Marine Environmental Research 121 (2016) 74–86

Contents lists available at ScienceDirect

Marine Environmental Research

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

Fish condition factor, peroxisome proliferator activated receptors and biotransformation responses in *Sarotherodon melanotheron* from a contaminated freshwater dam (Awba Dam) in Ibadan, Nigeria

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ARTICLE INFO

Article history:
Received 30 October 2015
Received in revised form
31 January 2016
Accepted 3 February 2016
Available online 8 February 2016

Keywords:
Environmental obesogens
CYP1A
PPARs
Tilapia
Condition factor
Aquatic environment
Developing country

ABSTRACT

The relationship between condition factor (CF), peroxisome proliferator-activated receptors (PPARs), phase 1 biotransformation (CYP1A isoforms) and contaminant burden has been studied in *Sarotherodon melanotheron* from a contaminated tropical freshwater dam (Awba Dam) and compared to a reference site (Modete Dam) in Southwest, Nigeria. A total of 89 fish (57 males and 32 females) was collected from Awba Dam and 95 fish (48 males and 47 females) from the reference site. In general, fish sampled from Awba Dam were bigger than reference site. Sediment samples were also collected from both sites for contaminant analysis. Expression of *ppar* and *cyp*1 isoforms was analyzed using validated real-time PCR, while CYP1A and PPAR protein levels were analyzed using immunochemical method with specific antibodies. CYP-mediated catalytic responses (EROD, MROD and BROD) were performed by biochemical methods. We observed significant increases in *ppar* and *cyp*1 isoforms mRNA in both male and female fish from Awba Dam, compared to the reference site. Catalytic activities of EROD, MROD and BROD paralleled cyp1 transcript levels. Sex-related differences in PPAR and CYP1A protein levels were also observed, showing higher CYP1A proteins in males, compared with females, and higher PPAR proteins in females compared with males. Principal component analysis (PCA) biplot showed positive relationships between biological responses (ppar isoforms), condition factor (CF) and sediment PCBs, PAHs, OCPs and heavy metal concentrations. The present study shows that *S. melanotheron* inhabiting Awba Dam are severely affected by different classes of environmental contaminants that target metabolic processes (PPAR) and biotransformation pathways (CYP1A) in male and female fish, compared to a reference site. Interestingly, fish from Awba Dam were exhibiting good growth (evidence by high CF values) that paralleled increases in the transcriptional activation of ppar and cyp1 isoforms, despite the high contaminant burdens, suggesting a possible contaminant-induced obesogenic effects.

1. Introduction

Environmental contaminants such as heavy metals, methoxychlorine, organochlorine compounds (OCPs) such as DDT (1,1,1-trichloro-2,2-bis(chlorophenyl)ethane), alkylphenol polyethoxylates, organotins, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and natural plant constituents (phytoestrogens), may produce estrogenic activities in laboratory animals (Pelissero et al., 1991, 1993 Arukwe et al., 1999; Celius et al., 1999). In general, a vast majority of these hormone mimicking contaminants are produced for specific purposes, such as pesticides, plasticizers, electrical coolants in transformers and other products (for review see, Caldwell, 1985; Ahlborg et al., 1995). Additional reasonable amounts of these compounds are generated as by-products during manufacturing or are breakdown products of some other chemical, and some, such as 17β-ethinylestradiol and diethylstilbestrol (DES), are used as drugs. Irrespective of their source or original intended use, a high amounts of these compounds end up in the aquatic environment due to physicochemical, hydrologic and atmospheric processes (Bjerregaard et al., 1998; Guardans and Gimeno, 1994).

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http://dx.doi.org/10.1016/j.marenvres.2016.02.002

Given that environmental contaminants occur as mixtures, chemical interactions after exposure to complex mixtures of environmental pollutants may have profound consequences on aquatic organisms. Exposure of organisms to contaminants may alter important energetic and biochemical pathways with various deleterious consequences (Jedamski-Grymlas et al., 1995; van der Oost et al., 2003; Kopecka-Pilarczyk and Correia, 2009; Arukwe et al., 2015; Adeogun et al., 2015a, b). For example, the effects of endocrine disrupting chemicals (EDCs) on reproduction have been reported in several invertebrate and vertebrate species (Brouwer et al., 1989; Isidori et al., 2010; Palermo et al., 2012). Recently, scientific attention has been focused on EDCs with the ability to interfere with thyroid function or lipid signaling and consequently produce metabolic disruption or incidence of metabolic diseases such as obesity and diabetes, through the activation of peroxisome proliferator activated receptors (PPARs) (Feige et al., 2007; Casals-Casas and Desvergne, 2011). The obesogen activity of several contaminants with established endocrine disruptive effects has been reported under laboratory conditions (Grun et al., 2006; Grun and Blumberg, 2009b; Penza et al., 2006; Alonso-Magdalena et al., 2006; Smink et al., 2008; Feige et al., 2007; Cocci et al., 2015). However, similar effects have not been documented in the field on wild fish populations from contaminated environments, which represent the ultimate sink for most of the EDCs. Recent studies have suggested a number of mechanisms linking EDCs to obesity which include, but not limited to – alterations in thyroid and steroid hormone function, elevated triglycerides and cholesterol levels, activation of PPARs, which play integral roles in adipocyte differentiation and energy storage (Desvergne et al., 2002; Janesick and Blumberg, 2011; Pereira-Fernandes et al., 2013). PPARs (α, β and γ isoforms) are nuclear receptors that regulate key molecular and cellular functions, including glucose and lipid metabolism, cell differentiation and adipogenesis (Latruffe et al., 2001; Tan et al., 2001; Casals-Casas and Desvergne, 2011).

It has been shown that persistent environmental contaminants, such as PCBs, PAHs, OCPs may produce an array of toxicological effects on multiple pathways in teleosts (Isidori et al., 2010; Palermo et al., 2012), including the activation of PPARs and aryl hydrocarbon receptor (AhR) mediated responses (Fang et al., 2012; Cocci et al., 2013). The AhR is a transcription factor belonging to the helix-loop-helix-PAS (bHLH-PER-ARNT-SIM) family of gene regulatory proteins (Gu et al., 2000). After ligand binding, the AhR dimerizes with AhR nuclear translocator (Arnt), translocating to the nucleus where the complex activates mRNA transcription of XRE (xenobiotic responsive elements) containing genes, including cytochrome P450s or CYPs (Gu et al., 2000). Particularly, the CYP1 (a, b and c) isoforms superfamily catalyzes the metabolism and detoxification of organic compounds and drugs (Nelson et al., 1996). In the aquatic ecosystem, the induction of CYP1A measured as 7-ethoxyresorufin-O-deethylase (EROD activity or CYP1A transcripts) has been widely used as a sensitive and convenient early warning signal of biological exposure to organic pollutants (Goksøyr and Förlin, 1992).

In fish, condition factor (CF) is generally regarded as a physiological index of fish growth (Fulton, 1902), representing a classical measure of health and condition as accepted by fish biologists for screening fish populations. CF is used to provide reliable information on fish populations living in sub-optimal environment and fish with CF > 1 is considered to be exhibiting isometric growth and in good health (Khan, 1999; van der Oost et al., 2003; Oliva-Paterna et al., 2003 Craig et al., 2005). CF if often complemented with organosomatic indices (ratios of organ weight to body weight), and both indices can vary naturally with food availability, state of sexual maturation, and life history stage (Barton et al., 2002). As such CF is considered a reliable representation of an organism's state of health depicting a correlation between physiological and environmental status, prompting the assumption that exposure to environmental contaminants may lead to significantly lower CF (van der Oost et al., 2003; Oliva-Paterna et al., 2003). The mechanistic aspects of many contaminant-mediated effects are often poorly understood in fish, and lack of information on the activation of nuclear receptor mediated pathways, phase 1 biotransformation response and its implications on the health and condition of fish chronically exposed to contaminants in the natural environment is needed, in order to incorporate environmental concentrations and biomarker responses into fish health studies. Therefore, the present study was undertaken with the aim of assessing chemical modulation of PPARs and CYP1-isoforms as indices of metabolic disruption in a tropical teleost (*Sarotherodon melanotheron*) from Awba Dam, Nigeria.

In developing countries such as Nigeria, environmental laws are poorly enforced and the rapid industrial development, agricultural activities, urbanization among others, have resulted in the discharge of a wide range of environmental contaminants into the aquatic system. Hence, high concentrations of environmental contaminants such as phthalates (Adeniyi et al., 2011; Arukwe et al., 2012; Adeogun et al., 2015a, b), PCBs (Adeyemi et al., 2009; Adeogun et al., 2013), PAHs (Obiakor et al., 2014; Asagbra et al., 2015); PBDEs (Adewuyi and Adeleye, 2013) have been reported in fish from inland waters, sediment and open dumpsites in Nigeria. The Awba Dam was selected for this study for unique reasons that include (a) the presence of point sources of pollution through effluents from University of Ibadan resident community, (b) the presence of tangible commercial fishing activity that covers the entire length of the dam, and (c) the domestic use of the dam water by the University of Ibadan community.

2. Materials and methods

2.1. Study site

Awba Dam is an enclosed watershed within the University of Ibadan, southwestern Nigeria at an altitude of 185 m above sea level and lies within latitude 7° 26′ to 7° 28′ N and longitude 3° 35′ to 3° 54′ E. The dam was constructed in 1964 by damning Awba stream at a point where it flowed through a natural valley and expanded to its present size in 1971 primarily for domestic water supply, fisheries and research. It is a small man-made lake with a surface area of 0.06 km^2, a maximum depth of 5.5 m, a storage capacity of 227 million liters of water and a treatment rate of 68 thousand liters per day (Ugwumba and Ugwumba, 1993). The dam presently serves as the second source of domestic water supply to the water treatment plant for the University community, and receives untreated effluents from staff and students' residents, Zoological garden, experimental waste waters from Faculties of Science and Technology laboratories and non-point sources resulting from erosion and leaching of chemicals from surrounding farmlands (Fig. 1). Three sampling stations (i.e. stations 1–3 (S1–3) in Fig. 1) were randomly chosen within the dam for fish and sediment collection to give a comprehensive overview of the effects of discharged effluents on fish population since there was unimpeded fish movement being a small reservoir.

2.2. Sample collection and preparation

2.2.1. Fish samples

Samples of adult stages (male and female) of 89 black jaw tilapia (*S. melanotheron*: 57 males and 32 females) were collected randomly twice a month with the aid of an artisanal fisherman using a combination of gill and cast nets (mesh sizes 50–55 mm)