Hepatic CYP1A Involved in Metabolism and Sequestration of PCDD, PCDF and Coplanar PCB Congeners in Common Cormorants

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Introduction

The planar halogenated aromatic hydrocarbons (PHAHs) including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) have been of great concern because of their worldwide distribution, high persistency and lipophilic nature. Such chemical properties allow these chemicals to facilitate accumulation through food web in higher trophic organisms. Previous reports indicated the impairments of reproductive performance and developmental deformities related to high accumulation of these congeners in avian species [1].

Toxicokinetic behavior of each dioxin-like congener is known to be dependent on factors including sex, life stage, tissue and metabolic capacity of organisms. In addition, dose/concentration, exposure route, absorption efficiency of certain chemicals may also be crucial factors, as reported in several laboratory studies [2]. Wildlife is chronically exposed to complex mixtures of dioxin-like compounds via the gastrointestinal tract, whereas laboratory animals, in most cases, are administered with single or repeated dose of a defined congener through various routes for a short period. The validity of such experimental approach for their toxicokinetics is completely unproven, and many questions still remain to be resolved.

Exposure to dioxin-like compounds activates the aryl hydrocarbon receptor (AHR) and regulates the transcription of cytochrome P450 (CYP) 1A and other target genes. Altered expression of CYP1A is linked with production of reactive oxygen species and metabolic activation of PHAHs [3]. Therefore, measurement of CYP expression levels is considered as a useful approach to assess the environmental exposure to dioxin-like compounds and their effects.

Common cormorants (*Phalacrocorax carbo*) contained considerable amount of persistent organochlorines such as dioxin-like compounds, PCBs and DDTs [4,5]. Our recent study verified contamination status of PCDD/DFs and Co-PCBs and immunochemically detected CYP1A-like protein in hepatic microsomal fraction using an anti-rat CYP1A1 polyclonal antibody [6]. However, no comprehensive data is available on whether CYP protein expressions are influenced by PCDD/DFs and Co-PCBs, and are involved in their toxicokinetics. This study therefore investigates the effects of PCDD/DFs and Co-PCBs on CYP protein expressions in Lake Biwa populations of common cormorants. The role of CYP proteins related to congener profiles of residue concentration and tissue distribution will also be discussed.

Methods and Materials

Chemical analysis: Data on the concentrations and toxic equivalents (TEQs) of PCDD/DF and Co-PCB congeners in twenty-six livers of common cormorants from the Lake Biwa reported elsewhere [6] were used in this study. To understand tissue distribution of these chemicals, 20 pectoral muscles, selected from the above 26 specimens, were also analyzed. Chemical analysis of the congeners was carried out using the standard method described by Environmental Agency of Japan, with some modifications [6].

Enzyme assay: Information on preparation of hepatic microsomal fractions and their protein concentrations were described elsewhere [6]. Detection and quantification of CYP1A-, CYP2B-, CYP2C- and CYP3A-like proteins in the liver microsomes were conducted by western blot analyses using polyclonal antibodies against rat CYP1A1, CYP2B1, CYP2C6 and CYP3A2, respectively.

Statistical treatment: To evaluate the relationships between TEQs of individual PHAH congeners, CYP staining intensities and liver/muscle concentration ratios, Spearman's rank correlation test was performed using StatView ver. 5.0. These measurements were logarithmically transformed before the statistical analyses. For values below quantification limit, half of the values was substituted. When more than 50% of the observations were below the quantification limit, statistical analyses were not conducted for the congener, and results were shown as " no data available (NA) ". *P* value of <0.05 was regarded as significant.

Results and Discussion

Induction of hepatic CYP1A by dioxins exposure: Cross-reactive proteins with anti-rat CYP1A1, CYP2B1, CYP2C6 and CYP3A2 were detected in all the hepatic microsomes of common cormorants. Correlation analyses were conducted in order to investigate whether the expression levels of hepatic CYP proteins were altered by exposure to PHAHs. The relationships between total TEQs and CYP protein levels showed that CYP1A-like protein significantly (p < 0.0001) increased with an increase in hepatic total TEQs, suggesting the induction of CYP1A by TEQs (Fig. 1). Since none of expression levels of CYP2B-, CYP2C- and CYP3A-like proteins was correlated with TEQs (data not shown), CYP1A was found to be specifically enhanced by PCDD/DFs and Co-PCBs.

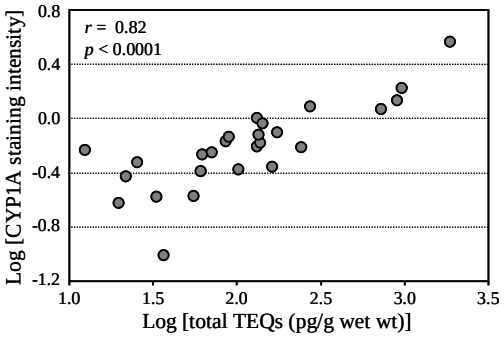

Fig. 1. Relationships between total TEQs and CYP1A staining intensity in the liver of common cormorants.

To verify the role of CYP1A related to accumulation and metabolism of each congener, relationships between TEQs of individual congeners and CYP1A-like protein levels were examined. TEQs from most congeners including PCB126 and 2,3,4,7,8-

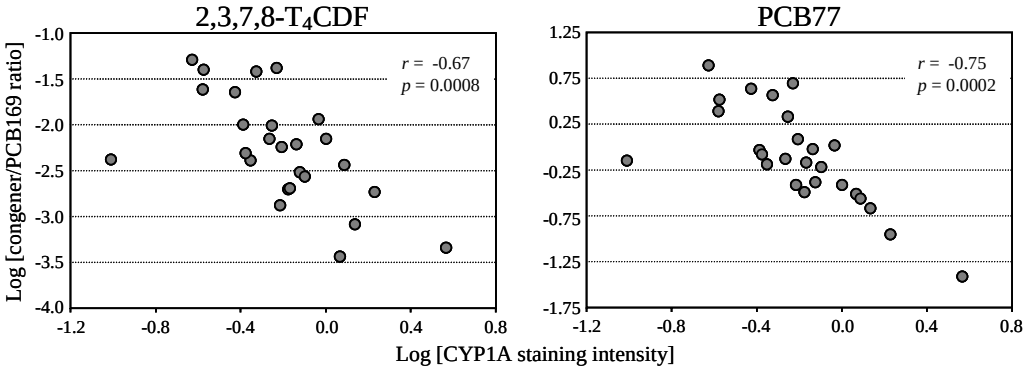

Fig. 2. Relationships between CYP1A staining intensities and concentration ratios (congeners/ PCB169) in the liver of common cormorants.

P_5CDF showed significant ($p < 0.0001$) positive correlations with CYP1A, whereas O_8CDD ($p = 0.024$) and lower chlorinated congeners, 2,3,7,8-T_4CDF ($p > 0.05$) and PCB77 ($p = 0.018$), exhibited relatively low correlations. Result from O_8CDD may reflect inefficient absorption from the gastrointestinal tract of this congener, supporting a study carried out using rat treated with this congener [7]. Regarding the lower levels of correlations of 2,3,7,8-T_4CDF and PCB77, preferential metabolism of these congeners by CYP1A induction may be speculated. To confirm this hypothesis, concentrations of 2,3,7,8-T_4CDF and PCB77 were normalized to a relatively recalcitrant congener, PCB169, and the relationships between CYP1A protein levels and the congener ratios were examined. Spearman's rank correlations revealed that CYP1A-like protein levels were negatively correlated with 2,3,7,8-T_4CDF/PCB169 ($p = 0.0008$) and PCB77/PCB169 ($p = 0.0002$) ratios (Fig. 2), whereas in the case of PCB126 ($p > 0.05$), the main contributor to total TEQs [6], no correlation was shown. These results indicate that 2,3,7,8-T_4CDF and PCB77 were preferentially metabolized by CYP1A induced by TEQs.

Hepatic sequestration of certain dioxin-like congeners: The tissue distribution of PHAHs has been extensively investigated by acute or subchronic studies using experimental animals, but still remains to be clarified in wildlife, which is chronically exposed to PHAHs. Tissue distribution following chronic exposure to environmental pollutants in wildlife could be in steady-state. Assuming the steady-state tissue distribution of PHAHs in common cormorants, concentration ratios of liver to pectoral muscle on a lipid weight basis were examined for individual congeners (Table 1). For most PCDD/DFs (except 2,3,7,8-T_4CDD, 1,2,3,7,8-P_5CDF, 1,2,3,7,8,9-H_6CDF, 1,2,3,4,7,8,9-H_7CDF and O_8CDF), liver/muscle ratios were greater than 1.0 in all specimens. O_8CDD had the highest liver/muscle ratios, reaching a maximum value of 30. Liver/muscle ratios for PCB77 and all mono-*ortho* Co-PCBs were less than or nearly equal to 1.0 in all specimens.

The liver/muscle concentration ratios varied among specimens for most congeners (Table 1). For example, the ratio for O_8CDD fluctuated between 2.0 and 30. As the variance of the liver/muscle ratios may be concentration-dependent, relationships between total TEQs in the liver and the liver/muscle ratios were analyzed for individual congeners using Spearman's rank correlation test (Table 2). Results showed that the liver/muscle ratios significantly increased with an increase in total TEQs for most PCDD/DFs, except congeners for which more than 50% of the observations were below the quantification limit, and for PCB81, PCB126, PCB169, PCB114, PCB118, PCB157, PCB167 and PCB189, suggesting their concentration-dependent hepatic sequestration in common cormorants.

Several studies have suggested that dose-dependent increase in their hepatic sequestration could be caused by hepatic microsomal binding protein, including CYP1A2 [8], which appeared to have specific binding affinity for certain 2,3,7,8-substituted PCDD/DF and Co-PCB congeners [9,10]. Further, to provide a plausible explanation on the concentration-dependent hepatic sequestration, relationships between CYP1A-like protein levels and liver/muscle ratios of individual congeners were analyzed. For all

Table 1 Liver to pectoral muscle concentration ratios of PCDDs, PCDFs and Co-PCBs in common cormorants from the Lake Biwa, Japan

PCDD/DFs	n [a]	Concentration ratio	Co-PCBs	n [a]	Concentration ratio
2,3,7,8-T_4CDD	20	0.79–2.1	PCB77	20	0.43–0.91
1,2,3,7,8-P_5CDD	20	1.2–4.7	PCB81	20	0.49–1.4
1,2,3,4,7,8-H_6CDD	19	1.9–8.3	PCB126	20	0.58–2.4
1,2,3,6,7,8-H_6CDD	20	1.3–11	PCB169	20	0.68–3.9
1,2,3,7,8,9-H_6CDD	18	1.2–6.0	PCB105	20	0.47–0.98
1,2,3,4,6,7,8-H_7CDD	19	2.0–16	PCB114	20	0.38–0.92
1,2,3,4,6,7,8,9-O_8CDD	20	2.0–30	PCB118	20	0.41–0.95
2,3,7,8-T_4CDF	18	1.1–13	PCB123	20	0.34–1.1
1,2,3,7,8-P_5CDF	7	0.95–2.0	PCB156	20	0.45–1.0
2,3,4,7,8-P_5CDF	20	2.5–14	PCB157	20	0.43–1.0
1,2,3,4,7,8-H_6CDF	19	1.9–10	PCB167	20	0.45–1.1
1,2,3,6,7,8-H_6CDF	19	1.3–8.0	PCB189	20	0.41–1.0
1,2,3,7,8,9-H_6CDF	4	0.57–2.0			
2,3,4,6,7,8-H_6CDF	19	1.4–6.5			
1,2,3,4,6,7,8-H_7CDF	17	1.6–12			
1,2,3,4,7,8,9-H_7CDF	3	0.23–4.5			
1,2,3,4,6,7,8,9-O_8CDF	4	0.25–1.3			

[a] The number of specimens in which the particular congener was detected both in the liver and pectoral muscle.

Table 2 Spearman's rank correlations between hepatic total TEQs or CYP1A staining intensity and liver to pectoral muscle concentration ratios of PCDDs, PCDFs and Co-PCBs in common cormorants from the Lake Biwa, Japan[a]

PCDD/DFs	n [b]	ΣTEQs	CYP1A	Co-PCBs	n [b]	ΣTEQs	CYP1A
2,3,7,8-T_4CDD	20	0.71^{**}	0.63^{**}	PCB77	20	0.018	-0.072
1,2,3,7,8-P_5CDD	20	0.62^{**}	0.51^{*}	PCB81	20	0.74^{**}	0.68^{**}
1,2,3,4,7,8-H_6CDD	19	0.57^{*}	0.49^{*}	PCB126	20	0.73^{**}	0.67^{**}
1,2,3,6,7,8-H_6CDD	20	0.73^{**}	0.70^{**}	PCB169	20	0.76^{***}	0.74^{**}
1,2,3,7,8,9-H_6CDD	18	0.73^{**}	0.52^{*}	PCB105	20	0.43	0.16
1,2,3,4,6,7,8-H_7CDD	19	0.64^{**}	0.54^{*}	PCB114	20	0.53^{*}	0.49^{*}
1,2,3,4,6,7,8,9-O_8CDD	20	0.76^{***}	0.74^{**}	PCB118	20	0.63^{**}	0.41
2,3,7,8-T_4CDF	18	0.85^{***}	0.73^{**}	PCB123	20	0.40	0.47^{*}
1,2,3,7,8-P_5CDF	7	NA	NA	PCB156	20	0.40	0.34
2,3,4,7,8-P_5CDF	20	0.59^{*}	0.49^{*}	PCB157	20	0.57^{*}	0.41
1,2,3,4,7,8-H_6CDF	19	0.68^{**}	0.53^{*}	PCB167	20	0.61^{**}	0.39
1,2,3,6,7,8-H_6CDF	19	0.75^{**}	0.56^{*}	PCB189	20	0.45^{*}	0.39
1,2,3,7,8,9-H_6CDF	4	NA	NA				
2,3,4,6,7,8-H_6CDF	19	0.40	0.25				
1,2,3,4,6,7,8-H_7CDF	17	0.50^{*}	0.38				
1,2,3,4,7,8,9-H_7CDF	3	NA	NA				
1,2,3,4,6,7,8,9-O_8CDF	4	NA	NA				

NA denotes no data available because more than 50% of the observations were below quantification limit. [a] Rho values (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). [b] The number of specimens in which the particular congener was detected both in the liver and pectoral muscle.

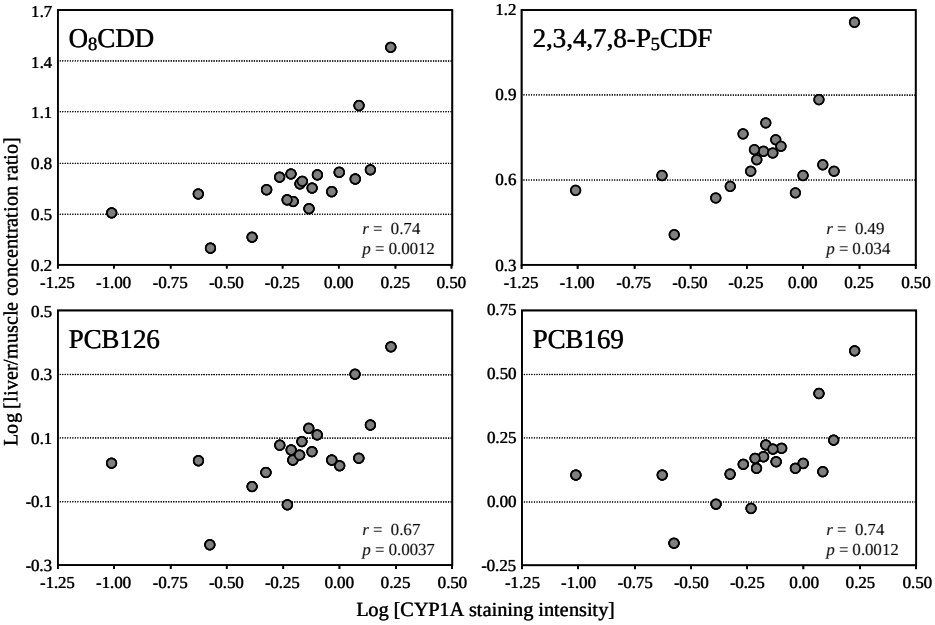

Fig. 3. Relationships between CYP1A staining intensities and liver/muscle concentration ratios in common cormorants.

2,3,7,8-substituted PCDDs, 2,3,7,8-T_4CDF, 2,3,4,7,8-P_5CDF, 1,2,3,4,7,8-H_6CDF, 1,2,3,6,7,8-H_6CDF, PCB81, PCB126, PCB169, PCB114 and PCB123, the liver/muscle ratios significantly increased with an increase in CYP1A levels (Table 2, Fig. 3), suggesting CYP1A-dependent hepatic sequestration of these congeners. Results from correlation analyses between CYP1A protein levels and liver/muscle ratios were roughly consistent with those of correlations between hepatic total TEQs and liver/muscle ratios except for mono-*ortho* Co-PCBs. No significant correlation between total TEQs or expression levels of CYP2B-, CYP2C- and CYP3A-like proteins and the liver/muscle ratios for any congener was observed. Therefore, certain PHAH congeners might be sequestered in the liver of wild cormorant population in a concentration-dependent manner through binding to induced CYP1A in accordance with results from studies using some experimental animals [2,8]. Our results exhibit that the profile of liver retention (liver/muscle concentration ratio) of congeners is different from AHR binding affinity in rat [11]. This indicates that AHR is unlikely to be a binding protein of PHAHs, but CYP1A may be involved.

Conclusions

This study demonstrates the induction of hepatic CYP1A protein by exposure to dioxin-like compounds in wild population of common cormorants, leading to the congener-specific hepatic sequestration and metabolism in CYP-dependent manner.

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