Comparative Biochemistry and Physiology, Part C 195 (2017) 78–90

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

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

Assessment of immune status of yellowfin seabream (*Acanthopagrus latus*) during short term exposure to phenanthrene

Mehrnaz Shirmohammadi [a,*], Negin Salamat [a], Mohammad Taghi Ronagh [a], Abdolali Movahedinia [a], Gholamreza Hamidian [b]

[a] Department of Marine Biology, Faculty of Marine Science, Khorramshahr University of Marine Science and Technology, Khorramshahr City, Khuzestan Province, Iran
[b] Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

ARTICLE INFO

Article history:
Received 6 February 2017
Received in revised form 23 February 2017
Accepted 23 February 2017
Available online 28 February 2017

Keywords:
Polycyclic aromatic hydrocarbons
Head kidney
Cortisol
Innate immunity
Bioaccumulation

ABSTRACT

The aim of the present investigation was to assess the immune status in yellowfin seabream (*Acanthopagrus latus*) exposed to different concentrations of phenanthrene (Phe) for 14 days. In addition, the Phe accumulation in the fish muscle was measured during the experiment. Fish were injected with different concentrations (0, 2, 20 and 40 mg/kg) of Phe and samples were taken from tissue and blood of fish 1, 4, 7 and 14 days after injection. Exposure of fish to Phe caused a significant decrease in white blood cells, C3 and C4 levels, lysosomal membrane stability, lysozyme activity after 4 days and antibacterial activity after 7 days of the experiment. In contrast, cortisol level significantly increased after 4 days. The concentration of Phe in fish muscle increased rapidly after 4 days. The main tissue changes observed in the head kidney including increase in melanomacrophage centers (MMCs), empty spaces between cells and hemorrhage. The degree of tissue changes ranged from normal to moderate in Phe-treated fish. The size and number of MMCs in treated fish were significantly higher than control. In conclusion, Phe toxicity in yellowfin seabream can induce increased cortisol level, tissue changes and immune suppression.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) represent one of the most abundant forms of organic pollutants which are found in a wide range of aquatic ecosystems (Wang et al., 2010). Phenanthrene (Phe) is one of the most dangerous PAH compounds for aquatic organisms. Many studies using Phe as a PAH-model have identified its activation and toxic pathways in fish (Sun et al., 2006; Prosser et al., 2011). PAHs accumulate in animal tissues; however, they do not accumulate to their full potential in the body because many of them can be metabolized in the liver (Allen and Moore, 2004). Nevertheless, these pollutants may result in reactive oxygen species (ROS) formation in the animal body which in turn can cause protein, DNA and lipid damage (Frenzilli et al., 2001; Regoli et al., 2004), enzyme inhibition, significant tissue damage and immune system debilitation (Hannam et al., 2010). On the other hand, due to the production of some intermediate compounds, PAHs may also affect on physiological processes, which consequently may disrupt production and secretion of circulating hormones such as cortisol (Brar et al., 2010).

The primary stress stimulates the hypothalamic–pituitary–interrenal (HPI) axis in fish, which lead to the production of cortisol and other corticosteroid hormones (Rastgar et al., 2016). Cortisol plays important role in the regulating of metabolic energy, stress responses (Belanger et al., 2001) and modulation of the immune system through apoptosis (Weyts et al., 1998).

The fish innate immune system, the first defense line including nonspecific cellular (e.g., phagocytic leukocytes) and humoral (e.g., complement system or lysozyme activity) parameters, maintains fish against different pathogens, even without prior exposure to them (Bols et al., 2001).Various components of the nonspecific immune system served as indicators of the stress response in teleost. The change in the immune status could lead to harmful effects on animal health (Yildiz and Altunay, 2011; Khaniyan et al., 2014). The complement system is composed of approximately 35 proteins in the plasma and on cell surfaces that play a major role in innate immunity and are responsible for the activation of the membrane attack complex and lytic activity against microorganisms (Boshra et al., 2006). The complement system acts as an important link between adaptive and innate immune system in fish

Abbreviations: PAHs, Polycyclic aromatic hydrocarbons; Phe, Phenanthrene; RSA, ROPME Sea Area; HPLC, High-Performance Liquid Chromatography; DTC, Degree of tissue change; WBCs, White blood cells; GVB, Gelatin-veronal buffer; TSA, Tryptic soy agar; ELISA, Enzyme-linked immunosorbent assay; ROS, Reactive oxygen species.

* Corresponding author at: Department of Marine Biology, Faculty of Marine Science, Khorramshahr University of Marine Science and Technology, Khorramshahr City, P.O.Box: 64199-669, Khuzestan Province, Iran.
E-mail addresses: shirmohammadim@yahoo.com (M. Shirmohammadi), Salamatnegin@yahoo.com (N. Salamat), mt.ronagh@yahoo.com (M.T. Ronagh), amovahedinia@yahoo.com (A. Movahedinia), ghamidian@yahoo.com (G. Hamidian).

http://dx.doi.org/10.1016/j.cbpc.2017.02.012
1532-0456/

(Holland and Lambris, 2002). C3 is the main component of the complement system. C4 is another component of complement system which plays an important role in the activation of the classic and lectin pathways (Boshra et al., 2006). Lysozyme is considered as a biomarker of the immune defense in both invertebrates and vertebrates (Abd-El-Rhman, 2009). It disrupts bacterial cell wall by hydrolyzing glycosidic linkages in the peptidoglycan layers (Grinde, 1989; Magnadottir, 2006). Lysozyme, an important antibacterial enzyme, acts against several species of Gram-positive and Gram-negative bacteria (Gopalakrishnan et al., 2009). Neutrophils and macrophages release lysozyme enzyme into the bloodstream during phagocytosis (Mock and Peters, 1990). High activity of this enzyme has been recorded in the lymphomyeloid tissue and plasma (Soltani and Pourgholam, 2007; Caruso et al., 2011).

Head kidney in most teleost acts as a hematopoietic, immune and endocrine organ. Changes in the tissue structure of head kidney in fish exposed to different pollutants impair its functional capacity which may threaten the survival of fish (Dangre et al., 2010; Salazar-Lugo et al., 2013). Size and number of Melanomacrophage centers (MMCs) have been used as suitable biomarkers of environmental stress (Leknes, 2007; Suresh, 2009). The increase in the size and number of melanomacrophage centers plays an important role in innate as well as adaptive immune system. It causes an increase in phagocytic activity against heterogeneous material such as cell particles and migration of lymphocytes to damage tissues during xenobiotic exposure (Kakkar, 2011). Van der Weiden et al. (1994) reported that intraperitoneal injection of 0.27 μg TCDD/kg or more to common carp (*Cyprinus carpio*) caused an increase in the number of MMC in the head kidney. Alteration in the number of MMCs in the hematopoietic tissues of fish is affected by different factors, such as disease process and bleeding (Mikul et al., 2008). An increase in MMCs in the head and trunk of the kidney was reported by Capkin et al. (2010) in the rainbow trout exposed to endosulfan for 96 h.

There is a few information on the immunosuppressive effects of PAHs in fish; however, it has been well documented in mammals (Page et al., 2002; Podechard et al., 2008). The information on bioaccumulation, immune system performance, tissue changes of head kidney and cortisol in PAH-exposed fish in the Persian Gulf is limited. According to Sinaei and Mashinchian (2014) water, sediment and fish in the north of the Persian Gulf are contaminated with a wide range of PAHs. Also, they reported that total PAH was ranged from 0.8–18.3 μg/l in the water and 113.5–3384.3 ng/g (d w) in the sediment of this region.

In the present study, yellowfin seabream (*Acanthopagrus latus*) was selected because of its wide geographic distribution and high commercial importance in the ROPME Sea Area (RSA). The present study aimed to assess bioaccumulation, stress (cortisol plasma level) and innate immune system parameters including total white blood cells (WBC) count, complement 3 (C3), complement 4 (C4), lysosomal membrane stability, lysozyme activity, antibacterial activity and head kidney histological alterations in yellowfin seabream exposed to Phe. Our results provided suggestions that could serve as early biological markers for evaluation of polluted aquatic ecosystems.

2. Materials and methods

2.1. Chemicals

Phe (98% pure) was purchased from Sigma (Code: P11409). C3, C4 and cortisol kits were purchased from Pars Azmun, Iran (Code: 505,035 and 506,035) and DIMETRA, Italy (Code: DKO001) respectively. Other chemicals were bought from Merck (Germany).

2.2. Fish maintenance

Immature yellowfin seabream (15.7 ± 0.2 cm mean body length and 81.1 ± 12.1 g mean body weight) were collected from the Musa creek, located in the northwest of the Persian Gulf, by trawling. Fish were kept in 300-l tanks filled with UV-treated running seawater (temperature 25 ± 1 °C, dissolved oxygen 7.12 ± 0.10 mg/l, pH 7.2 ± 0.06) for 10 days to acclimate to laboratory conditions. Animals were fed twice daily using commercial dry pellets (Dibaq-Diprotg S.A., Segovia, Spain) up to 24 h before the experiment. Water renewed daily at about 50% during the experimental period to reduce ammonia content and other waste materials. The chemical characteristics of the water were maintained constant during the experiment and fish were kept under photoperiod (12/12 h light/dark cycle). During acclimatization period no clinical sign was observed. Immature fish used in our study to remove possible effects of sex maturity in fish. Immune responses in fish were directly dependent on internal factors such as sex hormones changes. Sex hormones can modulate the development and function of the immune system (Wenger et al., 2011).

2.3. Experimental design

Fish were distributed into five experimental groups with three replicates (15 tanks) and each tank consisted of twelve fish: group 1 (control) with no injection, Group 2 were exposed to solvent (injected only with coconut oil), Groups 3 to 5 were injected with 2, 20 and 40 mg/kg-body weight of Phe. All fish were anesthetized with a 2-phenoxyethanol solution (0.2%) and weighed before injection. Then, groups 3 to 5 were intraperitoneally injected with coconut oil (10 μl/g-bw) containing Phe (2, 20 and 40 mg/kg-bw), while the solvent group received 10 μl g^{-1} coconut oil without Phe. Samplings were performed at different time intervals (1, 4, 7and 14 days) after injection. Choosing of different Phe concentrations was based on the previous studies on the PAHs toxicological effects on fish (Tintos et al., 2007; Nahrgang et al., 2009; Pathiratne and Hemachandra, 2010; Phalen et al., 2014). Different Phe concentrations were detected according the reported levels of PAHs in relation to pollution of sediments including such regions as the Elizabeth River, Virginia (21,200 μg/g total PAH), Newark Bay, New Jersey (1960 μg/g total PAH), the Black River, Ohio (1096 μg/g total PAH) and the huangpu River, Shanghai (5.8–11 μg/g) (Roberts et al., 1989; Baumann and Harshbarger, 1995; Huntley et al., 1995; Jinshu et al., 2004). Vossoughi et al. (2005) reported that total PAH was ranged from 11.2–89.6 mg/kg in the sediment of the Persian Gulf. Parang et al. (2013), detected a Phe concentration of 32 μg/g in Indian shrimp (*Penaeus indicus*) from Qeshm Island, The Persian Gulf. PAHs can quickly absorb on sediment because of their lipophilic nature, and can bioavailable to marine animals by food. Therefore, their biomagnification in food chain could be dangerous for animal health (Adamo et al., 1997; Parang et al., 2013). Also, high doses of Phe, used in the present study, purposed to display an actuated biological response in an acute exposure and a rapid peak in effects. It seems likely that similar biological response could occur due to exposure of fish to PAHs in the environment.

2.4. Blood and tissue sampling

The fish were euthanized by 2-phenoxyethanol solution (0.2%) and blood samples were taken from the caudal vein of three fish per tank using heparinized syringes at days 1, 4, 7 and 14. A part of each blood sample was used for leukocyte counting and lysosomal membrane stability analysis and the remaining was immediately centrifuged for 10 min. Then, plasma was separated and freezed at −80 °C for further analysis. Later, samples were taken from fish muscle and maintained in aluminum foil at −20 °C for further analysis of Phe accumulation. Also, samples were taken from the head kidney and fixed in 15% formalin buffer solution for 48 h for further histological study.

2.5. Phe concentrations in fish muscle samples

Phe concentration was analyzed in fish muscle according to the method described by Perugini et al. (2007). Two g of each freeze-dried