Ecotoxicology and Environmental Safety 108 (2014) 95–105

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effects of persistent organochlorine exposure on the liver transcriptome of the common minke whale (*Balaenoptera acutorostrata*) from the North Pacific

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ARTICLE INFO

Article history:
Received 18 March 2014
Received in revised form
20 June 2014
Accepted 20 June 2014
Available online 19 July 2014

Keywords:
Minke whale
Transcriptome
Organochlorines
Dioxins
Kynureninase

ABSTRACT

Hepatic concentrations of persistent organochlorines (OCs) were determined in the common minke whale (*Balaenoptera acutorostrata*) from the North Pacific. To investigate the effects of OCs on the transcriptome in the minke whale, the present study constructed a hepatic oligo array of this species where 985 unique oligonucleotides were spotted and further analyzed the relationship between the OC levels and gene expression profiles of liver tissues. The stepwise multiple linear regression analysis identified 32 genes that correlated with hepatic OC levels. The mRNA expression levels of seven cytochrome P450 (CYP) genes, *CYP1A1*, *1A2*, *2C78*, *2E1*, *3A72*, *4A35*, and *4V6* showed no clear correlations with the concentration of each OC, suggesting that the accumulated OCs in the liver did not reach levels that could alter CYP expression. Among the genes screened by the custom oligo array analysis, hepatic mRNA expression levels of 16 genes were further measured using quantitative real-time reverse transcription polymerase chain reaction. The mRNA levels of vitamin D-binding protein (DBP) were negatively correlated with non-*ortho* coplanar polychlorinated biphenyl (PCB) levels. Androgen receptor-associated coregulator 70 (ARA70) expression levels showed a significant positive correlation with concentrations of non-*ortho* coplanar PCB169. These correlations suggest that coplanar PCB-reduced DBP expression could suppress vitamin D receptor-mediated signaling cascades in peripheral tissues. Alternatively, the suppression of vitamin D receptor signaling cascade could be enhanced through competition with the androgen receptor signaling pathway for ARA70. In addition, a negative correlation between kynureninase and PCB169 levels was also observed, which suggest an enhanced accumulation of an endogenous aryl hydrocarbon receptor agonist, kynurenine in the minke whale population. Further studies are necessary to translate the changes in the transcriptome to toxicological outcomes including the disruption of the nervous and immune systems.

1. Introduction

Persistent organochlorine compounds (OCs) such as polychlorinated biphenyls (PCBs), 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane (DDT), and its metabolite, DDE, have been detected in a variety of environmental media (Iwata et al., 1993). Due to their high bioaccumulation and toxic potentials, the contamination of OCs in wildlife has been of great concern (Tanabe et al., 1994).

The levels of OCs in wild animal populations have been well documented; however, the information on its effects remains insufficient, especially at the molecular level. Regulatory programs in developed countries have started to incorporate tests and endpoints that capture the effects of chemicals on specific signaling pathways in animals (Ankley et al., 2010). Hence, 21st century ecological testing and screening programs for chemicals should be highly comprehensive and promptly implemented (Villeneuve and Garcia-Reyero, 2011). On the other hand, the majority of the methods used for ecological testing virtually relies on whole animal exposures that focus on its effects on survival, growth, and reproduction. These types of tests are resource-oriented and time-intensive; this makes it technically difficult to address wild species, particularly large-sized animals.

Animals react to chemical exposures by changing the expression patterns of their transcriptome (Van Aggelen et al., 2010;

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http://dx.doi.org/10.1016/j.ecoenv.2014.06.028
0147-6513/

Williams et al., 2003; Hirakawa et al., 2011). Thus, comprehensive monitoring of the transcriptome in animals of concern, also known as transcriptomics, will identify the critical targets of chemicals and reveal new mechanisms of action. For gene expression analysis, particularly at the transcriptome level, the use of the microarray technique has recently been recognized as the standard methodology and continues to improve in terms of efficiency, data enrichment, and cost (Van Aggelen et al., 2010). In toxicological studies, the microarray has been frequently used to comprehensively determine changes in gene expression profiles in organisms exposed to not only drugs and pharmaceuticals but also environmental contaminants. Hence, this technique could play an important role in screening chemicals and in assessing the health of animals. Molecular responses of murine models to OC exposure have been detected using microarrays (Zeytun et al., 2002; Boverhof et al., 2005, 2006; Vezina et al., 2004; Adeeko et al., 2003; Kopec et al., 2010). Such studies have identified genes responsive to OCs, as well as evaluated potential effects by associating histopathological features with specific gene expression profiles. These observations have thus facilitated the implementation of transcriptomics as a diagnostic tool in ecotoxicology (Van Aggelen et al., 2010). However, most ecotoxicology studies have used murine mammals that have been propagated in the laboratory and may thus inaccurately represent the gene expression profiles of wild animals that are subjected to chronic and/or long-term exposure to chemicals (Williams et al., 2003; Nakayama et al., 2006; Hirakawa et al., 2011), while inbred murine animals can diminish their genetic diversity which may contribute to significant differences in responses to chemical exposure. Comprehensive monitoring of changes in gene expression in wild species may lead to the development of molecular markers that aid in identifying causative chemicals and toxic mechanisms of action.

Our previous studies have revealed that common minke whales (*Balaenoptera acutorostrata*) from the North Pacific accumulate classical OCs including PCBs (sum of congeners), DDTs (*p,p′*-DDT, *p,p′*-DDD, and *p,p′*-DDE), hexachlorocyclohexanes (HCHs; α-, β-, and γ-HCH), chlordanes (CHLs; *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane) and hexachlorobenzene (HCB) in the liver and blubber (Aono et al., 1997; Niimi et al., 2005). Furthermore, by using a cDNA library generated from minke whale livers, we have identified seven cytochrome P450 (CYP) isozymes, including *CYP1A1* (Accession no. AB231891), *CYP1A2* (AB231892), *CYP2C78* (AB290008), *CYP2E1* (AB290010), *CYP3A72* (AB290009), *CYP4A35* (AB290011), and *CYP4V6* (AB290012) that might potentially respond to environmental contaminants such as OCs at the transcriptional level and be involved in their metabolism. We then determined their full-length cDNA sequences and hepatic mRNA expression levels (Kim et al., 2004; Niimi et al., 2005, 2007). In addition, we carried out correlation analyses between concentrations of each OC and mRNA expression levels of each *CYP* isozyme in the liver of the minke whale population from the North Pacific. The results showed no significant correlations between the *CYP* isozyme expression levels and the OC concentrations, which indicate that these CYP enzymes were not induced by OC exposure (Niimi et al., 2007). However, the effects of OCs on the hepatic expression of genes other than these *CYPs* in the common minke whales still remain unclear. Sequence data from the whale cDNA library could be potentially employed as a diagnostic tool such as microarray for evaluating chronic exposure of complex mixtures of OCs in the whale species because it could help to establish sensitive markers that identify pathways to adverse outcomes before they are manifested in the population.

In the present study, we constructed an oligo array of the common minke whale using sequence data from the hepatic cDNA library (Niimi et al., 2007) to investigate alterations in the expression of genes other than *CYPs* that may be associated with OC accumulation in the body. In addition to chemical analyses of PCBs, DDTs, HCHs, chlordanes, and HCB that have been so far reported (Niimi et al., 2005), we further analyzed dioxins and related compounds (DRCs), including polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and coplanar (dioxin-like) PCBs as aryl hydrocarbon receptor (AHR) agonists in the liver of minke whales. Statistical analyses of correlations between hepatic OC concentrations and gene expression levels on the custom oligo array were then executed. Based on the correlation analyses, we assessed the potential effects of OCs in the whale liver.

2. Materials and methods

2.1. Samples

Liver samples of the common minke whale employed in the present study were the same as those used in our previous studies (Niimi et al., 2005, 2007). In brief, nineteen male common minke whales (nine immature and ten mature) were collected from an area of the western North Pacific (35–45°N, 140–150°E) by JARPN II (Japanese Whale Research Program under Special Permit in the Western North Pacific-Phase II) in 2001. Livers of common minke whales were immediately removed on board the research vessel after capture. Only male liver samples were used to rule out the effect of sex on gene expression profiles. The livers wrapped with aluminum foil were frozen in liquid nitrogen and subsequently stored at −80 °C in the laboratory of Ehime University until total RNA preparation. The subsamples of the livers were stored at −20 °C until DRC analysis.

2.2. Construction of custom microarray

Hepatic cDNA library of the common minke whale was prepared for the construction of an oligo array (Niimi et al., 2005). A total of 6930 clones randomly selected from the library were screened, and 1991 cDNA clones with high sequence identities with genes deposited in GenBank were obtained. Unique 70-mer oligonucleotides for 985 genes were designed and spotted in duplicate onto a TaKaRa-Hubble slide glass (Takara Bio, Inc.). Spotted genes on the oligo array were categorized according to the gene annotation presented in Table 1.

Table 1
List of genes spotted onto our custom minke whale oligo array.

Gene species	*n*
Oncogene	6
Transcriptional factor	19
Translation initiation factor	12
Elongation factor	8
Receptor	45
Transporter	23
Immune system	15
Synthetase	42
Kinase	43
Phosphatase	19
Xenobiotic metabolizing enzyme	29
Antioxidant enzyme	4
Other enzymes	288
Homeobox	1
Growth factor	7
Heat shock protein	6
Ribosomal protein	13
Glycoprotein	14
KIAA	31
RIKEN	33
MGC	50
ATP-related protein	18
Haptoglobin	2
Selenoprotein	2
Cathepsin	5
Others	250
Total	985