Ecotoxicology and Environmental Safety 139 (2017) 18–26

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Negative effect of chronic cadmium exposure on growth, histology, ultrastructure, antioxidant and innate immune responses in the liver of zebrafish: Preventive role of blue light emitting diodes

Shuang-Shuang Yuan, Zhen-Ming Lv, Ai-Yi Zhu, Jia-Lang Zheng*, Chang-Wen Wu*

National Engineering Research Center of Marine Facilities Aquaculture, Zhejiang Ocean University, Zhoushan 316022, PR China

ARTICLE INFO

Keywords:
Spectra
Oxidative stress
Metal exposure
Non-specific immunity
Nrf2
NF-κB

ABSTRACT

The present study explored the possible preventive effects of blue light emitting diodes (LEDs) on cadmium (Cd)-induced oxidative stress and immunotoxicity in zebrafish. To this end, zebrafish were exposed to a white fluorescent bulb or blue LEDs (LDB, peak at 450 nm, at an irradiance of 0.9 W/m^2), and 0 or 30 $\mu g\ L^{-1}$ waterborne Cd for 5 weeks. Growth performance, survival rate, and hepatic histology, ultrastructure, antioxidant and innate immune responses were determined in zebrafish. Cd exposure alone reduced growth and survival rate, and induced oxidative damage and changes in histology and ultrastructure. However, Cd exposure in combination with LDB apparently relieved these negative effects. The alleviation of adverse effects might result from the up-regulation of antioxidant and innate immune genes at transcriptional, translational, or post-translational levels. Cd exposure alone dramatically enhanced mRNA levels of nuclear transcription factor κB (NF-κB) and E2-related factor (Nrf2). However, compared to Cd exposure alone, Cd exposure in combination with LDB apparently down-regulated both genes. Taken together, our results suggest that chronic Cd exposure induced a negative effect on zebrafish, possibly involved in NF-κB-induced immunotoxicity and Nrf2-induced oxidative stress. Finally, for the first time, our data demonstrated that LDB could protect fish against Cd toxicity.

1. Introduction

Cadmium (Cd) is a nonessential metal that is a source of contamination to the aquatic ecosystem (Van Dyk et al., 2007). The waterborne Cd level can reach 0.05 mg L^{-1} (Huang and Li, 2007), and even exceed 1 mg L^{-1} in some industrialized areas in China (Dong et al., 2015; Ma et al., 2008). In particular, Cd is a genotoxic metal that has been already classified as a human carcinogen, and is considered toxic to aquatic organisms by inducing the generation of oxidative stress and immunotoxicity (Zheng et al., 2016a, 2016b). In the case, some studies have started to focus on the search for interventions aimed at improving fish tolerance to metal toxicity by enhancing antioxidant defenses and immunity, which has been an area of interesting research. For example, early conditioning to mild metal stress primes fish to withstand episodes of high metal stress experienced later in life (Zheng et al., 2016c). Addition of waterborne calcium or humic substances mitigates the severity of Cd-induced injuries in fish (Meinelt et al., 2001). Decrease in water temperature, and an increase in salinity or dietborne nutrient or drug supplements also ameliorate metal toxicity in fish (Jiang et al., 2015; Park et al., 2014; Vergauwen et al., 2013; Zheng et al., 2015). However, to the best of knowledge, little information is available on the effect of changes in light spectrum composition of water on metal-induced toxicity.

Light emitting diodes (LEDs) affect the growth and development of fish by changing spectral composition (Migaud et al., 2007; Villamizar et al., 2009). The blue LEDs (LDB) are effective light sources, which play a positive effect in fish (Yeh et al., 2014). For example, LDB positively affect the growth and development of *Dicentrarchus labrax* larvae (Villamizar et al., 2009), inhibit oxidative stress and enhance immune function in starved *Amphiprion melanopus* (Choi et al., 2012), and relieve temperature-induced oxidative damage in *Carassius auratus* (Kim et al., 2014). Our recent study has also suggested that LDB promote fish innate immunity (Zheng et al., 2016d). Therefore, we hypothesize that LDB can protect fish liver against Cd-induced antioxidant and immune disturbances with an aim of elucidating measures that must be carried out to deal with metal toxicity in fish.

To test the hypothesis, zebrafish were exposed to Cd and LDB, singly or in combination for 5 weeks. The mRNA, protein, and/or activity levels of several related genes involved in oxidative stress and immune responses, growth, survival, histology, ultrastructure, lipid peroxidation

* Corresponding authors.
E-mail addresses: zhengjialang@aliyun.com (J.-L. Zheng), wucw2013@126.com (C.-W. Wu).

http://dx.doi.org/10.1016/j.ecoenv.2017.01.021
Received 31 October 2016; Received in revised form 9 January 2017; Accepted 12 January 2017
Available online 16 January 2017
0147-6513/

(LPO), and protein carbonylation (PC) were analyzed in zebrafish. The chosen genes included copper/zinc-superoxide dismutase (Cu/Zn-SOD), catalase (CAT), lysozyme (LZM), alkaline phosphatase (AKP), NF-E2-related factor 2 (Nrf2), and nuclear factor 'kappa-light-chain-enhancer' of activated B cells (NF-κB). Among these genes, Cu/Zn-SOD and CAT are considered to be the vital first line of defenses against oxidative stress by reducing the amount of reactive oxygen species (ROS) (Yu, 1994). LZM and AKP are vital markers for evaluating fish immunity owning to their important functions in the immune system (Alexander and Ingram, 1992; Zhou et al., 2012). Nrf2 and NF-κB control antioxidant- and immune-related genes through binding sequences in the promoter region of downstream genes (Jiang et al., 2015; Zeng et al., 2016). All of these genes are responsive to Cd and LDB exposure in the liver of zebrafish (Zheng et al., 2016a, 2016b, 2016d). However, to the best of our knowledge, few studies have been performed to evaluate the interplay between Cd and LDB. The present study is the first report about the effect of LDB on metal toxicity in fish, which would provide some information for dealing with metal toxicity and some strategic measures to promote the production of healthy fish.

2. Materials and methods

2.1. Experimental design

Female zebrafish (AB strain) about 4-weeks of age were obtained from Zhoushan fisheries research institute, Zhoushan, China. Prior to the experiment, zebrafish were acclimated under a white fluorescent bulb (a simulated natural photoperiod; SNP; 0.9 W/m^2) for 2 weeks. At the beginning of the trial, uniform-sized fish (initial body weight: 0.14 ± 0.03 g, mean ± SEM) were exposed to SNP (the control group) or blue LED (LDB, peak at 450 nm, 0.9 W/m^2), and 0 or 30 μg L^{-1} (equal to 0.27 μmol Cd L^{-1}, corresponding to 0.6% of the 96 h LC_{50}) waterborne Cd for 5 weeks. The values of irradiance and Cd concentration were set according to the study from Zheng et al. (2016c) and Zheng et al. (2016b). 70 fish (mean initial weight: 2.56 ± 0.27, means ± SEM) were stocked in each 20 L fiberglass tank. Each treatment was assigned to 4 tanks in a completely randomized design, with 16 tanks for the experiment. The fish were fed commercial Haid® diets (lipid, protein, Cd contents of 10.6%, 42.4%, 0.02 mg kg^{-1} on a dry matter basis, respectively) to apparent satiation three times daily. Water quality parameters were monitored once five days and continuous aeration to maintain the dissolved oxygen level near saturation. Water temperature was maintained at 25.8 ± 0.4 °C with a 12 L: 12D photoperiod. Dissolved oxygen and pH were 7.46 ± 0.34 mg L^{-1} and 7.84 ± 0.45, respectively. Cd concentrations for the control group and Cd exposed group were 0 and 29 ± 3 μg L^{-1}, respectively (equal to 0 for the control and 0.25 ± 0.02 μmol Cd L^{-1} for the Cd exposed group). The metal concentrations were measured using flame atomic absorption spectroscopy (FAAS).

2.2. Histological and ultrastructural observations

For histological observation, samples of liver were fixed for 24 h in 10% neutral buffered formalin. After dehydrated in graded ethanol concentrations and embedded in paraffin wax, sagittal sections (5 mm thick) were stained with hematoxylin/eosin (H & E), and then prepared for light microscopy (Zheng et al., 2011). The ultrastructural observations were conducted according to the methods from Zheng et al. (2016b). Generally, specimens of liver were prefixed by immersion in 2.5% glutaraldehyde in phosphate buffer solution (pH 7.2) at 4 °C. Post-fixation was in cold 1% aqueous osmium tetroxide for 1 h. They were rinsed again in phosphate buffer, dehydrated in a graded ethanol series, and embedded in epon-araldite mixture. Ultrathin sections were cut and stained with uranyl acetate and lead citrate, and examined under a HITACHI H-600 electron microscope.

2.3. LPO and PC analysis

Liver tissue was homogenized in an ice-cold 0.1 M phosphate buffered solution (PBS, pH 7.4) containing 1 mM of ethylene diamine tetraacetic acid (EDTA), 0.5 M of saccharose, 0.15 M of KCl, and 1 mM of dithiothreitol (DTT). The homogenates were centrifuged at 500× *g* for 15 min at 4 °C to precipitate large particles and the supernatants were centrifuged again at 12,000× *g* for 30 min at 4 °C. The supernatants were maintained at 4 °C for biochemical analysis.

Lipid peroxidation (LPO) was determined by the thiobarbituric reactive species (TBARS) assay according to the method described by Livingstone et al. (1990). Protein carbonylation (PC) were determined as previously described (Lushchak et al., 2005).

2.4. Enzymatic activities and protein level

The supernatants were directly applied to the assay of enzymatic activities and protein levels of Cu/Zn-SOD, CAT, AKP and LZM. Cu/Zn-SOD (EC 1.15.1.1) activity was measured according to the method of Beauchamp and Fridovich (1971) based on aerobic reduction of NBT at 535 nm by superoxide radicals. Catalase activity (CAT, EC 1.11.1.6) was determined by measuring the rate of disappearance of H_2O_2 according to methods described by Beutler (1982).

AKP and LZM activities were determined according to Zhou et al. (2012) and Engstad et al. (1992) respectively, using an assay kit (Nanjing Jiancheng Bioengineering Institute, China). The protein levels of Cu/Zn-SOD, CAT, AKP and LZM were detected using commercially fish enzyme-linked immunosorbent assay (ELISA) kits (Cusabio, Wuhan, China) in accordance with the manufacturer's instructions. Soluble protein content was determined according to the method of Bradford (1976) using bovine serum albumin (BSA) as a standard. All enzyme activities were expressed as U (units) per mg of soluble protein. All protein levels were expressed as ng per mg of soluble protein. Four biological replicates and two technical replicates were used in the analysis.

2.5. Expression levels of genes

Extractions of total RNA from liver and first strand cDNA synthesis were performed according to the methods in our recent study with slight modification (Zheng et al., 2013). Q-PCR reactions (20 μL) were performed in 96-well plates in an Applied Biosystems Prism 7500 Sequence Detection System (Applied Biosystems, USA) with SYBR® Premix Ex Taq™ Kit (Takara), containing 10 μL SYBR®Premix Ex Taq Master Mix, 2 μL of cDNA, and 0.2 μM of each primer. The primer sequences of each gene used in this analysis are given in Table 1. The

Table 1
Primers used for real-time PCR analysis from zebrafish.

Gene	Primer sequences (from 5′to 3′)	Size (bp)	Accession no.
Cu/Zn-SOD	F: GTCCGCACTTCAACCCTCA R: TCCTCATTGCCACCCTTCC	227	BC055516
CAT	F: CAAGGTCTGGTCCCATAAA R: TGACTGGTAGTTGGAGGTAA	217	BC051626
AKP	F: GAGCCAGCAGACCTGAACTA R: CAATGCGTCCACCTTCCA	138	BC052139
LZM	F: ACCCACCGAGTGCGTTCA R: TCAGCCCGTCCATTTTCA	197	BC162644
Nrf2	F: TCGGGTTTGTCCCTAGATG R: AGGTTTGGAGTGTCCGCTA	188	AB081314
NF-κB RelA	F: CAACGACACCACGAAAACG R: CGTCAGGAATCTTGAATGGGT	188	NM_001001839
β-Actin	F: CCACCACAGCCGAAAGAG R: GGATACCGCAAGATTCCATA	179	AF057040
GAPDH	F: GTAACTCCGCAGAAAAGCC R: CAAAAGAAACTAACACACAC	153	BC095386