TRANSFER POTENCY AND ACCUMULATION FEATURES OF HALOGENATED PHENOLIC COMPOUNDS INTO THE BRAIN OF FINLESS PORPOISES (*NEOPHOCAENA PHOCAENOIDES*)

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Introduction

Cetaceans position high in a marine food chain, and they accumulate higher levels of organohalogen contaminants such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs)[1-2]. Following the intake of PCBs and PBDEs through their diet, these compounds can be metabolized by cytochrome P450 monooxygenase enzymes (CYPs)[3-4] to form hydroxylated metabolites, OH-PCBs and OH-PBDEs. In addition, recent studies have shown that OH-PBDEs are also synthesized by marine organisms such as marine sponges and algae[5-6], as well as from their methoxylated analogs, MeO-PBDEs, which are also formed by marine organisms[7]. Some of these halogenated phenolics have chemical structures resembling thyroid hormones (T_4: thyroxine and T_3: triiodothyronine), and competitively bind to thyroid hormone transport protein (TTR: transthyretin)[8-9]. OH-PCBs and OH-PBDEs potentially cross the blood-brain barrier (BBB) and accumulate in the brain [10]. These congeners potentially exert toxicities such as endocrine-disruption, developmental defects and neurotoxicity[10-12]. *In vitro* studies have shown that prenatal exposure to 4OH-CB107 led to locomotor abnormalities, auditory loss and learning and memory disorder in rats[12]. Moreover, it is reported by the study using reporter gene assay that low doses of OH-PCBs (10^{-10} M) suppressed T_3-induced transcriptional activation of thyroid hormone receptors[13]. Although numerous studies on OH-PCBs in humans and rodents have been conducted, information on these metabolites in cetaceans is limited.

Finless porpoises are the small cetaceans inhabiting the semi-closed coastal environment along Japan, and their population decline due to human impact is of concern[14]. They accumulate higher levels of PCBs relative to other cetacean species[15-16], and our previous study showed that the major OH-PCBs found in finless porpoise blood were *tri*- to *penta*-chlorinated congeners and had T_3 or T_4 like structures[17]. So far, two studies reported OH-PCBs concentrations in cetacean brains[18-19], but these studies were not targeting low chlorinated congeners and T_3 like congeners because of methodological limitation for the removal of lipid-rich matrices of brain tissues. Thus, in this study, the extraction and clean up method necessary to analyze wide ranges of congeners in the brain of cetaceans was developed. Further, the present study investigated the accumulation features of *tri*- to *octa*-chlorinated OH-PCBs and OH-PBDEs in the brain of finless porpoises (*Neophocaena phocaenoides*) stranded or bycaught along Japanese coastal waters. Additionally, the transfer potencies of these halogenated phenolics into the brain were determined by comparing previously reported levels and congener profiles in the blood of the same porpoise samples.

Materials and methods

Sample collection

Finless porpoise carcasses stranded or bycaught during 2005-2010 along the Japanese coast were transported and stored in freezers at -25ºC at local universities and aquariums until biometric measurements and dissection were conducted. The brain samples were collected from 13 porpoises (male: *n*=10, female: *n*=3), and samples were stored in the Environmental Specimen Bank (*es*-BANK) at Ehime University, Japan, until the analyses were carried out.

Extraction and analytical methods

The extraction and clean up methods for the analyses of *tri*- to *octa*-congeners of OH-PCBs and OH-PBDEs in the brain of cetaceans were modified based on the methods described in detail elsewhere for blood samples[16, 20]. Briefly, approximately 5.0 g of brain sample was spiked with internal standards and denatured with 6 M HCl. Samples were extracted by liquid:liquid partitioning of 50% methyl *t*-butyl ether (MTBE)/hexane, and the organic phase was partitioned into neutral fraction containing PCBs, PBDEs and MeO-PBDEs and phenolic fraction containing OH-PCBs and OH-PBDEs with 1M KOH and 50% ethanol/H_2O. The neutral fraction was treated with sulfuric acid, washed with hexane-washed water, and cleaned up with gel permeation chromatography (GPC) and activated silica-gel column. The neutral fraction was then concentrated, and $^{13}C_{12}$-labeled hexabrominated diphenyl ether (BDE139) was added as a syringe spike. The phenolic phase was acidified to pH 2 with sulfuric acid and re-extracted with MTBE/hexane to obtain OH-PCBs and OH-PBDEs. The extracted solution was further cleaned up with liquid:liquid partitioning of 50% acetonitrile/hexane and the acetonitrile fraction was acidified to pH 2 with sulfuric acid, followed by extraction with 50% (MTBE)/hexane. The extracted solution was passed though a non-activated silica-gel column (5% H_2O) derivatized with trimethylsilyldiazomethane (TMSDS). The derivatized solution was cleaned up with GPC and activated silica-gel column. $^{13}C_{12}$-labeled CB77 and CB157 were added as syringe spikes. A total of 62 PCBs (*mono*- to *deca*-), 52 OH-PCBs (*tri*- to *octa*-), 42 PBDEs (*mono*- to *deca*-) and 24 OH-/MeO-PBDEs (*tri*- to *octa*-) congeners were analyzed. OH-PCBs and OH-PBDEs were determined as MeO-PCBs or MeO-PBDEs using high-resolution GC/MS. The statistical analyses were performed using Mann-Whitney U test and Spearman's rank correlation coefficient.

Results and discussion

Transfer potencies of OH-PCBs into the brain

OH-PCBs were detected in all the brain samples. OH-PCBs tended to accumulate at higher levels in the brain than in blood, yet no statistically significant difference was observed in the levels between these tissues. The median OH-PCBs level in the brain was 33 pg/g wet wt, comparable with the level found in blood (31 pg/g wet wt) (Fig. 1). The brain OH-PCBs level of this study was comparable to the levels reported by Kunisue *et al* (2007) in the brain of striped-dolphins and melon-headed whales, and approximately one order of magnitude lower than the levels of some other brain samples of striped-dolphins, melon-headed whales and finless porpoises[18] and the cerebellum gray matter of Atlantic white-sided dolphins reported by Montie *et al* (2009). There was a positive correlation between the levels in blood and brain samples ($p < 0.01$, r

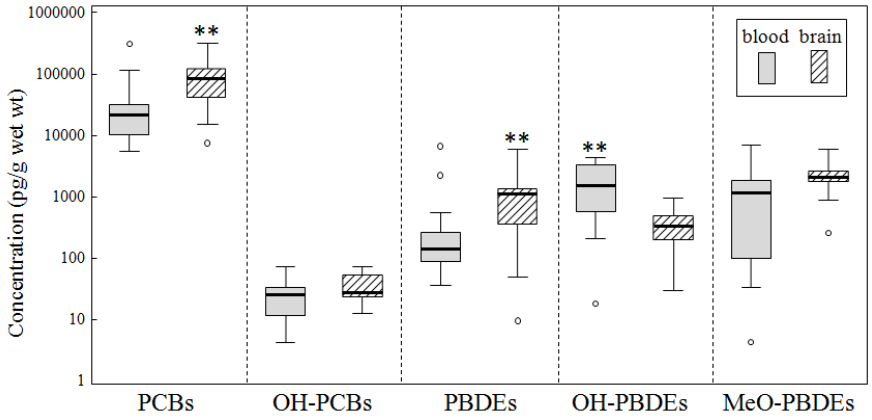

Fig. 1. Box-plot representing the concentrations of PCBs, OH-PCBs, PBDEs, OH-PBDEs and MeO-PBDEs in the blood and brain of finless porpoise (n = 13) stranded or bycaught along the Japanese coastal waters. The solid line in the middle of each plot marks the median, and the box shows 25th–75th percentiles. Error bars indicate 10th–90th percentiles. *: significantly greater than blood or brain level ($p < 0.01$).

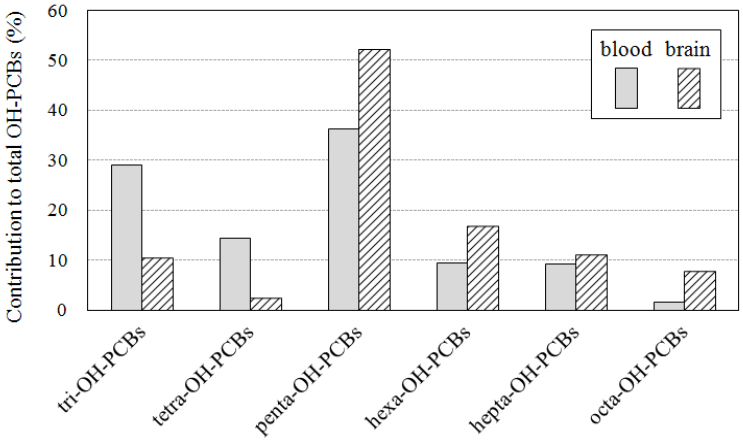

Fig. 2. OH-PCB homolog compositions in the blood and brain of finless porpoise (n = 13) stranded or bycaught along the Japanese coastal waters.

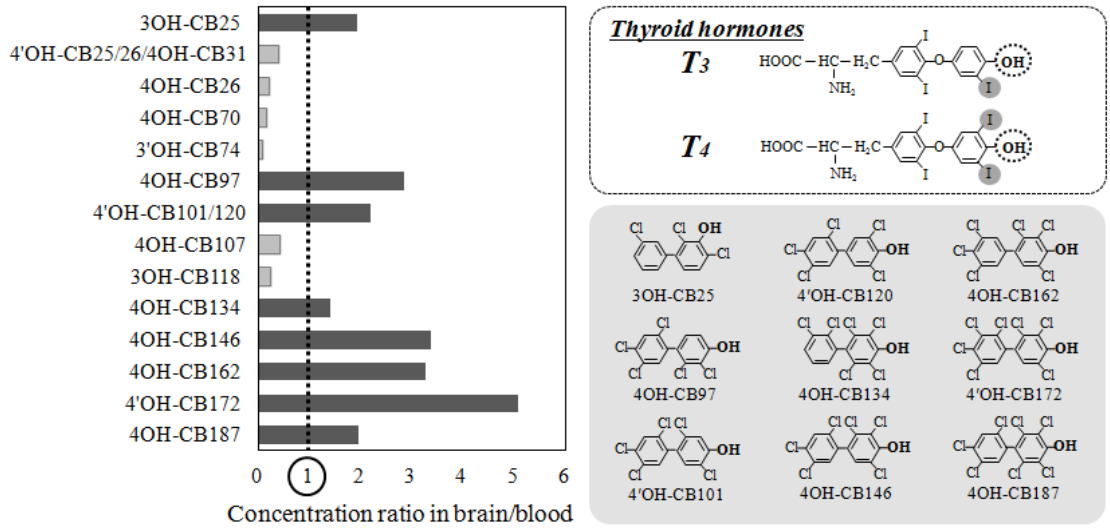

Fig. 3. Concentration ratios of OH-PCB congeners in brain/blood of finless porpoises (n = 13) stranded or bycaught along the Japanese coastal waters. Congeners with dark gray bars have the ratio greater than 1 and thus higher concentration in brain than in blood. Structures of thyroid hormones (T4, T3) and major OH-PCB congeners exceeding the concentration ratio of 1 are shown.

= 0.80), supporting that OH-PCBs cross BBB and transfer into the brain in a concentration dependent manner.

OH-PCBs congener profiles were different in the brain and blood samples, even though analogous compositions were observed for PCBs. In brain samples, *penta*-chlorinated congeners were dominant, accounting for 52% of the total concentration (Fig. 2). The rank order of contribution to sum of OH-PCBs was as follows: *penta-OH-PCBs* > *hexa-OH-PCBs* > *hepta-OH-PCBs* > *tri-OH-PCBs* > *octa-OH-PCBs* > *tetra-OH-PCBs*. In blood, on the other hand, *penta*-OH-PCBs comprised 36% of the total concentration, followed by *tri*- and *tetra*-chlorinated congeners (Fig. 2). From these results, specific transfer and accumulation of *penta*-chlorinated congeners in the brain was suspected. To further investigate congeners accumulating at higher concentration in the brain, the concentration ratios of congeners in the brain and blood were calculated. The congeners that have the ratio greater than 1 were 4OH-CB172, 4OH-CB146, 4OH-CB162, 4OH-CB97, 4′OH-CB101/120, 4OH-CB187, 3OH-CB25 and 4OH-CB134 (Fig. 3). All these congeners have thyroid hormone like structures with one or two Cl adjacent to *para*- or *meta*-substituted -OH. For these reasons, thyroid hormone disrupting effects of these congeners in the central nervous system is of concern.

Transfer potencies of OH-PBDEs into the brain

OH-PBDEs were also detected in all the samples, and the levels in brain samples were one order of magnitude higher than that of OH-PCBs (Fig. 1). However, contrary to OH-PCBs, the median OH-PBDEs level in the brain was significantly lower than in blood (p < 0.01) (brain: 350 pg/g wet wt, blood: 1,700 pg/g wet wt) (Fig. 1). One reason that might explain this phenomenon is the differences in binding affinities to TTR. The major constituent of OH-PBDEs, 6OH-BDE47, is reported to have a relatively low binding affinity with TTR[21], and this may account for the lower transfer potency of OH-PBDEs into the brain compared with OH-PCBs. Indeed, the proportion of 6OH-BDE47 in brain was lower than that in blood: 99% in blood and 95% in the brain. It might also be possible that the disposition of OH-PBDEs differs from OH-PCBs since OH-PBDEs have occurred in the marine environment since ancient times and cetaceans have been exposed to these compounds over time.

Transfer potencies of PCBs, PBDEs and MeO-PBDEs into the brain

Lipophilic compounds such as PCBs and PBDEs were accumulated at significantly higher levels in the brain than in blood (p < 0.01) (Fig. 1). Additionally, positive correlations were found between the levels in blood and

brain for PCBs and PBDEs ($p < 0.05$). From these results, it is suggested that the entry of PCBs and PBDEs into the brain is governed by the exposure levels to a certain extent.
As for MeO-PBDEs, the median level in brain sample was found to be comparable with that in blood (Fig. 1), and there was no correlation between the levels in the brain and blood. Although the blood MeO-PBDEs levels had the large ranges, the variations in the brain levels were notablly small. It might be possible that the entry of MeO-PBDEs into the brain is limited by some feedback systems such a threshold for these compounds, but at this point, no reasonable explanation is available.

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