Chemosphere 208 (2018) 907–915

Contents lists available at ScienceDirect

Chemosphere

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

Mitochondrial apoptotic pathway mediated the Zn-induced lipolysis in yellow catfish *Peteobagrus fulvidraco*

Dan-Dan Li [a], Zhi Luo [a, b, c, *], Shi-Cheng Ling [a], Kun Wu [a], Guang-Hui Chen [a], Jie Cheng [a]

[a] Key Laboratory of Freshwater Animal Breeding, Ministry of Agriculture, Fishery College, Huazhong Agricultural University, Wuhan, 430070, China
[b] Collaborative Innovation Center for Efficient and Health Production of Fisheries in Hunan Province, Changde, 415000, China
[c] Hubei Provincial Engineering Laboratory for Pond Aquaculture, Wuhan, 430070, China

HIGHLIGHTS

- Zn exposure induced the occurrence of apoptosis in the livers and hepatocytes of yellow catfish.
- Zn-induced apoptosis was mediated by intrinsic mitochondrial pathways.
- Zn-induced mitochondrial apoptotic pathways were related to mitochondrial permeability transition.
- Zn induced the mitochondrial-mediated apoptosis and resulted in hepatic lipolysis.
- Our study elucidates the importance of mitochondria-mediated apoptosis in Zn-induced lipolysis.

ARTICLE INFO

Article history:
Received 9 April 2018
Received in revised form
28 May 2018
Accepted 31 May 2018
Available online 8 June 2018

Handling Editor: Jim Lazorchak

Keywords:
Zn
Apoptosis
Mitochondrial apoptotic pathway
Lipid metabolism
Low vertebrates

ABSTRACT

In the study, effects of waterborne zinc (Zn) exposure on apoptosis were investigated, and the potential mechanism of apoptosis participating in the Zn-induced variations of lipid metabolism was explored in a low vertebrate, yellow catfish *Pelteobagrus fulvidraco*. We found that Zn induced occurrence of apoptosis of livers and hepatocytes in yellow catfish. Waterborne Zn also increased hepatic transcriptional levels of p53, cytochrome *c* (Cycs), caspase 3a (Casp3a) and caspase 3b (Casp3b) of yellow catfish. Zn increased caspase 3 activity and reduced the mitochondrial permeability transition (MTP) in yellow catfish hepatocytes. Z-VAD-fmk (caspase inhibitor) and CsA pretreatment (MTP inhibitor) attenuated the Zn-induced apoptosis and reduction in MTP. Z-VAD-fmk pretreatments attenuated the Zn-induced increase in transcriptional levels of p53, Cycs and Casp3b although the differences were not statistically significant between the Zn group and Zn + Z-VAD-fmk group. In contrast, Zn and N-acetylcysteine (NAC) did not significantly influence the reactive oxygen species (ROS) production. Zn significantly reduced triglyceride (TG) content, increased the activities of carnitine palmitoyltransferase 1 (CPT I), hormone-sensitive lipase (HSL) and adipose TAG lipase (ATGL), and the transcriptional levels of p53, Cycs and caspase 3b of the hepatocytes; these Zn-induced effects on TG contents, activities of CPT I, HSL and ATGL, and mRNA levels of p53, Cycs and caspase 3b could partly be reversed by Z-VAD-fmk, suggesting that Zn induced the mitochondrial-mediated apoptosis and reduced lipid accumulation. Taken together, our study demonstrated the importance of mitochondria-mediated apoptosis in Zn-induced lipolysis, which suggested a new mechanism for elucidating metal element influencing lipid metabolism.

Abbreviations: Apaf-1, apoptotic peptidase activating factor-1; ATGL, adipose TAG lipase; B2M, beta-2-microglobulin; Casp3a, caspase 3a; Casp3b, caspase 3b; Casp9, caspase 9; Cycs, cytochrome *c*; CPT I, carnitine palmitoyltransferase 1; CsA, cyclosporin A; ELFA, translation elongation factor; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; HPRT, hypoxanthine-guanine phosphoribosyltransferase; HSL, hormone-sensitive lipase; MMP, mitochondrial membrane potential; MPT, mitochondrial permeability transition; NAC, N-acetylcysteine; PPARα, peroxisome proliferators-activated receptor α; ROS, reactive oxygen species; RPL7, ribosomal protein L7; TBP, TATA-box-binding protein; TG, triglyceride; TUBA, tubulin alpha chain; UBCE, ubiquitin-conjugating enzyme; Zn, Zinc; Z-VAD-fmk, Benzyloxycarbonyl-Val-Ala-Asp(OMe)-fluoromethylketone.

* Corresponding author. Key Laboratory of Freshwater Animal Breeding, Ministry of Agriculture, Fishery College, Huazhong Agricultural University, Wuhan, 430070, China.
E-mail addresses: luozhi99@mail.hzau.edu.cn, luozhi99@aliyun.com (Z. Luo).

https://doi.org/10.1016/j.chemosphere.2018.05.200
0045-6535/

1. Introduction

Zinc (Zn) is an essential trace mineral and plays important roles in the maintenance of the structure and function of many proteins in vertebrates, including fish (Watanabe et al., 1997; King et al., 2016). However, excessive Zn will be toxic (Spry and Wood, 1989), and adversely influence growth and health, and result in the histopathological changes in fish (Dautremepuits et al., 2004; Zheng et al., 2011). Lipid is one of the main energy sources in fish. Our recent studies suggested that waterborne Zn influenced lipid metabolism and hepatic lipid accumulation of yellow catfish *Pelteobagrus fulvidraco* (Zheng et al., 2015; Song et al., 2017). However, further mechanism remained unknown.

Apoptosis is a highly regulated kind of programmed cell death (PCD). Studies found that Zn excess induced apoptosis in mammals and their cell lines (Chung et al., 2000; Franklin and Costello, 2009). Mitochondria play vital roles in cellular functions and affect many important biological progresses, including nutrient and energy metabolism. Mitochondria are also the sources of reactive oxygen species (ROS) and possess vital roles in apoptosis (Sousa and Soares, 2014). Studies suggested that the breakdown of the mitochondrial membrane potential (MMP) mediated Zn excess-induced apoptosis in cell lines, with concomitant release of the mitochondrial cytochrome *c* (Cycs) (Untergasser et al., 2000; Watjen et al., 2002). Then, the apoptosome, which consists of Cycs, apaf-1, and caspase 9, is formed, initiates the caspase cascade and activates caspase 3 (Green and Reed, 1998). Caspase 3 takes part in the digestion of numerous proteins, resulting in an apoptotic phenotype (Rabi and Banerjee, 2008). p53 is a transcription factor which plays a central role in apoptosis (Polyak et al., 1997). Studies demonstrated that Zn excess induced apoptosis in different cell lines; during the process, mitochondrial injury and oxidative stress mediated Zn-induced apoptosis (Jiang et al., 2001; Schrantz et al., 2001). Meantime, ROS accumulation decreases the MMP and increases the membrane permeability, thus resulting in the Cycs release into the cytoplasm (Molina-Jijon et al., 2011) and inducing apoptotic occurrence (Johnson et al., 1996). Accumulating evidence indicated that ROS generation was the modulator of apoptosis, and responsible for the regulation of Zn-induced apoptosis in many cell models (Provinciali et al., 2002; Mirandola et al., 2010).

In response to stress, cells will change their process of nutrient and energy metabolism (Buchakjian and Kornbluth, 2010). In mammals, studies indicated that apoptosis mediated the regulation of lipid metabolism (Blankenberg, 2008; Boren and Brindle, 2012). However, no such studies have been conducted in fish. Accordingly, here, we hypothesize that Zn activated mitochondrial apoptosis pathways and affected lipid metabolism, and ROS mediated the Zn -induced variation of lipid metabolism. To this end, we determined the effects of waterborne Zn on transcription of genes involved in apoptosis of the liver in yellow catfish. Using primary hepatocytes of yellow catfish, the relationship between apoptosis/oxidative stress and lipid metabolism by Zn addition was also explored. For the first time, our study demonstrated the importance of mitochondria-mediated apoptosis in Zn-induced reduction of lipid accumulation, which suggested a new mechanism for metal element exposure influencing lipid metabolism.

2. Materials and methods

2.1. Drug treatment

Zn was added as $ZnSO_4 \cdot 7H_2O$ (Shanghai, China), and its stock solution was prepared at 1 M concentration with sterile double-distilled water. Z-VAD-fmk (inhibitor of caspase), CsA (MMP inhibitor) and NAC (ROS inhibitor) were dissolved in DMSO. Their levels were chosen, based on our and other studies (Shrivastava et al., 2006; Sharma et al., 2012; Pan et al., 2018).

2.2. Experiment treatments

Two experiments were conducted, and they followed the ethical guidelines of Huazhong Agricultural University (HZAU) and all experimental protocols were approved by HZAU.

2.2.1. Expt. 1: in vivo study: investigating effects of waterborne Zn on the transcription of genes of apoptotic pathway

The experiment procedures have been described in details in our parallel studies (Song et al., 2017; Wei et al., 2017), and the dose and time for Zn exposure followed Song et al. (2017). Briefly, 216 healthy fish (body weight: 8.5 ± 0.4 g/fish) were randomly stocked in 9 tanks, 24 fish per tank. They were exposed to three nominal Zn levels of zero (control, without extra Zn addition), 3.85 μM Zn, and 7.69 μM Zn (0, 2.5 and 5% of the 96-h LC50 of Zn for *P. fulvidraco*, Zheng et al., 2013). Each treatment had triplicate tanks. All fish were fed twice daily with commercial diets. Zn concentrations in the tanks for three groups were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Wu et al., 2016), and the values were 0.1077 ± 0.015 μM, 3.892 ± 0.062 μM, 7.908 ± 1.077 μM, respectively. Water temperature ranged from 18.1 °C to 22.5 °C. Natural photoperiod was approximately 14L:10 D. Dissolved oxygen and pH were 7.04 ± 0.53 mg/L and 7.49 ± 0.23, respectively. The experiment continued for 28 days.

2.2.2. Expt. 2: In vitro study: using primary hepatocytes of yellow catfish to investigate the effect of Zn incubation on apoptosis and lipid metabolism

Hepatocytes were isolated from *P. fulvidraco* liver according to Zhuo et al. (2015) and Song et al. (2015). Z-VAD-fmk, NAC and CsA were used to explore the mechanism of apoptosis and oxidative stress mediating Zn affecting lipid metabolism. 8 groups were designed as follows: control, 90 μM Zn, 0.5 mM NAC, 5 μM CsA, 50 μM Z-VAD-fmk, 90 μM Zn + 0.5 mM NAC, 90 μM Zn + 5 μM CsA, 90 μM Zn + 50 μM Z-VAD-fmk. Each treatment was performed in triplicate. The hepatocytes were collected at 48 h for the following determination. Prior to the present study, we conducted the pilot trials to decide the optimal dose and time for the treatments.

2.3. Sample analysis

2.3.1. Ultrastructural observation and TUNEL assay

Ultrastructure analyses of livers were performed according to Song et al. (2013). We randomly examined 10 microscopic fields for each subsample, and the results from each observation were incorporated into the overall results. TUNEL (DNA fragmentation by Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling) assays were used to detect the apoptotic cells with the TUNEL Kit (Roche, Shanghai, China), and the analytical protocols were based on the method of Gupta et al. (2004). For quantifying the percentages of DNA damage, we randomly counted 100 cells in different areas (six fields in each slide) and the results were expressed as the percentage of apoptotic cells to total cell numbers.

2.3.2. Cell viability and TG content

We measured cell viability by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) according to Wu et al. (2016), and analyzed TG content in the hepatocytes by glycerol-3-phosphate oxidase with the commercial kits (Nanjing Jian-Cheng Bioengineering Institute, Nanjing, China).