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学 位 論 文 要 旨
Dissertation Summary

氏　　名 (Name)　Jean Yoo

論　文　名: **Assessment of the metabolic potential of polychlorinated biphenyls in the Caniformia, the Baikal seal and the beagle dog**
(Dissertation Title)

Polychlorinated biphenyl (PCB) congeners elicit a broad spectrum of biochemical and toxic responses in humans and laboratory animals including hepatic enzyme induction, acneform eruption, carcinogenicity, immunosuppression, and endocrine disruption. The high accumulation profile of the PCBs has been reported in the liver of carnivore mammals, but the mechanism of the PCB metabolism in such animals is not yet fully understood.

Cytochrome P450 monooxygenases (CYPs) form a superfamily of heme-containing isoenzymes that play a central role in the disposition of a wide variety of endogenous and exogenous compounds in Phase I reactions. Members of CYP1 and 2 families are involved in drug/xenobiotic metabolism and are preferentially expressed in the liver. It has been reported that for the Phase I metabolism of PCBs, isoenzymes of the CYP1 and 2 families are responsible for the insertion of OH group into the biphenyl ring of PCB congener. It is also known that CYP2 genes are highly diverse and exhibit broad substrate specificities among species. Knowledge on the molecular characterization of CYP2 in a variety of animals is needed for our understanding of the evolution and functional divergence of CYP2. However, the catalytic functions of these CYP genes in carnivore mammals have not yet been characterized.

Hydroxylated PCBs (OH-PCBs) are formed by oxidative metabolism of parental PCBs by CYPs mostly in the liver of animals. Due to the structural similarity of OH-PCBs with L-thyroxin (T4), toxicities of OH-PCBs include disturbances of thyroid hormone homeostasis and of resulting cerebral nervous system. OH-PCBs have been detected in the blood and liver of wildlife, but the levels and congener profiles are different among species, due to the species difference in the metabolic potency of PCBs by CYPs.

The aim of this study is to understand how PCBs are metabolized in the Caniformia, a suborder of the order Carnivora. As the representatives, this study focuses on the Baikal seal and the beagle dog. To provide more information on the metabolic potency of PCBs by CYPs in the liver of Baikal seals and dogs, *in vitro* metabolic assay by incubating the liver microsome with 62 PCB congeners and *in silico* docking analyses of these PCBs and CYPs were carried out.

The heterologous expression of the Baikal seal CYP 2B and 2C were performed in the yeast. The spectral analyses of 450 nm showed that the protein expression levels of CYP2B and 2C were 59.6 pmol/mg protein and 176 pmol/mg protein, respectively. Expression of these CYPs was further confirmed by western blot analyses. This suggests that the CYP2B and CYP2C are not pesudogenes, but protein-coding genes in the Baikal seal.

In vitro metabolism of 62 PCB congener mixtures was investigated by incubating them with liver microsomes of the Baikal seal. The *in vitro* metabolism assay revealed that the decreased ratios for CB3, CB4, PCB8, CB15, CB19, CB22, CB37, CB54, CB77, and CB105 were over 20%, suggesting preferential metabolism of low chlorinated PCBs by hepatic CYPs. Instead, formation of hydroxylated (OH-) PCBs was observed. 4OH-CB79 was detected at the highest amount among identified OH-PCB congeners, followed by 4′ OH-CB25/4OH-CB31 and 4′ OH-CB20. Some possible metabolic pathways were predicted; CB18 to 4′ OH-CB18, CB22 to 4′ OH-CB20, CB28 to 3′ OH-CB28 and 4′ OH-CB25/4OHCB31, CB70 to 4′ OH-CB72, and CB77 to 4OH-CB79. Total amount of OH-PCBs detected as identified and unidentified congeners accounted only for 3.75% of decreased PCBs, indicating that there are large unknown metabolic pathways of PCBs.

We then constructed CYP2A, 2B, and 2C *in silico* homology models, and carried out the docking simulation of these PCB congeners with CYP2s together with CYP1A1, 1A2 and 1B1 homology models prepared in our earlier study. The estimated distance from the Cl-unsubstituted carbon atom of docked PCB congeners to heme Fe in CYP proteins implied that Baikal seal CYP2A and 2B are involved in most of PCB metabolism and CYP1A2 and 1B1 are selective for some congeners. For CYP1A1 and CYP2C, the Cl-unsubstituted carbon of all PCB congeners failed to be positioned within 5Å from proximal to heme Fe due to steric hinderance of amino acid residues proximal to the heme. To explore factors involved in the CYP-dependent PCB metabolism, the relationships of structural properties and *in silico* docking parameters of PCBs with their decreased ratios in the *in vitro* metabolism assay were examined by principal component analysis (PCA). The statistical analysis showed that PCB decreased ratio was correlated with the number of substituted chlorines, hydrophobicity, and molecule bending of PCBs, and the distance from Cl-unsubstituted carbon at *para* position of docked PCBs to the heme Fe in CYP2B protein. Collectively, congener-specific metabolism of PCBs may be determined by the interaction with CYP2B in the liver of Baikal seals, although their metabolic pathways remain largely unknown.

The liver microsome protein concentration and CYP contents were increased when the dogs were treated with 12 PCB congeners and also with PB and 12 PCBs. In the PB+PCB groups all the AROD (MROD, EROD, PROD, and BROD) activities were increased compared to the control group, but in the PCB treated group increase was only observed for MROD, EROD, and PROD. Furthermore, by

the western blot results, we confirmed that CYP1A1, and 1A2 proteins were induced by PCB treatment (group 2 and 3), whereas CYP2B proteins was induced only in the group of PB+PCB treatment (group 3). In vivo metabolic assay performed by incubating 62 PCB congener mixtures with dog liver microsomes. By the PCB decreased ratio and the metabolic formation (OH-PCBs) we found that the metabolic potential of the 62 PCB congeners was increased in the in vivo PCB treated dogs, and more enhanced in the PB+PCB treated dogs. One clear metabolic pathway was the formation of 4'OH-CB79 from the parent compound of CB77. Also the present study demonstrated that the dog has a higher metabolic potency than the Baikal seal.