INDUCTION OF HEPATIC CYTOCHROME P450 ENZYME ACTIVITY BY COPLANAR PCBS IN BAIKAL SEAL (*Phoca sibirica*)

Yuka Okajima[1], Hisato Iwata[1], Shinsuke Tanabe[1], Masao Amano[2] and Nobuyuki Miyazaki[2]

[1]Center for Marine Environmental Studies, Ehime University, Tarumi 3-5-7, Matsuyama 790-8566, Japan
[2]Otsuchi Marine Research Center, Ocean Research Institute, The University of Tokyo, Akahama, Otsuchi-cho, Iwate 028-1102, Japan

Introduction

Planar halogenated aromatic hydrocarbons (PHAHs) such as polychlorinated dibenzo-*p*-dioxins and coplanar polychlorinated biphenyls (Co-PCBs) are ubiquitous contaminants.[1] Being biomagnified in the food web, PHAHs are notably accumulated in a variety of aquatic mammals.[2] Among the PHAHs, non- and mono-*ortho* Co- PCB congeners are particularly of great concern due to their higher residue levels than the other PHAHs, in spite of their relatively low potential of 2,3,7,8-TCDD toxic equivalency factors.

The induction of cytochrome P450 (CYP) enzymes is a responsive mechanism against exposure to xenochemicals. Exposure to PHAHs activates aryl hydrocarbon receptor (AhR), and stimulates the transcription of CYP1A and the other target genes. Various endo- and exogenous compounds, that may be potentially signalling molecules, are oxidized by CYP1A-mediated reactions into more hydrophilic and often less active or harmful metabolites. Through these processes modulated by AhR-mediated signalling or by altered CYP1A-mediated metabolism of signalling molecules, PHAH exposure may affect cell signalling, cell growth, and tumor promotion.[3] Therefore, level of CYP1A induced is considered as a biomarker of PHAHs exposure and their toxic responses.

In 1987-1988, outbreak of morbillivirus infection resulted in mass mortality of Baikal seals (*Phoca sibirica*). Immunosuppression elicited by chronic exposure to environmental contaminants was suspected to be associated with the virus-induced mass mortality.[4] The following chemical analyses in the Lake Baikal waters and seal tissues demonstrated the presence of high concentrations of PCBs.[5-7] However, no toxicological studies have been made so far in Baikal seal. The purpose of this study is to investigate the relationships between hepatic CYP enzyme activities and Co-PCB congener levels in Baikal seals.

Materials and Methods

Baikal seals were collected from Lake Baikal in May-June in 1992. Seals were immediately dissected on board after the measurements of biometry. Blubber samples were taken and stored in a freezer at -20℃ until chemical analysis. Liver samples were frozen in liquid nitrogen, and stored at -80℃ until microsome preparation. The age of animals were determined by counting dentinal and cemental growth layer in sections prepared from lower canine teeth. The analysis of Co-PCBs was performed following the procedures previously reported, and the concentrations have already been published elsewhere.[7] The 2,3,7,8-TCDD toxic equivalents (TEQs) derived from Co-PCBs were

calculated from mammalian toxic equivalency factors (TEFs)[8] and concentrations of non- (IUPAC 77, 126 and 169) and mono-*ortho* Co-PCB congeners (IUPAC 105, 118 and 156). Hepatic microsomal fractions were prepared according to the method of Guengerich.[9] Protein content was measured with the bicinchoninic acid assay. CYP content was determined using dithionite difference spectra of CO-treated sample.[10] Measurements of ethoxy-, methoxy, pentoxy- and benzyloxyresorufin-*O*-dealkylation activities (EROD, MROD, PROD and BROD) were followed by the method of Kennedy *et al*.[11]

Correlations between age, blubber thickness or CYP activity levels, and Co-PCB concentrations were examined by Spearman rank correlation. The Mann-Whitney U-test was used for the detection of statistical differences among groups.

Results and Discussion

Average TEQs of Co-PCB congeners in the blubber of male and female seals are shown in Table 1. In most of samples, the contribution of TEQs from individual congeners to total TEQs was the largest for non-*ortho* Co-PCB126 due to the high TEF (= 0.1). Mono-*ortho* PCB congener, PCB118, was secondarily contributed, and PCB77 and PCB169 were minimum contributors due to their lower TEF (= 0.0001) and trace levels of concentrations. While male animals sequentially revealed the positive correlation of total TEQs with age ($p < 0.05$), TEQs in females remained constant after the maturity due to the transfer of TEQs from mother to her pup through gestation and lactation. Activities of EROD, MROD, PROD and BROD were also shown in Table 1. These activities of seal liver microsomes were characterized by the high activity of EROD followed by MROD, BROD and PROD activities. Spearman rank correlation analysis showed that each enzyme activity had a strong positive correlation with the others (p <0.001), suggesting that these four enzyme

Table 1. TEQs of non- and mono-*ortho* Co-PCBs and CYP enzyme activities in Baikal seals

	Male		Female	
	Inmature	Mature	Inmature	Mature
TEQs (pg/g fat wt)				
n	7	8	6	19
non-*ortho*				
77	0.25 ± 0.16	0.90 ± 0.39	0.34 ± 0.29	0.55 ± 0.32
126	280 ± 150	400 ± 140	280 ± 290	390 ± 230
169	2.2 ± 1.2	4.3 ± 2.3	3.0 ± 1.1	3.3 ± 2.1
mono-*ortho*				
105	29 ± 16	110 ± 59	30 ± 19	42 ± 18
118	75 ± 43	280 ± 150	85 ± 54	110 ± 58
156	51 ± 33	130 ± 76	42 ± 23	89 ± 36
Total	430 ± 210	920 ± 410	440 ± 340	630 ± 300
CYP enzyme activities (pmol/min/mg protein)				
n	8	9	5	19
EROD	330 ± 150	280 ± 69	270 ± 53	300 ± 160
MROD	38 ± 18	36 ± 15	31 ± 5.5	32 ± 17
BROD	7.1 ± 3.5	4.7 ± 1.6	5.9 ± 1.0	6.0 ± 3.9
PROD	4.6 ± 1.4	3.7 ± 0.8	4.3 ± 0.5	4.6 ± 1.8

Note: Values are means ± SD

activities are catalyzed by one CYP isozyme. In our previous study using liver microsomes of largha (*Phoca largha*) and ribbon (*Phoca fasciata*) seals, significant positive correlations were

found between the enzyme activities and the expression levels of CYP1A protein cross-reacted with anti-rat CYP1A1 antisera, and anti-rat CYP1A2 antisera inhibited the enzyme activities by almost 100%.[12] In addition, our recent study could detect novel CYP1A1 cDNA fragments from livers of both species.[13] These studies indicate that such enzyme activities depend upon CYP1A isoform in Baikal seal.

The effect of time taken for postmortem liver treatment should be considered, when CYP enzyme activity is used in association with PHAH levels. Comparison of CYP activity between two groups that taken >2 hrs and <2 hrs for liver treatment revealed that EROD activities in >2 hrs group were significantly lower those in <2 hrs group (p <0.01). Therefore, the samples in <2 hrs group were only counted for the following consideration.

When relationship between blubber PHAH and hepatic CYP levels is discussed, considerable attention has to be paid for an assumption that PHAHs are in the equilibrium between blubber and liver, and that PHAH concentration in blubber reflects that in liver. In order to examine this, the effects of age and blubber thickness were taken into account in this study. Microsome fractions prepared from pups that were <0.5 years old revealed high hepatic EROD activities, while blubber from the same animals contained relatively low TEQs. Ecological surveys have recorded that Baikal seal pups are born in February-March, and the lactation period is 2-2.5 months. Considering that the pups collected in May-June may have been in or just after the lactation, intense exposure to Co-PCBs in the liver through lactation may have enhanced the expression levels of CYP1A prior to the equilibrium distribution of Co-PCBs between blubber and liver. The relationship between blubber thickness and PCB77 is shown in Figure 1. Although a significant negative correlation (p <0.01) was obtained, plots from three mature females, plotted as open circle (○), were outstanding for the concentration of PCB77 against the blubber thickness. One possibility explaining this phenomenon may be due to the effect of lactation. Rapid wasting of blubber during the lactation may have temporally raised Co-PCB concentrations in the blubber, which may reflect neither the levels of Co-PCBs nor the corresponding CYP1A in the liver. Thus, the data from animals that were speculated to be suckling pups and lactating mothers were eliminated for the following statistical analysis.

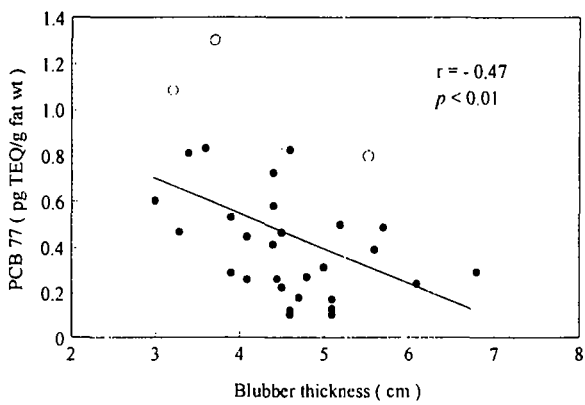

Fig. 1. Relationship between blubber thickness and PCB77 concentrations

Correlations between congener-specific TEQs and CYP-dependent enzyme activities are presented in Table 2. Significant correlations between PCB126, the greatest TEQ-contributed congener, and all enzyme activities were observed (p <0.05), whereas the other Co-PCB congeners were not correlated with the enzyme activities. This relationship suggests that CYP1A is congener-specifically induced by PCB126, implicating toxic effects in Baikal seal, and that the hepatic CYP1A expression level is a useful biomarker for the AhR agonist exposure.

In order to understand the direct effects of PHAH exposure in Baikal seal, the relationship between the levels of PHAH and CYP1A in the target tissue would be of more importance.

Table 2. Spearman rank correlations between CYP enzyme activities in liver microsomes and blubber concentrations of Co-PCBs

	non-*ortho* PCB			mono-*ortho* PCB		
	CB77	CB126	CB169	CB105	CB118	CB156
EROD	0.19	0.45*	-0.16	-0.02	0.08	0.24
MROD	0.34	0.55*	-0.13	0.08	0.23	0.34
BROD	0.34	0.54*	0.11	0.03	0.14	0.14
PROD	0.30	0.49*	0.10	-0.08	0.17	0.23

* $p < 0.05$

Acknowledgements

The authors would like to thank Dr. M. Grachev (Limnological Institute of the Siberian Division of the Academy of Sciences of Russia) for directing the present project supported by BICER (Baikal International Center for Ecological Research) and JABIRP (Japan Association for Baikal International Research Program). Our thanks also due to Dr. T. Kawai (National Institute for Environmental Study) and Prof. K. Numachi (Faculty of Marine Biology and Fishery Technology, Tokai University, Japan) for arranging and supporting the research.

This study was supported by Grants-in-aid for Scientific Research (to H.I.; Grant No., 09306021 and to S.T.; Grant Nos., 12308030 and 12055101) and for the Development of Innovative Technology (Destruction Processes of Dioxins/PCB and Bioassay Monitoring) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1. Iwata, H., Tanabe, S., Sakai, N., and Tatsukawa, R. (1993) Environ. Sci. Technol. 27, 1080-1098.
2. Tanabe, S., Iwata, H., and Tatsukawa, R. (1994) Sci. Total Environ. 154, 163-177.
3. Whitlock, J. P., Okino, S. T., Dong, L., Ko, H. P., Clarke-Katzenberg, R., Ma, Q., and Li, H. (1996) FASEB J. 10, 809-818.
4. Ross, P. S., de Swart, R. L., Reijnders, P. J. H, Van Loveren, H., Vos, J. G., and Osterhaus, A. D. M. E. (1995) Environ. Health Perspect. 103, 162-167.
5. Iwata, H., Tanabe, S., Ueda, K., and Tatsukawa, R. (1995) Environ. Sci. Technol. 29, 792-801.
6. Nakata, H., Tanabe, S., Tatsukawa, R., Amano, M., Miyazaki, N., and Petrov, E. A. (1995) Environ. Sci. Technol. 29, 2877-2885.
7. Nataka, H., Tanabe, S., Tatsukawa, R., Amano, M., Miyazaki, and N., Petrov, E. A. (1997) Environ. Pollut. 95, 57-65.
8. Van den Berg, M. *et al.* (1998) Environ. Health Perspect. 106, 775-792.
9. Guengerich, P. (1982) In: Principles and Methods of Toxicology (Hayes, A.W., Ed) Raven Press.
10. Omura, T. and Sato, R. (1964) J. Biol. Chem. 239, 2370-2378.
11. Kennedy, S. W., Jones, S. P., and Bastien, L. J. (1995) Anal. Biochem. 226, 362-370.
12. Chiba, I, Sakakibara, A., Iwata, H., and Tanabe, S. (2001) Environ. Toxicol. Chem. submitted.
13. Teramitsu, I., Yamamoto, Y., Chiba, I., Iwata, H., Tanabe, S., Fujise, Y., Kazusaka, A., Akahori, F., and Fujita, S. (2000) Aquat. Toxicol. 51, 145-153.