

BIOMARKER RESPONSES IN PERSIAN STURGEON (*ACIPENSER PERSICUS*) EXPOSED TO BENZO-A-PYRENE AND BETA-NAPHTHOFLAVONE

KATAYOON KARIMZADEH¹ and ASGAR ZAHMATKESH²

¹ Department of Marine Biology, Faculty of Sciences, Islamic Azad University, Branch of Lahijan 1616, Iran

² Department of Aquaculture, Mirza Koochak Khan Higher Education Center for Fisheries Science and Technology, Iran

Abstract - Biotransformation enzymes of xenobiotics (ethoxyresorufin-O-deethylase, cytochrome P4501A1 content and glutathione-S-transferase) were investigated in the liver of Persian Sturgeon (*Acipenser persicus*) after a 96-hour exposure to polycyclic aromatic hydrocarbons (PAHs), premutagenic benzo[a]pyrene (BaP) and beta-naphthoflavone (BNF). The fish were injected 10 mg/kg wet-body weight in corn oil for 96 hours every days. Ethoxyresorufin-O-deethylase activity (EROD) and glutathione s-transferase activity (GST) were measured in the fish liver. Cytochrome P4501A1 (CYP1A1) content was estimated by indirect enzyme-linked immunosorbent assay (ELISA). The response appeared as early as 12 hours post exposure. A time-dependent response was observed in the EROD activity, being significantly higher at 48 hours post exposure to 10 mg/kg of BaP. The greatest induction occurred in the fish treated with 10 mg/kg BaP, in which a 32.1-fold increase in EROD activity was observed. Results showed that EROD activity in *A. persicus* is significantly increased by BaP and BNF treatments. Both chemicals showed higher values of EROD activity compared to the liver CYP1A content. There was a rise in glutathione-S-transferase activity in fish exposed to BNF, but no increase was observed in fish treated with BaP. The results showed that hepatic CYP1A expression in terms of induction of EROD activity might be suited as a biomarker of organic contamination in aquatic environments and led to lower sensitivity of the second phase in the detoxification enzyme.

Key words: Biomarkers, *Acipenser persicus*, benzo(a)pyrene, beta-naphthoflavone

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) have been introduced into aquatic environments where they have been found to accumulate in several aquatic species (Hassanain et al., 2007). The ability to accumulate these compounds in the environment makes them deleterious to the aquatic ecosystem and consequently to humans who depend on aquatic products as a source of food. Due to their carcinogenic and mutagenic potentials, polycyclic aromatic hydrocarbons are important environmental pollutants (Holladay et

al., 1998; Barra et al., 2007; Hassanain et al., 2007). PAHs are also readily absorbed by fish and other aquatic animals during exposure to contaminated food, water and sediments, reaching levels higher than those in the ambient medium (Neff, 1985). Most living organisms have at least some ability to metabolize xenobiotics (Miller and Miller, 1981).

According to Miller and Miller (1981), the biotransformation of a xenobiotic in a fish is a major determinant of its toxicity distribution and ability to be excreted.

These transformations have been differentiated in two major phases. In phase I, the enzyme increases the polarity of substrate through oxidative, reductive or hydrolytic reactions. In Phase II, reactions include the conjugation of the xenobiotic along with polar endogenous constituents such as glucuronic acid, sulfate, glutathione and glutamate. In general, this conjugation dramatically improves solubility, which then promotes rapid excretion. Therefore, the degree of ecosystem contamination by toxic organic chemicals can be estimated by the analysis of biochemical changes (Nimmo, 1987). Fish are widely used for the assessment of the quality of an aquatic environment, and serve as bioindicators of environmental pollution (Adams et al., 1989).

The induction response of biotransformation enzymes in fish to xenobiotics such as organic pollutants was the main concern of early studies (Pesonen, 1992). Biotransformation enzymes are impacted by exposure to chemicals and they can be used as biomarkers to monitor aquatic contaminants (Arinc and Sen 1994; Winter et al., 2005).

According to Levin (1997), the sturgeon population of the Caspian Sea is the largest in the world and Iran is the second major country that produces this resource (Josupeit 1994; Pourkazemi et al., 2000). Nevertheless, there are evidence that the native population has declined by over 90% in the past three decades. According to the International Union for Conservation of Nature (IUCN) red list status, all sturgeon species in the Caspian Sea are currently considered as critically jeopardized (IUCN, 2010).

The Caspian Sea, one of the largest land-locked drainage areas in the world, is a terminal sink for water draining from several bordering countries including Russia, Iran, Azerbaijan, Turkmenistan and Kazakhstan (Kosarev and Yablonskaya, 1994). This contamination originates from riverine influx of industrial and domestic effluent, agricultural runoff and petrochemical contamination (Kaplin, 1995). Therefore, it is expected that these pollutants will have some adverse impact on the aquatic life, including sturgeon.

BaP and BNf are the most important pollutants that occur in many aquatic ecosystems (VanderOoest et al., 2003; Wang et al., 2006). However, no laboratory time-response studies related to applied biomarkers have been reported on *Acipenser persicus* so far. Therefore, the main objective of this study was to describe the effect of BaP and BNf on biotransformation enzyme activity in the Persian Sturgeon (*Acipenser persicus*).

MATERIALS AND METHODS

Chemicals

Resorufin, 7-ethoxyresorufin, benzo(a)pyrene (BaP), β -naphthoflavone (BNF), reduced β nicotinamide adenine dinucleotide phosphate (NADPH) and 1-chloro-2,4-dinitrobenzene (CDNB), were obtained from Sigma-Aldrich (Steinheim, Germany). All other chemicals were of analytical grade and were obtained from commercial sources.

Test animals and experimental design

Immature Persian Sturgeon (*A. persicus*) weighing 450-700g was obtained from the Shaid Beheshti Farms (Rasht, Iran). The fish were initially maintained in tanks containing 500 L of aerated seawater with natural photoperiod for 10 days. Sturgeon (n=6) per dose group were i.p. injected with 10 mg/kg of BaP and BNf in corn oil. Control fish received an equal volume of corn oil. This process was repeated every day until one day before sampling. Fish were fed once a day during the acclimation period using commercial pellet food.

The experiment was carried out in duplicate. The fish were killed and the livers were removed, washed with saline solution and immediately frozen in liquid nitrogen. The heart, gills, brain, muscle were taken and frozen (-70°C) for enzymatic analysis.

Liver microsomal preparation

Before analysis, a thawed sample of liver tissue (from 0.5 to 2.0g) was placed into a homogenization buffer

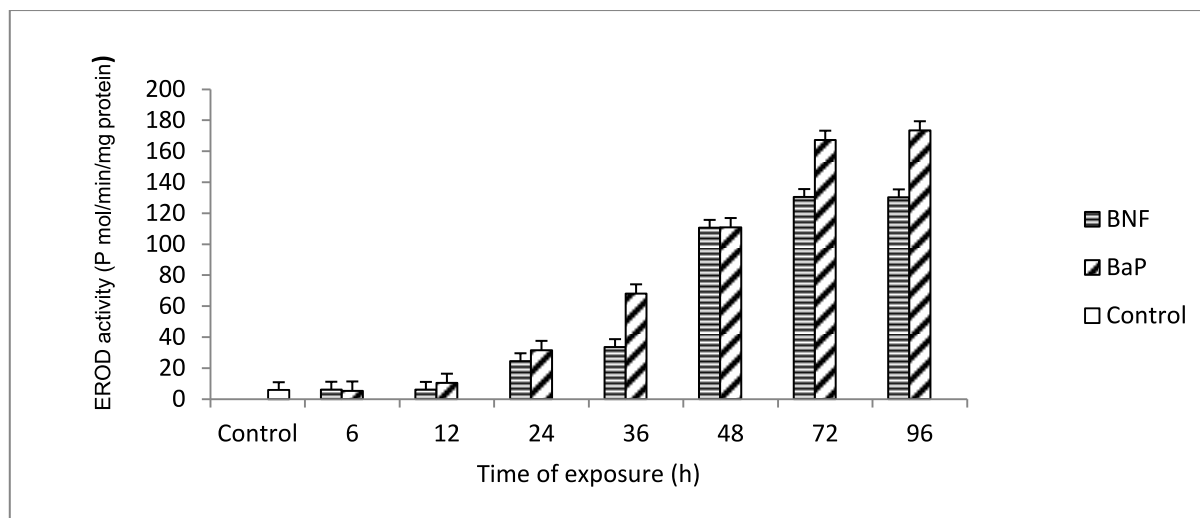


Fig. 1. Activities of hepatic EROD in *Acipenser persicus* after injections with BNF or BaP for 96 hours. The results are mean \pm SDE; n=6. Significantly different from Control.

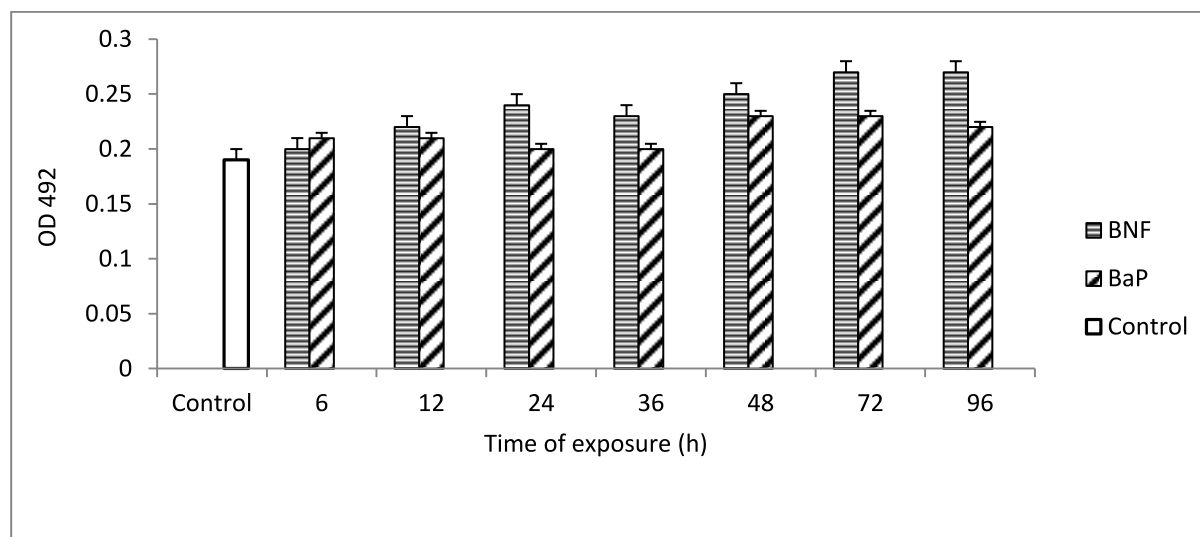


Fig. 2. The cytochrome P4501A1 content in *Acipenser persicus* after injections with BNF or BaP for 96 hours. The results are mean \pm SDE; n=6.

(1.15% KCl buffered with 0.01 mol/l Tris-HCl, pH 7.4). The homogenized samples were centrifuged at 10 000g for 20 min at a temperature of 4°C. The supernatant was then carefully pipetted into ultracentrifugation tubes and re-centrifuged at 100 000 g for 60 min at 4°C. This second supernatant was removed and the microsomal fraction was washed with a homogenizing buffer twice and then resuspended in

the buffer. This suspension was put into Eppendorf tubes and stored at -80°C until the enzymatic analysis could be performed.

EROD assay

7-Ethoxyresorufin-O-deethylase (EROD) activity was evaluated at 25°C by measuring the fluorescence

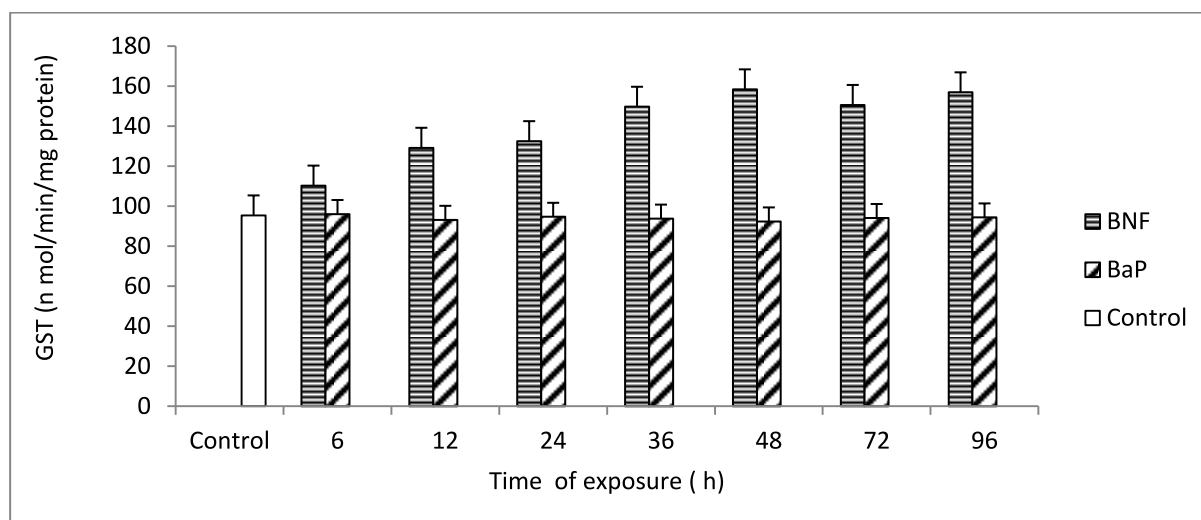


Fig. 3. The activities of hepatic glutathione S-transferase (GST) in *Acipenser persicus* after injections with BNF or BaP for 96 hours. The results are mean \pm SDE; n=6.

of the deethylated product, resorufin, following the procedures of Burke and Mayer (1974).

Cytochrome P4501A1 content

The cytochrome P4501A protein was assessed by a semi-quantitative indirect enzyme-linked immunosorbent assay (ELISA) following the method described of Goksüyür (1991) using a monoclonal antibody against cod liver P4501A1.

GST activity

GST activity was determined using the method of Habig et al. (1974). This was accompanied by an increase in absorbance at 340 nm due to the formation of the conjugate 1-chloro-2, 4-dinitrobenzene (CDNB), using as a substrate reduced glutathione (GSH). The reaction mixture was prepared by mixing 1.5 ml sodium phosphate buffer 0.1 M pH 6.5, 0.2 ml GSH 9.2 mM, 0.02 ml CDNB 0.1 M and 0.1 ml of the sample. The absorbance was measured at 340 nm and 25°C. The increase in absorbance was recorded for total 3 min. The reaction solution without the fish hepatic homogenates and blood lysates was used as blank.

Protein measurement

Protein concentrations were determined by the Bradford procedure (1976) using bovine serum albumin as standard.

Statistical analysis

Mean and standard deviation for descriptive analysis of data was used and data were analyzed by analysis of variance (ANOVA), using SPSS 10.0 software to determine the comparisons. The significance of results was ascertained at p -value less than 0.05.

RESULTS

Time-related activity and levels of cytochrome P4501A in sturgeon exposed to BNF and BaP (from 10 mg/kg body weight) were observed; both EROD and ELISA values increased with increasing time of exposure.

Cytochrome P4501A1 levels showed lower responsiveness when compared to EROD activity measurements after BNF treatment in *A. persicus* (Fig.1). There was 21.4-fold increase in EROD activ-

ity compared to a 1.37-fold increase in CYP1A1 content in this species treated with BNF for 72 h (Fig. 2). Although similar results were obtained after BaP exposure in this fish, EROD activity was increased significantly after 48 h post exposure. There was 32.1-fold increase in EROD activity and 1.2-fold increase in CYP1A1 level in *A. persicus* (Fig. 1 and Fig. 2).

The activity of glutathione-s-transferase in *Acipenser persicus* exposed to 10 mg/kg BNF for 96 h was increased 1.7-fold, while there was no any change in GST activity in fish treated with BaP and the control (Fig. 3).

DISCUSSION

Biotransformation of a wide range of xenobiotics was performed by a CYP1A subfamily, whose catalytic is expressed as activity of EROD. The effect of the PAH compounds, β -naphthoflavone and benzo(a)pyrene on fish CYP1A1 was previously assessed (Zapata-Perez et al., 2002; Gobi and Regoli 2004; Mdegela et al., 2005; Hassanain et al. 2007).

In this study, *A. persicus* exposed to BNF and BaP exhibited a significant liver EROD activity increase for the all the experimental exposures with a maximum value (21.4- and 32.1-folds, respectively) increase after 12 h exposure.

The effect of (BaP) on CYP1A1 was evaluated in *Acipenser persicus*. There was a time-dependent effect on EROD activities in fish liver after 12 h induction of both xenobiotics and maximum value was achieved at 72 h. In previous studies, more or less similar responses as a result of BNF or BaP were obtained, such as Arctic charr, *Salvelinus alpinus* (Wolker et al., 1996), European eel, *Anguilla anguilla* (Gobi and Regoli, 2004), Beluga, *Huso huso* (Karimzadeh et al., 2006) and Egyptian fish, *Oreochromis niloticus* and *Clarias gariepinus* (Hassanain et al., 2007). During the induction of biotransformation enzyme phase I, cytochrome P4501A1 as the xenobiotic could be bound to aryl hydrocarbon receptor. As a result of binding, the gene coding for CYP1A1 and synthesis of CYP4501A1 will be increased (Okey et al., 1994).

This information reveals the response of EROD activity to PAHs (BNF or BaP) that were considered in the current study. There was a higher response of EROD activity compared to CYP4501A1 level measurements after BNF or BaP exposure in *Acipenser persicus*. Zapata-Perez et al (2002) and Hassanian et al., (2007) showed similar results in CYP1A1 level in Tilapias and *Oreochromis niloticus*, respectively. Similar results on CYP1A1 levels were also recorded in different fish (Frasco and Guilhermino, 2002).

In this study, EROD activity induction was accompanied by an increase in CYP1A1 content but some previous studies stated that EROD activity does not lead to new synthesis of enzyme proteins (Jimenez and Stegeman 1990; Hassanian et al., 2007).

Many laboratory experiments have shown an increased level of GST activity following the exposure of various fish species to organic substances (Statinsk et al., 2005). There was an increase in GST activity in liver tissue following exposure to β -Naphthoflavone for 96 hour in guppy, *Poecilia reticulata* (Frasco and Guilhermino, 2002). An increase in GST activity after a 3-day exposure to BaP in Africa catfish (*Clarias gariepinus*) has been observed (Mdegela et al., 2005).

Total activity in liver tissue exposed to β -Naphthoflavone and benzo(a)pyrene showed a minimum increase in total GST activity at low concentrations of both xenobiotics in chub, *Leuciscus cephalus* (Krea et al., 2007).

An increase in the total GST activity by a factor of 1.3 was observed in the liver of *Acipenser persicus*, but in the 96-hour exposure to BaP, there were no changes in GST activity. This could be explained by first or second metabolites that are produced during biotransformation phases. These metabolites could be accumulated and may cause the inhibition of total GST activity (Krea et al., 2007).

EROD and total GST enzyme activities are biotransformation enzymes of exogenous substances. Both are used as biomarkers of aquatic contami-

nants. There have been several studies in laboratory experiments that have shown an increased level of both biomarkers following exposure to xenobiotics in various different fish species. As the results of field conditions are not always similar laboratory results, it is necessary to investigate a combination for a final assessment of biomonitoring aquatic pollution.

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