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Aryl hydrocarbon receptor (AHR) and AHR nuclear translocator (ARNT) expression in Baikal seal (*Pusa sibirica*) and association with 2,3,7,8-TCDD toxic equivalents and CYP1 expression levels ☆

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Abstract

Most toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related planar halogenated aromatic hydrocarbons (PHAHs) are mediated by ligand-activated aryl hydrocarbon receptor (AHR) signaling pathway. To understand the regulation mechanism of AHR and AHR nuclear translocator (ARNT) expression in wild Baikal seal (*Pusa sibirica*) population contaminated by PHAHs, the present study investigated hepatic mRNA expression levels of AHR and its heterodimer, ARNT genes, in association with biological index (age, gender and body weight), PHAH accumulation and expression levels of cytochrome P450 (CYP) 1A and 1B. While there was no gender difference, the AHR mRNA expression levels were increased with ages ($p=0.014$) and body weights ($p=0.015$), indicating that AHR expression might be affected by these biological factors. The AHR mRNA expression levels exhibited significant positive correlations with total TEQs and most of individual congener TEQs derived from polychorinated dibenzo-*p*-dioxins, dibenzofurans and non-*ortho* coplanar polychorinated biphenyls (PCBs), indicating the transcriptional up-regulation of AHR expression by these congeners. On the other hand, there was no significant correlation between individual TEQs from mono-*ortho* coplanar PCBs and AHR expression. These results imply the structure-related transcriptional activity of AHR among PHAHs congeners. AHR mRNA levels showed positive correlations with both CYP1A protein ($p=0.039$) and CYP1A1 mRNA expression levels ($p=0.046$). In contrast to AHR expression, neither the total nor individual congener TEQs influenced ARNT at the transcriptional level. ARNT mRNA showed significant negative correlations with CYP1A/1B protein ($p=0.027$ and $p=0.006$) and CYP1A1 mRNA expression levels ($p=0.039$), implying the existence of different transcriptional regulation between AHR and ARNT genes and negative regulation by CYP1A/1B-mediated signaling pathways. The present findings may render significant insight on the basic mechanisms underlying regulation of AHR and ARNT expressions associated with biological factors and PHAH exposure in wild mammalian populations.

Keywords: Aryl hydrocarbon receptor; AHR nuclear translocator; TEQs; CYP1A; CYP1B; Baikal seal

1. Introduction

Exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related planar halogenated aromatic hydrocarbons (PHAHs) poses numerous adverse effects including hepatic damage, immune dysfunction, teratogenesis and carcino-

☆ The nucleotide sequence of Baikal seal ARNT has been deposited in the DDBJ/EMBL/GenBank database under accession number AB201467.

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genesis in both laboratory and wild animals (Pohjanvirta and Tuomisto, 1994; Okey et al., 1994; Tanabe et al., 1994; de Swart et al., 1996). Numerous studies indicate that such toxic effects of PHAHs are mediated by the ligand-activated aryl hydrocarbon receptor (AHR) and induction of its battery genes, such as cytochrome P450 (CYP) 1A1, 1A2 and 1B1 isozymes (Fernandez-Salguero et al., 1995, 1996; Teraoka et al., 2003).

The AHR belongs to the basic-helix–loop–helix/Per-ARNT-Sim (bHLH-PAS) family of transcriptional regulation proteins. The ligand binding to AHR leads to its translocation into the nucleus, where it forms a heterodimer with AHR nuclear translocator (ARNT). The heterodimer complex activates the transcription of multiple target genes including CYP1A/1B through the interaction with a specific DNA response element, known as xenobiotic response element (XRE), located in the promoter of these genes (reviewed by Hahn, 1998a).

Sustained activation of the AHR–ARNT signaling pathway by PHAHs leads to a variety of pathophysiological effects through changes of gene expression profiles involved in cell growth and differentiation (Schmidt and Bradfield, 1996). The induction of CYP 1A/1B genes has been used as a model system to assess the PHAH exposure (Stegeman et al., 2001; Chiba et al., 2002) and to define the molecular mechanism of the AHR–ARNT signaling pathway (Besselink et al., 1998; Teraoka et al., 2003). However, the molecular mechanisms underlying the regulation of AHR and ARNT genes and the effects that their expression levels may elicit are poorly understood.

The AHR expression level appears to be regulated in cellular-, tissue-, species-, developmental-, and chemical-specific manner (FitzGerald et al., 1996; Abbott et al., 1995; Yamamoto et al., 2004). For example, AHR mRNA levels were not altered by TCDD exposure in Hepa-1 and LS180, although the content of AHR protein dropped rapidly after exposure (Giannone et al., 1998). In mouse hepa-1c17 cells, TCDD treatment decreased the AHR expression levels (Pollenz, 1996). Significant reduction in the concentration of AHR protein was observed in most of the TCDD-exposed (10 μg/kg/day) tissues of female Sprague–Dawley rats (Pollenz et al., 1998). In contrast, up-regulation of hepatic AHR levels occur in rodents treated with a 5 μg/kg/day dose of TCDD (Franc et al., 2001a). Other chemicals, such as Aroclor 1254 (Denomme et al., 1986) and phenobarbital (Okey and Vella, 1984), were also shown to elevate AHR levels in rats. Levels of ARNT mRNA were comparatively more stable to TCDD exposure. After treatment of 5 μg/kg dose of TCDD, a small drop at day 1 was observed in Long–Evans (L–E) and Sprague–Dawley (SD) rats (Franc et al., 2001a). L–E rats also showed a decrease in ARNT mRNA at day 10 following 50 μg/kg dose of TCDD. Several studies have also suggested that the differential responses of AHR and ARNT expression could be influenced by administered dose and duration.

Regarding the quantitative relationship of AHR and CYP1, the mRNA levels of CYP1A1 from the blood of healthy subjects correlated with that of AHR (Hayashi et al., 1994). According to Lin et al. (2003), AHR levels were positively correlated with CYP1B1 levels in cultivated human lymphocytes treated with DMSO or benzanthracene (BA) but negatively correlated with CYP1A1 levels in the DMSO-treated cells. ARNT levels were also associated with CYP1B1 levels in BA-treated cells. In a mutant cell, Hepa 1c1c7 C12 cell, which possesses AHR at ~10% of the levels found in Hepa 1c1c7 wild-type cells, 7-ethoxyreorufin *O*-deethylase (EROD) and 7-methoxyresorufin *O*-demethylation (MROD) activity were not detectable even after the treatment of AHR ligands, implying that CYP1A expression would be associated with AHR expression levels (Elbekai and El-Kadi, 2004).

To date, the majority of research on the regulation of AHR and ARNT has been conducted in laboratory animals, such as mice and rats, and established with a single or repeated dose of a particular PHAH congener at acute or sub-acute levels in short-time experiments. However, validity of such experimental designs in elucidating the effects of contaminants in the environment is still unproven. On the other hand, there is less information regarding effects of PHAH exposure on regulation and expression of AHR and ARNT in wildlife populations. Molecular and biochemical studies on AHR/ARNT-regulation in wildlife which are chronically exposed to TCDD and PHAHs can contribute to our understanding of the effects related to the AHR–ARNT signaling pathway at substantial residue levels of those chemicals and to reducing uncertainty in interspecies extrapolation.

Baikal seals (*Pusa sibirica*), an aquatic mammal species in the Baikal, Russia, accumulate considerable levels of PHAHs in their tissues (Nakata et al., 1995; Iwata et al., 2004). This seal species is thought to be highly sensitive to the toxic effects of these chemicals, as suggested by an outbreak of morbillivirus infection that resulted in mass mortality (Osterhaus et al., 1995). To understand the susceptibility of aquatic mammals to PHAHs, we investigated molecular characterization and binding affinity of AHRs in harbor and Baikal seals (Kim and Hahn, 2002; Kim et al., 2002). This indicated that these seals might be sensitive to PHAH effects, due to the high TCDD-binding affinity of seal AHR.

Apart from the TCDD-binding affinity of AHR, alteration in AHR and ARNT expression levels could potentially modify the ultimate response to PHAHs in seals. Consequently, species differences in regulation of AHR and ARNT expressions may lead to species-specific susceptibility (reviewed in Hahn, 1998b). Therefore, more diligent efforts for unraveling the regulatory mechanism of AHR and ARNT expression and toxicological consequences driven by their expression levels are necessary to estimate the risk of PHAHs in seal species with high accuracy.