition reported previously [11].

Results and Discussion

OH-PCBs were detected in all the brain samples of melon-headed whales and striped dolphins analyzed and levels of OH-P_5CBs, -H_6CB, -H_7CBs, and O_8CBs including identified and unknown peaks were in the range of 20-290 and 21-330 pg/g wet wt., respectively (Table 1). Concentrations of PCBs in the brain of melon-headed whales and striped dolphins ranged from 7.4 to 160 ng/g wet wt. and from 30 to 620 ng/g wet wt., respectively. When OH-PCBs/PCBs ratios were examined, the values in melon-headed whale and striped dolphin cerebrums were relatively lower than those in blood of humans and wildlife reported previously [5-7] and in the brain of polar bear [8] (Fig. 1), indicating lower transport and residual properties of OH-PCBs into the brain compared with PCBs and/or poor metabolic capacity for PCBs in these odontocetes.

Among the identified OH-P_5CB, OH-H_6CB, and OH-H_7CB congeners, 4'OH-CB101/120, 4OH-CB107/4'OH-CB108, 4OH-CB146, and 4OH-CB187, 3'OH-CB182/183, and 4'OH-CB172

were predominant in melon-headed whale and striped dolphin cerebrums. Generally, these metabolites have been detected in blood of humans and wildlife reported previously [5-8]. However, unknown isomers were dominant among OH-P_5CBs and -H_6CBs in the brain of these odontocete species. These results imply that predominant OH-PCBs isomers in blood are not necessarily transported into the brain.

Table 1. Concentrations of OH-PCBs (pg/g wet wt.) and PCBs (ng/g wet wt.) in the brain of odontocetes stranded along Japanese coast

Species	Melon-headed whale (*Peponocephala electra*)					Striped dolphin (*Stenella coeruleoalba*)		
Sex	Male	Female	Female	Female	Female	Male	Male	Female
Body length (cm)	222	241	228	231	236	238	231	223
4'OH-CB101/120	4.7	5.8	15	19	<1.0	12	23	<1.0
3'OH-CB118	2.9	3.7	12	11	<1.0	5.6	10	<1.0
4OH-CB107/4'OH-CB108	2.3	6.4	12	11	<1.0	20	14	<1.0
Unknown OH-P_5CB [a]	50	45	64	120	16	140	150	13
Total OH-P_5CB	60	61	100	160	16	180	200	13
4OH-CB134	<1.0	<1.0	3.9	3.4	<1.0	<1.0	3.1	<1.0
4OH-CB146	<1.0	<1.0	8.4	9.3	<1.0	2.3	5.5	2.9
3'OH-CB138	<1.0	<1.0	5.0	4.9	<1.0	1.7	1.3	<1.0
4'OH-CB130	<1.0	<1.0	3.3	2.9	<1.0	<1.0	<1.0	<1.0
4'OH-CB159	<1.0	<1.0	5.8	7.5	<1.0	<1.0	1.0	<1.0
Unknown OH-H_6CB [b]	22	26	47	50	3.7	67	71	4.4
Total OH-H_6CB	22	26	74	78	3.7	71	82	7.3
3'OH-CB184	<1.0	<1.0	4.0	2.4	<1.0	<1.0	2.6	<1.0
4OH-CB178	<1.0	<1.0	4.7	3.4	<1.0	<1.0	3.1	<1.0
3'OH-CB182/183	<1.0	<1.0	9.9	6.6	<1.0	<1.0	5.7	<1.0
4OH-CB187	<1.0	3.8	9.3	7.8	<1.0	2.2	5.0	<1.0
4OH-CB177	<1.0	<1.0	4.6	3.3	<1.0	<1.0	2.3	<1.0
3'OH-CB180	<1.0	<1.0	5.0	4.1	<1.0	2.0	3.7	<1.0
4'OH-CB172	<1.0	3.5	7.8	7.5	<1.0	7.1	13	<1.0
Unknown OH-H_7CB [c]	<1.0	7.1	8.3	4.5	<1.0	<1.0	2.2	<1.0
Total OH-H_7CB	<1.0	14	54	40	<1.0	11	38	<1.0
4OH-CB202	<1.0	<1.0	2.5	2.0	<1.0	<1.0	2.1	<1.0
4'OH-CB201	<1.0	<1.0	2.3	2.0	<1.0	<1.0	1.7	<1.0
4'OH-CB198/3'OH-CB203	<1.0	<1.0	5.8	5.0	<1.0	<1.0	4.2	<1.0
4'OH-CB199	<1.0	<1.0	3.0	2.5	<1.0	<1.0	2.1	<1.0
Unknown OH-O_8CB [d]	<1.0	<1.0	2.4	1.9	<1.0	<1.0	1.9	<1.0
Total OH-O_8CB	<1.0	<1.0	16	13	<1.0	<1.0	12	<1.0
Total OH-PCBs	82	100	250	290	20	260	330	20
Total PCBs	48	110	93	160	7.4	490	620	30

[a] 7 (Melon-headed whale) and 6 (Striped dolphin) isomers were identified.
[b] 9 (Melon-headed whale) and 12 (Striped dolphin) isomers were identified.
[c] 2 (Melon-headed whale) and 1 (Striped dolphin) isomers were identified.
[d] 1 isomer were identified.

When the concentrations of OH-PCBs in melon-headed whale and striped dolphin cerebrums were compared with the 4'OH-CB106 level (0.1 nM) suppressed T3-induced transactivation by TR

in cell lines [9], total concentrations of OH-PCBs in the brain exceeded the suppressed value except in one sample. This implies that these odontocetes may be susceptible to neurotoxic effects by OH-PCBs .

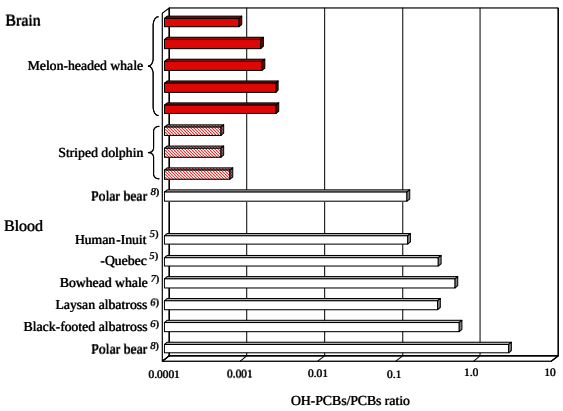

Fig. 1. OH-PCBs/PCBs ratios in the brain of melon-headed whales and striped dolphins and in blood and brain of human and wildlife reported elsewhere. *5-7*) References cited.

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