Aquatic Toxicology 92 (2009) 86–94

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Chronic exposure to low concentration of arsenic is immunotoxic to fish: Role of head kidney macrophages as biomarkers of arsenic toxicity to *Clarias batrachus*

Soma Datta[a], Debabrata Ghosh[a,1], Dhira Rani Saha[b], Shelley Bhattacharaya[c], Shibnath Mazumder[a,*]

[a] *Immunobiology Laboratory, School of Life Sciences, Visva Bharati University, Santiniketan 731 235, India*
[b] *Microscopy Laboratory, National Institute of Cholera and Enteric Diseases, P-33, Scheme XM, C.I.T. Road, Beliaghata, Kolkata 700 010, India*
[c] *Environmental Toxicology Laboratory, School of Life Sciences, Visva Bharati University, Santiniketan 731 235, India*

ARTICLE INFO

Article history:
Received 14 November 2008
Received in revised form 10 January 2009
Accepted 13 January 2009

Keywords:
Arsenic
Head kidney
Macrophage
Immunotoxicity
Clarias batrachus

ABSTRACT

The present study was aimed at elucidating the effect of chronic low-level arsenic exposure on the head kidney (HK) of *Clarias batrachus* and at determining the changes in head kidney macrophage (HKM) activity in response to arsenic exposure. Chronic exposure (30 days) to arsenic (As_2O_3, 0.50 μM) led to significant increase in arsenic content in the HK accompanied by reduction in both HKM number and head kidney somatic index (HKSI). Arsenic induced HK hypertrophy, reduction in melano-macrophage population and increased hemosiderin accumulation. Transmission electron microscopy of 30 days exposed HKM revealed prominent endoplasmic reticulum, chromatin condensation and loss in structural integrity of nuclear membrane. Head kidney macrophages from exposed fish demonstrated significant levels of superoxide anions but on infection with *Aeromonas hydrophila* were unable to clear the intracellular bacteria and died. Exposure–challenge experiments with *A. hydrophila* revealed that chronic exposure to micromolar concentration of arsenic interfered with the phagocytic potential of HKM, helped in intracellular survival of the ingested bacteria inside the HKM inducing significant HKM cytotoxicity. The immunosuppressive effect of arsenic was further evident from the ability of *A. hydrophila* to colonize and disseminate efficiently in exposed fish. Enzyme linked immunosorbent assay indicated that chronic exposure to arsenic suppressed the production of pro-inflammatory 'IL-1β like' factors from HKM. It is concluded that arsenic even at very low concentration is immunotoxic to fish and the changes observed in HKM may provide a useful early biomarker of low-level xenobiotic exposure.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

The semimetal arsenic (As) is one of the most hazardous substances released in the aquatic environment as a result of both geogenic and anthropogenic processes (ATSDR, 2002). In nature arsenic can exist in the inorganic and organic form and the inorganic form has been found to be more toxic (Liao et al., 2004).

Arsenic is known to affect different physiological processes in mammals. It has been observed that exposure to high concentrations of arsenic is lethal to most organisms and chronic exposure to low concentrations of this toxicant is responsible for several disease conditions (Hughes, 2002). There are reports describing that intake of arsenic through drinking water causes melanosis, keratosis and hyperpigmentation in humans (Guha Majumdar et al., 1998). Arsenic can induce genotoxicity through generation of reactive oxygen species (ROS) and lipid peroxidation (Kannan and Jain, 2000). It can inhibit enzyme functions, cause cellular GSH depletion, and induce DNA oxidation (Lynn et al., 1997). Arsenic also inhibits Iκ-B kinase (Roussel and Barchowsky, 2000), promotes the activation of AP-1, and up-regulates mitogen activated protein kinases (Cavigelli et al., 1996). There are also reports on arsenic inhibiting cellular metabolism, affecting mitochondrial respiration and synthesis of adenosine triphosphate (Abernathy et al., 1999). Other effects of arsenic on mammals include activation of estrogen receptors, inhibition of angiogenesis, induction of heat shock proteins and oxidation of glutathione (Bernstam and Nriagu, 2000). Arsenic is immunotoxic exhibiting its effects on a variety of immune responses, such as reducing delayed hypersensitivity reactions, modulation of co-receptor expression and release of lymphokines, inhibiting mitogen activated T cell proliferation and increasing free intracellular Ca^{2+} production (Galicia et al., 2003; Goytia-Acevedo et al., 2003).

Aquatic habitats are the final sink for many chemicals and water can serve as the vehicle for exposure to many toxic agents. The occurrence of pollutants in aquatic environments influences the immune system and compromises the health and survival of fish

* Corresponding author. Tel.: +91 3463 261268; fax: +91 3463 261176.
E-mail address: shibnath1@yahoo.co.in (S. Mazumder).
[1] Present address: Department of Biochemistry, Uniformed Services University of Health, Sciences, Bethesda, MD 20814, USA.

0166-445X/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2009.01.002

(Sweet and Zelikoff, 2001). In the aquatic milieu arsenic exists either in arsenite (As^{3+}) or arsenate (As^{5+}) form which is inter-converted through redox and methylation reactions. Fish appeared to have evolved different mechanisms for biotransformation of arsenic to less toxic forms, which are then readily excreted (Bears et al., 2006). Since the assessment of xenobiotic contamination of the aquatic environment is based on results of tissue analysis, fish are considered as important organisms for assessing contamination of the aquatic ecosystem (Gernhöfer et al., 2001).

Our knowledge on the effect of arsenic on the fish immune system is relatively limited. Fish offer a number of advantages over the currently available immunotoxicological mammalian models because they are not only amenable to laboratory and field studies but also provide a large repository of immune cells (Zelikoff et al., 1991). It has been observed that, although arsenic accumulates primarily in retina, liver and kidney of fish, it can interfere with the fish immune system by suppressing antibody and cytokine production (Ghosh et al., 2007). It has also been reported that short-term exposure of fish to non-lethal concentration of arsenic can induce time-dependent and tissue-specific changes in B cell and T cell functioning, making them susceptible to infections (Liao et al., 2004; Ghosh et al., 2006).

The innate immune response in fish consists of a variety of immune defense mechanisms and serves as the first line of defense against invading pathogens (Magor and Magor, 2001). In teleosts head kidney (HK) represents the main immunocompetent organ and head kidney macrophages (HKMs) are important components of the fish innate immune system (Fishelson, 2005). The HKM engulf invading pathogens and destroy them by producing reactive oxygen species (Nayak et al., 2007). Fish macrophages also help in the release of several pro-inflammatory cytokines important for the initiation of host immune responses (Pressley et al., 2005; Lage et al., 2006). There are several reports on the role of arsenic on mammalian macrophage functioning but only few detailed studies on how arsenic interacts with the innate immune system and affects the immunopathological status of the fish. Studies on zebrafish revealed that arsenic inhibited synthesis of macrophage-derived cytokines like TNFα and IFN-γ thereby compromising the antiviral responses (Lage et al., 2006). Exposure of fish to various concentrations of arsenic also affected the phagocytic potential of macrophages and helped in the dissemination and persistence of viral and bacterial pathogens into distant host tissues (Ghosh et al., 2007; Nayak et al., 2007). Together, these results suggest that, in addition to lymphocytes fish macrophages may constitute sensitive targets of arsenic.

Earlier studies on the effects of arsenic on the innate immune system of fish were either conducted *in vitro* or even when conducted *in vivo* used exposure levels not reported in nature (Hermann and Kim, 2005; Ghosh et al., 2006). In the present study we have made an attempt to understand the effects of micromolar arsenic exposure on the innate immune responses of *Clarias batrachus* by studying the structural and functional alterations in HK and HKM. *C. batrachus* was used as a model because of its availability round the year, ability to adapt to laboratory conditions and its easily identifiable immune organs. The present study was therefore designed to unravel the effect of non-lethal arsenic exposure on the innate immune responses of *C. batrachus* in terms of ecotoxicological risk assessment.

2. Material and methods

2.1. Bacterial strains

The pathogenic strain of *Aeromonas hydrophila* used in this study was isolated from naturally infected fish (Majumdar et al., 2006). The bacteria were grown to mid-log phase in brain heart infusion broth (BHI, Hi-Media, India) at 30 °C, harvested by centrifugation at 10,000 × *g* for 15 min at 4 °C, washed with phosphate buffered saline and used for experimental work.

2.2. Animal care and maintenance

Healthy *C. batrachus* (50–70 g) obtained locally were maintained in 50-L glass tanks (six to seven fish in each tank) under natural photoperiod. The fish were fed on boiled chicken liver ad libitum every day and excess food was always removed from the tanks. Fish were acclimated to the laboratory conditions for 15 days prior to exposure to arsenic. Diseased fish or fish showing any abnormal behavior were removed immediately from the tanks.

2.3. Arsenic exposure

The method for preparation of arsenic stock solution for *C. batrachus* has been described earlier (Ghosh et al., 2006). In the present study fish were exposed to arsenic for 1 day and 30 days. An exposure concentration corresponding to 0.50 μM of arsenic was selected which is non-lethal to *C. batrachus* and also relevant to environmental level of contamination (CEPA, 1993; ATSDR, 1997; Bears et al., 2006). The water quality parameters viz. pH, hardness, dissolved oxygen content were checked regularly and the arsenic concentration in experimental tanks maintained as described earlier (Ghosh et al., 2006). Unexposed fish were maintained in separate tanks without arsenic under identical conditions. Fish were sacrificed using an overdose of MS 222 (Sigma, USA).

2.4. Atomic absorption spectrophotometry

Head kidneys from unexposed and exposed *C. batrachus* were weighed and digested in a quartz beaker using nitric acid: perchloric acid (5:1) mixture in a closed chamber (Takatsu and Uchiumi, 1998). The digested samples were diluted to 5 mL with purified water in a calibrated flask and arsenic content in the tissue measured using atomic absorption spectrophotometer (PerkinElmer, USA). To confirm the analytical performance for arsenic detection, National Institute of Standards and Technology (NIST) standard reference material (SRM) citrus leaf (SRM 1577) and oyster tissue (SRM 1566) were analyzed (Table 1). The mean recovery of arsenic was 89.2% and detection limit of arsenic was about 0.75 ng mL^{-1} as a solution which is within the ranges reported earlier (Takatsu and Uchiumi, 1998).

2.5. Determination of head kidney somatic index

Unexposed and exposed *C. batrachus* were sacrificed and their body weights recorded. Head kidney was removed carefully, the fresh weight recorded and alterations in the head kidney somatic index (HKSI) calculated and compared with the values obtained from unexposed fish (Ghosh et al., 2007).

$$\text{HKSI} = \frac{\text{Wt. of HK}}{\text{Wt. of fish}} \times 100$$

2.6. Separation of HKM

Head kidneys from unexposed and exposed fish were removed aseptically and placed in RPMI-1640 (Gibco, USA) supplemented with 1% penicillin–streptomycin and dissociated by passing through 100-μm wire mesh (Sigma). The single cell suspension thus obtained was again washed and layered on 34/51% Percoll gradient (Sigma) (Secombes, 1990) and centrifuged at 400 × *g* at 4 °C for 20 min. The phagocyte-rich fraction appearing above 34/51

Download English Version:

https://daneshyari.com/en/article/4530590

Download Persian Version:

https://daneshyari.com/article/4530590

Daneshyari.com