Tissue Distribution of polychlorinated biphenyls and their metabolites in dog and cat

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Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants and have been detected in the environment and humans and wildlife. PCBs are known to elicit an array of toxic effects including thyroid hormone (TH) homeostasis, immune dysfunction and reproductive toxicity. Recent studies have shown that toxic effects are induced by their metabolites such as hydroxylated PCBs (OH-PCBs) and methylsulfonyl-PCBs ($MeSO_2$-PCBs) as well as PCBs. OH-PCBs are produced through oxidative metabolism of PCBs by cytochrome P450 monooxygenases (CYPs)(phase I metabolism) in the liver. *In vitro* studies reported that some OH-PCB congeners have neurotoxicities including inhibition of TH-dependent extension of Purkinje cell dendrites [1,2] and suppression of T_3-induced transcription of TTR [3]. The occurrence of OH-PCBs in the brain of animals such as marine mammals and birds [4–6] raise concerns over their potential neurotoxicological effects. However, in our knowledge, the residual levels of PCBs and their metabolites in the brain of terrestrial mammals have not been investigated. $MeSO_2$-PCBs are produced after glutathione conjugation of PCB metabolite intermediate [7]. Although it is important for understanding of PCB metabolic capacity and elimination rate to investigate $MeSO_2$-PCB accumulation features, there are few studies on $MeSO_2$-PCB accumulation features in terrestrial mammals.

Previous studies suggested that terrestrial mammals, especially carnivores, have higher PCB metabolic capacity than marine mammals [8,9]. In addition, it is known that cat has specific metabolism for PCBs compared with caniformia such as dog, fox and raccoon [8]. However, there are few studies on the occurrence of PCBs and their metabolites in liver and bile terrestrial mammals. The present study elucidated the accumulation features of PCBs and their metabolites such as OH-PCBs and $MeSO_2$-PCBs in brain, liver and bile samples of dogs and cats.

Materials and methods

The animal specimens collected in this study were cats (*Felis silvestris catus*, 5 males, 5 females) killed in traffic accidents in Ehime, Japan during 2008 - 2011 and stray dogs (*Canis lupus familiaris*, 6 males, 4 females) euthanized as part of mammalian pest control programs in Osaka, Japan in 2009. The brain and liver samples were obtained after dissection.

The extraction and clean-up methods for PCBs and OH-PCBs in liver, bile and brain samples have been described elsewhere [10]. $MeSO_2$-PCBs were analyzed in liver and bile samples using the method by Gebbink et al. (2008) with several modifications. Identification and quantification of PCBs and OH-PCBs were performed using GC (Agilent 6890)/high-resolution MS (JEOL JMS-800D, Japan) in electron ionization mode, and $MeSO_2$-PCBs were quantified with external standards using GC (SHIMADZU GC 2010 Ultra)/MS (SHIMADZU GCMS-QP2010 Ultra) operating in electron capture negative ionization mode.

Results and discussion

PCBs were detected in all samples analyzed in this study. Median ΣPCBs concentrations in the brain, liver and bile of dogs were 220 pg/g wet wt. (range 35-2,700 pg/g wet wt.), 1,000 (range 83 - 9,900 pg/g wet wt.) and 800 (range 140 - 1,400 pg/g wet wt.), respectively (Fig.1). The concentrations in the brain, liver and bile of cats were 3,500 pg/g wet wt. (range 810 - 140,000 pg/g wet wt.), 4,600 (range 1,200 - 94,000 pg/g wet wt.) and 37,000 (range 740 - 74,000 pg/g wet wt.), respectively (Fig.1). ΣPCB concentrations in cat livers and brains were significantly higher than in dogs ($p < 0.05$), consistent with the finding of previous studies on PCBs in other tissues of dogs and cats [8, 11]. These results can be explained by not only higher PCB metabolic capacity in dogs, but also higher PCB concentrations in cat food than in dog food in Japan [12, 13]. The compositions of PCB congeners in dogs and cats tissues were different. Hexa-chlorinated PCBs were predominant in the brain, liver and bile of dogs. The dominant congeners were CB180, CB206 and CB170. PCB accumulation profiles in various cat tissues were also similar, with hexa- and hepta-chlorinated PCBs as predominant homologues, CB153, CB138 and CB187 as predominant congener. Congener profiles of PCBs in the tissue samples in this study were similar to those reported for blood of cats and dogs [8].

OH-PCBs were detected all of the samples in this study. Median ΣOH-PCBs concentrations in the brain, liver and bile of dogs were 20 pg/g wet wt. (range 6.0-120 pg/g wet wt.), 37 (range 20 - 290 pg/g wet wt.) and 680 (range 390 - 710 pg/g wet wt.), respectively (Fig.1). For cats, the concentrations were 35 pg/g wet wt. (range 7.9 - 450 pg/g wet wt.), 210 (range 34 - 3,000 pg/g wet wt.) and 730 (range 130 - 1,300 pg/g wet wt.) for brain, liver and bile, respectively (Fig.1). ΣOH-PCBs concentrations in the brain and liver of cats were higher than in dogs. On the other hand, levels of OH-PCBs in the bile of dogs and cats were comparable. However, their homologue profiles of OH-PCB congeners in dogs and cats were different. Higher-chlorinated OH-PCBs dominated in dog tissues, with some tissue-specific differences. In dog brain and liver, hexa- and octa-chlorinated OH-PCBs were predominant, and 4-OH-CB199, 4-OH-CB193, 4-OH-CB202 were the dominant congeners. Penta- and hepta-chlorinated OH-PCBs were predominant in the bile of dogs, and the dominant congeners were 3-OH-CB180, 4-OH-CB108/107 and 3-OH-CB138. On the other hand, the homologue profiles of OH-PCBs in all cat tissues were dominated by lower-chlorinated (tri- to penta-) OH-PCBs. However, the dominant congeners were different for each tissue types, the major OH-PCB congeners identified in brain of cats were 4-OH-CB101/120 and 4-OH-CB107/108. The molecular structures of the OH-PCBs are similar to T4 and T3 where the OH group is adjacent to a chlorine atom, suggesting that these congeners bind to transthyretin in the blood and might be transported to the brain. 4'-OH-CB72 and 4-OH-CB107/108 were predominant in cat liver. In the cat bile, 4-OH-CB101/120 and 4'-OH-CB25/26/4-OH-CB31 were dominant congeners. Moreover, higher-chlorinated OH-PCB congeners such as 5OH-CB183 and 4OH-CB172 were detected in the bile of cats, but not detected in the brain and liver. These results implied that cats preferentially excreted these higher-chlorinated OH-PCBs as hydroxylated metabolites.

$MeSO_2$-PCBs were detected in six dog and two cat liver samples in this study. In the bile samples, $MeSO_2$-PCBs were below limit of quantitation except for the cat bile pool sample. In contrast to the case of PCBs and OH-PCBs, the concentrations of $MeSO_2$-PCBs in liver samples of cat (<MDL - 46 pg/g wet wt.) and dogs (<MDL - 1300 pg/g wet wt.) were not significantly different (Fig.1). 4-$MeSO_2$-CB132 accounted for 56 - 100 % of the total $MeSO_2$-PCBs in dog livers. $MeSO_2$-PCBs congeners detected in cat livers were not detected in dog livers such as 4$MeSO_2$-CB91, 4$MeSO_2$-CB101 and 4$MeSO_2$-CB49. These results indicate that cats may have different conjugation capacity of phase II compared with dogs.

The present study evaluated potential toxicity of OH-PCBs toward the brain by comparing the concentrations of OH-PCBs in brain of dogs and cats to affected mouse neuron cells *in vitro* (Fig. 2). The ΣOH-PCB concentrations in the brain of five dogs and seven cats were higher than the OH-PCB levels that affected development of mouse Purkinje cells in vitro [1]. In two dogs and five cats, the levels of ΣOH-PCBs in brain exceeded the concentration reported to cause inhibition of T3-mediated gene expression in mouse neuron cells [3]. Moreover, ΣOH-PCB concentrations in one cat exceeded the levels that inhibited neuronal cell development [2]. The concentrations of T4-like OH-PCBs showed similar trend to ΣOH-PCBs levels, and a part of T4-like OH-PCB concentrations in brain of dogs and cats were above levels affected to neurons. In contrast to ΣOH-PCBs and T4-like OH-PCBs, T3-like OH-PCB levels in five cat brains were higher than affected OH-PCB levels, and those in dog brain were less than these value. Therefore, these compounds may negatively affect the brain of dogs and cats, especially, cats have the potential to be affected.

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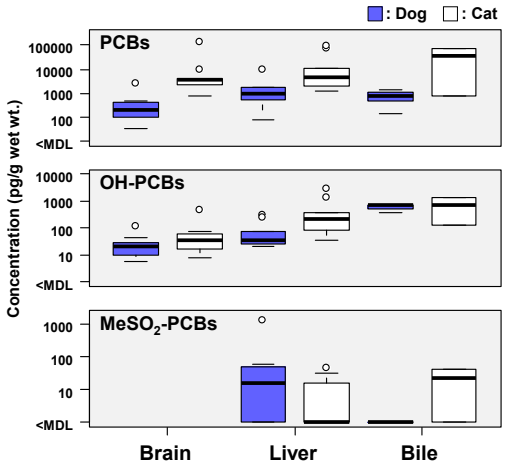

Fig. 1. Concentrations of PCBs and their metabolites in brain, liver and bile of dogs and cats.

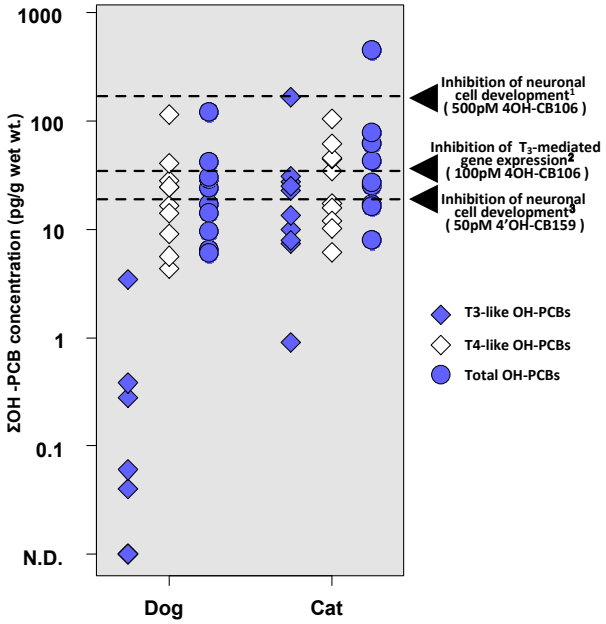

Fig. 2. Comparing the levels of OH-PCBs detected in brain of dogs and cats with concentrations that affected mouse neuron cells *in vitro*.